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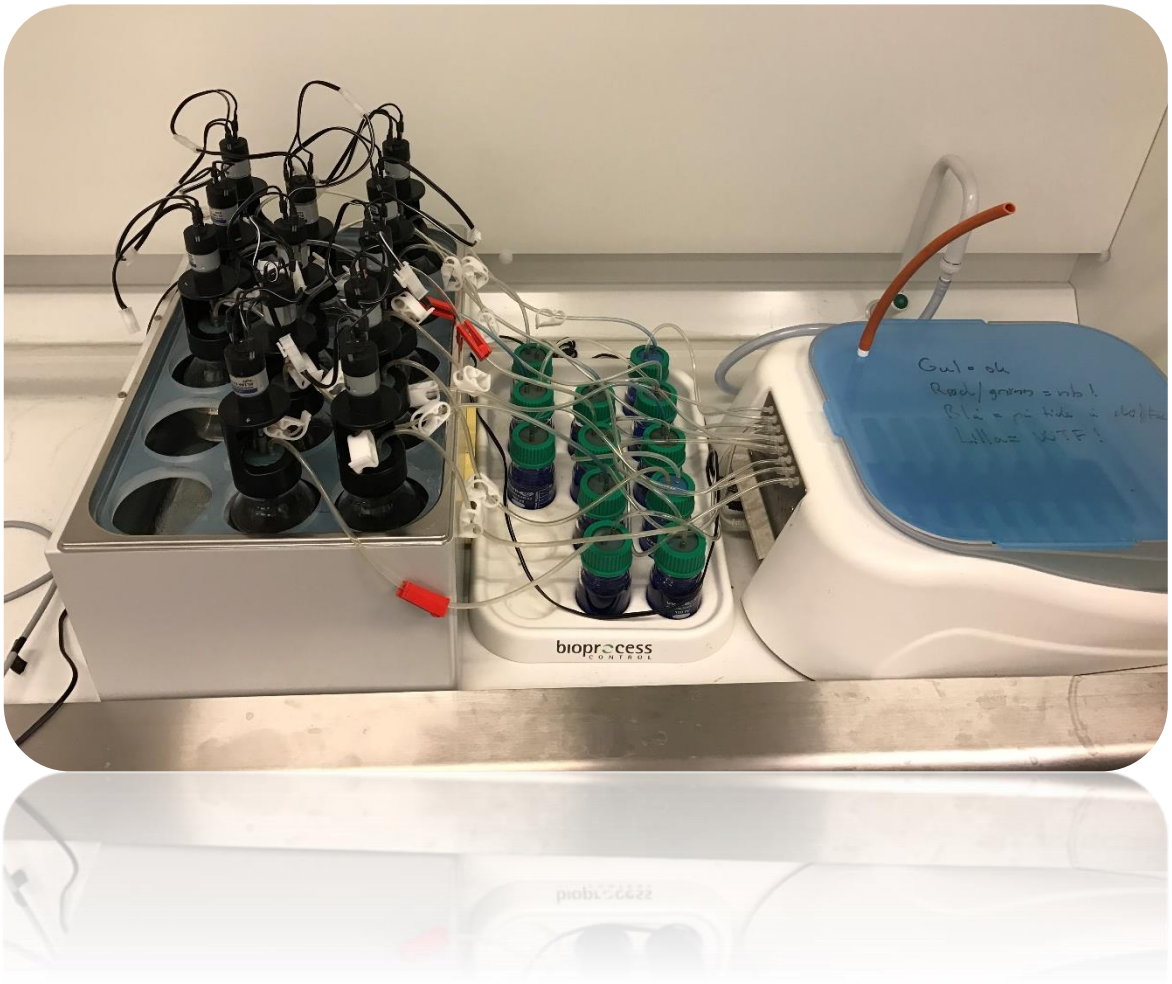
BIOGAS POTENTIAL OF MUNICIPAL SLUDGE, INDUSTRIAL WASTE, LIVESTOCK MANURE, AND PRELIMINARY STUDY OF RØYSLAND FARM BIOGAS PLANT

MASTER'S THESIS



Ricky Dalys Rakotonomenjanahary

Abstract

Anaerobic digestion involves a series of metabolic reactions in which complex substrates in the feed are reduced to a mixture having as main products methane and carbon dioxide. Biochemical methane potential (BMP) of wastewater sludge, manure, food waste and industrial waste samples have been tested by using an automated methane potential test system II, in batch system, at 35°C. In addition, the effectiveness of the farm scale Røysland biogas plant has been investigated by analysing the COD and VS removal, the VFA, pH and alkalinity variations for a period of 3 months (January to April 2017). The farm itself is equipped with one up-flow anaerobic sludge blanket reactor (UASB, R1) and one anaerobic baffled reactor (ABR, R2).

High BMP was found in mixed food waste (0.672 m^3 CH_4/kg VS), Grødaland primary sludge (0.756 m^3 CH_4/kg VS) and in influent R1 (0.614 m^3 CH_4/kg VS). The specific methanogenic activity of the granules/inoculum, the extent of fat fraction (thus COD/VS ratio), the environmental conditions, as well as the availability of substrates are the main parameters influencing the methane potential.

Analysis of the manure percolate of R1 has shown a low VFA concentration and higher pH in post-starting of the reactor (period 1, from 1 to 69 days) compared to a high VFA and a lower pH found in period 2 (from 85 to 139 days). Period 3 (from 161 and further) where restarting of the reactor occurred has shown a low VFA and high pH, which is similar as found in period 1. Also, a significant increase in COD and VS was found from period 1 to period 2, which led to an increase in the organic loading rate (OLR). The change in the type of feed manure might be the reason behind the COD increase.

Performance issue was observed concerning the COD/VS removal in R1, which upset the process. Bad hydraulics leading to a channelling of substrate distribution), and/or bad quality granule might have caused the low biogas production, far from the total estimated potential. While a high alkalinity prevented reactor souring due to high VFA observed in the sample.

The methane potential results were within expectation. Feedstock manures are full of potential compared to other organic materials, in addition to the potential of phosphorous and nitrogen recovery.

Keywords: anaerobic digestion, biochemical methane potential, livestock manure, municipal sludge, biogas production, UASB reactor

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Abbreviations

AB: Acetogenic bacteria

ABR: anaerobic baffled reactor

AeWT: Aerobic wastewater treatment

AnDi: Anaerobic digestion

BMP: Biochemical methane potential

BPC: Bioprocess Control Sweden

COD: Chemical oxygen demand

d: day

IVAR: Interkommunalt Vann, Avløp og Renovasjon

meq/L : milliequivalent per litre

Nl/Nml: Normal litre/millilitre

RA: Recirculation amount

RR: Recirculation rate

SRB: Sulphate reducing bacteria

ST: Storage tank

UASB: Upflow anaerobic sludge blanket

VFAs: Volatile fatty acids

1. INTRODUCTION

Anaerobic digestion (AnDi) is referred to the fermentation process in which organic material is degraded and biogas composed mainly of methane and carbon dioxide is produced. The process occurs in many locations where organic material is available and redox potential is low (zero oxygen). The overall process is rather complex microbiologically as it involves a mixed of microbial communities such as hydrolytic and fermentative bacteria, acetogens, acidogenic bacteria, methanogens and sulphate reducing bacteria (Henze, 2008). AnDi is considered as one of the oldest forms of wastewater treatment, yet because of the complex ecosystem involved, it has continued to be the subject of research and new process development (Grady et al., 2011).

Adding to the consciousness about the crucial importance of a clean environment, the need for attaining sustainability in society grew considerably in the last half century. Citizens are more aware about the public health risks of wastes, wastewaters and polluted surface water. A growing group of specialists in the field of waste and wastewater treatment is convinced that aerobic wastewater treatment (AeWT) as core wastewater treatment method needs to be substituted by anaerobic digestion (AnDi) and anaerobic wastewater treatments, supplemented with the adequate complementary methods (Lettinga, 2010).

