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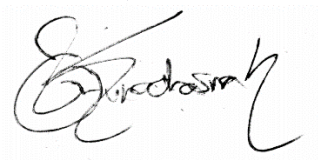
Author: Kobina Andam Quansah	 (signature of author)

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ANAEROBIC TREATMENT OF MUNICIPAL WASTEWATER AT MESOPHILIC (16℃) AND PSYCHROPHILIC (2.5℃) TEMPERATURES USING BENCH SCALE UPFLOW ANAEROBIC SLUDGE BLANKET (UASB) REACTORS

MASTER'S THESIS

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Abstract

The limitation of high TSS wastewater treatment is hydrolysis. At low temperatures, the hydrolytic rate limitation is exacerbated. This project investigates the operational conditions required to improve the hydrolytic rate of mesophilic wastewater at 16℃ and the potential for psychrophilic wastewater treatment at 2.5℃. Parameters studied in this thesis include Methane Production rate, Methane Yield, COD Removal, VFA removal and Upflow velocity. At 16℃ OLRs between 2.5 and 6.4 gCOD/L·d were investigated as well as upflow velocities of **23**, **42** and **68** m/d. Maximum CODRE and Methane Yield were **44%** and **68%** respectively. Average CODRE was low at **33%** and this was attributed to granule-substrate incompatibility.

The possibility of anaerobic wastewater treatment at 2.5℃ was tested and confirmed with an average sCODRE of **31%** and an average Methane Yield of **83%** was achieved during an average OLR of 5.42gCOD/L·d. The organic loading limit for treatment of Grødaland wastewater was below 8gCOD/L·d. A mixture of granules from different sources was more effective at treating wastewater than singly sourced granules. Optimal sCODRE was 32% and occurred at an OLR of 2.45gCOD/L.d

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Abbreviations

ADM1	Anaerobic Digestion Model No.1
AMB	Acetoclastic Methanogenic Bacteria
ATP	Adenosine Triphosphate
AWT	Anaerobic Wastewater Treatment
COD	Chemical Oxygen Demand
CODRE	COD Removal Efficiency
sCOD	Soluble COD
pCOD	Particulate COD
DAF	Dissolved Air Flotation
ECP	Extracellular Polymers
EGSB	Expanded Granular Sludge Blanket
DO	Dissolved Oxygen
F/M	Food Mass Ratio
HAc	Acetic Acid
HMB	Hydrogenotrophic Methanogenic Bacteria
HRT	Hydraulic Retention Time
IVAR	*Interkommunalt Vann Avløp og Renovasjon*
LCFA	Long Chain Fatty Acid
MPB	Methane Producing Bacteria
OHPB	Obligate Hydrogen Producing Bacteria
OLR	Organic Loading Rate
ORP	Oxygen Reduction Potential
RE	Removal Efficiency
SRB	Sulphate Reducing Bacteria
SCFA	Short Chain Fatty Acid
SRT	Solid/Sludge Retention Time
STP	Standard Temperature and Pressure

TN	Total Nitrogen
TP	Total Phosphorous
UASB	Up-flow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solid
WWTP	Wastewater Treatment Plant

1.0 Introduction

The purpose of wastewater treatment is to expedite the natural process of water purification. Primary and secondary sewage treatment have been a necessity since the humankind's urbanization. The simplest, oldest and commonest primary sewage treatment process is the Fosses Moura's tank developed in France in the 1860s. English engineers later modified this system into the septic tank in 1895. During this period, depth and trickling filters were the secondary treatment options available. However, these primary and secondary treatment methods were slow and ineffective. [1, 2]

In the 1910s, Ardern and Locket published a paper describing all elements of the activated sludge process. This process accelerated sewage treatment. The system provided air for ordinary heterotrophic organisms (OHO's) in a CSTR. It also incorporated a secondary settler to sediment and recycle the OHO's. Higher "treatment" parameters were met at the expense of energy used for aeration. High energy prices in the 1970s rendered aerobic methods unattractive. Scientists began research into energy-saving alternatives to aerobic wastewater treatment. Lettinga and company eventually invented the UASB reactor in the mid-1970s. The UASB system accomplished both requirements of speed and energy recovery. UASB equivalents of aerobic methods were also compact and simpler to operate and maintain. Thus, UASB reactors accomplish the main criteria of modern wastewater treatment, which goes beyond mere primary, secondary and tertiary pollutant removal.

Modern wastewater treatment is driven by effluent quality and cost minimization, but energy and resource recovery. In this regard, anaerobic treatments have been shown as more economically beneficial than aerobic treatments[3]. Particularly, the UASB process reduces production and operational costs by eliminating the use of complex infrastructure. The little energy used in running these reactors can

usually be recovered in the form of biogas. To date, up to 70% of full-scale water anaerobic sewage treatment installations worldwide are based on UASB reactors [4]. However, temperature has been the major limitation on the geographic potential of this technology.

UASB reactors adequately treat wastewater in tropical temperatures (>30°C). Their efficiency is improved when used in conjunction with other technologies like anaerobic filters, UASB-digesters, UASB-septic tank or two-stage UASB systems[5]. However, standalone operation of UASB reactors under low temperature mesophilic and psychrophilic conditions is an unattractive option due to the inhibition of treatment efficiency and lower biogas potential.

2.0 Background

This chapter describes the processes involved in anaerobic digestion. It focuses on process mechanisms that contribute to ideal and suboptimal anaerobic digestion. Operational control measures to mitigate suboptimal conditions are also presented.

2.1 Anaerobic Digestion and the Anaerobic Digestion Model (ADM1)

Anaerobic treatment is a process by which microorganisms convert biodegradable organic materials in the absence of oxygen. “Anaerobic” can be considered as having an Oxygen Reduction Potential (ORP) <-200mV. And according to the Anaerobic Digestion Model Number 1 (ADM1), there are four main steps involved in anaerobic digestions[6]. These are: Disintegration and Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis (Fig 1.) A complex consortium of respective microbial guilds mediates the reactions/conversion of organic matter primarily into carbon dioxide and methane. The anaerobic conversion mechanisms in non-sludge systems, like AWT treatment, is characterized by the same conversion reactions and the growth of similar microbial guilds as described by the ADM1. [6, 7]

2.1.1 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.[6]” Disintegration involves the breakdown of complex composite organic material into polysaccharides, proteins and lipids. Hydrolysis uses the products of disintegration as substrates and degrades them into soluble organic substances such as monosaccharides, amino acids and fatty acids respectively. Facultative and obligate anaerobes hydrolyze compounds by releasing extracellular enzymes to perform hydrolysis. [8]

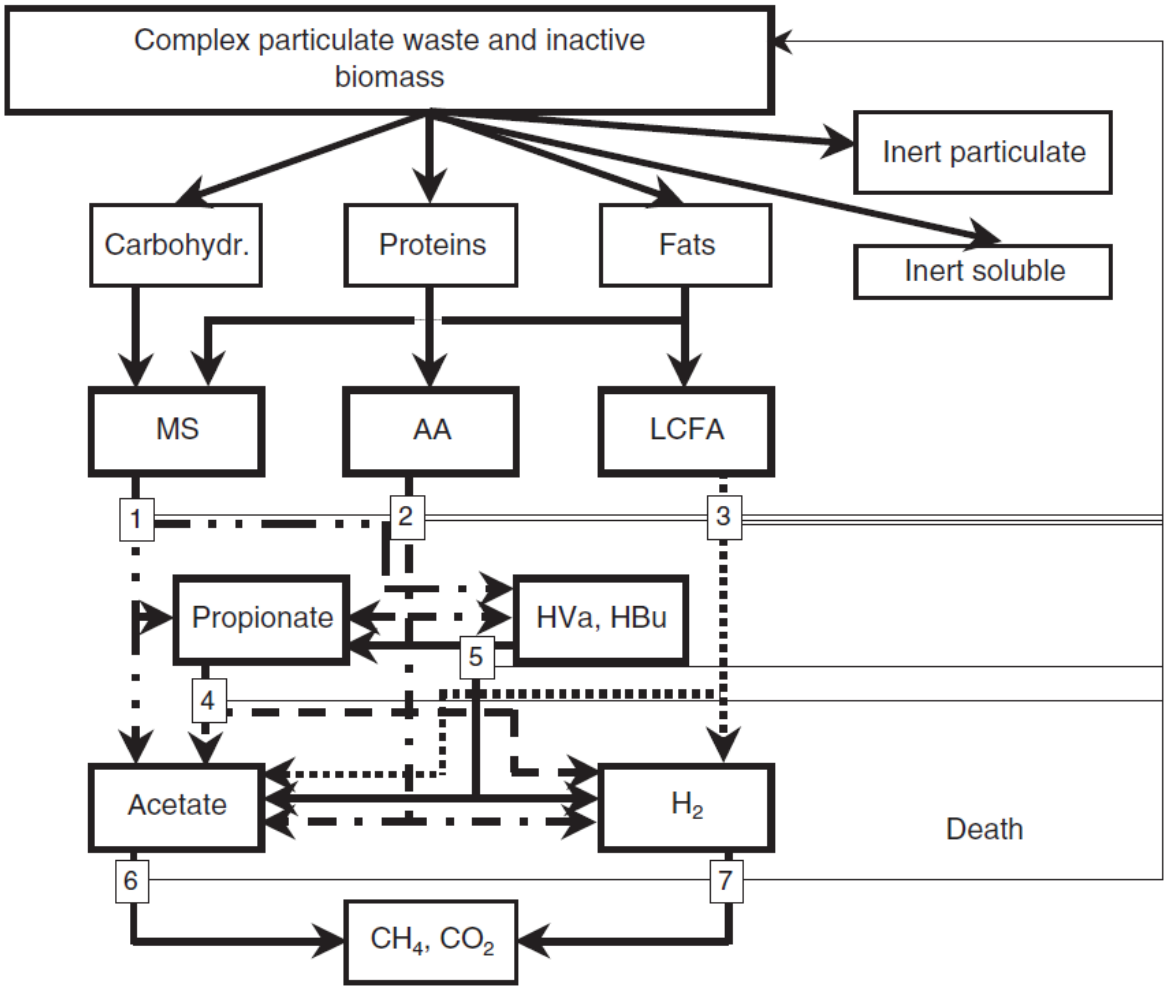

Figure 2.1 *ADM1 Model including biochemical processes: 1.Acidogenesis from carbohydrates, 2.Acidogenesis from amino acids, 3.Acetogenesis from LCFA, 4.Acetogenesis from propionate, 5.Acetogenesis from butyrate and valerate, 6.Aceticlastic methanogenesis and 7.Hydrogenotrophic methanogenesis [6]*

The ADM1 conceptualizes hydrolysis using two possible models: (a) The organisms secrete enzymes to the bulk liquid where they are adsorbed onto a particle or react with a soluble substrate (Jain et al, 1992). (b) The organisms attach to a particle, produce enzymes in its vicinity and benefit from soluble products released by the enzymatic reaction [6].

Several extracellular enzymes have been characterized. Cellulases, amylases and glucanases depolymerize polysaccharides into monosaccharides. Proteases

degrade proteins into peptides. Lipases convert fats and oils into fatty acids. A few species of hydrolyzing bacteria and their preferred substrates (Table 2.1)

The type and concentration of substrate controls enzyme kinetics and hydrolytic order. If extracellular enzyme concentration exceeds the number of adsorption sites of particulate substrates, hydrolysis can be described as a first-order reaction [8, 9]. Table 1 shows the kinetic coefficients of the first-order rate of hydrolysis of different substrates under different conditions.

Table 2.1 Substrate affinities of common hydrolytic bacteria present in anaerobic consortium [8]

Protease-producing bacteria	Lipase-producing bacteria
Clostridium proteolyticum	*Butyrivibrio*
Eubacterium sp.	*Clostridium* sp.
Peptococcus anaerobicus	*Anaerovibirio lipolytica* sp.

For particulate organic matter, hydrolysis follows a first order reaction as described by the following equations.

$$\frac{dS}{dt} = -kS \qquad \frac{dP}{dt} = \alpha kS \tag{1}$$

where S, k, P and α represent substrate concentration, first-order reaction coefficient, product concentration and conversion coefficient, respectively. These equations can be integrated and expressed as

$$P = P_o + \alpha S_o(1 - e^{-kt}) \tag{2}$$

Where P_o and S_o represent initial product and substrate concentrations.

Michaelis-Menten kinetics, expressed below, can be applied to hydrolysis of soluble substrates. According to Goel et al. (1998), soluble starch hydrolysis follows this model.

$$\frac{dS}{dt} = k.E\frac{S}{K_m+S} = V_m\frac{S}{K_m+S} \tag{3}$$

where k, E, S, K_m and V_m are maximum hydrolysis rate constant, Hydrolase concentration, Substrate concentration, half-saturation rate coefficient and maximum hydrolysis rate respectively. E is proportional to the biomass concentration. Thus, the maximum hydrolysis rate of soluble substrates is achieved when $S >> K_m$.

Hydrolysis is considered the rate-limiting step in anaerobic digestion of feed containing any of the hydrolytic precursors to monosaccharides, fatty acids and amino acids[10]. Table 2.2 presents a collection of these first order hydrolytic rate coefficients.

Table 2.2 First order rate coefficients of hydrolysis of different substrates [10]

Substrate	k (day^{-1})	T(°C)	References
Carbohydrates	0.025-0.2	55	Chris et al. (2000)
Proteins	0.015-0.075	55	Chris et al. (2000)
Lipids	0.005-0.010	55	Chris et al. (2000)
Carbohydrates	0.5-2.0		Garcia-Heras (2003)
Lipids	0.1-0.7		Garcia-Heras (2003)
Proteins	0.25-0.8		Garcia-Heras (2003)
Lipids	0.76		Shimizu et al. (2002)
Lipids	0.63	25	Masse et al. (2002)
Slaughterhouse waste	0.35	35	Lokshina et al. (2003)
Household solid waste	0.1	37	Vavilin and Angelidaki (2005)
Crops and crop residues	0.009-0.094	35	Lehtomaki et al. (2005)
Municipal solid waste	0.1	15	Bolzonella et al. (2005)
Kitchen waste	0.34	35	Liebetrau et al. (2004)
Cellulose	0.04-0.13		Gujer and Zender (1983)
Cellulose	0.066	35	Liebetrau et al. (2004)
Office paper	0.036	35	Vavilin et al. (2004)
Cardboard	0.046	35	Vavilin et al. (2004)
Newsprint	0.057	35	Vavilin et al. (2004)
Food waste	0.55	37	Vavilin et al. (2004)
Forest soil	0.54	30	Lokshina and Vavilin (1999)
Forest soil	0.09-0.31	20	Lokshina and Vavilin (1999)
Primary sludge	0.4-1.2	35	O'Rourke (1968)
Primary sludge	0.99	35	Ristow et al. (2006)
Secondary sludge	0.17-0.60	35	Ghosh (1981)
Proteins (gelatine)	0.65	55	Flotats et al. (2006)
Cattle manure	0.13	55	
Pig manure	0.1	28	Vavilin et al. (1997)

2.1.2 Acidogenesis

Acidogenesis (fermentation) is an intracellular process. Acidogenic bacteria (AB) convert small dissolved organic compounds into fermentative products. These fermentative products include:

- Volatile Fatty Acids (VFA's): Acetate, Propionate, Butyrate, Formic and Lactic Acid
- Alcohols
- CO_2, H_2, NH_3 and H_2S

Fermentation of sugar and amino acid convert amino acids into acetate, propionate, butyrate, CO_2 and hydrogen. LCFA fermentation yields the same products except for propionate and butyrate, but a greater fraction of LCFAs are converted to hydrogen than sugars and amino acids[8].

The Stickland reaction describes the conversion of amino acids into VFA's. The process occurs when anaerobic oxidizing bacteria perform deammonification on amino acids to produce VFA's and H_2. The produced H_2 is consumed during reductive deammonification. Both reactions produce ammonium which functions as a proton acceptor within the system, thus reducing pH and providing alkalinity. [7]

Acidifying reactions have the highest Gibbs free energy ($\Delta_f G^\circ$) of amongst the four anaerobic processes. Prevailing H_2 concentrations determine ΔG° of acidogenic reactions using sucrose as a substrate. Moreover, H_2 scavenging organisms such as hydrogenotrophic methanogenic bacteria (HMB) regulate H_2 concentrations. Therefore, HMB determine ΔG of acidogenesis to an extent. Low HMB activity causes H_2 accumulation. This results in the production of more reduced products such as propionate and butyrate. However, adequate H_2 scavenging produces high acetate concentrations, which mitigates acidification.

Acetate production is the purpose of the third step of the ADM1. This makes it the more desirable VFA of the three aforementioned during acidogenesis. [7]

Acidogenesis has the fastest conversion rate amongst the ADM1 processes. Due to high ΔG, acidifying reactions yield five times more biomass than Methane Producing Bacteria (MPB). Also, acetogenic growth rates are up to twenty times faster than methanogenic growth rates. Moreover, acidogenic bacteria are active down to a pH of about 4. Thus, they can cause reactor souring in conditions of low methanogenic scavenging. [7]

Table 2.3 Kinetic growth and conversion rates of Acidogenesis and Methanogenesis [11]

Process	Conversion Rate, gCOD/gVSS.d	Y, gVSS/gCOD	Ks, mgCOD/L	μ_m
Acidogenesis	13	0.15	200	2
Methanogenesis	3	0.03	30	0.12
Overall	2	0.03-0.18		0.12

2.1.3 Acetogenesis

Acetogenic Bacteria convert all other VFA's produced from acidogenesis into acetate, hydrogen and carbon dioxide. The most common acetogenic substrates include propionate and butyrate. Lactate, Ethanol, Methanol, H_2 and CO_2 can also be homoacetogenically converted acetate. LCFAs are converted to acetate following beta-oxidation.

Acetogens are obligate hydrogen producers and as such undergo inhibition under high H_2 concentration. Syntrophic associations between H_2 producing acetogens and H_2 consuming MPB are thus required to ensure the growth of acetogens through interspecies hydrogen transfer. To ensure the proper functioning of an anaerobic bioreactor, hydrogen partial pressures will have to remain between 10^{-4} to 10^{-6} atm (Figure 2.2). This ensures that the degradation of ethanol, butyrate or propionate are exergonic and yield energy for acetogenic metabolism.

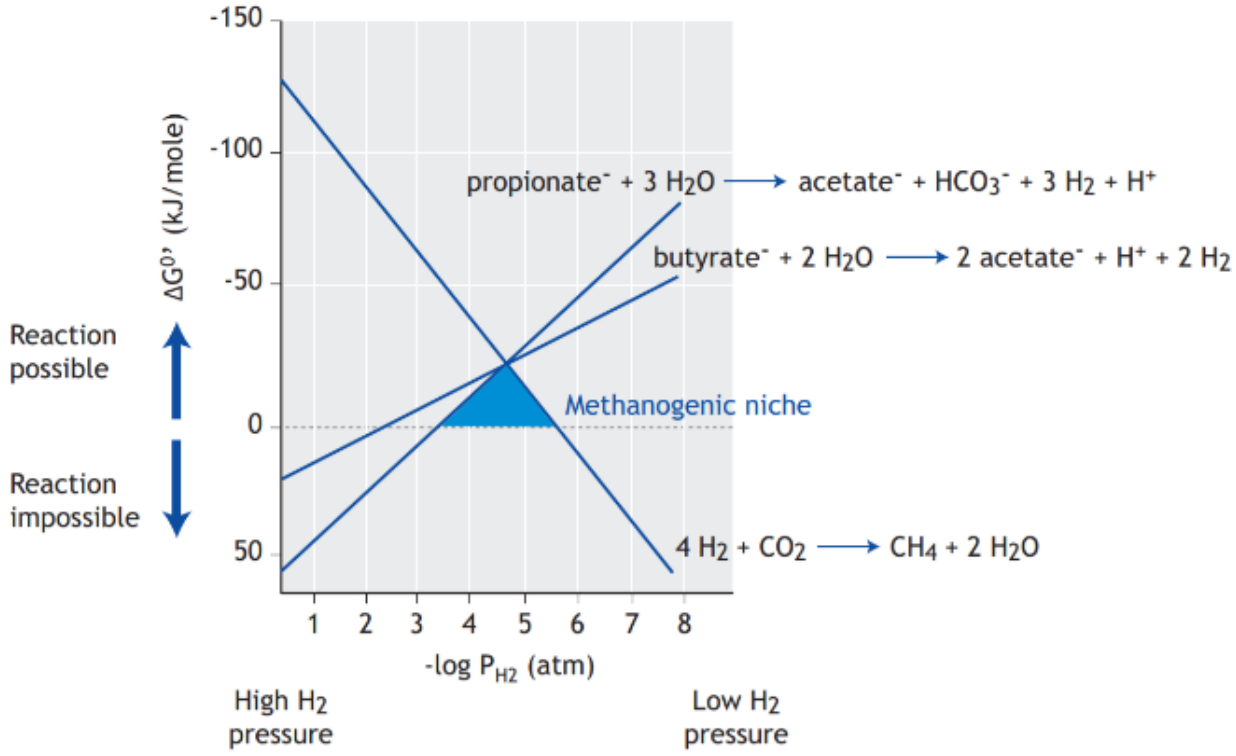

Figure 2.2 Free Energy Change as a function of H_2 partial pressure (Optimal methanogenic activity occurs within the blue highlight) [11]

Acetogenesis from ethanol, butyrate, propionate and palmitate will not occur spontaneously, as ΔG>0. However, an effective uptake of hydrogen by MPB or sulfate reducing bacteria will promote acetogenesis [11]. The ΔG values of acidogenesis are outlined under different stoichiometric conditions in Table 2.4.