Anaerobic fermentation and oxidation processes are nowadays used primarily for the treatment of waste sludge and high-strength organic wastes (Tchobanoglous et al., 2003). Anaerobic technology is also applied to a various of agro-industrial wastewaters such as food industry, beverage, alcohol distillery, pulp and paper industry, pharmaceutical industry, etc… (Henze, 2008). It is advantageous because of the lower biomass yields, the cut in energy cost due to aeration (which is fundamental in AeWT), and because energy, in the form of methane, can be recovered from the biological conversion of organic substrates. The lower biomass production by a factor of about 6 to 8 times reduces cost for sludge processing and disposal. Thus, the cost for nutrient addition is much less for anaerobic processes because less biomass is produced (Tchobanoglous et al., 2003). Also, it is in the interest of operators of AnDi plants to maximise methane production whilst concomitantly reducing the chemical oxygen demand (COD) of the digested material (Ward et al., 2008).

One sector where AnDi is currently growing is the agricultural and farming industries. For livestock farmers, AnDi represents not only an alternative source of energy but also an income when the biogas is being upgraded. Upgrading the biogas not only helps reducing greenhouse gas emissions as it replaces the use of fossil fuels, but also reduces the emissions from the

agricultural sector. Other advantages are: (1) the possibility to use organic fertilizer instead of mineral fertilizers, helping to reduce emissions from the mineral fertilizer production, and (2) the possibility to reuse the phosphorus and other resources contained in the manure.

The use of biogas can vary within different countries. For instance, in Norway (2008), the biogas produced was allocated: for electricity (18 %), for heating (53 %), for flaring (19 %), for upgrading as fuel (2 %) and the rest was unknown. The trend in upgrading biogas for fuel use was expected to increase. Now, the number of buses using biomethane (i.e. upgraded biogas) has experienced a strong growth in recent years (the trend is also observed in Europe). In addition to buses, several light and heavy vehicles are fuelled with biogas. Several companies like AGA and Lyse operate filling stations and assure the distribution of biogas (Miljødirektoratet, 2013).

The success of a wastewater treatment is based upon an adequate design and a proper reactor operation. The type of wastewater and its characteristics are important in the evaluation and design of anaerobic processes (Tchobanoglous et al., 2003). For any AnDi processes, a good design requires the best available information about the substrates used, their degradability, methane potential/yield and COD recovery. Those information provide a better understanding of the quality of the raw gas produced from wastewater treatment (Safitri, 2016), and can be used to optimise the process. Thus, experimental testing is needed to investigate the specific methane production of different substrates.

The main objectives of this study are (1) to estimate and compare the methane potential of different organic wastes by using an automated methane potential test system (AMPTS II) and (2) to evaluate the process effectiveness of the full-scale up-flow anaerobic sludge blanket (UASB) reactor, by monitoring the COD removal efficiency as well as the biogas production.

For a complete understanding of the process, a literature review has been performed which is presented within this report. This will lead to a future perspective on the importance of biogas potential determination and the various factors affecting the process. The methods performed during the laboratory experiments are also presented, as well as the results obtained from the study. Later, a discussion section is presented, which reviews the quality of this study compared to previous studies, highlighting confirmed assumption and/or close any gaps.

2. LITERATURE REVIEW AND THEORETICAL BACKGROUND

2.1. Anaerobic digestion process

Anaerobic digestion is a process found in many naturally occurring anaerobic environments including watercourses, sediments, waterlogged soils and the mammalian gut. It can also be applied to a wide range of feedstocks including industrial and municipal wastewaters, agricultural, municipal, food industry wastes, and plant residues (Ward et al., 2008). Generally, four successive stages (figure 1) are involved in the overall anaerobic oxidation of a waste: (1) hydrolysis, (2) acidogenesis (also known as fermentation), (3) acetogenesis and (4) methanogenesis (Henze, 2008); Tchobanoglous et al. (2003). These steps will be further described in the sections below.

2. 1. 1. Hydrolysis

The first step where the particulate materials (biopolymers) are converted to soluble compounds which can then be hydrolysed further to simple monomers (sugars, fatty acids, amino acids) that are used by bacteria performing fermentation, is termed hydrolysis (Tchobanoglous et al., 2003). The polymeric materials are degraded through the action of an exo-enzymes, resulting in simple compounds that can cross the cell barrier (Henze, 2008).

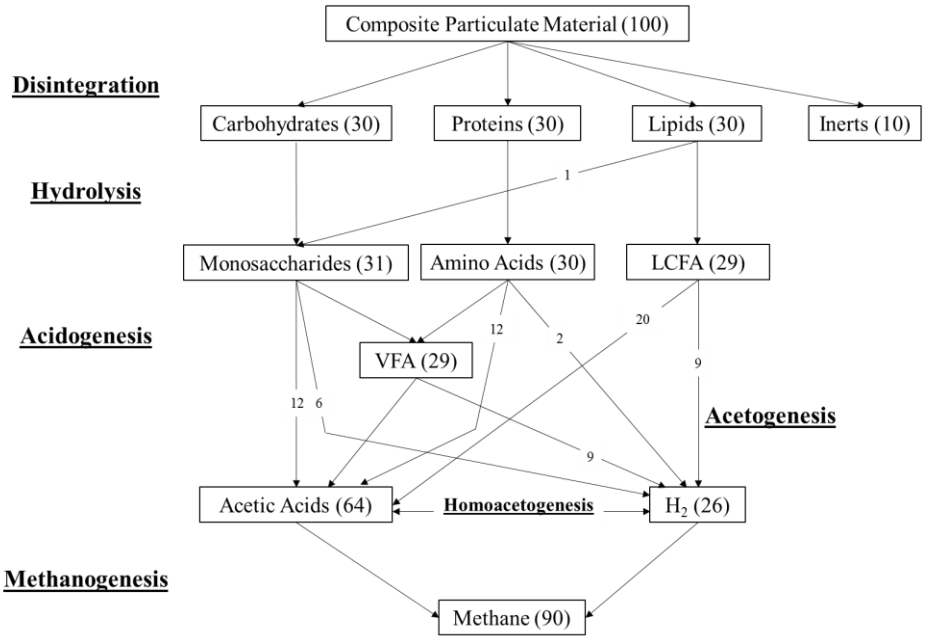

Figure 1: COD flow for anaerobic processes (expressed on percentage)

Adapted from (Batstone, 2002; Henze, 2008; Madigan et al., 2012)

During the hydrolysis process, proteins are being hydrolyzed to amino acids, lipids to long chain fatty acids (LCFA) and polysaccharide to simple sugars (figure 1). Hydrolysis is generally considered as the rate-limiting step during the anaerobic digestion of complex substrates such as semi-solid substrates and wastewaters with a high suspended solid to COD ratio. The process is considered as highly sensitive to temperature fluctuation (Henze, 2008).

2. 1. 2. Acidogenesis (fermentation)

During the acidogenesis step, the soluble hydrolysis products: simple sugar, LCFA and amino acids are diffused through the bacterial cells. They are fermented or anaerobically oxidized into several simple compounds which are then excreted. The compounds produced during this phase include volatile fatty acids (VFAs), i.e. acetate and higher organic acids such as propionate and butyrate, as well as alcohols, lactic acid, CO_2, H_2, NH_3 and H_2S. Oxidation process is predominant during this phase (Henze, 2008).

Acidogenesis is performed by a large group of hydrolytic and non-hydrolytic organisms. About 1% of all known bacteria are (facultative) fermenters. Non-methanogenic bacteria responsible for hydrolysis and fermentation are *Clostridium spp*, *Lactobacillus*, *Actinomices* and *Escherichia coli* (Tchobanoglous et al., 2003). As VFAs and carbonic acid constitute the main end products from sugars and proteins, fermentative organisms are usually termed as acidifying or acidogenic microorganisms, therefore the process is called acidogenesis (Henze, 2008).

The type and extent of each fermentation products (VFAs, HCO_3^-, H_2, H^+, etc…) are highly dependent on the efficiency of the process inside the reactor. As shown in table 1, removing H_2 will result in acetate as being the main end product (Henze, 2008). Hydrogenotrophic methanogens act as H_2 scavengers through a syntrophic relationship termed “interspecies hydrogen transfer".

Table 1: Acidogenic reactions with sucrose as substrate and the corresponding free energy change (ΔG°’) at 25°C (Henze, 2008)

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

Accumulation of VFAs into the reactor may result into an inhibition of methanogens. The produced acid will cause a pH drop once the alkalinity is consumed, which results in a high

concentration of non-dissolved VFAs. The fact that acidifiers are active even at low pH (4), means that reactor souring (pH drops to 4-5) can and will occur when the methanogenic capacity of the system is trespassed (Henze, 2008).

2. 1. 3. Acetogenesis (intermediary acid production)

The VFAs, other than acetate, which are produced in the acidogenesis are further converted to acetate, hydrogen gas and carbon dioxide by acetogenic bacteria (AB). The most important substrates are propionate and butyrate. But also lactate, ethanol, methanol, and even H_2 and CO_2 are (homo) acetogenically converted to acetate (homoacetogenesis step presented in figure 1). VFAs are converted by specific AB following the so-called "Beta-oxidation" in which acetate moieties are split from the aliphatic chain. LCFAs with uneven C atoms also yield propionate next to acetate.

A narrow association between the H_2-producing AB and the H_2-consuming (methanogens) is required to regulate the H_2 level in their environment. Keeping the H_2 at the right level has a crucial importance as the acetogenic reactions are thermodynamically unfavourable (positive $\Delta G°'$ as shown on table 2). However, under stabilised digestion conditions the hydrogen partial pressure is maintained at a sufficiently low level. This can be achieved by an effective uptake of the hydrogen by methanogens or sulphate reducing bacteria. Methanogenic bacteria usually utilize molecular hydrogen in the anaerobic digester so rapidly that the hydrogen partial pressure drops below 10^{-4} atm, which is enough to ensure the actual occurrence of the hydrogen producing acetogenic reaction (Henze, 2008).

Table 2: Stoichiometry and change of free energy for some acetogenic reactions, assuming neutral pH, a temperatire of 25°C and a pressure of 1 atm.

Water is regarded as pure liquid, and all soluble compounds have an activity of 1 mol/kg (Henze, 2008).

Compound	**Reactions**	**$\Delta G°'$ (kj/mol)**
Lactate	$CH_3CHOHCOO^- + 2H_2O$ ------> $CH_3COO^- + HCO_3^- + H^+ + 2H_2$	- 4.2
Ethanol	$CH_3CH_2COH + H_2O$ ------> $CH_3COO^- + 4HCO_3^- + H^+ + 2H_2$	+ 9.6
Butyrate	$CH_3CH_2CH_2COO^- + 2H_2O$ ------> $2CH_3COO^- + H^+ + 2H_2$	+ 48.1
Propionate	$CH_3CH_2COO^- + 3H_2O$ ------> $CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+ 76.1
Methanol	$4CH_3OH + 2CO_2$ ------> $3CH_3COOH + 2H_2O$	- 2.9
Hydrogen-CO_2	$2HCO_3^- + 4H_2 + H^+$ ------> $CH_3COO^- + 4H_2O$	- 70.6
Palmitate	$CH_3\text{-}(CH_2)_{14}\text{-}COO^- + 14H_2O$ ------> $8CH_3COO^- + 7H^+ + 14H_2$	+ 345.6

This interdependence means that the degradation of higher fatty acids and alcohols largely depends on the activity of electron scavenging organism (i.e. due to reduction of the quantity of electron acceptor) such as methanogenic bacteria. Microbial associations in which a H_2-producing organism can grow only in the presence of a H_2-consuming organism are called syntrophic association. The coupling of formation and use of H_2 is called interspecies hydrogen transfer. In a properly functioning methane-producing installation, the partial hydrogen pressure will not exceed 10^{-4} atm and usually between 10^{-4} to 10^{-6} atm. At such low hydrogen concentration, the degradation of ethanol, butyrate or propionate becomes exergonic and will yield energy for the acetogens (Henze, 2008).

2. 1. 4. Methanogenesis

Methanogenesis is carried out by a group of organisms collectively known as methanogens. Two groups of methanogenic organisms are involved in methane production. One group, termed *aceticlastic methanogens*, split acetate into methane and carbon dioxide. The second group, termed *hydrogen-utilizing methanogens*, use hydrogen as the electron donor and CO_2 as the electron acceptor to produce methane (figure 1). However, the acetic acid will be converted to methane, so the impact of this reaction is minor. About 72% of the methane produced in anaerobic digestion is from acetate fermentation, the remaining 28% comes from the CO_2 reduction (Tchobanoglous et al., 2003). It is only in this stage that the influent COD is converted to a gaseous form that automatically leaves the reactor system (Henze, 2008).

Methanogens are obligate anaerobes with a narrow substrate spectrum (Henze, 2008; Madigan et al., 2012). In addition to CO_2 plus H_2 and acetic acid, methanogens can ferment formic acid, methanol, methylamine, and a few other simple compounds to form CH_4, but they are probably not as important in nature as acetic acid, CO_2 and H_2 (Madigan et al., 2012). Many of the methanogenic organisms identified in anaerobic digesters are similar to those found in the stomachs of ruminant animals and in organic sediments taken from lakes and rivers. The principal genera identified at mesophilic conditions include the rods (*Methanobacterium, methanobacillus*) and spheres (*Methanothrix*, *Methanococcus*, *and Methanosarcina*). *Methanosarcina* and *Methanothrix* are the only organisms able to use acetate to produce methane and carbon dioxide. The other organisms oxidize hydrogen with carbon dioxide as the electron acceptor to produce methane (Tchobanoglous et al., 2003).

The growth rate of aceticlastic methanogens is very low, resulting in doubling times of several days or even more. The extremely low growth rates explain why anaerobic reactors require a

very long start-up time with unadapted seed material and why high sludge concentrations are pursued. Hydrogenotrophic methanogens have a much higher maximum growth rate than the aceticlastic bacteria with doubling times of 4 to 12 hours (table 3). Because of this feature and despite the very delicate acetogenic reaction step discussed in the previous section, anaerobic high rate reactor systems exert a remarkable stability under varying conditions (Henze, 2008).

Table 3: Most important methanogenic reactions and the corresponding free energy change

Functional step	**Reactions**	**ΔG°' (kj/mol)**	**u_{max} 1/d**
Acetotrophic methanogenesis	$CH_3COO^- + H_2O$ ------> $CH_4 + HCO_3^-$	- 31	0.12[a] 0.71[b]
Hydrogeneotrophic methanogenesis	$CO_2 + 4H_2$ ------> $CH_4 + 2H_2O$	- 131	2.85

[a] *Methanosarcina* and [b] *Methanosaeta*

In the presence of specific inorganic electron acceptors like nitrate, sulphate or sulphite, the production of methane will decrease due to the reduction of the amount of hydrogen available for the methanogenesis process. The following reactions may occur:

$10H + 2H^+ + 2NO_3^- \rightarrow N_2 + 6H_2O$

$8H + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^-$ (Henze, 2008)

2. 1. 5. Process kinetics

Bacterial conversion rates, including anaerobic processes, can be described by using the Monod equation:

$$\mu_s = \mu_{max} \frac{S}{K_S + S}$$

where μ_s is the specific growth rate, μ_{max} (1/h) is the maximum specific growth rate, S (mg/l) is the limiting substrate concentration and K_S (mg/l) is the half saturation coefficient (the substrate concentration at which μ_s is equal to half of μ_{max}).