Table 2.4 Stoichiometry and free energy change for Acetogenic reactions at STP[11]

Substrate	Reaction	ΔG˚, kJ/mol
Lactate	$CH_3CHOHCOO^-+2H_2O \rightarrow CH_3COO^-+HCO_3^-+H^+ + 2H_2$	-4.2
Ethanol	$CH_3CH_2OH+H_2O \rightarrow CH_3COO^- + H^+ + 2\ H_2$	+9.6
Butyrate	$CH_3CH_2CH_2COO^-+2\ H_2O \rightarrow 2CH_3COO^-+H^++2H_2$	+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
H_2-CO_2	$2HCO_3^-+4H_2+H^+ \rightarrow CH_3COO^-+4H_2O$	-70.3
Palmitate	$CH_3^-(CH_2)_{14}\text{-}COO^-+14H_2O \rightarrow 8CH_3COO^-+7H^+ + 14H_2$	+345.6

2.1.4 Methanogenesis

MPB perform the final stage of AWT treatment. Certain MPBs can use substrates such as acetate, methylamines, methanol, formate and H_2/CO_2 or CO. Acetoclastic methanogenic bacteria (AMB) produce methane from acetate whereas hydrogenotrophic methanogenic bacteria (HMB) produce methane using carbon dioxide and hydrogen as the terminal electron acceptor. However, AMBs usually accounts for about 70% of methane production, even with its low growth rate resulting in doubling times of several. AMB growth rate limits reactor startup times. HMB, however, exhibit much higher growth rates. They improve the stability of methane production by controlling the upper limit of the hydrogen window required for acetogenesis. [11]

Methanosarcina and Methanosaeta are the only two genera within order Methanosarcinales that can use acetate to produce methane. However, methanosarcina has higher substrate compatibility due to its ability to convert acetate, H_2/CO_2, methylamines, methanol and formate. Also, methanosarcina handles increases in acetate concentration more effectively, thus, increasing digestion stability. Methanosaeta can only convert acetate and has a lower μmax. However, methanosaeta is usually dominant within anaerobic reactors due to low acetate concentration and long SRT.

2.2 Chemical Oxygen Demand

Chemical Oxygen Demand (COD) is the measure of the reductive potential of organic matter (pollutants) in a wastewater sample using oxygen as the oxidizing agent. In this text, any oxidizable organic matter will be referred to as a pollutant. These oxidizable pollutants are measured as a concentration in mg/L. Untreated effluents with high COD provide aerobic bacteria with the macronutrients necessary for proliferation. This causes eutrophication of receiving waters.

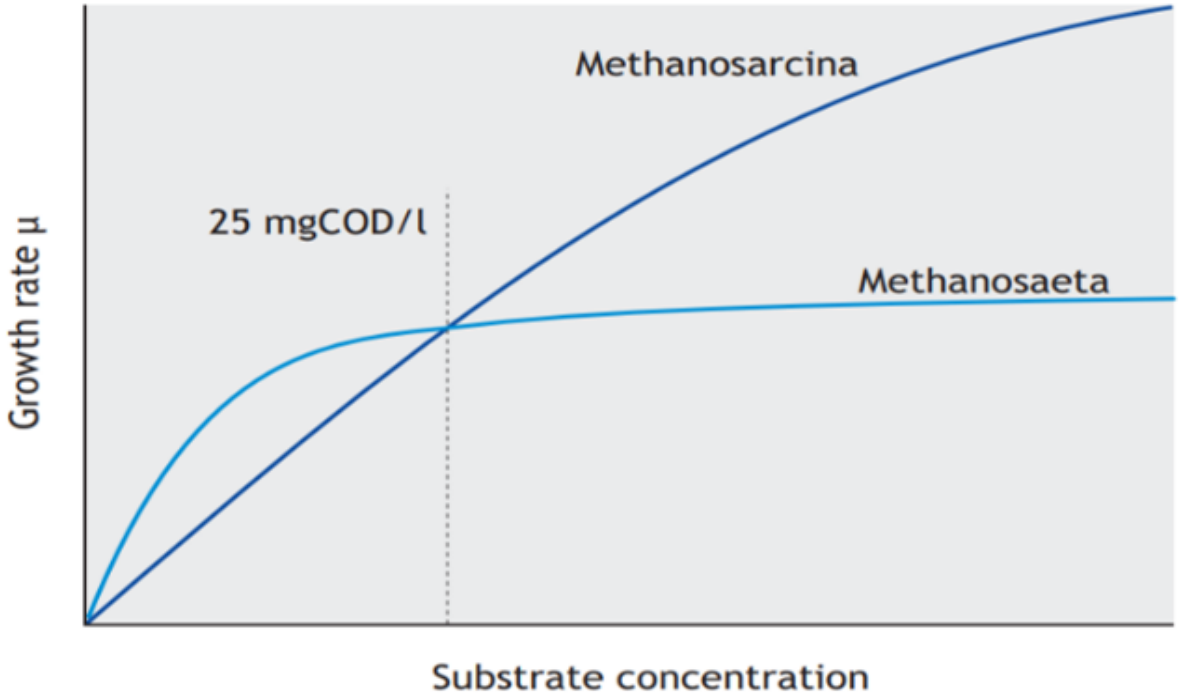

Figure 2.3 Growth kinetics of different types of MPB[11]

Theoretical COD is defined as the amount of oxygen required to completely oxidize a unit quantity of oxidizable pollutant. COD of a simple substrate can be calculated as such.

$$C_aH_bO_C + \frac{1}{4}(4a + b - 2c)O_2 \rightarrow aCO_2 + \frac{b}{2}H_2O,$$

COD is generally used for estimation of organic matter but is seldom equal BOD. BOD is a more specific measure of oxidizable organic matter biochemically available to microbes. However, COD is the preferred test because it takes roughly 2.5 hours to complete whilst BOD takes 5 or more days to complete.

For nitrogenous, phosphorous and/or sulfur containing organic substrates, different equations have been formulated simply by stoichiometrically balancing the reactants and products obtained when the substrate is fully oxidized.

For more complex substrates containing a wide range of molecules, theoretical COD is used as a preliminary estimation. Glucose is a commonly used substrate

for this purpose. 6 mol O_2 are required to fully oxidize 1 mol of glucose. Conversion from molar stoichiometric coefficients to mass units yields 1.067 gCOD/g glucose.

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$$

In this study, the COD of methane will be used.

$$CH_4 + 4O_2 \rightarrow CO_2 + 2H_2O$$

ThCOD(CH_4) = 4gCOD/gCH_4.

If a completely biodegradable compound $C_aH_bO_CN_d$, is completely converted by anaerobic microbes without the production of biomass, the theoretical amounts of methane gas (and CO_2) can be calculated using the Buswell Equation.

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

2.2.1 COD Fraction

COD can be fractionated based on biodegradability and/or solubility. Colloidal and particulate biodegradable COD (pbCOD) substrates require disintegration and hydrolysis before they can diffuse through bacterial cell membranes to be metabolized. Along with colloidal biodegradable COD, particulate biodegradable COD (pbCOD) is classified as slowly biodegradable COD. Soluble biodegradable COD (sbCOD) can immediately diffuse through the cell membrane for use in metabolism. Particulate non-biodegradable COD (pnbCOD) becomes temporarily or permanently trapped (depending on reactor type) in sludge through adsorption and enmeshment. Dissolved non-biodegradable COD (snbCOD) is not converted biochemically inside the reactor. Chemical reactions, such as precipitation, can capture snbCOD. However, precipitated snbCOD will

have to be removed through sludge wasting. Therefore, influents are rarely 100% biodegradable.

Table 2.5 COD Fractionation according to particle size

	Biodegradable	**Non-biodegradable**
Dissolved	Complex VFA's	Non-biodegradable
Particulate	Particulate Colloidal	Non-biodegradable

2.2.2 Bacterial Growth Curve

Bacterial growth occurs in four phases. The **Lag Phase** is the period between inoculation and growth where bacteria adapt physiologically to a new medium. The **Exponential Growth** phase is the period where bacteria undergo a constant rate of binary fission. Ideally, bacteria in this phase will grow at their maximum specific growth rate (μ_{max}) due to the presence of excess substrate concentrations. The **Stationary Phase** is characterized by equal rates of replication and death. In this phase, bacterial growth is nutrient-limited. Also, waste products, including toxic metabolites begin to accumulate. The **Death Phase** is the final stage, where death rate exceeds replication rate. A constant decline in biomass is observed.

During the operation of a reactor, bacteria will ideally spend most of their life between the exponential growth and stationary phase. During the exponential growth phase, the rate of bacterial replication will vary inversely to the doubling time. A first order reaction can be used to describe this phenomenon.

$$\frac{dX}{dt} = \frac{ln(2)}{t_g} X \tag{4}$$

$$\frac{dX}{dt} = \mu \cdot X \tag{5}$$

Where μ represents the specific growth rate. This is dependent on the concentration of all limiting factors of metabolism. These include macronutrient

concentration (Carbon source, Nitrogen, Phosphorus) and electron acceptor concentrations. The specific growth rate is most commonly expressed using the Monod equation. Monod kinetics are illustrated in Figure 2.4

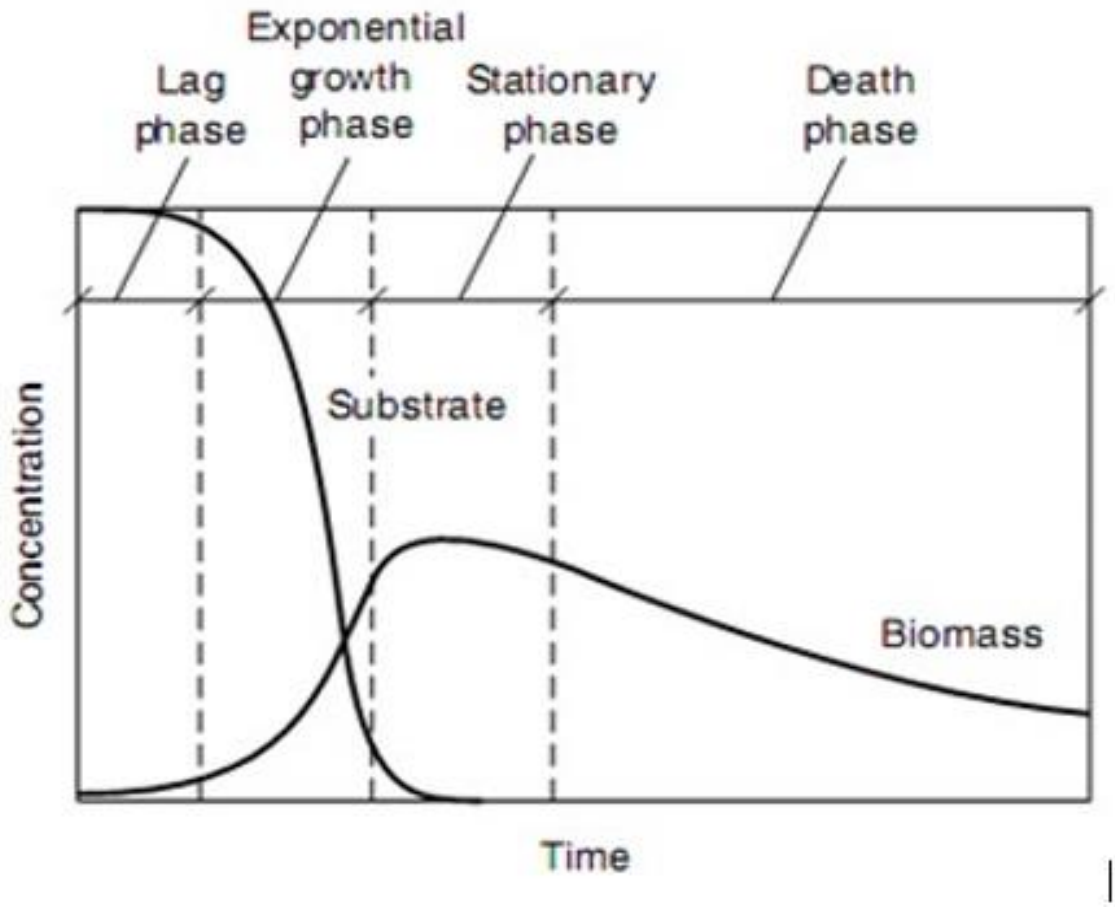

Figure 2.4 Standard Bacterial Growth and Substrate Utilization curve[8]

Here K_s is the half-limiting substrate concentration. K_s is the substrate concentration where the specific growth rate is half the maximum growth rate. Monod equation is a switch-function and maximum growth rate is approached asymptotically when substrate concentration is much higher than half-limiting substrate concentration[8].

$$\mu = \mu_{max} \cdot \frac{S}{K_s+S} \qquad \frac{dX}{dt} = \mu_{max} \cdot \frac{S}{K_s+S} \cdot X \qquad (6)$$

Biomass synthesis yield (Y) is the ratio of the amount of biomass produced to the amount of substrate consumed (g biomass/ g substrate)

$$Y = \frac{X}{S} = \frac{dX}{dS} \quad (7)$$

However, not all converted substrate is used for growth. In anaerobic processes, the by-product of substrate conversion is reduced electron donors. Methane accounts for the greatest fraction of COD-containing reduced product in AWT. The quantity of methane produced is related to substrate conversion as follows[8].

$$\frac{\mathrm{dM}}{\mathrm{dt}} = (1 - \mathrm{Y})\frac{\mathrm{dS}}{\mathrm{dt}} = (1 - \mathrm{Y})\frac{\mu \mathrm{X}}{\mathrm{Y}} \quad (8)$$

2.2.3 Methane Production

Microbial methane production rate is related to organic matter supply. Based on the expected fraction of biodegradable substrate, the rate of methane production can be predicted. These include flow rate, COD concentrations and hydraulic retention time.

$$COD_{feed} = COD_{effluent} + COD_{gas} + COD_{accumulated\ (biomass)} \quad (9)$$

Accumulated COD can be estimated using the theoretical biomass synthesis yield, under the assumption that bacterial VSS has a composition of $C_5H_7O_2N$. This yields a value of 1.42 kgCOD/kgVSS[8]. When newly grown COD and final CH_4 production are inserted into Equation 9, a COD balance can be made. However, endogenous respiration and death make COD available again in the effluent. As bioreactors reach steady state under these conditions, COD accumulation can be removed from Equation 8.

$$\mathrm{Y} = \frac{\mathrm{COD(CH_4)} + \mathrm{COD(V_{DM})}}{\mathrm{COD_{removed}}} \quad (10)$$

Available methane yield as COD captured within methane gas must be recalculated since a fraction of methane is lost as dissolved methane at 16℃. This correction can be made by subtracting dissolved methane COD from removed COD as such.

$$Y_A = \frac{COD(CH_4)}{COD_{removed} - COD(V_{DM}, COD)} \quad (11)$$

$$V_{DM}(L\ CH_4) = \%CH4 \times \beta \times V_R \quad (12)$$

$$COD(V_{DM}) = \frac{64(\frac{gCOD}{L})}{V_{DM}(L\ CH_4)} \quad (13)$$

where Y=Methane Yield, Y_A=Available methane Yield, V_{DM}=Dissolved Methane

Where V_{DM} is the volume of dissolved methane (L), $\%CH_4$ is the methane concentration in reactor headspace, β is methane Bunsen solubility coefficient at 16 ℃ (0.03768) and 2.5℃ (0.04955) and V_R is the reactor volume [12]. At low methane fractions, the difference between available methane yield and methane yield is barely noticeable and vice versa.

2.2.4 COD Balance

COD conservation follows the laws of conservation of mass and charge due to redox reactions which do no net effect on charge magnitudes. During anaerobic respiration, COD is only rearranged through electron and reverse electron transport. Complex organic compounds are broken down into simple intermediaries and mineralized to CH_4 and CO_2. All inlet COD is converted to CH_4 minus the quantity used for growth. COD is the simplest method of preparing a mass balance as a control tool to operate anaerobic systems.

$COD_{in}=COD_{out}$

COD equivalents can be calculated for bacterial VSS by assuming an estimated composition of 1.42 kgCOD/kgVSS.

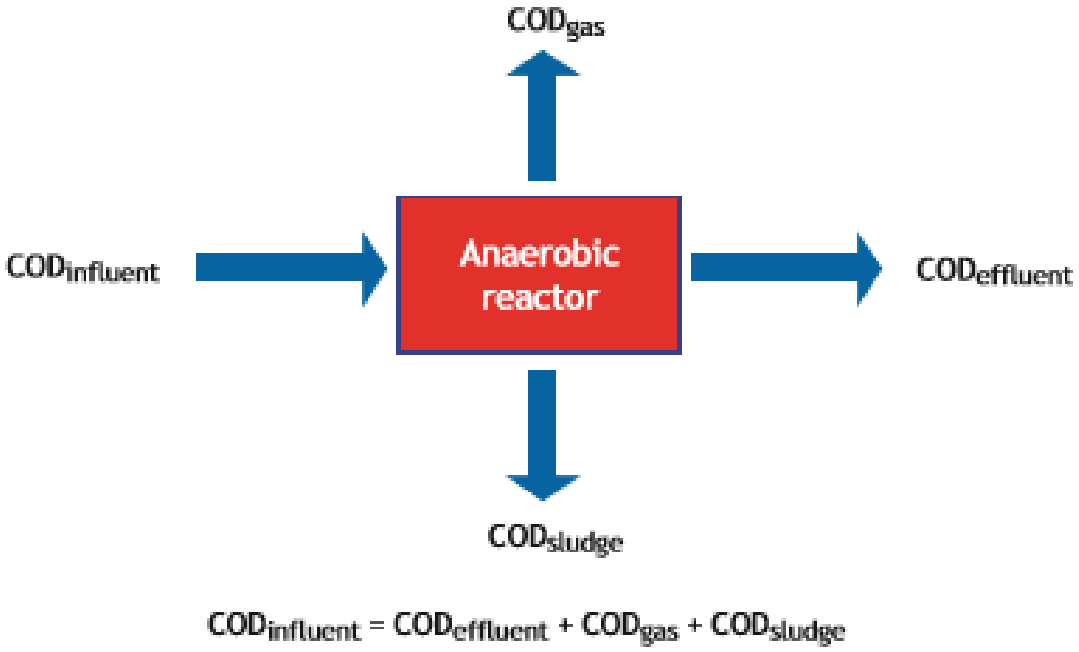

Figure 2.5 Diagram illustrating the conservation of COD[7]

2.3 Factors Affecting Anaerobic Processes

Anaerobic Processes are either stimulated or inhibited by physico-chemical parameters. Effective anaerobic production relies on the operational control of these of these parameters.

2.3.1 Solids Retention Time

Solids Retention Time is the average length of time that a particle remains inside the reactor. This factor mainly is affected by upflow velocity and particle settling characteristics. In CSTR systems without settling and sludge recycle, SRT is equal to Hydraulic Retention time (HRT). In UASB, Expanded Granular Sludge Bed (EGSB) and fluidized bed reactors, SRT is independent of HRT.

Hydrolysis of carbohydrates and proteins, acidogenesis and acetoclastic methanogenesis are the faster biochemical processes and require SRT's of less than 5 days. Thus, using an SRT of 5 days is enough to produce methane. However, 6 or more days are required for LCFA, butyric acid and propionic acid oxidation. Therefore, an SRT of below 6 days could lead to temporary or permanent reactor souring from VFA or LCFA accumulation respectively. Even if SRT is maintained at 12 days, optimal methane production will not be obtained as methanosaeta bacteria require greater than 12 days to begin methanogenesis with higher SRT's yielding higher production rates.

2.3.2 Organic Loading Rate (OLR)

Organic Loading Rate is the flux of organic matter supplied to the bioreactor per unit volume per unit time. At constant reactor volume, OLR is inversely proportional to HRT. Each anaerobic treatment method has an optimal OLR range. Also, anaerobic reactors can either be hydraulically or organically limited. HRT and OLR are inversely proportional. Medium and high strength wastewater are organically limited. High diffusion coefficients can be maintained inside the

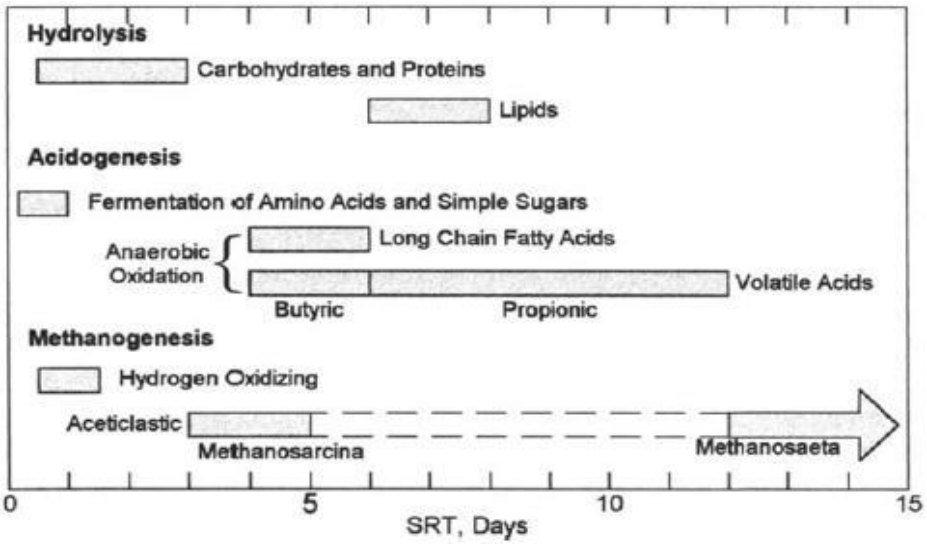

Figure 2.6 Typical SRT ranges for biochemical anaerobic substrate conversions at 35°C[7]

reactor even in the case of low volumetric flow rate. Excessive OLRs result in VFA accumulation due to disproportionately high acidogenic rate in comparison to methanogenesis. This results in pH reduction and inhibition of MPB activity.

Underloading can either be caused by low feed flow rate or low substrate concentration. In either case, a lower bulk substrate concentration is established. This affects substrate concentration gradient, which is the driving force behind diffusion across anaerobic cell membranes.

$$\mathrm{OLR} = \frac{\mathrm{Q \cdot S_i}}{\mathrm{V}} = \frac{\mathrm{S_i}}{\mathrm{HRT}} \tag{14}$$

where Q = flow rate(L/d), S_i = feed concentration(gCOD/L), V = reactor volume (L)

2.3.3 Temperature

Biochemical and enzymatic processes are generally affected by temperature[13]. Most processes involved in the biodegradation of organic matter require more energy to proceed at psychrophilic temperatures than at mesophilic temperatures. Some exceptions include hydrogenotrophic sulfate reduction, hydrogenotrophic methane production and acetate formation from hydrogen.