Because of the relatively low free energy change for anaerobic reactions, growth yield coefficients are considerably lower than the corresponding values for aerobic oxidation. Typical synthesis yield and endogenous decay coefficients for fermentation and methanogenic anaerobic reactions are Y=0.06 and 0.03 g VSS/g COD and b= 0.02 and 0.08 g VSS/g VSS.d, respectively (Tchobanoglous et al., 2014).

The flow of COD through acidogenesis to methane production starts with hydrolysis of colloidal and solid particles (Henze, 2008). Two rate-limiting concepts are important: (1) the hydrolysis conversion rate and (2) the soluble substrate utilization rate for fermentation and methanogenesis (Tchobanoglous et al., 2014). Acidogenesis is the most rapid conversion step in the anaerobic food chain (Henze, 2008). The production kinetics for VFAs are faster than the corresponding utilization and methane production kinetics for methanogens (Tchobanoglous et al., 2014).

2.2. Reactor design considerations

The basic requirements of an anaerobic digester design are: to allow for a continuously high and sustainable organic load rate, a short hydraulic retention time (to minimize reactor volume) and to produce the maximum volume of methane. Most anaerobic digesters are mixed to ensure efficient transfer of organic material to the active microbial biomass, to release gas bubbles trapped in the medium and to prevent sedimentation of denser particulate material. Mixing systems not only affect the digestion process but are often expensive to install, maintain, and run. Therefore, an efficient mixing system will be beneficial in terms of productivity and cost. A certain degree of mixing is necessary for presenting substrate to the bacteria, but excessive mixing can reduce biogas production (that negative effect is still unclear) (Ward et al., 2008).

Immobilisation of microbial biomass can involve the use of an inert or degradable medium to which the microbial populations attach, for example anaerobic filter reactors. Another method of immobilising biomass is to take advantage of the natural tendency of cells to form dense granules which settle in the digester, for example the UASB reactors. Both methods reduce the quantity of microorganisms washed out from the digester. For a degradable support materials, they can act as part of the feedstock. (Ward et al., 2008). As a biofilm carrier, straw in a packed bed was found to have a greater methane production than a glass packed bed or suspended plastic carriers (Andersson & Björnsson, 2002).

2. 2. 1. Up-flow anaerobic sludge blanket (UASB) reactor

The UASB reactor is the most successfully-used high rate anaerobic technology for treating several types of wastewater. Its success can be attributed to its capability for retaining a high concentration of sludge, meanwhile efficient solids, liquids and water phase separation is attained. The UASB reactor consists of a circular or rectangular tank in which waste (water or slurry) flows in an upward direction through an activated anaerobic sludge bed which occupies about half the volume of the reactor and consists of highly settleable granules or flocs. During

the passage through the anaerobic sludge the treatment process takes place by solids entrapment and organic matter conversion into biogas and sludge. The produced biogas bubbles automatically rise to the top of the reactor, carrying water and solid particles, i.e. biological sludge and residual solids. The released gases are captured in an inverted cone or related structure and directed to the gas outlet, while the solid particles drop back to the top of the sludge blanket (Henze, 2008). This applies primarily for reactors equipped with GLSS (Gas-liquid-solids separator) device, but the basic process behind gas-solid-liquid separation is the same.

2. 2. 2. Solids Retention Time (SRT)

SRT can be defined as the average time a solid particle spends inside the reactor. It is a fundamental design and operating parameter for all anaerobic processes, which should be above a minimum value in order to maintain the methanogenic conversion capacity of the sludge. The SRT is determined by dividing the mass of solids in the reactor by the solids removed daily primarily through the effluent and wasting (as presented below).

$$SRT = \frac{X.V}{Q_e X_e + Q_w X_w} \quad \begin{matrix}\text{[Total biomass present in the reactor]} \\ \text{[Total biomass withdrawn from the reactor per day]}\end{matrix}$$

Where: SRT = solids retention time, d

X = concentration of the inlet viable biomass (kg/m^3)

V = reactor volume, m^3

Q_e = effluent flowrate, m^3/d

X_e = concentration of the effluent biomass (kg/m^3)

Q_w = wasting sludge flowrate, m^3/d

X_w = concentration of the wasted sludge biomass (kg/m^3)

SRT follows directly from its relationship to the specific growth rate of the biomass in the bioreactor:

$$\frac{1}{SRT} = \mu_s - K_d = \mu_{max}\frac{S}{K_S + S} - K_d$$

Where K_d is the decay rate

Thus, at high growth rate, a lower SRT can be used. In general, SRT values greater than 20 days are needed for anaerobic processes at 30°C for effective treatment performance, with much higher SRT values at lower temperatures (Tchobanoglous et al., 2003).

2. 2. 3. Upward liquid velocity

The upward liquid velocity (V_{upw}) is primarily dependent on the sludge mass as well as the settling velocity of the sludge/granules. A sufficient velocity to lift-up the sludge blanket requires a sufficient, evenly distributed influent flow rate. The inflow rate is directly linked to the HRT as well as the SRT, because in order to reach a necessary sludge mass, an appropriate SRT is needed. Therefore, the required SRT sets limits to applicable V_{upw} as well as to the specific biogas loading resulting from the anaerobic conversion process (Lettinga G., 1991). Ideally, the average V_{upw} in the UASB reactor's cross-sectional area and the clarification section at the top should be in the range of 0.5 – 1.0 m/h. Higher hydraulic loadings may lead to non-desired loss of biomass if flocculent type of sludge accumulates during reactor operation. The latter may happen, for instance, during the first start-up when the reactor is seeded with non-adapted seed material like digested sewage sludge or during the anaerobic treatment of domestic sewage. The up-flow velocity can be calculated as following:

$$V_{upw} = \frac{Q_{inf}}{A}$$

Where A is the cross-sectional area ($A = \pi r^2 = \pi d^2/4$) and Q_{inf} the influent flow rate.

Generally, for UASB reactors, and particularly for those operating with non-granular sludge, a maximum up-flow velocity of 1 m/h is considered. When a higher up-flow velocity can be tolerated as is the case when good quality granular sludge is cultivated, the reactor volume can be reduced (Henze, 2008) or loading can be increased.

2. 2. 4. Organic loading rate (OLR)

OLR indicates how much organic matters are loaded per m^3 of digester volume and unit of time (kg COD/m^3.d). It can be calculated as followed:

$$OLR = \frac{Q.C_{in}}{V}$$

Where C_{in} is the influent concentration, kg COD/m^3, Q is flow rate and V is the reactor volume.

If the loading rate is too high, the active biomass can be stressed, due to the increase in VFA concentration which cause pH reduction and consequently the inhibition of methanogens (Henze, 2008). Consequently, low or no biogas production takes place and the process upset occurs. Therefore, at high VFAs concentration, methanogens will not be able to metabolise the excess acetate produced by the acetogenic organisms until the methanogenic organisms has reached a sufficient number. This is true for feedstocks which are rapidly hydrolysed, while a

slowly-degradable feedstock, the hydrolysis phase is more likely to be the limiting step. However, inhibitors of methanogenesis such as excessive fatty acids, hydrogen sulphide, and ammonia are toxic only in their non-ionised forms(Ward et al., 2008).

2.3. Factors affecting performance

In anaerobic digestion, the control of the process is usually performed by measuring the VFA, pH, alkalinity and gas production. Generally, change in VFA concentration is the most sensitive parameter. The reason is because the primary cause of digester failure hinges on imbalance between acidogenic, acetogenic and methanogenic organisms (Lahav O. & R., 2000). Besides the methanogenic association, other group of bacteria such as sulphate reducing bacteria (SRB) is present which can compete with the methanogens for methanogenic substrates (Henze, 2008).

2. 3. 1. Volatile fatty acids

As previously mentioned, methanogens serve as a hydrogen sink that allows the fermentations reactions to proceed. If process upsets occur, the propionate and butyrate fermentation will be slowed with the accumulation of volatile fatty acids in the anaerobic reactor and a possible reduction in pH (Tchobanoglous et al., 2003).

VFAs are a key intermediate in the process of anaerobic digestion and are also capable of inhibiting methanogenesis in high concentrations (Ward et al., 2008). Anaerobic processes will alter the pH, particularly the production of fatty acids, and it has been found that fermentation of glucose is inhibited at total VFA concentrations above 4 $g.l^{-1}$ (Siegert & Banks, 2005). Acetic acid is usually present in higher concentrations than other fatty acids during anaerobic digestion (Wang et al., 1999). As an increase in fatty acids concentration can be indicative of an organic overload of the digester (Ward et al., 2008), thus, fatty acids, particularly butyrate and isobutyrate together have been found to be particularly good indicators of the process stability (Ahring et al., 1995).

2. 3. 2. Toxic sulphides

SRB present in anaerobic digesters can cause problems when a significant amount of sulphate is present. The main product of the sulphate reduction, the hydrogen sulphide (H_2S), is very toxic to methanogenic bacteria (Hazen et al.), AB and even SRB (Henze, 2008; Reis et al., 1992). SRB can use several of the methanogenic intermediates (H_2, formate, acetate, methanol, pyruvate) when sulphate, sulphite or thiosulphate are present. Other donors such as lactate, LCFA, benzoate, butyrate, propionate, etc…) are more restricted for some special environment. For instance lactate and pyruvate are widely used by species found in freshwater anoxic

environments, while acetate and longer-chain fatty acids are used by marine SRB (Madigan et al., 2012).

Since, the main intermediates (H_2/CH_3COO^-) can be used by both SRB, methanogens and/or obligate hydrogen producing bacteria, and as they operate under the same environmental conditions, they will compete for the same substrates. A lower overall treatment efficiency (i.e. same COD treatment efficiency but with lower methane yield) will occur as a lower quantity of COD is reduced to CH_4 when sulphate is present. Therefore, the methane yield per unit of degraded COD will decrease, which will negatively affect the overall energy balance of the process (Henze, 2008).

Moreover, the quality of the biogas is reduced since a part of the produced sulphide ends up as H_2S in the biogas. Also, the produced sulphide has a bad smell and can cause corrosion problems to pipes, engines and boilers. This can have a huge impact on the maintenance costs of installation. If sulphide is not being removed, it can upset the efficiency of an aerobic post-treatment system. Removal of H_2S from the biogas is therefore usually required (Henze, 2008). Adding iron at controlled amount to form iron sulphide precipitate can solve the issue (Tchobanoglous et al., 2003).

2. 3. 3. Temperature

Most anaerobic digestion processes are operated in the mesophilic range (30 to 40°C (Madigan et al., 2012)), while in the meantime there is an increased interested on thermophilic digestion (Tchobanoglous et al., 2003) with an optimum temperature at 55°C (Ward et al., 2008). A change from mesophilic to thermophilic temperatures (or vice versa) can result in a sharp decrease in biogas production rate until the necessary populations have increased in number (Ward et al., 2008). During batch digestion of vegetable waste and wood chips, more rapid degradation of fatty acids was found at 55°C than at 38°C, and also 95% of the methane yield was realised after 11 days under thermophilic conditions compared to 27 days under mesophilic conditions (Hegde & Pullammmanappallil, 2007). Other experiments (Parawira et al., 2007) (Fang & C., 1999) have shown that mesophilic temperature digesters have improved degradation rates compared with thermophilic digesters.

Even with an increase in methane yield or production rate in the thermophilic process, it should balance against the energy requirement maintaining the reactor at the higher temperature. This might not be a big issue if the biogas produced is used for the generation of electricity, as heating the reactor is accomplished by routing the waste heat from the gas engines to heat exchangers

within the reactor, and the engines generally produce more heat than the reactor requires (Ward et al., 2008).

Another consideration is that the optimum temperature for methanogenesis may not necessarily be the optimum for other processes in anaerobic digestion, such as hydrolysis or acidification. Thus, multi-stage digesters could be possibly be used for temperature optimisation of the separate processes taking place in the respective tanks (Ward et al., 2008).

2. 3. 4. pH

Anaerobic processes are sensitive to pH and inhibitory substances. A pH value near neutral (pH between 6.8 and7.2 (Ward et al., 2008)) is preferred and below 6.8 the methanogenic activity is inhibited. Because of the high CO_2 content in the gases developed in anaerobic processes (30 to 35 percent CO_2), a high alkalinity is needed to assure pH near neutrality (Tchobanoglous et al., 2003). Although the optimal pH of methanogenesis is around pH 7.0 (Kim et al., 2003a) , the optimum pH of hydrolysis and acidogenesis have been reported as being between pH 5.5 and 6.5 (Yu & Fang, 2002). This is an important reason why some designers prefer the separation of the hydrolysis/acidification and acetogenesis/methanogenesis processes in two-stage processes (Ward et al., 2008).

To maintain the pH in the system at optimum, the buffering capacity should be at a sufficient level. The natural tendency of anaerobic process to produce alkalinity through breakdown of organic material (see section 2.3.5 below) can overcome this issue (Tchobanoglous et al., 2003). However, when a significant pH drop occurs, maintaining optimal pH is best accomplished by reducing the organic loading rate, although a more rapid approach is the addition of strong bases or carbonate salts to remove carbon dioxide from the gas space and convert it to bicarbonate, or alternatively bicarbonate can be added directly (Guwy et al., 1997). Direct bicarbonate addition is more accurate as converting carbon dioxide to bicarbonate will require a time lag for gas equilibrium to occur which could result in over-dosing. It has also been demonstrated that the inoculum-to-feed ratio can be modified to maintain a constant pH (Gunaseelan, 1995).

2. 3. 5. Alkalinity

Alkalinity in wastewater results from the presence of the hydroxides [OH^-], carbonates, and bicarbonates [CO_3^{2-}], and bicarbonates [HCO_3^-] of elements such as calcium, magnesium, sodium, potassium, and ammonia. As mentioned above, alkalinity helps to resist changes in pH caused by the addition of acids.

An alkalinity concentration in the range of 3000 to 5000 mg/l as $CaCO_3$ is often found in wastewater. For sludge digestion sufficient alkalinity is produced by the breakdown of protein and amino acids to produce NH_3, which combines with CO_2 and H_2O to form alkalinity as $NH_4(HCO_3)$ (Tchobanoglous et al., 2003).

2.4. Expected methane production and COD balance

Unlike aerobic system, the COD in anaerobic process is only re-arranged, not destroyed. The organic material is transformed into intermediate products and further mineralized as CH_4 and CO_2. Generally, the COD entering a system must end up into the three different outlets: gaseous (as CH_4), liquid (effluent COD) or solid (sludge COD) (figure 2). Each COD types can be calculated based on the basic influent characteristics, the COD loading, and the degradation and conversion of the COD (Henze, 2008).