The effect of temperature on the growth/conversion rates of bacteria can be described using the following equation

$$\mu_{m(T)} = \mu_{m(20)} \Theta^{T-20} \tag{14}$$

Mechanistically, higher temperatures allow for lower activation energies, lower fluid viscosities and higher diffusion coefficients. However, cell components begin to denature above a certain temperature, beyond which enzymatic activity rapidly reduces. Conversely, lower temperatures restrict the movement of cell

components, reduce diffusion across cell membranes and increase activation energy requirement. This slows down enzymatic reactions. Temperature also has a significant effect on the partial pressure of H_2 in reactors, thus influencing the kinetics of syntrophic metabolism.[13, 14]

Different classes of organisms can be distinguished according to their optimal operating temperature range. However, there are crossovers between the three classifications.

Table 2.6 Relative diffusivities of pure water[13]

Temperature (C)	10	20	30	40	50	60
D/D30	0.57	0.77	1.00	1.26	1.55	1.88

Values were calculated using eqn1 and the viscosity of pure water at various temperatures

As a result, AWT treatment at full-scale operation was restricted to temperatures above 18℃. Nevertheless, research has shown that it is still possible to perform anaerobic treatment below 18℃ by providing sufficiently high SRT's[13].

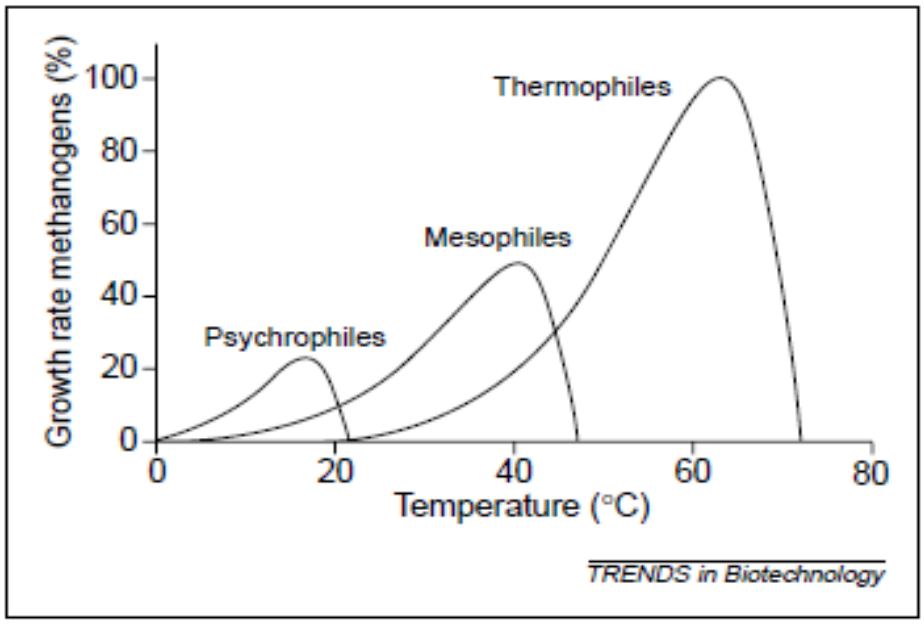

Figure 2.7. Growth rates of different classes of MPB at varying temperatures [13]

2.3.4 Transient Capacity

A sudden increase in feed COD concentration can affect reactor performance. Transient capacity is the ability of a reactor to quickly accommodate changes in feed COD concentrations without exhibiting any major instabilities that cause increase in operational inhibition. Transient capacity is calculated using the acetate capacity number (ACN). [8]

$$ACN = \frac{V_{max}}{V_{plt}} \quad (14)$$

Where V_{max} (gCOD-Acetate/m^3·d) = maximum acetate utilization rate by biomass

V_{plt} (gCOD-Acetate/m^3·d) = average daily acetate utilization rate

V_{max} depends on SRT, average COD loading rate history and acetoclastic MPB population

2.3.5 pH

The optimal pH range differs slightly between the consortium of anaerobic bacteria. The optimal pH for maximum methane production is approximately 7.0. Outside the range of 6-8 pH units, very little activity can be observed. Hydrogen ion concentration is one of the most influential factors that affect both acidogenic bacteria and MPB. MPB are especially affected as they rely on reverse electron flow to provide energy for metabolism. [7]

VFA accumulation reduces pH below the tolerable range of the anaerobic bacteria involved in methanogenesis. Excess alkalinity is always required to buffer VFA production and maintain pH stability. pH control can be accomplished using sodium carbonate, sodium bicarbonate, sodium hydroxide and calcium hydroxide. Amongst these chemicals' sodium bicarbonate is the weakest base and it shifts pH towards the desired value without disturbing the chemical balance of the microbial community.

2.4 Inhibitors

This section outlines the major inhibitors that limit growth and conversion rates of anaerobic bacteria. Literature shows considerable variation in reported inhibitory concentrations of different substances. The main reasons for these variations are:

- the complexity of the anaerobic digestion process
- mechanisms such as synergism, antagonism, acclimation and complexing which may significantly affect inhibition.[15]

2.4.1 VFAs

Volatile Fatty Acids are a necessary precursor for methanogenesis. Higher VFA concentrations lead to higher methane production if pH remains at near neutral values. This occurs because of neutralization by reactor alkalinity. However, when VFA production rate exceeds VFA conversion into methane, the buffering capacity of available alkalinity runs out. This causes pH to reduce below applicable range for MPB. [8] Research shows that neither butyric nor acetic acid inhibited methanogenesis at concentrations up to 10,000 mg/L. Propanoic acid became inhibitory around concentrations of 6,000 mg/L. As a result, it has been suggested that non-ionized forms of VFA's are responsible for inhibition.[15]

2.4.2 Ammonia

Ammonia is produced by the degradation of nitrogenous substances such as proteins and amides (mainly urea). Inhibition by ammonia involves changes in intracellular pH, an increase of maintenance energy requirement and inhibition of certain enzyme reactions. In aqueous solution, ammonia exists either as free ammonia (FA) or ionically associated as ammonium (NH_4^+). FA is hydrophobic and may diffuse into the cell causing proton imbalance as well as potassium deficiency. MPB are the most susceptible to free ammonia inhibition.[15, 16]

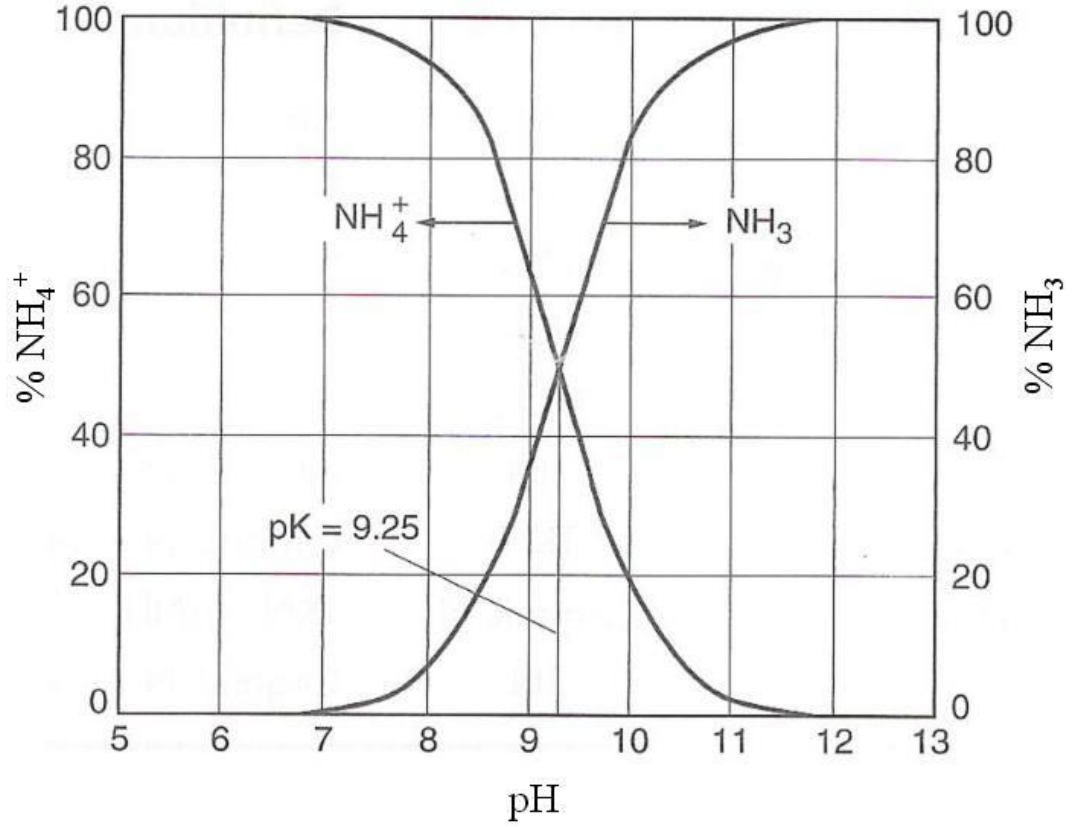

Figure 2.8 Ammonium and Ammonia distribution as a function of pH[17]

2.4.3 Sulfide

Sulfide exhibits two forms of inhibition in the anaerobic treatment process. Primary inhibition occurs due to competition between Sulfate Reducing Bacteria (SRB) and the desired anaerobic microbial consortium. Secondary inhibition results from the toxicity of sulfide to the microbial community. There are two major forms of SRB. Complete oxidizers convert acetate into CO_2 and HCO_3^-, while incomplete oxidizers convert lactate to acetate and CO_2. [15]

SRB exhibit a diverse range of metabolic pathways. Branched-chain fatty acids, Long-chain fatty acids, Alcohols, Organic Acids and aromatic compounds are some of the substrates SRB can degrade. SRB have an affinity for organic electron donors which is arranged as follows. H_2>propionate>other electron donors. SRB compete with MPB, acetogens or fermentative microorganisms for

available H_2 or VFAs (acetate, propionate and butyrate) in anaerobic systems. However, SRB are unable to compete with acidogenic bacteria due to their high growth rate. Sulfide concentration feeds back into the competition between SRB and other anaerobes through secondary inhibition.[15]

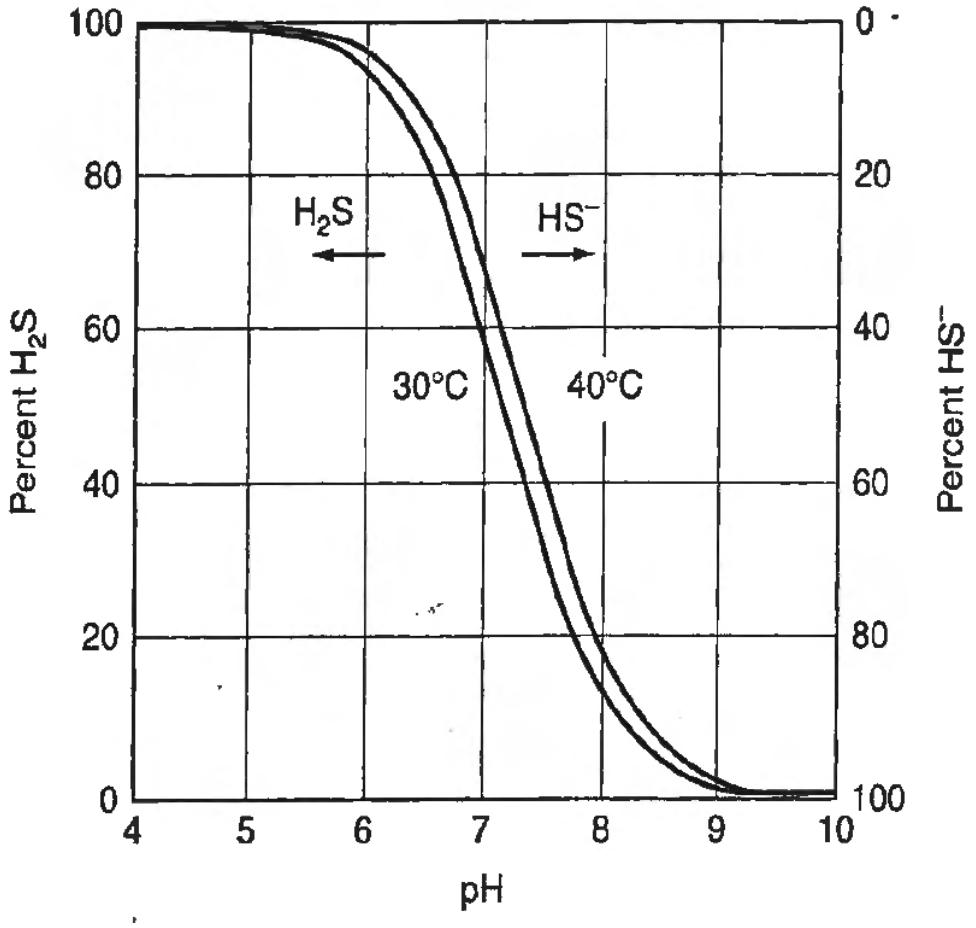

Figure 2.9 Distribution of sulfide in the form of H_2S as a function of pH[8]

COD/SO_4^- ratios are important in determining the outcome of competition between SRB and desired anaerobes. SRB have a higher affinity for propionate and a higher growth rate than propionate using acetogens [18]. However, the reverse is true regarding their competition with butyrate and ethanol utilizing acetogens. Higher COD/SO_4^- promotes higher butyrate and ethanol concentrations and favors the growth of non-SRB acetogenic bacteria. SRB usually outcompete HMB as a result of their lower required hydrogen threshold.

Studies show contradictory results on the outcome of competition between SRB and AMB. [7, 19]

The optimal level of sulfur varies from 1-25 mg/L for MPB[20]. Inhibitory sulfide levels reported in literature range from 100-800 mg/L dissolved sulfide or 50-400 mg/L undissociated H_2S. Dilution, Stripping and acclimation are some methods of controlling sulfur toxicity. H_2S is slightly soluble in water and will exist in equilibrium between the liquid and gas partition. This results in the reduction of methane quality due to the odor and toxicity of H_2S. [15]

2.4.4 Light Metal Cations

Light metal cations are responsible for the salinity of water and wastewater samples. Aluminum, Calcium, Magnesium, Potassium and Sodium can be made available in a reactor from the breakdown of organic matter or during the addition of pH adjustment chemicals. They are required in small amounts just like any other nutrient, for the growth of anaerobic microbes and as such, they affect growth rates according to their bulk concentrations. [15]

Synergism, Potentiation of toxicity of one cation by another below the stimulatory threshold and Antagonism are all possible interactions between light metal cations. [15]

Table 2.7 Light Metal Cations Stimulatory and Inhibitory Concentrations[7]

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

2.4.5 Heavy Metals

Only soluble heavy metals are inhibitory to the anaerobic treatment process. Table 2.8 details the concentrations corresponding to the IC_{50} of anaerobic treatment. Heavy metal inhibition can easily be prevented by sulfide precipitation. Approximately 0.5 mg of sulfide is required to precipitate 1mg of heavy metals. Ensuring that the ratio of total sulfide concentration (produced + added) to total heavy metal concentration is key to preventing heavy metal inhibition. Added sulfide is usually present in the form of ferrous sulfide, as ferrous iron is much less inhibitory than other heavy metals. Furthermore, sulfide precipitates of the more inhibitory heavy metals are less soluble than ferrous sulfide. Thus, low concentrations will be maintained in the reactor by residual iron. Excess iron will precipitate as iron carbonate at a pH of 6.4 or above. [15]

However, heavy metal ion precipitation in acidic conditions can lead to the evolution of H_2S fumes and the formation of colloidal precipitates that cause separation problems in either the settling or filtration process. Therefore, it is essential that neutral to alkaline conditions are maintained in the reactor. [21]

Table 2.8 Typical Heavy Metal IC_{50} Concentrations[7]

Cation	Concentration (mg/L)
Fe^{2+}	1 to 10
Zn^{2+}	10^{-4}
Cd^{2+}	10^{-8}
Cu^{+}	10^{-12}
Cu^{2+}	10^{-16}

2.5 Nutrients

The macronutrient elements C, H, O, N and P are required for all microbial growth. These macronutrients are required to supply the raw materials for the biosynthesis of cells. In addition, some macronutrients serve as energy sources

for metabolic reactions and electron acceptors for released electrons during these reactions. The composition of different bacterial cells is hard to exactly quantify. However, the general proportions of nutrients between the cells of different microbes are relatively constant and can be represented as $C_5H_7NO_2$ or more accurately $C_{12}H_{87}O_{23}N_{12}P$ (to include phosphorus). The amount of nitrogen and phosphorus used in anaerobic treatment is much less in comparison to aerobic methods. This results from the lower overall biomass synthesis yield of microbes involved in anaerobic treatment. From literature, the optimal C: N: P ratios for aerobic and anaerobic treatment are 100:5:1 and 250:5:1. [7, 8]

2.6 UASB Reactor

The UASB Reactor is the commonest anaerobic treatment technology in use. They are commonly used in the treatment of high strength municipal and industrial wastewaters. [22] In UASB reactors, bacteria can naturally aggregate in flocs and granules to form good settling properties. They are less susceptible to wash-out.

Wastewater is fed through the bottom of the reactor and flows upward through a sludge blanket. The upflow velocity is controlled such that it does not exceed the settling velocity of the biomass. This results in SRT's in excess of 30 days for the sludge blanket involved in the wastewater treatment [8]. Small influent particles are retained by entrapment and adsorption to the sludge blanket where they undergo disintegration and hydrolysis into readily biodegradable COD (rbCOD). Effluents are in the form of treated wastewater and produced biogas. Both effluents exit close to the top of the reactor. A specially designed compartment for gas collection is present at the top of the reactor. The schematic diagram of a UASB reactor is presented int Figure 2.10

UASB Reactors can accommodate OLRs of between 5 and 20 $kg/m^3 \cdot d$. Upflow velocities may vary from 1 to 6 m/h with reactor heights between 5 and 20m for

large scale use. Inadequate mixing is a common problem with UASB reactors. The Expanded Granular Sludge Bed Reactor (EGSB) was designed to solve this problem. The EGSB reactor is a taller version of the UASB reactor which allows for sludge particles to be slowed down by gravity and fluid drag such that they cannot reach the effluent level even at relatively high upflow velocities. As such, upflow velocities between 4 and 10 m/h were applied. However, EGSB reactors re not as effective at colloidal and particulate solids capture as UASB reactors. [8]

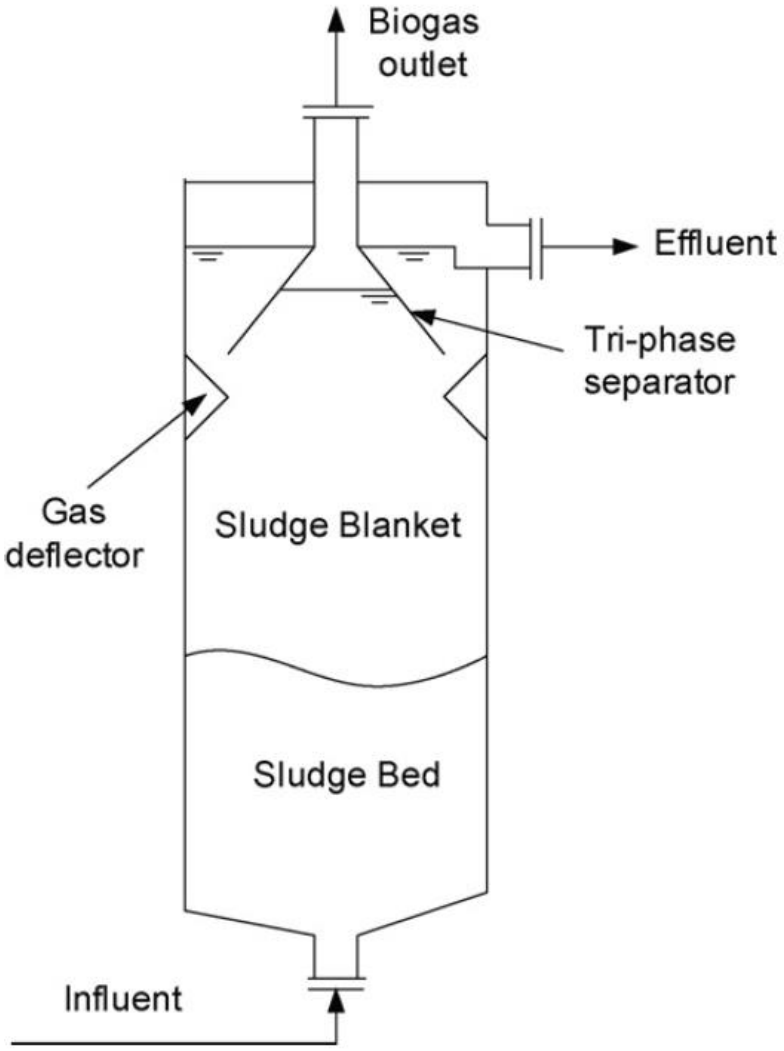

Figure 2.10 Vertical cross section of a UASB reactor

2.6.1 Granular Sludge Characteristics

The success of UASB reactors can be attributed to the formation of sludge granules. Much higher loading rates can be applied to UASB reactors than conventional activated sludge reactors due to two main factors. These are the superior settling characteristics of granular sludge and their high specific methanogen activity. Settling velocities of 60m/h are common in UASB reactors, whereas upflow velocities are usually kept below 2m/h. This allows for the decoupling of solids retention time and hydraulic retention time without the need of a secondary settler. [23]

Specific methanogenic yields of granular sludge in excess of 2kgCOD/kgVSS·d have been recorded. Studies have shown that acetogen colonies are closely linked hydrogenotrophic methanogenic archaea allowing for effective interspecies hydrogen transfer resulting in higher conversion rates [23]. Several studies have studied granule structure and the consensus is that substrate type and concentration determine the structure and arrangement of the microbial consortium in the granule. MacLeod et al proposed a three-layered concentric structure for sucrose-degrading granules; the outer layer, middle and inner layer consisting of acidogenic bacteria, syntrophic microcolonies and MPB respectively[24]. Guiot et al confirmed this observation for glucose-degrading granules[25]. [26]

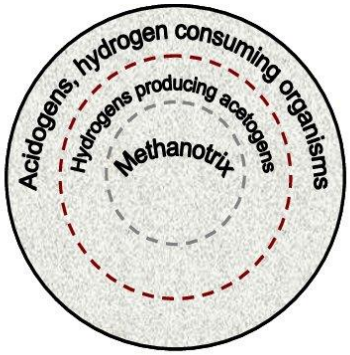

Figure 2.11 Layered microbial guilds (Adapted from McLeod et al.,1990)[27]