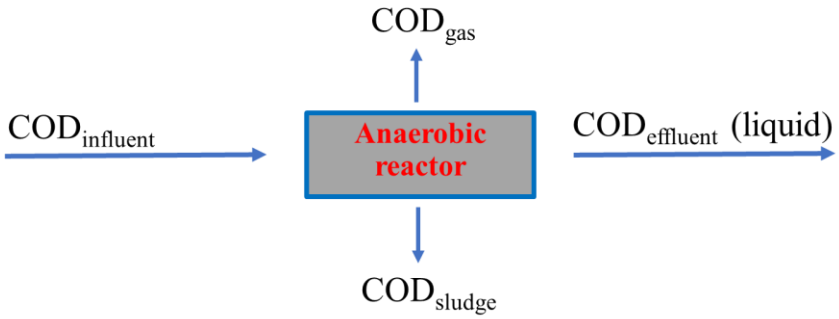

Figure 2: COD balance of an anaerobic reactor

As described previously, the COD converted (i.e. the solid COD transformed into gaseous COD) in anaerobic reactor is accounted for by the methane production. Considering that the oxidation of methane has a COD of 64 g O_2/mole CH_4, and the volume of methane per mole at standard conditions (0°C and 1 atm) is 22.4 L, the theoretical CH_4 production is estimated as following:

$$V_{CH_4} = \frac{22{,}4 \frac{l\ CH_4}{mole\ CH_4}}{64 \frac{g\ COD}{mole\ CH_4}} = 0{,}35\ \frac{l\ CH_4}{g\ COD}$$

Therefore, theoretically, 1 kg of COD can be converted in 0,35 m^3 CH_4 (Henze, 2008; Tchobanoglous et al., 2003). For any other experimental conditions, the quantity of methane is determined by using the universal gas law (equation below) to determine the volume of gas occupied by one mole of CH_4 at the temperature in question (Tchobanoglous et al., 2003).

$$V = \frac{nRT}{P}$$

where V= volume occupied by the gas (L), n = moles of gas (mole), R = universal gas law constant (0.082057 atm.L/mole.K), T = temperature K (273,15 +°C) and P = absolute pressure, (atm).

3. MATERIALS AND METHODS

This chapter will present the origin of the different substrates/sludges tested, the setting up and characteristic of the automated methane potential tests, as well as the analytical methods performed during the study.

3. 1. Substrates

Substrates from food factory, wastewater plants and a farm-scale biogas plant in Rogaland county were analysed. The characteristics of each waste sources are presented in this section. Grødaland, Vik and SNJ WWTPs are operated by IVAR (Interkommunalt Vann, Avløp og Renovasjon).

3. 1. 1. Sentralrenseanlegg Nord-Jæren (SNJ)

SNJ is an underground WWTP, with an expected upgrading capacity for 400 000 p.e. in 2016. It is located at Mekjarvik in Randaberg, Norway. It receives wastewater (sewage and surface water) from municipalities Randaberg, Stavanger, Sola, Sandnes and Gjesdal. It is a chemical wastewater treatment plant, with an anaerobic sludge digestion and treatment, a dewatering and drying plant and an odour treatment plant (Razafimanantsoa, 2010). For wastewater treatment section, pre-treatment consists of screening and sand/grit removal. Then followed by flocculation and sedimentation for suspended solids removal and treatment of sludge.

3. 1. 2. Grødaland and Vik WWTP

Grødaland WWTP treats sewage from industry and private households in the southern region of Rogaland. The total load is about 120 000 p.e. where 3 000 p.e. only are from people (living at Varhaug), and the rest is food industry; dairy, slaughter house and chicken processing. Treatment consists of flotation using a polymer as coagulant for suspended solid (SS) removal (usually 60% SS removal). Biological treatment using sequencing batch reactor (SBR) is used for removal of dissolved chemical oxygen demand (COD).

In 2016, biogas production was estimated to be 3 331 125 m^3, which is 33 000 000 kwh produced in terms of power. A simplified sketch of the different processes is presented in the figure 3 below.

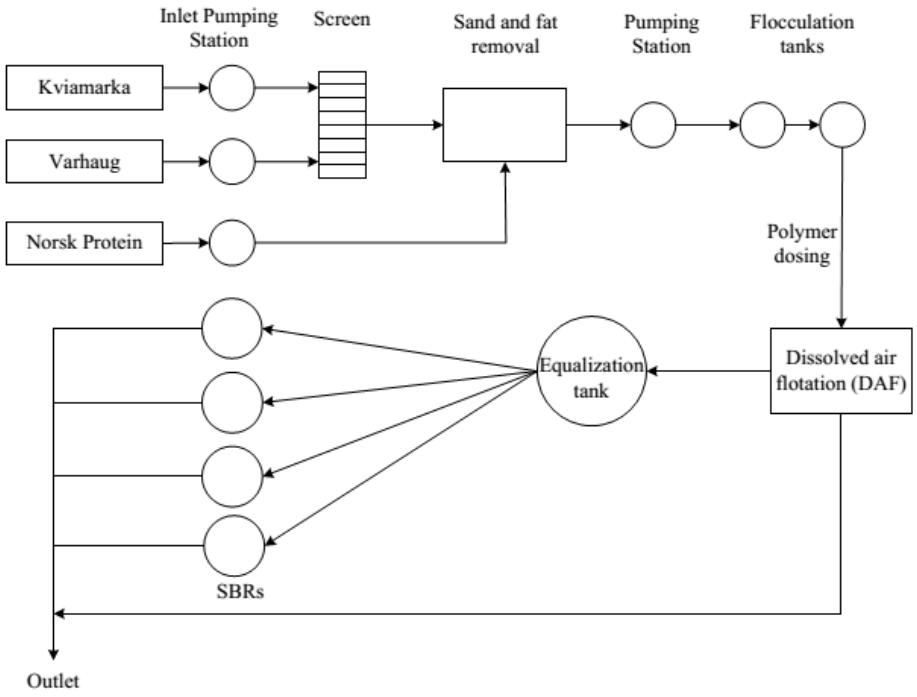

Figure 3: Process configuration in IVAR (Safitri, 2016)

Vik WWTP is an activated sludge plant loaded at about 70 000 p.e., where about 15 000 p.e. of the load is from houses, the rest is from dairy (Q-meieri) and potato industry (Hoff factory).

3. 1. 3. Røysland Biogas

Røysland Biogas is a farm-based biogas plant located in Voll (Rogaland), Norway. Cattle, pig and chicken manures from the farm is being handled by the farm-scale biogas plant. The manure production from the farm is estimated to be 8 m^3/d. The plant is equipped with one UASB reactor and one anaerobic baffled reactor, respectively labelled R1 and R2, both with granular sludge. The designed biogas production capacity is about 300 m^3 biogas/d. The reactor R1 (21 m^3) treats a percolate mixture of cow, chicken and sometimes pig manure, while R2 (10 m^3) is handling pig manure (the slurry supernatant) only. A control room with a control system monitors the entire biogas plant. From the control, all operation (flow rate, pumping) can be adjusted.

Three containers filled with dry manure (70 -77 % dry matter) are connected to R1. A sprinkling system on the top part of the containers disperses water into the dry manure to create a liquid manure (will be termed from here as percolate), which will be collected at the bottom. A filter

(mesh: 0,5 cm) is installed at the bottom of the container to sieve the solids part of the manure from the percolate. The latter is transferred into a storage tank (ST), and then pumped into R1 for digestion. The effluent from R1 is pumped back into ST which is then either pumped back to the containers and sprinkled over the semi-dry manure or returned into R1. The latter establishes a recirculation system around R1 (see figure 4).

Pig manure used as feed for R2 is collected into a storage tank, located underground below a pig barn. A filter is separating the slurry and the filtrate manure compartments. The filtrate is then directly pumped into R2, where it is being digested. The digestate produced is used in agriculture, as fertilizer.

The temperature inside the reactors is to be kept between 37-38°C. Heat is generated from a heater pump (and is distributed through a spiral steal inside the reactor). Heat can also be collected from underground by using a glycol solution. In addition, the plant is planning to generate heat from a stream nearby the farm.

Samples were transported from the plant to the university of Stavanger for analysis. The chemical characterization of the substrates and their biogas production potential were determined in laboratory testing. Investigation on the farm based biogas plant lasted for 3 months, from 17/01/2017 to 20/04/2017.

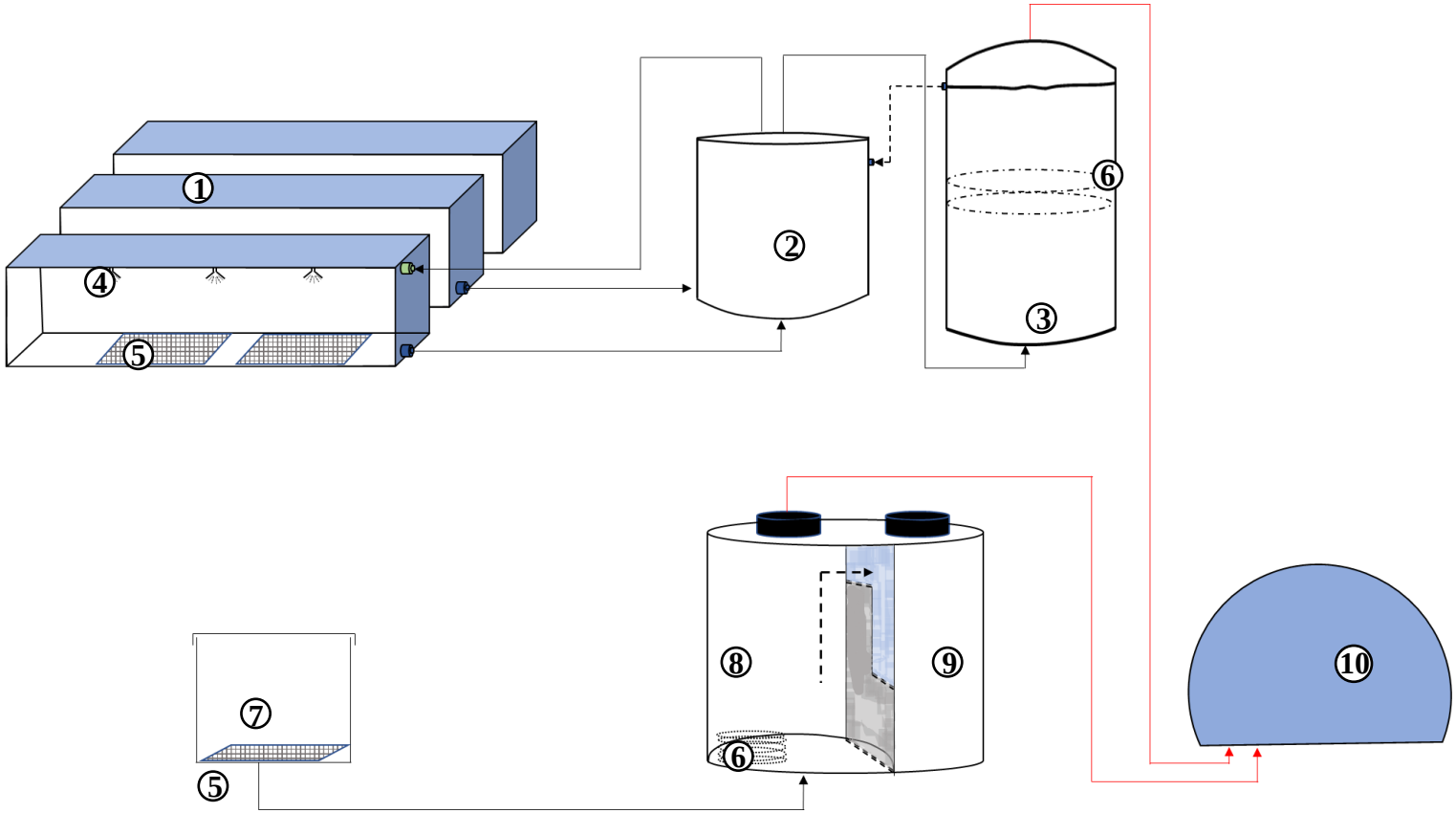

Figure 4: Simplified process flow diagram of Røysland biogas

-----> Liquid + solid flow **- - - - > Effluent flow** **------> Gas flow**

1. Manure storage (Containers), 40-50 m^3
2. Liquid manure equalization tank, 10 m^3
3. R1, 21 m^3
4. Water spreading device
5. Steel Filter, 0.5 cm mesh
6. Spiral heating system
7. Pig manure storage
8. R2, 10 m^3
9. Heat exchanger compartment
10. Gas holder, 100 m^3

3. 2. Biogas potential determination (AMPTS test)

This section will describe the characteristics of the equipment used for testing the methane potential, as well as the preparation of the samples and the conditions for starting up the batch test. A special section is described under experiment settings to highlight the proper set-up of the experiment reactors according to the guidelines from the AMPTS II software.

3. 2. 1. Anaerobic Methane Potential Test System II unit

AMPTS II produced by Bioprocess Control Sweden AB (BPC) is used for methane potential testing. It has been developed for on-line measurements of ultra-low biogas and biomethane flows produced from the anaerobic digestion of any biological degradable substrate (both solid and liquid form) at laboratory scale. It is composed by four units: sample incubation unit, CO_2-fixation unit, gas volume measuring device and AMPTS II software (figure 5).

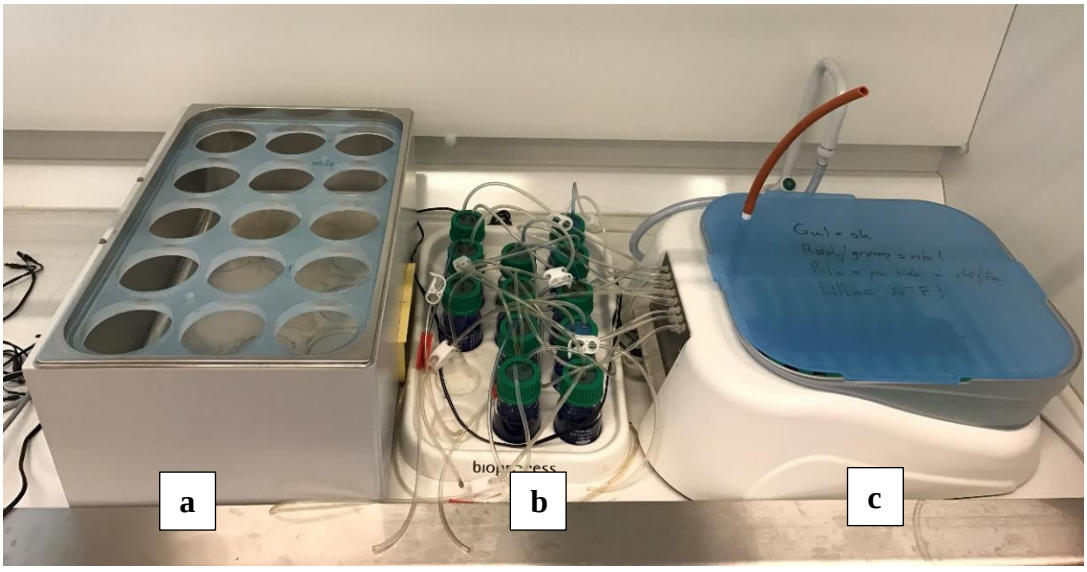

Figure 5: AMPTS II: Sample incubation unit (a), CO_2 fixation unit (b) and gas volume measuring device (c)

One of the major advantage of this instrument is that the analysis and data recording are fully automatic during the long incubating period (usually from 14 to 30 days).

Gas stripping is usually applied into the sample bottles prior each test. The mixed oxygen free gas (N_2 and CO_2) remove any remaining gas in the headspace of the bottles, thus, establishing an anaerobic condition of the inoculum. This will reduce the overestimation of methane production of the sample.

3. 2. 2. CO2 fixation unit preparation

Carbon dioxide fixing liquid were prepared for all CO_2 trap bottles (volume, 100 ml). The volume of the NaOH solution were set to 80 ml for each bottle (as recommended by BPC). This unit has a fixing efficiency more than 98%.

A 3 M NaOH solution were prepared before starting up the operation, with an estimated volume of 1.5 l, in order to fill all the 15 bottles. The necessary amount required were 180 g of NaOH, which was mixed with approximately ¾ of the volume of distilled water. The remaining ¼ amount of water is added after the chemical is completely dissolved, and a constant mixing of the solution is applied all along the preparation.