(Fluorescence in situ Hybridization) FISH has been used to study the layered structure of UASB granules along with the pH, H_2, ORP and CH_4 variations with increasing distance from granule surface. [22]

Most methane was produced within the inner layers of the sludge granule. H_2 was be produced closer to the surface, but its concentration rapidly dropped in the middle layer. The inverse proportionality of CH_4 production and H_2 consumption as the distance from granule surface decreases suggests consumption of H_2 by HMB and the diffusion of H_2 into granule center. [22, 26]

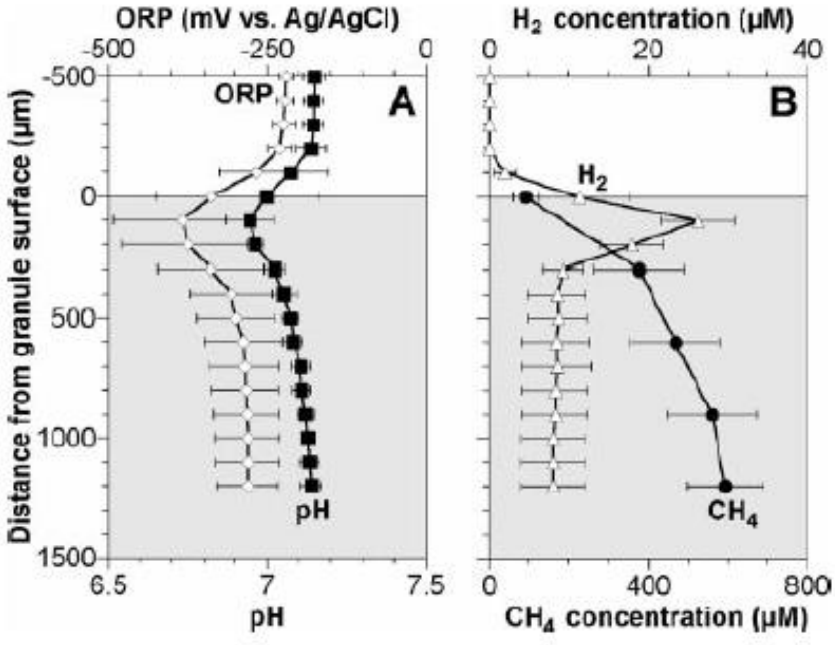

Figure 2.12 Granule depth vs i. ORP and pH ii. H_2 and CH_4 concentration, from UASB reactor at 35℃ after 1 year of operation [22]

However, non-layered granules have also been observed. However, a study by Grotenhuis et al. observed that propionate, ethanol and sugar-refinery wastewaters exhibited no layered structures[28]. Fang et al compared granules obtained from a cannery, slaughterhouse and two breweries were studied using

Fluorescence in situ Hybridization (FISH), Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM). Results are shown in Table 2.9

Table 2.9 Substrate specific differences between granules studied[26]

Property	Cannery	Protein	Brewery	Brewery 2
Microbial Density	Very high	Low	High	High
Layered structure	Yes	No	Some	Yes
Microbial Diversity	4 main types	Many types	3 main types	4 main types
Acidogenic bacteria	Around edge	Clumped throughout granule	Not found	Around edge
Syntrophic groups	In center	Throughout granule	Over 100-200m	Over 100-200
Methanosarcina	Outer 200	Some on edge	Not found	Not found
Methanosaeta	In center	Filamentous, throughout granule	Low numbers in outer 100-200	Med. Numbers in outer 100-200

2.6.2 UASB Rector Applications in Municipal Wastewater Treatment

The application of UASB technology in raw sewage and sludge treatment under low temperature conditions has been studied in the Netherlands since 1976. Lettinga et al operated these UASB reactors at HRT of 14-17h. They recorded CODRE of 65-85% at 20°C and 55-75% between 13-17°C. In 1986, de Man et al that the anaerobic treatment of raw low strength (500-700mg/L) wastewater can achieve CODRE and BODRE of 50-70% and 40-60% respectively at 12-18°C with HRTs of 7-12h. [29]

Mahmoud, N studied high strength (>1200mg/L) sewage treatment in a one stage UASB reactor to assess system response to Mediterranean climatic seasonal

temperature fluctuation. The study incorporated a “hot” and “cold” phase to describe periods of high and low temperatures. This study was conducted in Jordan, where average winter-summer temperature variation is 15-25°C. CODRE averaged 55% and 32% respectively during hot and cold phases respectively. [29] Moreover, numerous studies seem to indicate that the complexity of the substrate mix is the most significant limitation to psychrophilic wastewater treatment. Raw sewage poses a sever issue in this regard as it is quite rich in complex organic matter.

As of 2018, about 800 full scale UASB reactors had been installed worldwide amongst approximately 1200 other anaerobic technologies. [30] However, post-treatment of wastewater is usually necessary to achieve standards for discharge in surface waters. This is because UASB reactors are not designed to remove the remaining organic matter, nutrients and pathogens. Table 2.10 and Table 2.11 present various UASB applications on sewage and low strength wastewater along with a few performance and operational parameters.

Table 2.10 Anaerobic Treatment of Sewage under psychrophilic conditions (<15C)

Reactor type	Influent	Concentration (g COD dm^{-3})	OLR (kg COD m^{-3} d^{-1})	Temperature (°C)	HRT (h)	Efficiency (%)	Refs
UASB[a]	Row	0.3	0.6	8–20	12	67	50
UASB[a]	Row	0.4	1.2	11–12	8	30–50	51
AF[b]	Row	0.53	1.8	13–15	6	35–55	52
UASB[a]	Row	0.19–1.18	0.6–3.5	12–20	7–8	30–75	53
UASB[a]	Row	0.465	0.62	12–18	18	73	54
FB[c]	Row	0.76	8.9	10	1.7–2.3	53–85	55
EGSB[d]	Pre-settled	0.3	4.5	9–11	2.1	20–48	48
UASB + EGSB	Row	0.32–0.51	1.6–2.5	8–13	5	45–57	Wang K., 1994
AF + AH[e]	Row	0.46–0.53	0.9–1.1	13	12	70	56
UASB	Row	0.15–0.6	0.8–3.1	13–25	4.7	64–70	57

[a]Upflow anaerobic sludge blanket reactor. [b]Anaerobic filter. [c]Fluidized bed reactor. [d]Single stage expanded granular sludge bed reactor. [e]Anaerobic filter + anaerobic hybrid reactor.

These processes are accomplished using conventional systems such as maturation ponds, waste stabilization ponds, polishing ponds, constructed wetlands, rotating

biological contactors, moving bed biofilm reactors, downflow hanging sponge and advanced oxidative processes. UASB-Activated sludge technology is an effective combination for municipal wastewater treatment. It is economically efficient and less complex than other methods. Moreover, this system oxidizes dissolved methane. [31]

Table 2.11 Anaerobic treatment of low strength wastewaters under psychrophilic conditions (<20°C)

Reactor type	Influent	Concentration (g COD dm^{-3})	OLR (kg COD m^{-3} d^{-1})	Temperature (°C)	HRT (h)	Efficiency (%)	Refs
AAFEB[a]	Glucose	0.2–0.6	4–16	10	1–6	40–80	9
ASF[b]	Peptone	0.2[h]	0.64	5–10	7.5	27–35	14
UASB[c]	Sugar Vinasse	0.2–0.4	0.7–6.5	8	1.5–14	32–65	27
EGSB[d]	VFA	2.6	2.0	12	32	50	27
UASB[c]	Beef consommé	1.4–7.0	2–10	10	16	49–80	28
EGSB[d]	VFA	0.5–0.8	10–12	10–12	1.6–2.5	90	16
ASBR[e]	Dry milk	0.6	0.6–2.4	5–10	6	65–85	37
EGSB[f]	Malting	0.2–1.8	3–12	10–15	3.5	67–78	38
EGSB[f]	VFA	0.5–0.9	5–12	4–8	2–4	90	18
UASB[c]	Wine Vinasse	1.2–5.2	0.3–7.3	4–11	12–38	15–92	39
UASB[g]	Wine Vinasse	1.1–5.4	0.8–5.5	4–10	19–31	16–80	39

[a]Anaerobic attached film expanded bed reactor. [b]Anaerobic submerged filter tank. [c]Upflow anaerobic sludge blanket reactor. [d]Single stage expanded granular sludge bed reactor. [e]Anaerobic sequencing batch reactor. [f]Two stage expanded granular sludge bed reactor. [g]Two stage upflow anaerobic sludge blanket reactor. [h]g BOD dm^{-3}.

2.7 Knowledge Gaps

More research is required toward the effect of VFA removal and upflow velocity VFA removal on the UASB system. As hydrolysis is the limiting and alterations in upflow velocity may affect hydrolytic rate, this thesis will seek to. The positive and negative effects of upflow velocity need to be determined. The limits of psychrophilic AWT treatment also need to be investigated. Most studies that have sought to ascertain the practicability of psychrophilic AWT have relied on VFA-based wastewater samples. Real WW samples have only been studied to temperatures of 5.5˚C Research must minimum temperature and maximum applicable OLR at a the studied temperature.

2.8 Specific Objectives

This thesis investigates the treatment of medium and high strength wastewater treatment at low temperature using UASB technology. The main objective was to determine overall reactor performance of a UASB reactors at mesophilic temperatures using average inlet wastewater temperatures (16℃) and psychrophilic temperatures (2.5℃) of IVAR Renseanlegg Grødaland (IVAR Wastewater Treatment Plant, Grødaland) of 16℃. Lab-scale experiments were performed to fulfil the specific objectives of determining:

- Effect of upflow velocity on the conversion efficiency of mesophilic (16℃) UASB reactors under OLR conditions between 3 and 7gCOD/L·d and variable wastewater conditions.
- Maximum practical sCODRE, Methane production and Methane Yield during treatment of Grødaland DAF-filtered wastewater at 2.5℃.
- OLR limitation for treatment of wastewater at 2.5℃
- Theoretical optimal OLR range for sCODRE maximization 2.5℃

3.0 Materials and Methods

This section explains the experimental methods used to determine the efficiency of anaerobic treatment and methane production using municipal wastewater from DAF effluent, IVAR Grødaland. This project investigated reactor performance with a strong focus on the influence of inlet VFA on multiple other performance parameters such as removal efficiency, VSS production, VFA removal rate and changes in alkalinity. All experiments were performed at the University of Stavanger in fulfilment of a master's thesis project.

3.1 Granule Source

Reactor A granules were sourced initially from a BIOPAQ®IC UASB reactor at Norsk Skog Saugbrugs pulp and paper mill (Halden, Norway). Norsk Skog operates a 1,500 ton per day paper mill. The granules were then used in a pilot plant for dilute cow manure and swine supernatant biodigestion.

Reactor B granules were a mixture of a. Norsk Skog pulp and paper mill, b. Pilot plant for treating dilute cow manure and swine supernatant and c. Hydrocarbon-oil containing wastewater at Bamble Industrial Park, Telemark.[32]

3.2 Wastewater Source

Wastewater was sourced from Grødaland wastewater treatment plant, which was designed to receive effluents from 150,000 people and receive these effluents from three different locations namely: Kviamarka (dairy production plant), Norsk Protein (meat by-product processors) and Varhaug municipality.

3.3 Reactor Configuration

A 1.5L **polyethylene** pre-inoculate reactor (Reactor B) was installed in a fume chamber. The reactor consisted of an external cooling jacket kept at 2.5℃ by a thermo-heating circulator. 25L batches of primary domestic effluent were

continuously fed to the reactor from a refrigerator kept at 8℃. Using an adjustable-flow peristaltic pump (ISMATEC ISM4408), the feed was pumped from the container into the UASB reactor. Liquid effluent was drained into a sink. Produced biogas was transported upward due to influent upflow liquid pressure. Biogas was transported through a gas counter and stripped of carbon dioxide with 3M NaOH gas to absorb CO_2. The remainder of biogas was recorded by a second gas counter under the assumption that methane constituted the greatest fraction and impurities were negligible. This reactor was operated for 48 days in completion of a research project began started by Safitri, Anissa Sukma

A 3L **polyethylene** UASB reactor (Reactor A) constructed was installed in a fume chamber and operated for 99 days at 16℃ with the same configuration as Reactor B.Figure 3.1 details experimental reactor configuration. Reactor design specifics are detailed in Appendix 1.

Table 3.1 Equipment Specification for devices used with both Reactors

Equipment	**Manufacturer**	**Specification**	
Pump (Feed)	Ismatec	Type	: peristaltic pump
		Channel	: 4 adjustable channels
		Model	: Reglo ICC
		Flowrate	: 0 – 43 ml/min
Pump (Recirculation)	Heidolph	Type	: Peristaltic pump
		Model	:Pumpdrive PD-501
		Flowrate	:5-120rpm
Gas Counters	Ritter	Model	: MGC-1 V3.3 PMMA
		Gas flowrate	: 1 ml/h- 1 l/h
		Max. pressure	: 100 mbar
		Min. pressure	: 5 mbar
Thermo-heating circulator	Laud Alpha	Model	: RA 8 LCK 1907
		Temperature range	: -25 to 100 °C
		Heater capacity:	: 230 V; 50/60 Hz; 1.5 kW
		Max. pressure	: 0.2 bar
		Max. flowrate	: 15 l/min
		Bath volume	: 20 liters

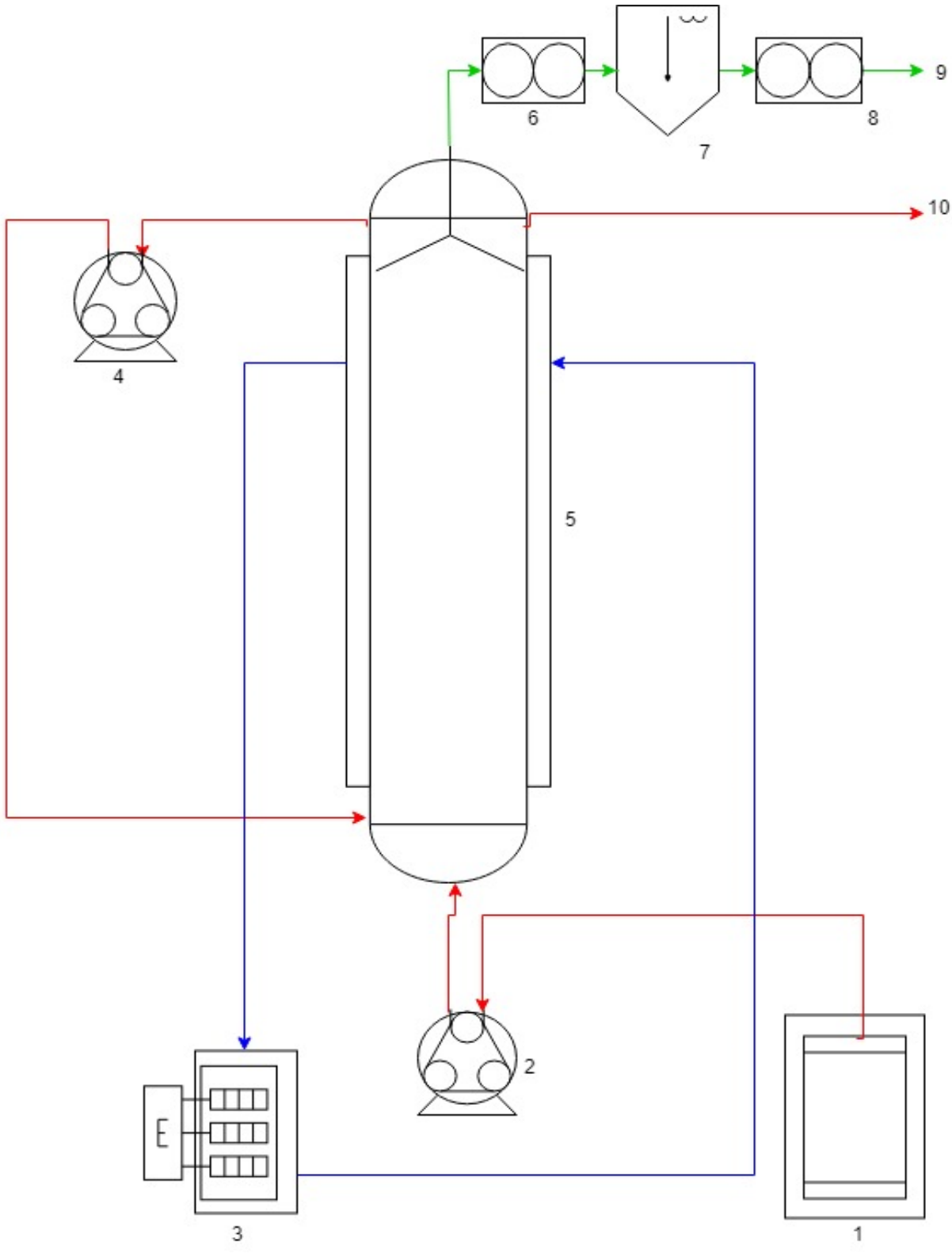

Figure 3.1 UASB Reactor Configuration (1. Refrigerated feed, 2. Peristaltic feed Pump, 3. Thermostatic Cooler, 4. Peristaltic recirculating pump, 5. Jacketed UASB reactor, 6. Biogas counter, 7. CO_2 stripper, 8. CH_4 counter, 9. Gas effluent, 10. Liquid effluent.

3.4 Analytical Methods

Analytical methods used in the process are detailed in this section. Most methods follow standard experimental protocols.

3.4.1 Experimental Process

Feed samples were taken and analyzed batchwise. Effluent samples were taken once a day on weekdays only. Every sample taken was homogenized by shaking to ensure equal particle size distribution. Dilution was performed with distilled water. Four tests were performed to check reactor performance relating to the conversion of COD in the form of VFA's and non-VFA based substrate. All tests are performed on both feed and effluent. Dissolved COD is tested daily, along with Alkalinity, VF and TSS.

3.4.2 Chemical Oxygen Demand

The Hach Method outlined [33] and Merck manual was used to determine COD concentrations. Merck Spectrosquant reaction cells (COD Cat. No. 109773) with upper and lower limits of 100-1500 mg/L respectively were used. The Merck Spectroquant TR 620 thermoreactor and Spectroquant Pharo 300 spectrophotometer were used for digestion and analysis. [34]

Dissolved COD was performed using the same method as stated above. However, samples were filtered through a 1.5µm GC filter.

3.4.3 Total Suspended Solids

Filters were kept in laboratory oven (Termaks TS 9135). Filters were removed and desiccated before use to prevent the filter from absorbing moisture during cooling. Filters were then weighed on a laboratory scale (OHAUS AX523) to determine initial mass. The filtration step described in Section 1.4.1 was then performed using weighed filters. Used filters were then placed in a laboratory

oven again for 1 hour to evaporate water content. Filters were desiccated for 30 minutes and weighed a second time to determine final filter weight. The difference in filter weights was divided by the quantity of the sample taken to determine TSS.

3.4.4 Volatile Fatty Acids and Alkalinity

Volatile Fatty Acids and alkalinity were determined using 5-point titration. The TitroLine 5000 Autotitrator was used in conjunction with Titra 5 software.

20ml of the sample were measured and diluted to 50ml with distilled water. Each diluted sample was placed on a magnetic stirrer at low rotation speed (<100 rpm) to prevent CO_2 absorption or loss through rapid mixing. Initial pH of the diluted sample is measured and titrated to pH values of 6.7, 5.9, 5.2 and 4.3. If the initial pH was below 6.9, 0.1 M NaOH (Product No. 106498, Merck) was added to adjust pH greater than or equal to 6.9. The volume of titrant added per titration was recorded cumulatively from initial pH to 4.3. Measurements were fed into Titra 5 software to generate VFA in acetic acid equivalent concentration (mg/L) and alkalinity in calcium carbonate equivalents (mg/L).

3.5 Reactor A Startup and Operation of Reactor A and B

The initial setup of the UASB reactor involved hydraulic stabilization and steady state operation with respect to biogas production and COD removal at a constant organic loading rate. The preliminary operation was performed using tap water to ensure the proper functioning of instruments and a system boundary devoid of leakages.

Approximately 0.5L of sludge was poured into Reactor A, thus reducing the effective reactor volume to 2.5 L. The reactor was then started at OLR 2.5 gCOD/L·d. OLR was maintained and modified in response to performance. Three upflow velocities were investigated. Phase 1:42-46m/d (Day 1 to 71) Phase

2:23m/d (Day 72-91) and Phase 3:68m/d (Day 92-99). After reactor acclimatization, step 2 involved reactor operation until a pseudo steady state was achieved. OLR was increased from 2.5gCOD/L to 4.5gCOD/L. Step 2 was repeated to OLRs of 6.4, 4.2 and 4.9gCOD/L·d. (Figure 3.2)

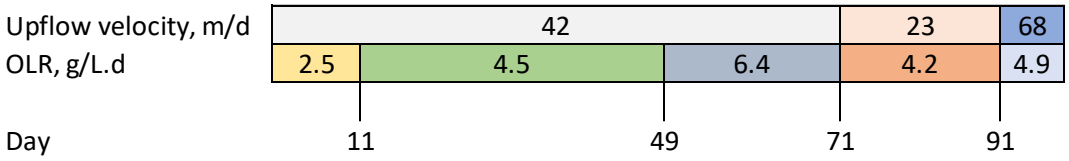

Figure 3.2 Reactor A Operation timeline

Reactor B was operated from day 99 to 137. Reactor B contained 0.5 L granulated sludge, thus reducing the effective reactor volume to 1.0 L. However, results from this experiment include results from day 1 at which were performed by Safitri, Anissa Sukma. In Reactor B, a similar stepwise increase of OLR was performed in the order: 1.7, 3.3, 5.4 and 8.1 gCOD/L·d., where 5.4gCOD/L coincided with day 99.

OLR was only increased when steady state was obtained. During operation, alkalinity in the form of $NaHCO_3$ was added to keep pH close to 7.

4.0 Results

This chapter highlights results obtained from the performed experiments. Overall reactor performance of Reactor A and B were measured using four key parameters. COD Removal Efficiency, COD Balance and Methane Production and VFA Removal. Results from analysis of granular sludge in Reactor A are also presented in this chapter. Raw data used for the computation of these results are presented in the Appendix. Outliers were removed using Pierces criterion and lines of best fit were determined using the method of least squares.

4.1 Reactor A

This section presents findings on Reactor A performance indicators.

4.1.1 COD Removal and COD Balance

Overall average COD Removal Efficiency (CODRE) in Reactor A was 35% (±1.5). During initial reactor acclimatization using acetate, CODRE averaged 38% (±3.5). The highest tCODRE above COD Balance of 85% was 47% and occurred at OLR of 6.42gCOD/L·d and HRT of 5.0h. Average overall sCODRE was 27%. Upflow velocities were compared to averaged pCODRE and sCODRE (Figure 4.1)

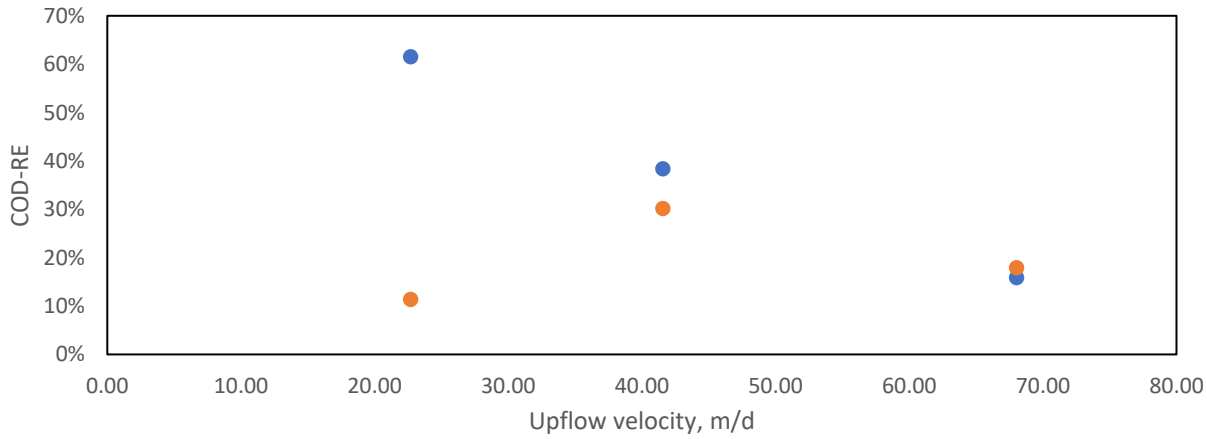

Figure 4.1 Dissolved (orange) and Particulate (blue) CODRE vs Upflow velocity

Lowest upflow velocity of 23m/d yielded an 11% (±4.0) sCODRE and a 62% (±9.3) pCODRE while the highest upflow velocity yielded a 16% (±38%) pCODRE and an 18% (±6.0%) sCODRE. 40-42m/d yielded a 30% (±7.0%) pCODRE and a 30% (2.9%) sCODRE.

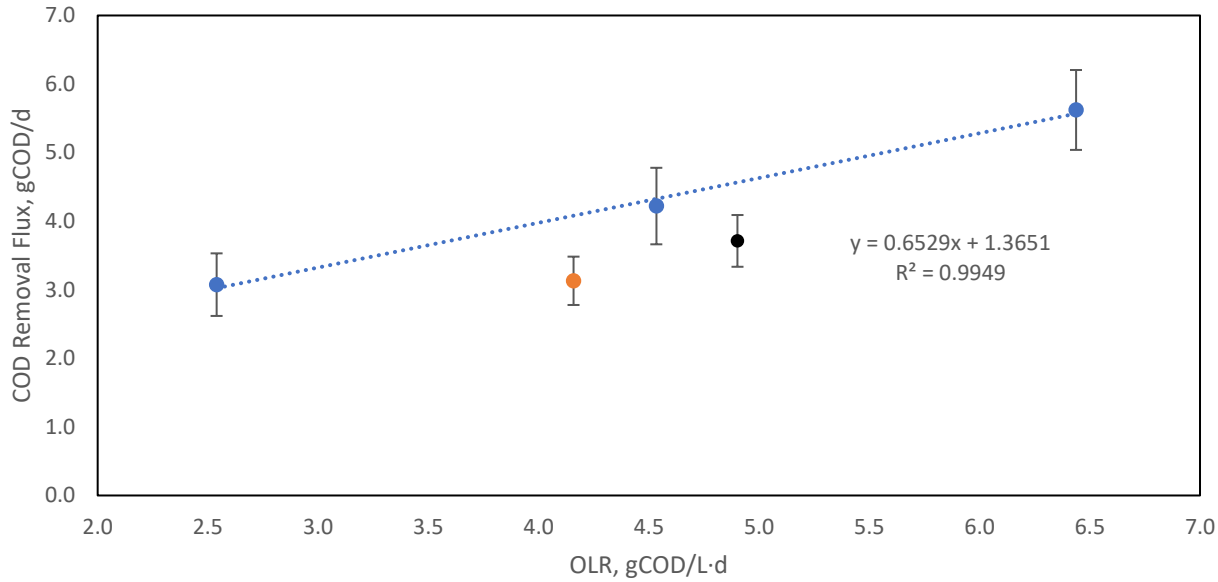

Figure 4.2 COD Removal Flux vs OLR at different upflow velocities (Blue: 40-42m/d, Orange:23m/d, Black:68m/d)

Figure 4.2 illustrates the dependence of total flux of COD removed on OLR. The trendline in Figure 4.2 describes the proportional increase in COD removal with increasing organic loading rate at upflow velocities of between 40 and 42 m/d. Upflow velocities of 23m/d and 68m/d are also presented to compare COD removals at higher and lower upflow velocities. COD Removal Flux at upflow velocity of 68m/d and 23md/ were 3.71 gCOD/d and 3.13gCOD/d, both lower than expected values at their respective OLRs.

Overall average COD Balance was 87% (±1.7). Maximum and minimum COD Balances were 113% and 58%. They occurred on days 52 and 56 respectively. Figure 4.3 illustrates COD balance variation over the course of reactor operation.

An apparent inverse proportionality was observed between COD balance and tCODRE.

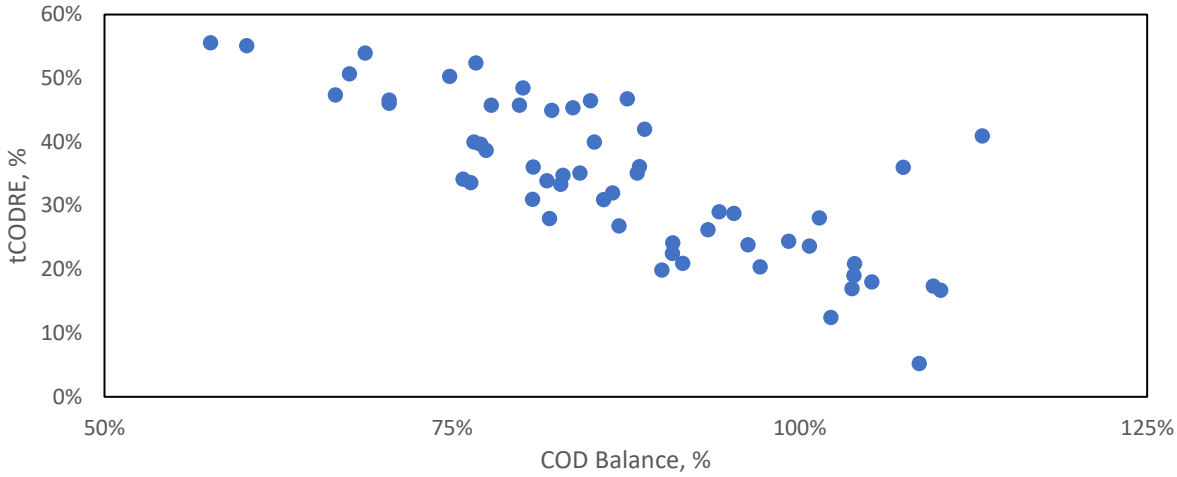

Figure 4.3COD removal efficiency vs COD Balance

4.1.2 Biogas Production

Methane fraction around median day 6 was 54% (±10) during reactor acclimatization. A steady increase was observed until day 35 where Methane fraction stabilized above 85%. Averaged methane fraction readings are presented in Figure 4.4. Methane fraction remained stable throughout period of reactor operation.

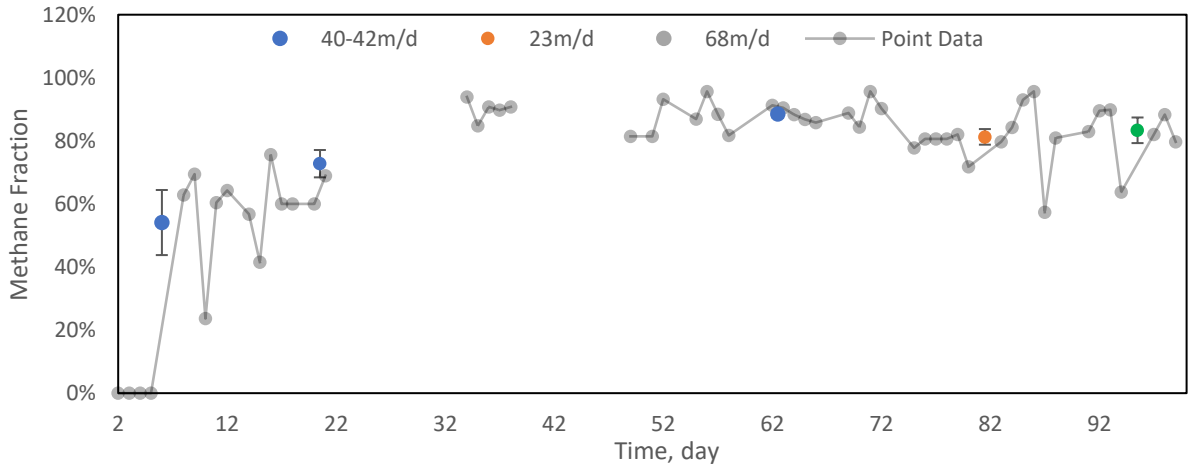

Figure 4.4 Methane fraction vs time

Figure 4.5 presents the variation of methane production with increasing OLR. A linear correlation was drawn between the two variables at constant upflow velocity with a correlation coefficient of 0.993. The correlation is described by the equation G_M=0.161X+0.638, where G_M = methane production rate (gCOD/d) and X=OLR(gCOD/L·d). Methane Production at 2.5, 4.5, 6.4, 4.2 and 4.9 gCOD/L·d were 1.06 (±0.17), 1.34 (±0.019), 1.69 (±0.47), 0.94 (±0.16) and 2.40 (±0.40) gCOD/d. Highest average specific methane production was 1.05 (±0.16) gCOD/L·d at OLR of 4.9g/L·d.

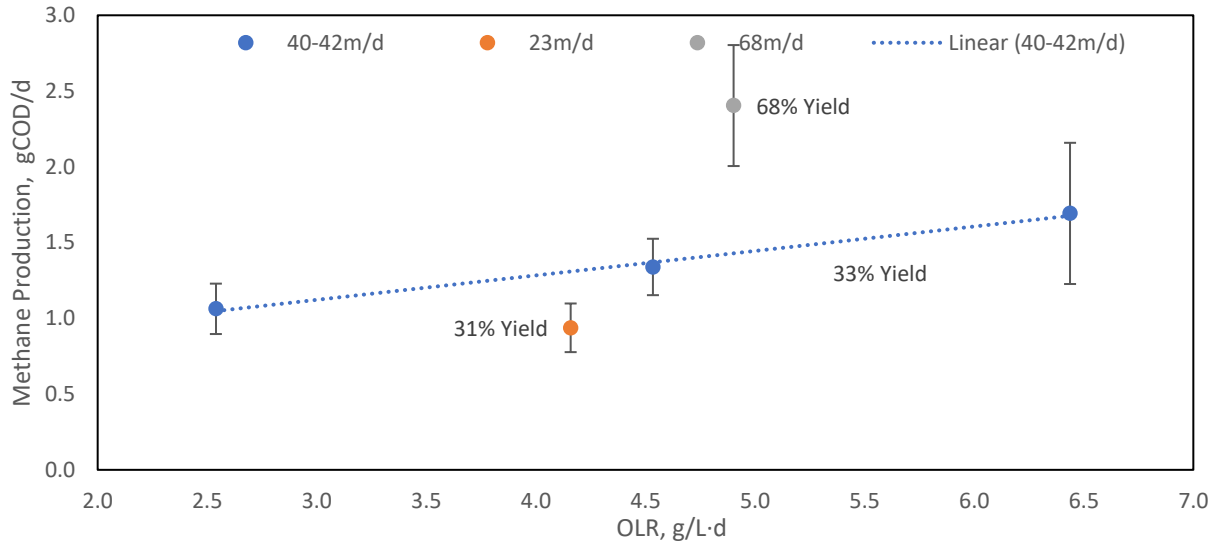

Figure 4.5 Methane Production vs OLR at different upflow velocities (Blue: 40-42m/d, Orange:23m/d, Black:68m/d)

4.1.3 VFA Removal

Average effluent VFA/HAc ratio remained below 0.2 during wastewater treatment and averaged 0.56 during reactor acclimatization. Figure 4.6 describes the removal of VFA in relation to the removal of COD and the variation of this ratio with changing upflow velocity at between OLR of 4 and 5gCOD/L·d and comparable dissolved COD fraction of 0.66 (±0.20) VFA removal in Figure 4.5 is normalized against absolute COD removed. Upflow velocities of 23 m/d, 42

m/d and 68m/d yielded VFA/COD removal factors of 0.10 (±0.06), 0.14 (±0.06) and 0.28 (±0.10) respectively. During wastewater treatment, the highest VFA accumulation during of 86.96mg/L VFA in outlet was observed at OLR of 6.4gCOD/L·d and upflow velocity of 42m/d. The lowest VFA accumulation of 0.10mg/L VFA was recorded at OLR of 4.9g/L·d and upflow velocity of 68m/d.

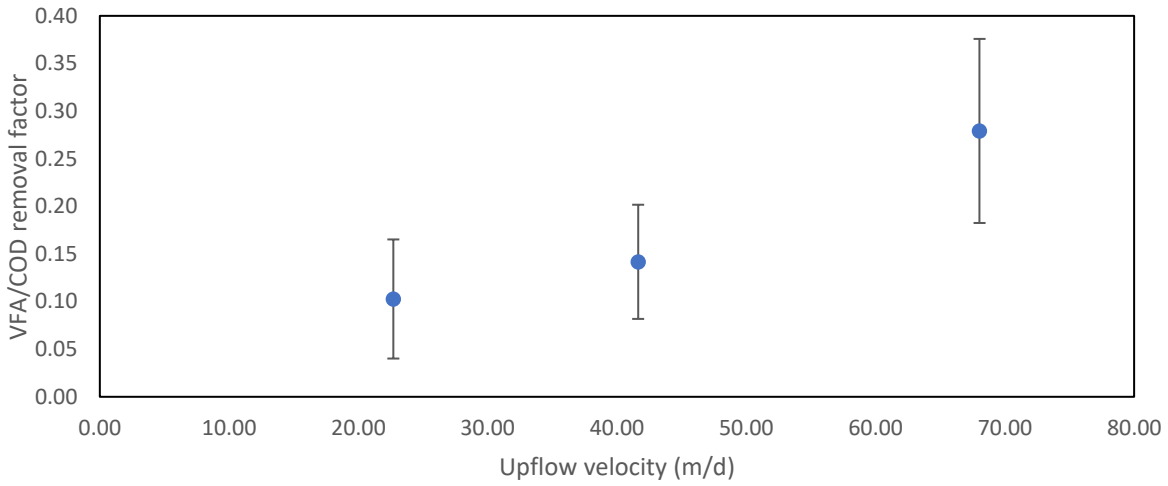

Figure 4.6 Upflow Velocity vs Ratio of Absolute VFA/COD removed

4.1.4 Granule Density

Granule Density Measured on TS was determined as 1.35 g/L. VS/TS ratio was 0.863, thus Granule VS density was determined as 1.165g/L.

4.2 Reactor B

In this section, the performance of the 1.0L UASB reactor fed medium strength wastewater of approximately 1002.0 ± 133mg/L is presented. Average sCOD, pH and Alkalinity were 836.11(±45.5)mgCOD/L, 7.38 (±0.05) and 216.85 (±17.25)mgVFA/L

4.2.1 COD Removal and COD Balance

The point data and median averages of sCODRE are illustrated in Figure 4.9. sCODRE average 29.31% on day 19, peaked at 30.68% on day 84. sCODRE reduced to 26.08% and subsequently to 23.40% on day 125 and 142 respectively. The highest recorded tCODRE of 63% occurred on day 106 at OLR of 5 gCOD/L·d and HRT of 3.6h. CODRE Overall tCODRE in Reactor B over the period of investigation was 36%, Overall sCODRE for Reactor B was fairly constant at approximately throughout 28% (Figure 4.10).

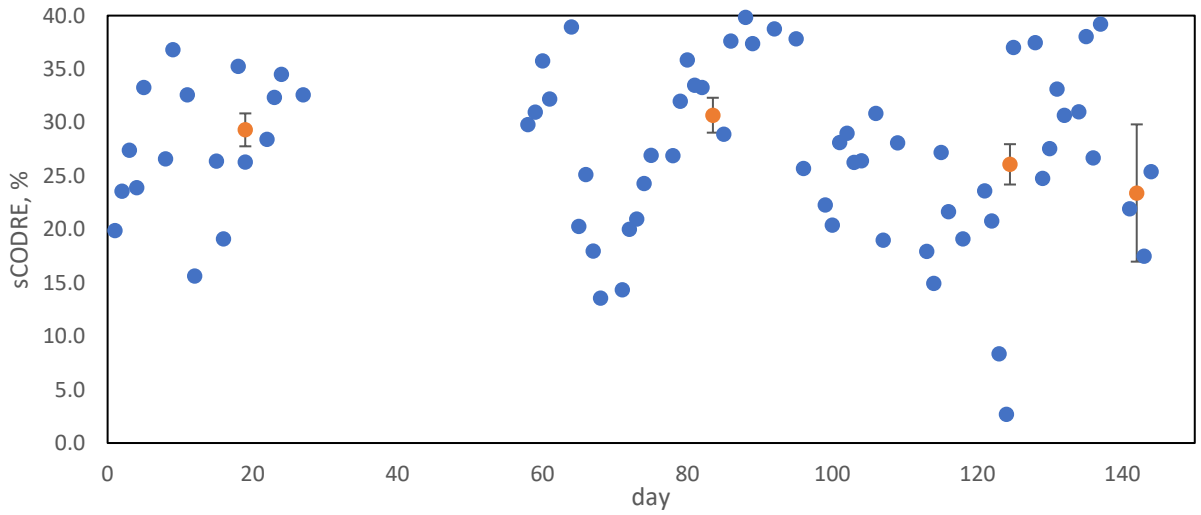

Figure 4.7 sCODRE (%) vs time profile of Reactor A

COD Balance showed an apparent inverse proportionality to sCODRE with maximum and minimum COD Balance values at 105.2% and 72.5%. COD

Balances at OLRs of 1.71,3.25 and 5.42gCOD/L·d were 82.48%(±1.61),82%(±0.96) and 85%(±1.69).

To determine the theoretical limit of soluble COD removal for medium strength wastewater at 2.5℃, COD Removal Flux was plotted against OLR

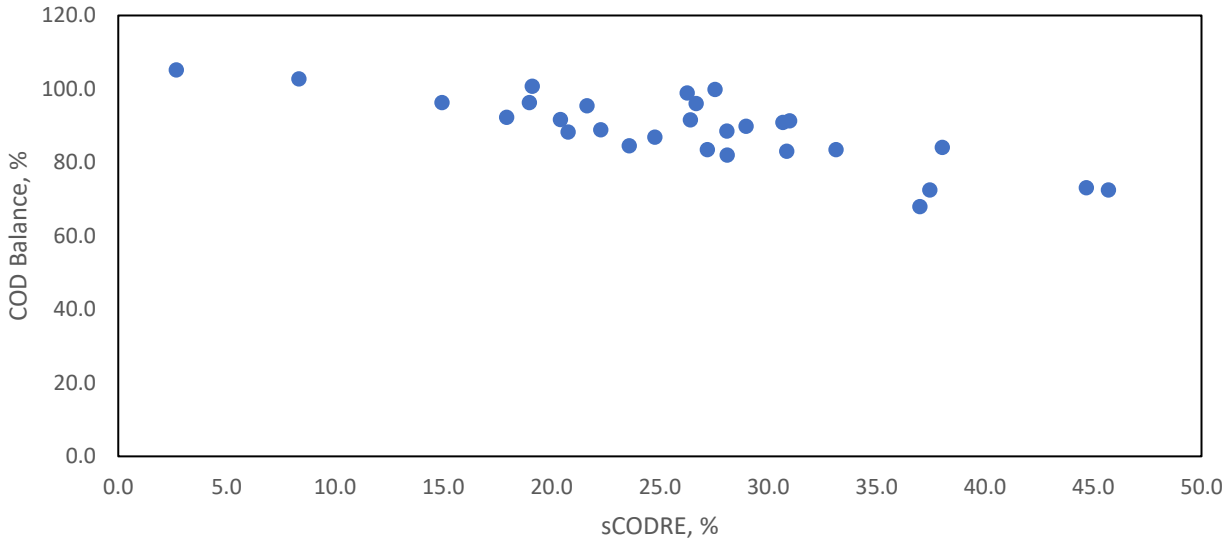

Figure 4.8 Variation of sCOD Balance with sCODRE of Reator B

COD Removal flux showed a direct proportionality to OLR (Figure 4.10). A logarithmic trendline was fitted and showed a correlation coefficient of 0.999 along the following equation. CODr=812.6ln(OLR)+59.1. Determination of trend was performed in absence of OLR of 8.07gCOD/L·d since effecting this OLR quickly caused reactor to shut down. COD removal fluxes at OLR of 1.71, 3.25 and 5.42gCOD/L·d were 501.45 (±34.78), 1000.75 (±55.61) and 1442.10 (±119.18) mgCOD removed/d.

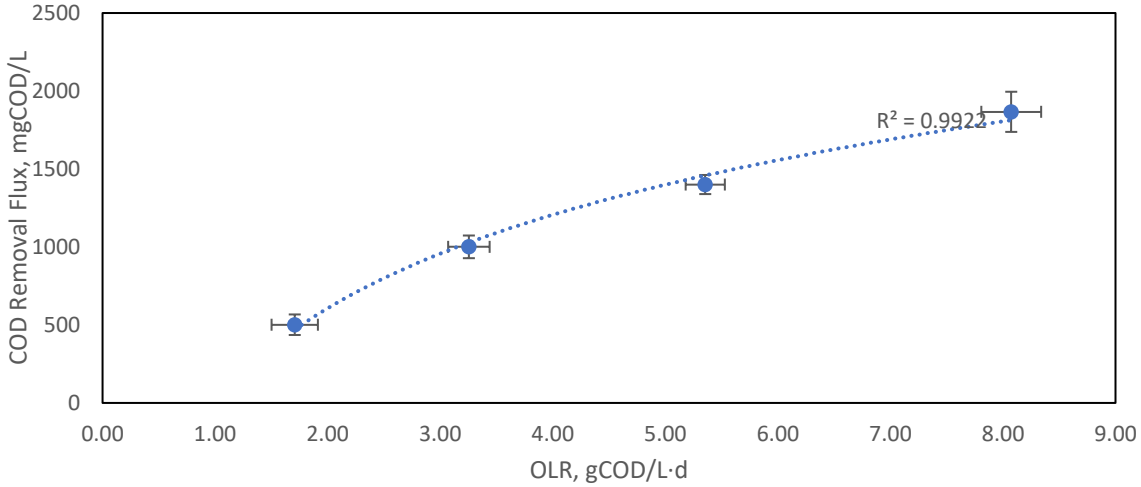

Figure 4.9 COD Removal Flux vs OLR to determine theoretical organic limitation of Reactor B

Theoretical optimal sCODRE was calculated using the equation, the average sCOD of and computing inlet outlet and removal flux. Average wastewater inlet sCOD concentration at Grødaland WWTP from OLR of 1.71 to 5.42gCOD/L was considered for this calculation. The theoretical optimal sCODRE calculated is approx. 32% and it occurs at approximately 2.43gsCOD/L·d. 15%sCODRE was used as a cutoff for OLR extrapolation and which occurred at 14.93gCOD/L·d. Operable OLR for Reactor B is shown in Figure 4.10

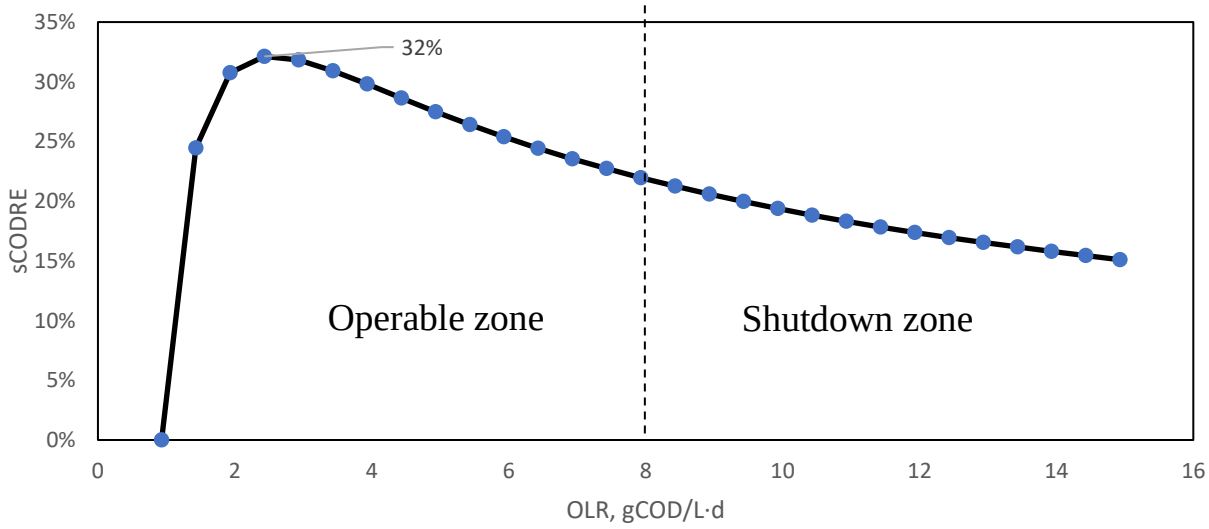

Figure 4.10 Theoretical Optimal OLR for sCOD in Reactor B

4.2.2 Biogas Production

A direct proportionality was observed between sCOD concentration and Methane Production. The highest and lowest methane productions of 1.36 and 0.27 gCOD/d were recorded at 1573 and 490.5 mg sCOD/L respectively. Both of these measurements were recorded at averaged OLR of 5.42gsCOD/L·d. A direct proportionality was also observed between Methane Production and OLR (Figure 4.15) Methane production rates at 1.71, 3.25 and 5.42gCOD/L·d were 0.34 (±0.027), 0.58 (±0.044) and 0.95(±0.056) gCOD/d. The maximum average methane production in Reactor B was 1.44 (±0.12)gCOD/L·d and occurred at OLR of 5.42

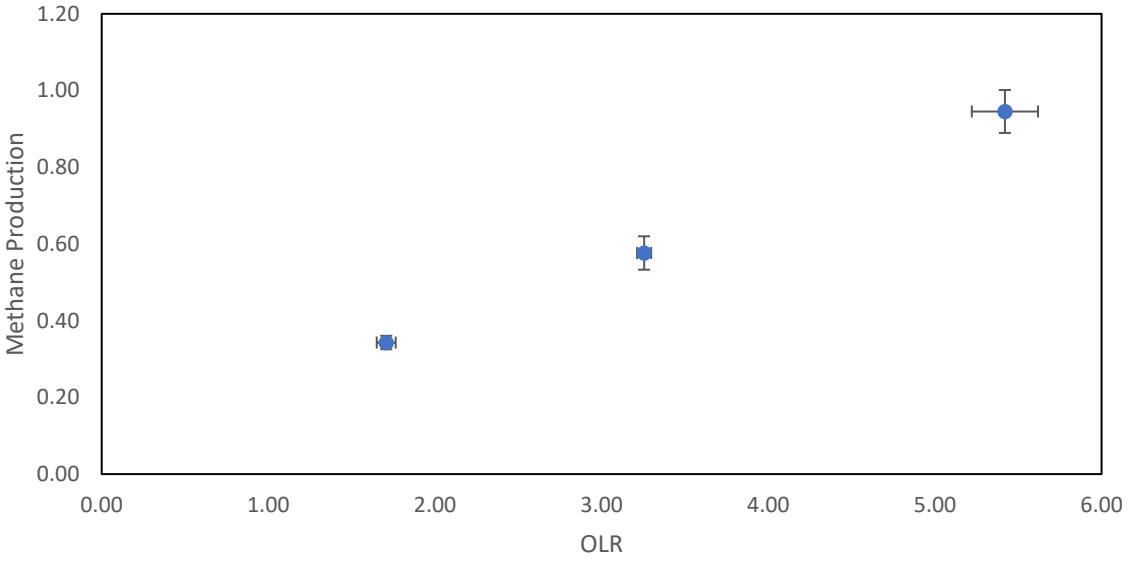

Figure 4.11 The Dependence of Methane Production on OLR in Reactor B

4.2.3 VFA Removal

An inverse proportionality was observed between reduction in VFA concentration and HRT as well as a direct proportionality between VFA concentration and flux of VFA removed. Figure 4.13 presents these findings. Flux of VFA removed at HRT of 3.15, 4.71 and 18.92 h were 700.89 (±212.99), 508.55(±109.55) and 135.55(±40.96) mg/d respectively while reduction of VFA

concentrations were 91.90(±27.42), 96.75(±17.98) and 107.42 (±29.57)mg/L respectively.

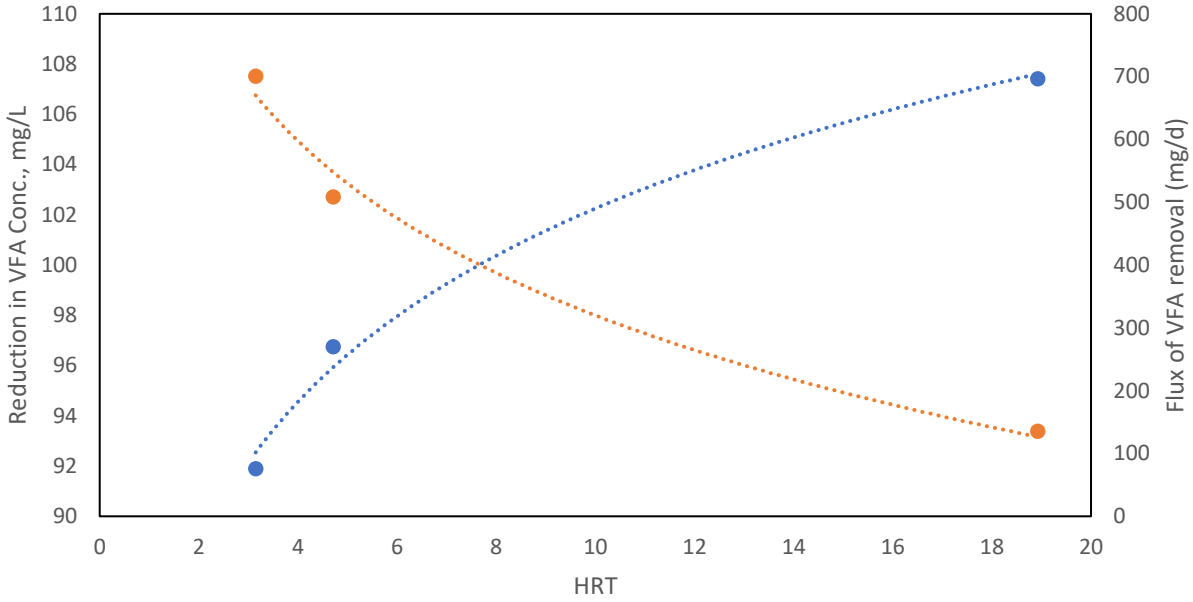

Figure 4.12 Effect of Feed pH on Average VFA Removal (Blue– Reduction in VFA concentration, Orange – VFA flux removed)

5.0 Discussion

This chapter discusses how different independent performance characteristics affected measured operational efficiencies of the bioreactor. Independent parameters are characterized as such due to their independence from the conditions inside the reactor, though they may still depend on other wastewater characteristics. Independent inlet characteristics include inlet COD, TSS, pH, OLR, Upflow velocity and hydraulic retention time. Measured performance indicators include Total Methane Production, Methane Fraction, Methane Yield, COD Removal Efficiency, VFA removal and COD Balance. Also, relations were drawn between dependent characteristics as they also contribute to mechanistic changes within the reactor.

5.1 COD Removal and COD Balance

In Reactor A, average overall CODRE of 35% was much lower than listed values in literature. Van Lier et al reported CODRE of 68% for the treatment of municipal wastewater at 14℃. Seghezzo operated pilot scale UASB reactors with OLR of 0.6gCOD/L·d. at inlet tCOD of 153mgCOD/L at a temperature of 17℃. Similar studies have reported higher COD removal efficiencies for both higher and lower strength wastewater than investigated in this project. Major points in this section discuss potential reasons for low overall COD removal.

On Reactor A startup, a suspension of yeast extract, acetate and sodium bicarbonate were used as the substrate. Studies conducted by Shen et al. (1993) showed that the maximum amount of carbohydrates extracted from granules by adding yeast extract to increase Carbon to Nitrogen (C/N) ratio. When feed was substituted for sewage, COD immediately reduced due to the lower C/N ratio. Also, acetate is the final intermediary substrate before acetoclastic methanogenesis. Thus, methane production using acetate is faster than methane production from the complex substrates in real wastewater. This may have been

the reason why the lowest OLR value of 2.5gCOD/L·d showed the highest tCODRE of 48%, even though no trend was observed between OLR and tCODRE.

pCODRE and sCODRE were markedly affected by upflow velocity. When upflow velocity was reduced, pCODRE increased at the expense of sCODRE. However, when upflow velocity was increased, pCODRE reduced, with no significant change in sCOD was recorded. This suggests that the reduction of upflow velocity fostered conditions that caused long-term reactor inhibition to sCOD removal. This can be explained by LCFA accumulation, which is further explained in the Section 5.5.

Within the range of OLR investigated in Reactor B, OLR had little effect on COD Removal Efficiency due to the inverse nature of OLR and HRT at constant COD concentration (Figure 4.7). As COD flux increased with increasing OLR, HRT reduced, thereby reducing granule-substrate contact time. However, an increase in the flux of sCOD removed was observed in the range of OLR investigated (Figure 4.9). Optimal sCOD removal occurred at 32% (Figure 4.10). This low COD removal is due to very low temperature which limits enzymatic activity, mass transfer and free movement of particulates.

From Figure 4.3 and Figure 4.8, there is an apparent inverse proportionality between COD removals and COD Balances in both reactor A and B. This suggests either a sampling error or an operational error. Settleable solids tended to adhere to bottom and inner wall of the sampling can. Vigorous homogenization could detach and resuspend them such that their COD content would contribute to feed COD. However, within a few minutes to hours, settleable solids would resettle before they could be transported into the reactor. As such, low COD Balances would coincide with high CODRE and vice versa. Homogenization can lead to the resuspension of settleable solids. Also, Henze et al. (2008) discussed

COD Balance gaps in high LCFA containing water. Often, gaps in COD balance can be attributed to the formation of oxidized anions like SO_4^{2-} and NO_3^-. Entrapment or accumulation of fat and LCFA from wastewater can also alter COD balance by drastically changing the COD/VSS ratio. Under LCFA-accumulative conditions, high removal efficiencies coincide with low CH_4 production causing gaps in COD balance. Also, considering that our wastewater was sourced from a DAF-treated combination of municipal slaughterhouse effluents, high lipid concentrations might have been present within the reactor. Lipids typically take a maximum of 8 days for complete hydrolysis.

5.2 Methane Production

From Figure 4.5, it can be observed that gas production was a function of OLR in Reactor A. At increasing OLR, tCOD removed increased as can be observed in Figure 4.2. With increasing tCOD removed, a corresponding increase in gas production is expected. Also, from Figure 4.3, we can assume using the previous establishments in this paragraph that treatment of high strength wastewater would produce more gas due to higher COD removal efficiency. Methane yield was largely determined by upflow velocity as shown in Figure 4.5. At OLR values between 2.5 and 6.4, upflow velocity of up to 42 m/d did not affect methane yield. However, at upflow of 68m/d, average methane yield increased to 68%.

Before improving recirculation in Reactor A, gas stagnation was be observed on the far side of the reactor opposite feeding port. This can be explained by liquid channeling along the inner feed-side wall surface. Intermittent rapid recirculation was required to release gas particles. After mixing improvement was made, gas bubbles were immediately disengaged on formation, thus preventing entrapment., Recirculation improved the distribution of organic matter and improved substrate-granule contact. Consequently, more gas was produced. More accurate and frequent readings could also be made.

Expected total COD removal was 4.56gCOD/d at OLR of 4.9gCOD/d (Figure 4.2). This value was estimated from trendline established at the upflow velocity of 40-42m/d. However, the measured total COD removal was 3.71gCOD/d. This was 19% lower than expected value due to the lower particulate removal. Assuming a similar upflow velocity was applied at an OLR of 6.4 gCOD/L·d, 4.56gCOD/d could theoretically have been removed. At 68% methane yield, this would amount to 3.1gCOD/L.

Reactor B showed a linear relation between methane production and OLR was observed (Figure 4.11). Safitri (2016) studied the biogas production rate of wastewater from Grødaland WWTP using different the Reactor B sludge mix and observed a power trend between OLR of 0 and 16gCOD/L·d [32]. However, for the narrow band of OLR investigated in this study, the linear least squares method produced the highest correlation coefficient. Applying the theoretical optimal OLR for sCODRE to linear Methane production trend yields 0.454gCOD/d. Biogas production at similar OLR values were also similar in Reactor A and B. 1.05 and 1.44 gCOD/L·d at 4.9 and 5.4gCOD/L·d respectively for Reactor A and B. This suggests superior performance of Reactor B in spite of the low temperature limitation.

5.3 Granule Source

In 1994, Schmidt and Ahring studied ECP in granular sludge from different reactors. Their studies showed that granules grown on simple substrates like acetate and VFA exhibited higher protein to polysaccharide (PN/PS) ratios of between 6.5 and 8.5. Sugar factory, paper mill and fish meal PN/PS were all below 6.1 and decreased with increasing complexity. [35] In a 2019 study, Dube and Guiot compared PN/PS ratios from a cheese factory, a fruit juice factory and a pulp/paper mill. They observed that granules from the pulp and paper mill had PN/PS ratios of 6 as compared to 3 in the other two factories. This also coincided

with a higher soluble fraction in the pulp and paper mill as compared to the other two factories which support the claim that simpler substrates requiring less hydrolysis yield higher PN/PS ratios. Relative microbial diversities were also different between different sludges suggesting the influence of environmental selection pressure[23] [36]. Thus, without knowing the source of granules, PN/PS ratio may serve as an indicator of the complexity of granulation-substrate.

A study conducted by *Batstone et al*,2004 observed that granules fed high protein-containing wastewater have had a lower number of microbes per unit volume (as observed by SEM) and lack a layering of structure. This has been attributed more to the particulate nature of wastewater than its proteinaceous nature. High TSS concentrations were observed in wastewater fractionation experiments as well as high particulate COD fractions and concentrations[37]. Also, Fang (2000) states that granulation does not entail random aggregation of suspended particles. Bacteria search for strategic positions for optimal supply of substrates and removal of products. Once a layered structure is generated, bacteria proliferate to a size that only stops when interfacial bacterial-mixed liquor area reduces to a critical level in relation to initial hydrolysis or fermentation that occurs at granule surface. [38] Thus, granules generated during the degradation of simple carbohydrates exhibit more stratification than complex composite-aggregated granules. The open porous, non-stratified system observed in composite-fed granules can be explained by the selection of bacteria close to the water-granule interface.

In the case of this study, Reactor A sludge granules likely exhibited similar relative microbial diversity to paper mill effluent granules as this is where granulation was performed. This resulted in suboptimal hydrolytic and methanogenic performance in the presence of a different and more complex substrates mix. Also, reselection and redistribution of the microbial consortium is unlikely due to the stratified and compact structure of sludge granules. This

resulted in a permanently suboptimal system design for our wastewater source. This is reflected in the net increase in VFA between feed and effluent during acetate treatment followed by the net reduction of VFA during sewage treatment from day 10. The net VFA decrease was accompanied by a reduction in CODRE suggesting that hydrolysis was the rate limiting step. Reactor B granules were partially sourced from a hydrocarbon-oil containing wastewater facility. This exposed them to higher fatty acid concentrations during granulation. This may have offered protection against LCFA inhibition. The diversity of syntrophic microbes in Reactor B may have been the cause for comparable sCODRE to Reactor A, even at a lower temperature.

5.4 VFA

The first and second rate-limiting processes in anaerobic digestion are the production of VFA's by disintegration, hydrolysis and acidogenesis and the removal of VFA's by acetogenesis and methanogenesis. The complete conversion of all COD required a minimum of hydrolysis and the production of methane requires a minimum of VFA conversion. Thus, these two rates can be compared by considering the VFA removal flux to COD removal flux of the system (VFA_r/COD_r). The lowest VFA_r/CDO_r at upflow velocity of 23m/d was caused by high solids accumulation rate that led to higher complex composite and polymeric substrate concentration. As a result, the bacterial consortium released hydrolytic enzymes that converted and liberated acidogenic substrates. High VFA production was exacerbated by insufficient reactor mixing, which promoted low VFA consumption. Figure 4.9 also suggests low methane production is recorded under the conditions present at low VFA_r/COD_r and vice versa. It is important to note that VFA_r/COD_r showed no relation to initial VFA concentration or OLR.

However, methane yield and methane production were both dependent on VFA_r/COD_r.

At lower temperatures, however, VFA_r/COD_r could not be compared due to lack of tCOD data points. Nevertheless, two relations were compared considering that VFA removal requires two minimal criteria to occur. Granule-substrate contact time and pH stability. Thus, the relation between average pH and VFA removal was compared to the relation between average HRT and VFA removal in Reactor B. VFA removal was highly dependent on HRT. This was not observed in Reactor A. Higher VFA removals in Reactor B resulted from the higher average initial VFA concentrations. Thus, methanogenic potential of wastewater fed to Reactor B was higher than methanogenic rate in reactor A due to lack of hydrolyzed acidogenic substrates in reactor A.

5.5 Environmental Conditions

A yellowish brown colour that intensified with time was observed in the reactor A. In addition, wastewater frequently foamed during feed homogenization. Buoyant filamentous bacteria occasionaly accumulated on top of sludge granules. The yellow tint can be explained by the occurrence of ferrous iron-containing slaughterhouse feeds. The buoyant filamentous bacteria could indicate the presence of sphaerotilus-leptothrix group of organisms also known as sewage fungus. These bacteria oxidize and deposit reduced iron along with their mucilaginous filaments. For instance, Sphaerotilus natans is involved in the oxidizing of both iron and reduction of sulfide. Black deposits in feed pipes into the reactor also suggested iron presence as well as the oxidizing of reduced compounds such as sulfide into iron sulfide. These organisms are obligate aerobes but will survive under low oxygen conditions (<0.3ppm). Thus, the occurrence of

these filamentous bacteria served only as an indication for the occurrence of iron or sulfide in feed during sample storage. [39]

Also, studies have shown that LCFAs cause long-term inhibition to the growth of methanogenic bacteria as they have a similar cell wall to gram-positive bacteria. LCFAs exhibit acute toxicity towards anaerobic consortium by adsorbing to granule surfaces and interfering with transport. In addition, the low density fats are known to float biomass and cause washout of smaller more susceptible granules. LCFAs also aid in the precipitation of Ca^{2+} and Mg^{2+}, thus preventing their bioavailability to anaerobic biomass. Oleic acid, Lauric acid and Myristic acid exhibit very low IC_{50} of 4.35 mM (1.21 mg/L), 4.8 mM (0.96 mg/L) and 4.8mM (1.10 mg/L) [40, 41]. Washout and long-term inhibition may have reduced potential for COD removal.

On the 3rd of March, during effluent collection for a parallel study on pathogen removal, NaOH from gas stripping apparatus was sucked into the reactor by backpressure. Reactor maintained a pH of 11 for at least 30 minutes. However, granules managed to survive high Na^{+} concentration, as well as the high pH.

5.6 Energy Potential

Assuming 1kgCOD:1.5kWh of electricity at 40% electricity conversion efficiency and 60% conversion to heat (Henze et al., 2000), upscaled versions of reactors A and B operated in Grødaland WWTP could produce 1.267 and 1.215 MWh of electricity along with 1.901MWh and 1.823MWh of heat per day. An upscaled version of Reactor B operated at optimal OLR for CODRE could produce 1.162MWh of electricity and 1.743MWh of heat per day.

According to the 2012 energy census, Statistik Sentralbyrå (SSB) determined that the average Norwegian household used 16,044 kWh/year or 43.96 kWh/d of electricity[42, 43]. Theoretically, upscaled versions of Reactor A, Reactor B and

optimized Reactor C could power approximately 29, 28 and 26 households respectively. A detailed description of the calculations is described in Appendix D.

5.7 Hydrodynamic Condition

This section describes the condition of the UASB reactors during operation. Granule characteristics and Gas liquid boundary are discussed regarding their effect on the behavior of the reactor.

In Reactor A, the gas liquid separator was very unstable during large and abrupt COD changes in the feed during sample replacement. This could be attributed to the system's transient capacity. During these periods, granule resuspension and washout were observed. Studies have shown that smaller granules have higher SMA due to the higher specific surface area. [37] The washout of smaller granules contributed to the gradual reduction in tCODRE. Between day 92 and 99, upflow velocity was increased and subsequent expansion of the sludge bed was observed. Due to the narrow inlet tube, upflow velocity was largest through the center of the horizontal cross section of the sludge bed. This motion of granules increased agitation within the reactor and contributed to the doubling of methane yield.

Reactor B exhibited a seemingly higher transient capacity than Reactor A as less periods of gas liquid interface instability were encountered. Reactor B was also fitted with a mesh to prevent washout of granules. The mesh retained smaller granules leading to higher specific surface area availability to for substrate diffusion and enzymatic release.

6.0 Conclusion

At consistent pCOD concentration, VFAr/CODr indicated higher methanogenic activity in reactor A. Upflow velocity can be used to modify the balance between the rate of methanogenesis and the rate of hydrolysis, although granule washout must be controlled. Sludge accumulation leads toan increase in higher hydrolytic rate, but limits methanogenic rate due to low upflow velocity.

Granulation environment is an important factor for selecting ideal granules. Granules produced from Norsk Skog Saugbrugs showed a similar sCODRE and methane production rate as compared to a mixture of hydrocarbon-oil, manure treatment and Norsk Skog sourced granules. This was due to the incompatibility between granules and the proteinaceous substrate as well as the diversity of microbes in Reactor B.

Psychrophilic wastewater treatment was successfully implemented on Grødaland DAF treated to an sCODRE of 30.7% at OLR of 3.25gCOD/L·d and HRT of 4.71h. The theoretical optimal COD Removal Efficiency was determined at 32% at OLR of 2.43mgsCOD/L·d and HRT of 8.3h assuming average inlet sCOD concentrations. Psychrophilic wastewater treatment was shown to be possible below OLR of 8gCOD/L·d to produce an average of 1.05gCOD/L·d of methane at maximum applied OLR of 5.42gCOD/L.d.

7.0 Recommendations

Characterization of lumped components to avoid uncertainty would be an improvement to this research. Gas chromatography can be used to improve the characterization of biogas components. This would be useful to determine potential H_2S and hydrogen production. VFA characterization would also improve.

The effect of upflow velocity on TSS removal should be investigated at constant OLR and different inlet TSS concentrations. Daily grab samples can be analyzed at the same time to determine VFARE/CODRE at different inlet TSS concentrations. The experiment can then be repeated at different OLRs to determine the best upflow velocity to apply at any given OLR and TSS concentration to achieve maximum biogas production and the required TSS removal efficiency. Accurate and precise TSS measurements and pCOD measurements should be made to delineate between inlet VSS and inlet TSS. The effect of particle size density can also be determined as a secondary study.

The effect of protein-granulated sludge granules can also be investigated on carbohydrate rich wastewater. This can determine whether their porous structure and higher surface area will have a significant positive effect on reactor performance variables.

8.0 References

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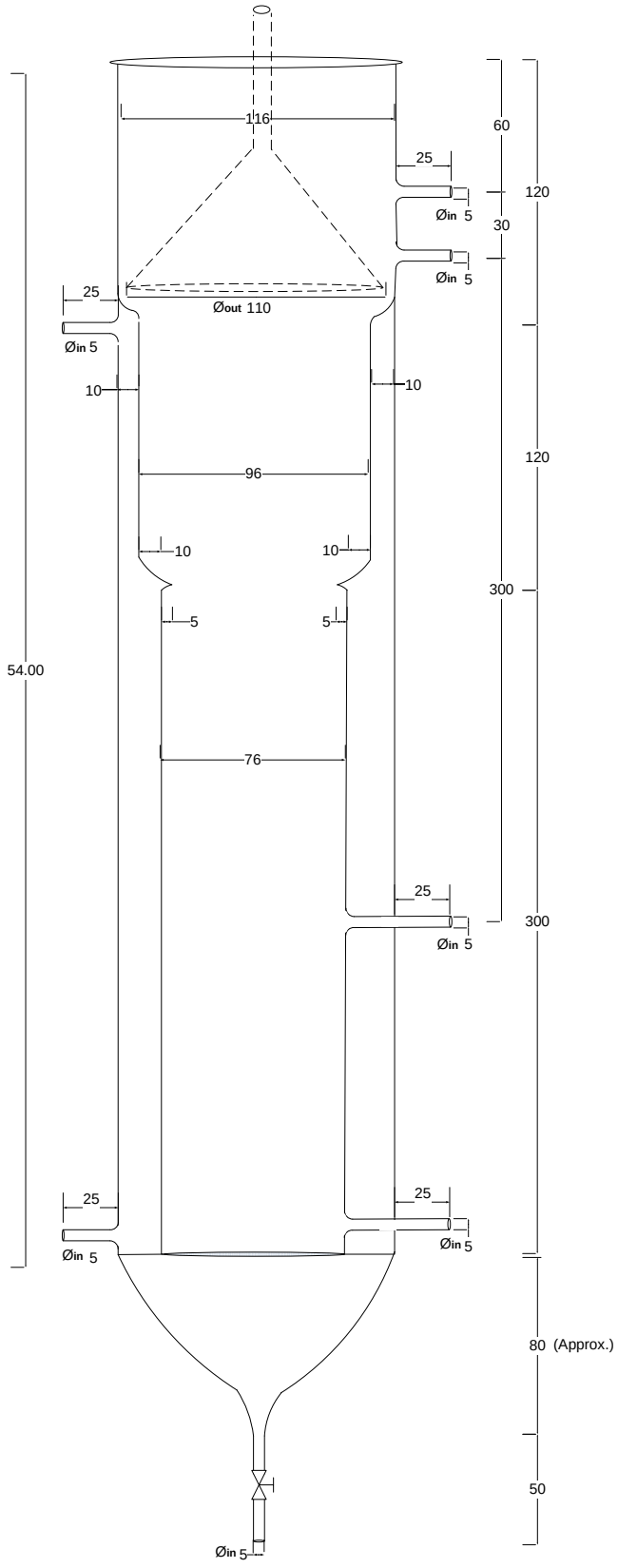
Appendices

Appendix A Reactor Design Details

Reactor A Design Details



Parameter(s)	Unit(s)	Range(s)	Chosen Value(s)	Detail(s)
Proposed Reactor Volume (V)	L		3	
COD Concentration (Cin)	mg/L	700 - 1000	1000	Grødaland WWTP
Organic Loading Rate (OLR)	kg COD/m^3.d	3 - 4	4	Henze *et al.* (2008)
Upflow Velocity (v)	m/h	0.5 – 1.0	1	Henze *et al.* (2008)
Slope GLSS	°	45-60	60	Verstraete et al (2009)
The overlap (deflector : GLSS)		1:10	1:10	Verstraete et al (2009)
Methane Production (γ)	m^3 CH_4/kgCOD	0.4	0.35	Tchobanoglous *et al.* (2012)

Reactor Design Calculations

- Inlet flow rate, Qin

$$V = \frac{Cin\,.\,Qin}{OLR}$$

$$Qin = \frac{V.OLR}{Cin} = \frac{3\ x\ 10^{-3} m^3 x\ 4\ \frac{kg\ COD}{m^3.d}}{1.0x10^{-3}\ \frac{kg\ COD}{L}} = 12\ L/d = 8.33\ mL/min$$

- Cross sectional area, A

$$A = \frac{Qin + Q_R}{v}$$

Q_R is Recirculation flow rate

$$Q_R = 10\ Qin = 120\ L/d = 83.33\ mL/min$$

$$A = \frac{11\ Qin}{v} = \frac{11\ x\ 12\ x \frac{10^{-3} m^3}{d}}{1\frac{m}{h} x \frac{24h}{d}} = 5.5\ x10^{-3}\ m^2$$

- Reactor diameter, D

$$D = \sqrt{\frac{4A}{\pi}}$$

$$D = \sqrt{4\ x \frac{5.5\ x\ 10^{-3}}{\pi}} = 0.084\ m = 8.4\ cm$$

- Reactor height, h

$$h = \frac{V}{A} = \frac{3\ x\ 10^{-3}}{5.5\ x\ 10^{-3}} m = 0.54\ m = 54\ cm$$

- Hydraulic Retention Time, HRT

$$HRT = \frac{V}{Qin} = \frac{3\ x\ 10^{-3} m^3}{12\ x10^{-3}\ m^3/d} = 0.25\ d = 6\ hours$$

- Gas Production, Gp

$$Gp = \gamma . OLR . V = 0.35\ \frac{m^3 CH_4}{kg\ COD}\ x\ 4\ \frac{kg\ COD}{m^3 . d}\ x\ 3\ L = 4.2\ L\ CH_4/day$$

Assumption: 70 – 90 % COD removal/conversion

*Reactor Designed by Kommedal, Roald

Appendix B Reactor Performance Data Points

Reactor A Daily Data Point

Day	Flow rate, ml/min	OLR, g/L·d	HRT, h	Meth rate, gCOD/d	pH	ALK	VFA	tCOD	sCOD	pH	ALK	VFA	tCOD	sCOD	Meth fraction, %	Meth Yield, %	CODRE, %
1	4.00	**2.68**	12.50	0.98	7.44	224.7	262.9	1162	1162.0	7.38	301.2	280.9	742	742.0	**0%**	**0%**	**36%**
2	4.00	**2.67**	12.50	0.00	7.34	133.2	262.2	1161	1161.0	7.38	170.0	294.0	930	930.0	**0%**	**0%**	**20%**
3	5.00	**3.00**	10.00	0.00	7.34	128.5	352.2	1040	1040.0	7.76	429.9	190.7	690	690.0	**0%**	**0%**	**34%**
4	5.00	**3.01**	10.00	0.00	7.21	81.5	410.6	1044	1044.0	7.37	481.4	89.4	687	687.0	**0%**	**0%**	**34%**
5	5.00	**3.15**	10.00	0.00	7.21	97.6	303.0	1093	1093.0	7.08	347.0	250.0	787	787.0	**0%**	**0%**	**28%**
8	5.00	**2.98**	10.00	1.01	6.70	91.1	383.8	1033	1033.0	7.27	281.4	188.6	560	560.0	**63%**	**30%**	**46%**
9	5.00	**3.21**	10.00	1.54	7.50	590.9	87.3	1116	1116.0	7.50	356.5	368.3	531	531.0	**69%**	**37%**	**52%**
10	5.00	**1.96**	10.00	0.77	7.89	541.6	116.0	681	517.5	7.62	777.4	93.8	369	177.0	**24%**	**34%**	**46%**
11	5.00	**2.01**	10.00	0.93	7.70	628.4	14.6	697	644.0	7.68	710.6	142.4	359	322.0	**60%**	**38%**	**48%**
12	6.90	**4.01**	7.25	0.62	7.36	721.7	210.6	1008	642.0	7.41	669.1	79.9	618	414.0	**64%**	**16%**	**39%**
14	6.90	**4.22**	7.25	0.90	7.88	565.6	86.5	1062	542.0	7.70	1144.0	0.0	722	426.0	**57%**	**27%**	**32%**
15	8.20	**4.42**	6.10	0.43	7.79	178.3	0.0	935	487.5	7.59	172.8	17.8	684	400.0	**42%**	**14%**	**27%**
16	8.20	**4.42**	6.10	0.68	7.69	506.5	130.9	935	487.5	7.73	742.6	0.0	623	426.0	**76%**	**19%**	**33%**
17	8.20	**4.03**	6.10	1.03	7.74	600.1	116.4	854	590.0	7.57	719.0	50.0	650	426.0	**60%**	**43%**	**24%**
18	8.20	**4.03**	6.10	0.97	7.74	600.1	116.4	854	590.0	7.64	583.7	70.6	630	420.0	**60%**	**37%**	**26%**
20	8.20	**3.30**	6.10	0.89	7.42	697.1	44.2	699	520.0	7.47	711.0	1.4	580	430.0	**60%**	**63%**	**17%**
21	9.00	**4.27**	5.56	0.99	7.96	691.1	58.1	823	558.0	7.80	763.8	109.3	534	438.0	**69%**	**27%**	**35%**
34	8.33	**3.64**	6.00	1.90	7.33	425.2	1.3	759		8.11			440		**94%**	**50%**	**42%**
35	8.33	**3.82**	6.00	2.05	7.33	379.1	16.1	796		7.15	294.8	78.4	426		**85%**	**46%**	**46%**
36	12.50	**6.34**	4.00	2.02	7.28	293.1	216.5	880	800.0	7.55	350.4	25.7	405	270.0	**91%**	**24%**	**54%**
37	11.00	**6.50**	4.55	2.46	7.16	336.7	66.7	1026	720.0	7.30	427.3	33.0	510	297.0	**90%**	**30%**	**50%**
38	14.00	**6.45**	3.57	2.46	7.16	336.7	66.7	800	600.0				480	165.0	**91%**	**38%**	**40%**
49	8.50	**4.03**	5.88	1.35	7.96	691.1	58.1	823	418.5	7.80	763.8	109.3	534	328.5	**81%**	**38%**	**35%**
51	6.40	**5.80**	7.81	1.21	7.29	500.3	137.4	1573	708.0	7.63	643.0	69.9	775	522.0	**81%**	**16%**	**51%**

52	8.84	**6.50**	5.66		7.29	500.3	137.4	1276	708.0	7.48	551.3	33.7	753	522.0	**93%**		**41%**
55	14.00	**5.57**	3.57	0.70	7.65	601.8	211.4	691	427.0	7.98	624.8	55.1	524	415.6	**87%**	**21%**	**24%**
56	10.00	**6.42**	5.00	0.51	7.50	601.8	211.4	1114	742.5		886.2	82.9	495	442.5	**96%**	**6%**	**56%**
57	10.00	**6.42**	5.00	0.86	7.87	669.6	34.2	1114	742.5	7.81	581.4	0.0	500	442.5	**88%**	**10%**	**55%**
58	10.00	**6.42**	5.00	1.13	7.35	509.9	72.7	1114	742.5	7.48	509.9	72.7	595	235.0	**82%**	**15%**	**47%**
62	7.50	**6.50**	6.67	0.29	7.46	712.1	215.8	1505	1212.0	7.61	865.3	91.2	1030	789.0	**91%**	**6%**	**31%**
63	7.70	**6.67**	6.49	2.16	7.62	734.0	0.0	1505	1212.0	7.50	928.5	78.1	1218	846.0	**90%**	**68%**	**19%**
64	7.20	**6.52**	6.94	2.16	7.41	764.2	299.8	1573	1162.5	7.54	922.0	185.0	1289	904.5	**88%**	**73%**	**18%**
65	8.00	**6.91**	6.25	2.48	7.38	838.1	244.3	1500	1047.4	7.48	921.3	155.7	1145	682.9	**87%**	**61%**	**24%**
66	8.00	**6.57**	6.25	2.44	7.38	838.1	244.3	1425	1127.0	7.59	960.4	117.4	1127	957.0	**86%**	**71%**	**21%**
69	8.40	**7.02**	5.95	0.45	7.30	798.8	240.9	1450	1006.0	7.52	964.1	159.0	1146	717.0	**89%**	**12%**	**21%**
70	8.40	**6.41**	5.95	1.07	8.05	915.6	72.7	1324	804.0	8.47	1114.1	90.1	799	634.5	**84%**	**17%**	**40%**
71	8.40	**6.41**	5.95	1.05	8.30	1032.0	0.0	1324	804.0	8.35	1077.4	26.2	714	549.0	**96%**	**14%**	**46%**
72	5.20	**3.94**	9.62	2.41	7.80	954.8	27.8	1316.0	828.0	7.77	905.9	60.9	700	595.5	**90%**	**52%**	**47%**
75	5.80	**4.07**	8.62	0.48	7.56	666.1	155.6	1218	871.5	7.91	1243.7	93.2	1066	691	**78%**	**38%**	**12%**
76	5.80	**4.07**	8.62	1.35	7.56	666.1	156	1218	871.5	7.56	1137.7	77.5	864	650	**81%**	**46%**	**29%**
77	5.80	**4.07**	8.62	1.75	7.45	666.1	156	1218	871.5	7.84	988.1	40.5	670	649	**81%**	**38%**	**45%**
78	5.90	**4.08**	8.47	0.59	7.20	1149.5	10.9	1202	631.5	7.80	968.4	0	794	705	**81%**	**17%**	**34%**
79	6.00	**4.15**	8.33	0.72	7.30	982	43.4	1202	631.5	7.29	1045	0	768	697.5	**82%**	**19%**	**36%**
80	6.00	**4.11**	8.33	0.68	7.36			1190	660	7.89	1242	0	714	545	**72%**	**16%**	**40%**
83	6.00	**4.11**	8.33	0.41	7.36			1190	660	7.64	1248.4	47.7	626	575	**80%**	**8%**	**47%**
84	8.00	**4.81**	6.25	0.41	7.60	980	70.7	1044	874	7.70	1082.2	4.8	809	688	**84%**	**15%**	**23%**
85	8.40	**5.31**	5.95	1.00	7.36	974.3	123.3	1098	781.5	7.48	981.3	0.4	874	708	**93%**	**37%**	**20%**
86	8.00	**4.78**	6.25	0.82	7.33	1021.3	0	1037	727.5	7.64	932.3	92.6	716	716	**96%**	**22%**	**31%**
87	6.70	**3.67**	7.46	0.35	7.58	952.8	136.6	951	784	7.64	1365.5	0	901	656	**57%**	**73%**	**5%**
88	8.40	**3.58**	5.95	0.69	6.90	421.4	28.2	739	345	7.13	426.9	27.2	482	379.5	**81%**	**22%**	**35%**
91	8.00	**3.46**	6.25	1.47	7.13	402.1	0.1	750	422.25	7.36	456.3	5.4	619.5	346.5	**83%**	**98%**	**17%**
92	10.20	**5.26**	4.90	2.51	7.34	538	19.5	896	350.25	7.42	527	0	489.5	360	**90%**	**42%**	**45%**
93	10.20	**5.05**	4.90	4.23	7.25	492.9	23	859	368.25	7.40	556	0	549.5	360.75	**90%**	**93%**	**36%**
94	13.20	**5.14**	3.79	2.51	7.24	555.5	95.4	676	486	7.31	632.2	0	486	336	**64%**	**69%**	**28%**
97	8.80	**4.75**	5.68	1.62	7.81	805.7	144.7	938	795.0	7.72	707.8	0.0	709.0	579.0	**82%**	**56%**	**24%**

98	8.80	**4.60**	5.68	1.94	7.36	254.1	35.7	907	843.0	7.43	273.9	0.6	755.0	586.5	**88%**	**101%**	**17%**
99	8.20	**4.61**	6.10	1.62	7.34	264.4	52.8	976	856.5	7.40	295.3	0	695	684	**80%**	**49%**	**29%**

Reactor B Daily Data Points

Days	CODs ,mg/l	Qin ,ml/min	OLR, gCOD/L·d	HRT (h)	pHf	VFAf mg/L	Alkf, mg/L	pHeff	VFAeff, mg/L	Alk, mg/L	Methane Fraction, %	Methane Produced, gCOD/d	CODse, mg/L	CODs, %	COD Balance, %	Methane Yield, %
1	1247	1	1.8	16.7	7	302	236	7	306.6	421	78.7	0.37	999	19.9	92.9	103%
2	1354	1	1.9	16.7	7.1	302	256.7	6.8	352.6	401	80.2	0.37	1035	23.6	88.2	80%
3	1102	1	1.6	16.7	7.1	123	459.6	6.9	325.3	351	88.2	0.31	800	27.4	83.2	71%
4	1305	1	1.9	16.7	6.8	123	569	7	126	216	80.1	0.37	993	23.9	88.3	82%
5	1265	1	1.8	16.7	6.7	201	478.3	7.1	103.6	312.9	79.9	0.56	844	33.3	89.5	92%
8	1575	1	2.3	16.7	7.1	132	596	7.3	120	667	79.8	0.4	1156	26.6	84.8	66%
9	1725	0.7	1.7	23.8	7	378.2	398.9	7	166.3	449.7	81.2	0.25	1090	36.8	69.6	39%
10	1689	0.7	1.7	23.8	6.9	319.5	370.5	6.9	296.2	397	80.3	0.32	989	41.4	69	45%
11	1896	0.7	1.9	23.8	6.9	288.4	388.2	6.8	99.1	567.6	82	0.39	1278	32.6	80.5	63%
12	1172	1	1.7	16.7	6.9	376.6	299.4	6.9	46	467.2	80.1	0.22	989	15.6	89.3	85%
15	1220	0.7	1.2	23.8	7	209.4	500.5	7.1	106.4	492.1	83.2	0.28	898	26.4	84.9	86%
16	1220	0.7	1.2	23.8	6.9	219.1	489.2	7.1	96.8	504.6	84.5	0.22	987	19.1	87.7	95%
17	1422	0.7	1.4	23.8	6.9	248	298	7.1	56	378	85.2	0.33	841	40.9	72.7	57%
18	1234	1	1.8	16.7	7	341	213	7	178	344	87.2	0.29	799	35.3	73.3	47%
19	1012	1	1.5	16.7	6.9	347	333	7	145	454	83.3	0.32	746	26.3	86	83%
22	1168	1	1.7	16.7	6.8	132	488.9	7	109.8	510.6	85.2	0.39	836	28.4	86.3	81%
23	1296	1	1.9	16.7	6.8	201.5	408	7.2	0	423.6	86.2	0.36	877	32.3	79.3	59%
24	1209	1	1.7	16.7	6.8	289	398	7.2	0	578	84.4	0.38	792	34.5	79.5	64%

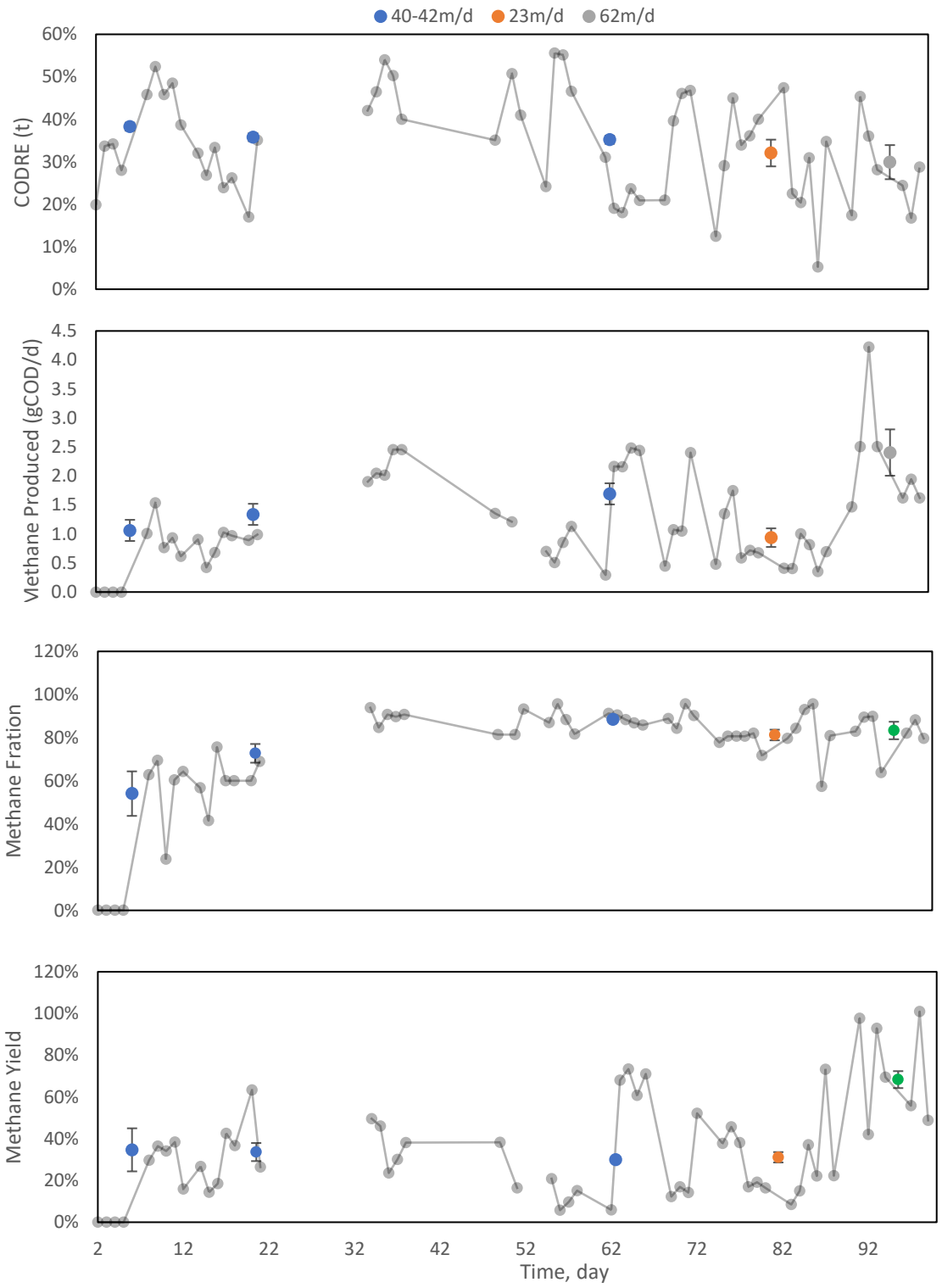
27	1169	1	1.7	16.7	7	142	461	7	0	569	83.9	0.39	788	32.6	82.3	71%
58	1269	2	3.7	8.3	7	301	356.8	7.1	110	486.9	82.3	0.47	891	29.8	79.3	43%
59	1385	1.5	3	11.1	7.1	246	256.9	7.2	105.7	463	83.7	0.67	956	31	86.7	72%
60	1197	2	3.4	8.3	6.9	278.6	487.6	7	119.8	403.9	82.9	0.92	769	35.8	86.8	74%
61	1243	1.7	3	9.8	7	227.6	430.5	7.1	109.2	429.6	84.2	0.53	843	32.2	80.7	54%
64	1459	1.7	3.6	9.8	7.6	138.7	397.8	7.4	79.9	417.9	82.8	0.84	891	38.9	80.7	60%
65	390	6	3.4	2.8	7	116	489	7	306.6	421	83.8	0.37	311	20.3	86.5	54%
66	414	6	3.6	2.8	7.4	87.5	379	7.7	33.7	542	82	0.33	310	25.1	80.3	37%
67	401	6	3.5	2.8	7.3	99.2	409.7	7.3	0	580	79.7	0.33	329	18	87.7	54%
68	420	6	3.6	2.8	7.4	147	435	7.5	34	528	84	0.24	363	13.6	89.3	49%
71	279	7	2.8	2.4	8.1	89.9	497	7.8	0	592.5	86	0.35	239	14.3	93	86%
72	295	7	3	2.4	8.3	69.2	513	8.2	13.4	578.5	89	0.4	236	20	88.7	67%
73	401	5.3	3.1	3.1	7.9	53.3	467.4	7.6	38	550.4	86.6	0.35	317	20.9	85.8	54%
74	412	5.3	3.1	3.1	7.4	23.6	500.2	7.3	0	596.3	85.6	0.42	312	24.3	84.6	55%
75	405	5.3	3.1	3.1	7.2	56.2	514	7.6	12.3	512.4	88.2	0.42	296	26.9	82.1	50%
78	409	5.3	3.1	3.1	7.3	57.2	522.6	7.5	23.2	577.3	85.1	0.42	299	26.9	82	50%
79	422	5.3	3.2	3.1	7.3	46.9	508.6	7.2	0	603.1	86.2	0.7	287	32	85.3	68%
80	435	5.3	3.3	3.1	7.4	60.3	513.3	7	0	614.2	87.2	0.47	279	35.9	74.2	40%
81	433	5.2	3.2	3.2	7	153	452	7.1	0	512	88.5	0.42	288	33.5	75.1	38%
82	445	5.2	3.3	3.2	7.1	174	348	7.1	12	501	87.6	0.42	297	33.3	75.1	38%
85	607.5	3.5	3.1	4.8	7	191	263.8	7.1	25.1	453.2	84.2	0.5	432	28.9	82.9	57%
86	642	3.6	3.3	4.6	7.6	193.6	162.1	6.6	252.1	368.6	84.2	1.02	400.5	37.6	88.7	81%
87	652.5	3.5	3.3	4.8	6.9	241.1	254.9	7	0	480.1	85.7	1.1	373.5	42.8	86.6	79%
88	663	3.7	3.5	4.5	7.3	136.1	360.5	6.8	42.1	473.9	83.3	0.82	399	39.8	79.4	58%
89	606	3.5	3.1	4.8	6.8	102.3	369.5	6.9	0	505.3	84.3	0.78	379.5	37.4	83.5	68%
92	600	3.7	3.2	4.5	7.2	162.7	422.2	6.7	0	572.4	82.8	0.8	367.5	38.8	81.9	65%
93	645	3.7	3.4	4.5	7	145.9	420.9	7.1	0	583.8	82.6	0.68	372	42.3	73.3	47%
94	715	3	3.1	5.6	7.1	122	436	7	25	509.6	83.1	0.67	421	41.1	76	53%

95	690	3.1	3.1	5.4	7.3	421.5	450.5	6.7	90.2	492.5	82.7	0.72	429	37.8	81.1	62%
96	700.5	5.3	5.3	3.1	8.1	341.6	516.2	6.7	406.7	410.8	81.70%	0.65	520.5	25.7	83.8	47%
99	723	6.5	6.8	2.6	7.6	566.9	499.2	6.847	163.8	550	82.05%	0.89	562	22.3	88.9	59%
100	495	7	5	2.4	8.2	445.4	621.8	6.963	416.4	661.2	82.39%	0.74	394	20.4	91.7	73%
101	498	7	5	2.4	8.2	462.2	613.3	6.872	315	662.8	82.30%	0.65	358	28.1	82	46%
102	754	4.6	5	3.6	6.8	425.7	307	7.506	481.2	479.4	82.41%	1.08	535.5	29	89.9	75%
103	750	4.8	5.2	3.5	7.3	497.6	543.7	6.78	292.6	557	84.08%	1.45	553	26.3	98.9	106%
104	721.5	4.8	5	3.5	7.3	546.1	474.5	6.78	316.4	603	81.38%	1.04	531	26.4	91.6	79%
106	685.5	5.5	5.5	3	7.3	331.4	527.1	6.8	237.1	637.5	81.93%	0.91	474	30.9	83.1	53%
107	648	5.3	4.9	3.1	7.6	627.3	564.3	6.8	386.6	309.8	81.81%	0.89	525	19	96.3	95%
108	691.5	5	5	3.3	7	207.8	276.5	7.5	0	438.4	81.32%	1.03	382.5	44.7	73.2	46%
109	705	5	5.1	3.3	7	208.3	197.5	7.4	61.1	477.7	81.32%	0.98	507	28.1	88.5	69%
113	418.5	8	4.8	2.1	7.2	0	437.9	7.5	7.9	401	79.80%	0.63	343.5	17.9	92.3	73%
114	522	8.3	6.2	2	7.4	106.1	369.8	7.4	232.9	340.2	85.17%	0.84	444	14.9	96.3	90%
115	838.5	8.3	10	2	7	206.2	314.6	7.1	71.3	422.9	80.62%	1.21	610.5	27.2	83.5	44%
116	769.5	4.5	5	3.7	7.2	211.5	423.3	7.4	121.8	449.2	80.07%	0.99	603	21.6	95.4	92%
118	471	7.1	4.8	2.3	7.3	0	473	7.4	87.4	471	79.81%	1.1	381	19.1	100.8	119%
121	523.5	7	5.3	2.4	7.3	370.9	300	7.3	186.5	286.6	86.21%	0.57	400	23.6	84.6	46%
122	469.5	7	4.7	2.4	7.4	157	0	7.6	0	527.5	81.23%	0.57	372	20.8	88.3	58%
123	456	8	5.3	2.1	7.3	140.5	358	7.4	218.7	363.6	80.89%	0.72	418	8.3	102.7	165%
124	448.5	8	5.2	2.1	7.6	46.5	446.6	7.2	0	540.4	80.80%	0.54	436.5	2.7	105.2	394%
125	490.5	7.6	5.4	2.2		0	458.8	7.6	0.8	511.8	79.72%	0.41	309	37	68	21%
128	606	7	6.1	2.4	7.2	159.2	344.1	7.4	0	533.1	80.27%	0.75	379	37.5	72.5	33%
129	444	6	3.8	2.8	7.5	55.8	453.9	7.5	0	426.9	80.23%	0.59	334	24.8	86.9	62%
130	933	3.7	5	4.5	7.6	169.8	509.6	7.7	70.6	656.4	79.59%	1.5	676	27.5	99.9	110%
131	996	3.7	5.3	4.5	7.7	200.5	522.8	7.7	17.6	699.6	79.51%	1.02	666	33.1	83.5	58%
132	939	3.7	5	4.5	7.3	18.7	599.9	7.7	144.9	634	91.14%	1.22	651	30.7	90.9	80%
134	936	3.7	5	4.5	7.3	215.7	459.3	7.7	259.2	489.3	79.11%	1.25	646	31	91.4	81%

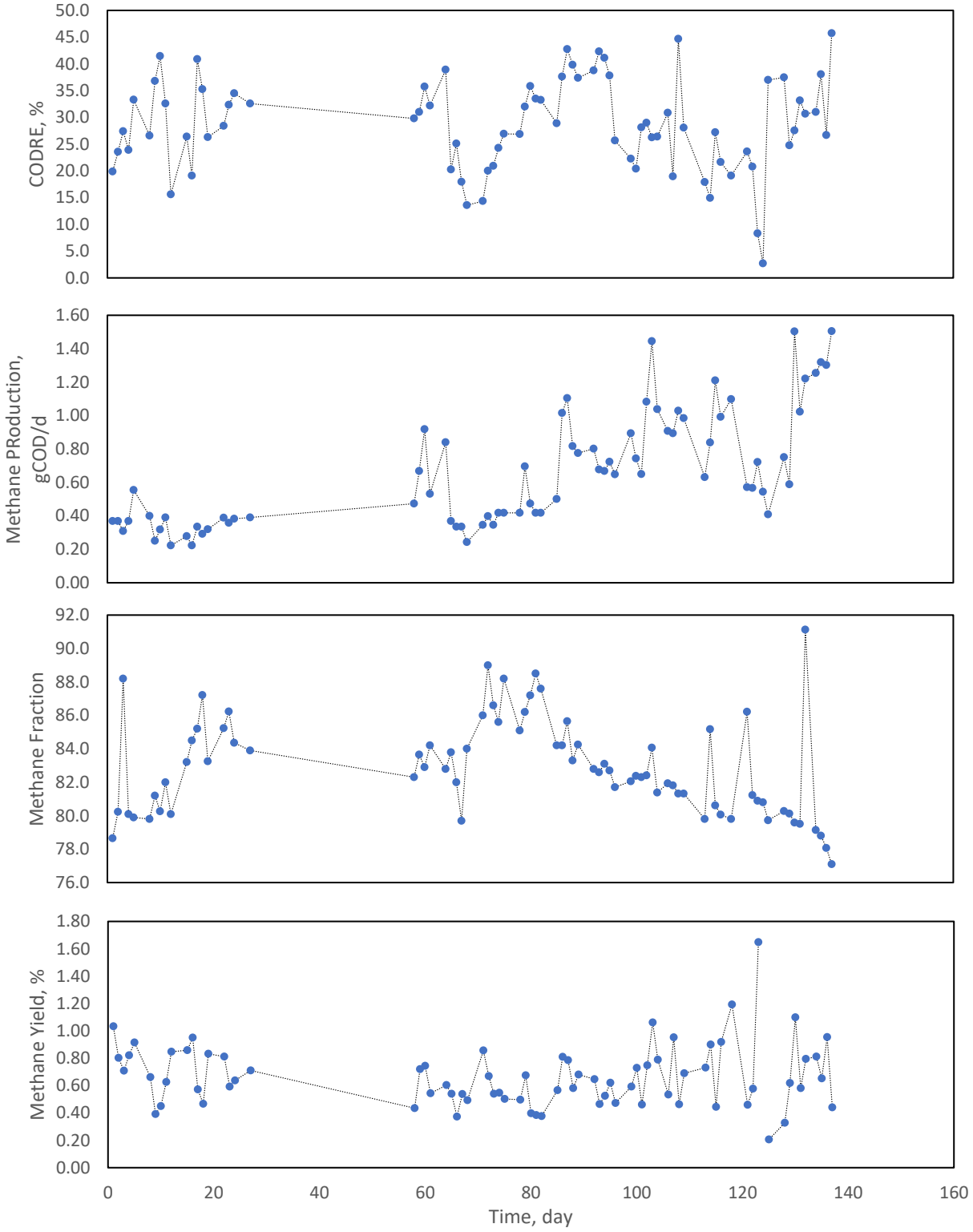
135	999	3.7	5.3	4.5	7.5	589.6	137.3	7.5	103.2	741	78.88%	1.32	619	38	84.1	65%
136	960	3.7	5.1	4.5	7.6	32	657.7	7.6	156.8	623.6	77.97%	1.3	704	26.7	96.1	95%
137	1162	3.3	5.5	5.1	6.9	172.1	549.7	6.9	521.3	433.3	77.76%	1.26	706.5	39.2	81.1	58%
138	1573	3.3	7.5	5.1	6.9	541	489.2	7.1	367.7	672.2	77.11%	1.5	854	45.7	72.5	44%
141	1669.5	3.3	7.9	5.1	7.2	494.3	514.9	7.1	272.9	328.7	75.75%	1.69	1303.5	21.9	97.6	97%
143	852	5.9	7.2	2.8	7.3	194.6	443.8	7.5	124.9	505.3	75.70%	0.97	703	17.5	94	77%
144	981	6.6	9.3	2.5	7.6	237.8	177.3	7.6	33.7	543	70.78%	0.7	732	25.4	80.6	29%
147	1296	4.5	8.4	3.7	7.2	232.1	479.2	7.3	537.3	644.4			1212	6.5		

Appendix C Graphical Reactor Performance Profiles

Reactor A Graphs



Reactor B Graphs



Appendix D Energy Recovery Calculations

Parameter	Mark(s)		
Conversion [a.]	13.5MJ CH_4: 1kg COD: 1.5kWh electricity and 2.25kWh heat at 40% electric conversion efficiency		
Reactor	A	B	B optimal
OLR, gCOD/L·d [b.]	4.9	5.42	2.43
Methane produced, gCOD/d [b.]	2.40	0.95	0.454
Flowrate into IVAR, L/d [c.]	5,000,000		
Flowrate into Reactor, L/d	14.2	5.86	2.93
Reactor scale factor	3.5×10^5	8.53×10^5	2.706×10^6
Electricity generation, MWh.d	1.267	1.215	1.162
Heat generation, MWh.d	1.901	1.823	1.743

a. Conversion: Henze et al (2008)
b. Reactor average data
c. IVAR average hydraulic loading rate

Reactor A

$$Electricity, A\left(\frac{MWh}{d}\right) = 3.5\times10^5 \times 2.40\frac{gCOD}{d}\times\frac{kgCOD}{1000\ gCOD}\times 1.5\times\frac{MWh}{1000\ kWh}$$

$$Electricity, A\left(\frac{MWh}{d}\right) = 1.267 MWh/d$$

$$Heat, A\left(\frac{MWh}{d}\right) = \frac{1.267}{0.4} = 1.901\ MWh/d$$

Appendix E Theoretical Optimal OLR for maximum CODRE Calculation

$$COD\ flux\ removed\ (CODrem), mg/d\ = 812.6ln(OLR) + 59.1$$

$$Average\ sCODi\ =\ 0.861\ mg/L$$

$$COD\ flux\ in\ (\frac{mg}{d}) =\ sCODi\ (mg/L)\ \times\ Flow\ rate\ (L/d)$$

$$COD\ flux\ out\ (\frac{mg}{d}) =\ COD\ flux\ in\ -\ COD\ flux\ removed$$

$$Removal\ Efficiency\ (\%)\ = \frac{COD\ flux\ removed}{COD\ flux\ in} \times 100\%$$

Graph of Removal Efficiency to OLR was generated from table below

Flow rate (m/d)	tCODin mg/L	OLR, g/L.d	tCODrem, mg/d	tCOD flux, mg/d	tCODrem flux, mg/d	tCOD out flux, mg/d	tCODout, mg/L	%RE
1.17	0.86	1.00	8.09	1.00	0.01	0.99	0.85	1%
1.75	0.86	1.50	358.42	1.50	0.36	1.14	0.65	24%
2.33	0.86	2.00	606.97	2.00	0.61	1.39	0.60	30%
2.91	0.86	2.50	799.77	2.50	0.80	1.70	0.58	32%
3.50	0.86	3.00	957.30	3.00	0.96	2.04	0.58	32%
4.08	0.86	3.50	1090.48	3.50	1.09	2.41	0.59	31%
4.66	0.86	4.00	1205.85	4.00	1.21	2.79	0.60	30%
5.25	0.86	4.50	1307.62	4.50	1.31	3.19	0.61	29%
5.83	0.86	5.00	1398.65	5.00	1.40	3.60	0.62	28%
6.41	0.86	5.50	1481.00	5.50	1.48	4.02	0.63	27%
6.99	0.86	6.00	1556.18	6.00	1.56	4.44	0.64	26%
7.58	0.86	6.50	1625.33	6.50	1.63	4.87	0.64	25%
8.16	0.86	7.00	1689.36	7.00	1.69	5.31	0.65	24%
8.74	0.86	7.50	1748.97	7.50	1.75	5.75	0.66	23%
9.33	0.86	8.00	1804.73	8.00	1.80	6.20	0.66	23%
9.91	0.86	8.50	1857.11	8.50	1.86	6.64	0.67	22%
10.49	0.86	9.00	1906.50	9.00	1.91	7.09	0.68	21%
11.07	0.86	9.50	1953.21	9.50	1.95	7.55	0.68	21%
11.66	0.86	10.00	1997.53	10.00	2.00	8.00	0.69	20%
12.24	0.86	10.50	2039.68	10.50	2.04	8.46	0.69	19%
12.82	0.86	11.00	2079.88	11.00	2.08	8.92	0.70	19%
13.41	0.86	11.50	2118.28	11.50	2.12	9.38	0.70	18%
13.99	0.86	12.00	2155.05	12.00	2.16	9.84	0.70	18%
14.57	0.86	12.50	2190.32	12.50	2.19	10.31	0.71	18%
15.15	0.86	13.00	2224.21	13.00	2.22	10.78	0.71	17%
15.74	0.86	13.50	2256.82	13.50	2.26	11.24	0.71	17%
16.32	0.86	14.00	2288.24	14.00	2.29	11.71	0.72	16%
16.90	0.86	14.50	2318.56	14.50	2.32	12.18	0.72	16%
17.49	0.86	15.00	2347.85	15.00	2.35	12.65	0.72	16%