A 0.9% Thymolphthalein pH-indicator solution was also prepared by adding 40 mg of Thymolphthalein into in 9 ml ethanol 99,5%, followed by addition of 1 ml water. The two solutions were mixed together, with a ratio of 5 ml of the 0.4 % indicator solution per 1 liter 3 M NaOH solution. Then all CO_2 trap bottles were filled with 80 ml of the prepared CO_2-fixation solution. The NaOH solution bottle was changed when the colour change was encountered while running the experiment in order to avoid overestimation of CO_2 into the biogas produced.

As recommended by BPC, the motor speed should only be set at a value higher than 75% to prolong the life time of the motors. During the experiment, mostly, the speed adjustment was set 90% or higher to allow all the motor to turn. This was done to reduce the power loss that was encountered within the wire setup, as the power was constantly dropping from motor 01 to 15. Thus, the 10 first motors rotated almost at the same speed, while the 5 last ones were much slower.

Mixers on time of 30 s and 180 s mixers off time were applied for all cells. The water bath was filled with distilled water (up to the required level) every two days to avoid huge temperature fluctuation during the digestion process. The test was stopped when CH_4 production was negligible (less than 5 Nml/day).

3. 2. 3. Sample preparation

Before starting any BMP test, the biomass has been characterized regarding to total and volatile solids. The inoculum to substrate ratio (based on VS) was then calculated. At the BCP laboratory, inoculum-to-substrate ratios of 3:2 – 2:1 are normally used. The amount of inoculum (m_{IS}) and substrate (m_{SS}) in the sample were also calculated. A total amount of maximum 400 g liquid per reactor (500 ml bottle) is normally used in the BPC-laboratory to avoid problems if foaming occurs. The amount of inoculum can be either constant in all the bottles or it can

vary from one sample bottle to another one. For a certain VS ratio, the necessary amounts of substrate and inoculum needed are automatically calculated by the AMPTS II software (for instance under section 3.2.5, Method 2).

If several substrates are to be tested with the same inoculum, the amount of inoculum in accordance with the substrate with the lowest VS value (see formulas below) is calculated if the amount of inoculum should be kept constant in all bottles. This amount of inoculum is then subsequently used for the other substrates, and the amount of substrate is then adjusted so that the desired VS ratio is achieved. In this way, only one set of blanks (corresponding to the amount of inoculum added in each reactor) can be used. However, some of the reactors will then contain a total volume smaller than 400 g. If the same amount is desired in all bottles, these reactors may be filled up to 400 g using distilled water.

Ratio 2:1 $$\frac{m_{IS} * VS_I}{m_{SS} * VS_S} = 2$$

Ratio 3:2 $$\frac{m_{IS} * VS_I}{m_{SS} * VS_S} = 3$$

If a ratio of 2:1 and a total amount of 400 g are chosen: $\frac{m_{IS} * VS_I}{m_{SS} * VS_S} = 2$

Thus, $m_{ss} + m_{IS} = 400$ g ====> $m_{ss} = 400$ g- m_{IS}

3. 2. 4. Starting up of the batch test

Two methods have been used to start up the experiment. One required that the same amount of inoculum is used in all reactors (Method 1 below), and the second used different amount of inoculum as well as substrates according to the experiment guidelines given by the software. In both methods, the tests were expected to give a comparable result. All experiment scenarios can be found in appendix 1.

3. 2. 5. 1. *Method 1*

Although, a triplicate was recommended, duplicate of each combination inoculum-substrate was performed. An example of the test scenario for this method is shown in table 4. The blank (same as the inoculum) was the digester sludge from SNJ. Acetic acid was used as control, as it is directly converted to methane. The inoculum for the second experiment (see appendix 1 for the scenario) was taken from the digester sludge in the wastewater treatment plant in Grødaland.

Table 4: Experiment 1 scenario

Cell	Name	Inoculum	Substrate
1	Blank 1	350 ml	0
2	Blank 2	350 ml	0
3	Primary sludge 1	350 ml	40 ml
4	Primary sludge 2	350 ml	40 ml
5	Food Waste 1	350 ml	40 ml
6	Food Waste 2	350 ml	40 ml
7	Potato diluted 1	350 ml	80 ml
8	Potato diluted 2	350 ml	80ml
9	Potato solid 1	350 ml	20 ml/20 g
10	Potato solid 2	350 ml	20 ml/20 g
11	Acetic acid 1	350 ml	0.9 ml
12	Acetic acid 2	350 ml	0.9 ml

The amount of inoculum needed was measured by a one litre graduated cylinder. Then added into the glass bottle, and followed by adding the right amount of substrate. Incubating temperature was set at 35°C. After all components and connection of the AMPTS were set up, the online monitoring was set on. The data collection lasted for at least a period of 2 weeks.

3. 2. 5. 2. *Method 2*

Differently from the previous method, the amount of inoculum and substrates used were according to the guidelines given by the AMPTS II software (table 5). The inoculum to substrate ratio calculated previously was used as input.

Table 5: Recommended Inoculum and substrate amount from the AMPTS software

Experiment 4			
Cell	**Name**	**Inoculum (granule)**	**Substrate**
1	Granule 1	400 ml	0
2	Granule 2	400 ml	0
3	Granule 3	400 ml	0
4	Influent R1,1	95.16 ml	304.84 ml
5	Influent R1,2	95.16 ml	304.84 ml
6	Influent R1,3	95.16 ml	304.84 ml
7	From R1,1	82.47 ml	295.08 ml
8	From R1,2	82.47 ml	295.08 ml
9	Effluent R1,1	94.41 ml	305.59 ml
10	Effluent R1,2	94.41 ml	305.59 ml

COD, TS and VS were measured prior running the reactor, because the values were used as input into the software to estimate the appropriate amount of inoculum and substrate that need to be used. It is assumed that the program will compensate the difference in amount of substrate/inoculum used in each bottle test, when displaying the result for all the samples.

3. 3. Biochemical methane potential (BMP) calculation

BMP (m^3 CH_4/ kg VS or COD) is defined as the volume of methane produced per amount of organic substrate material added to the reactor. As inoculum is added into the substrate, the amount of methane produced by the inoculum in question must be subtracted from the total methane produced to get the true production from the substrates. BMP can be expressed as:

$$BMP = \frac{V_S - V_I}{m_{VS,sS}}$$

where: V_S is the accumulated volume of methane from the reactor containing the sample (inoculum + substrate), V_I is the volume of biomethane coming from the inoculum present in the sample bottle (usually the blank), and $m_{VS,Ss}$ is the amount of organic material of substrate contained in the sample bottle, COD is often used instead of VS as unit.

Generally, the accumulated gas volumes of the last obtained values are used (for V_S and V_I). A separate analysis performed before and at the end of the test will generate the COD and VS reduction of all substrates (see analytical methods section below).

When different inoculum concentrations were used, a more appropriate formula was utilized. The methane from the individual substrates has been calculated as following:

$$BMP = \frac{V_S - V_I}{m_{VS,sS}} == \frac{V_S - V_B \frac{m_{VS,IS}}{m_{VS,IB}}}{m_{VS,sS}} = \frac{V_S - V_I \frac{m_{IS}}{m_{IB}}}{m_{VS,sS}}$$

Where: V_B: biomethane originating from the inoculum, ml

V_S: biomethane originating from the sample, ml

$m_{VS,IS}$: amount of dry organic material in the sample, g COD or VS

$m_{VS,IB}$: amount of dry organic material in the blank, g COD or VS

m_{IS}: amount of inoculum in the sample, g COD or VS

m_{IB}: amount of inoculum in the blank, g COD or VS

3. 4. Analytical methods

COD, pH, TS (total solid), TVS (Total volatile solid), VFAs and nutrients content (phosphorous and ammonium-nitrogen) were measured during the study.

3. 4. 1. pH

Measurement of pH was performed upon sample arrival at the laboratory using WTW multi 340i with a pH-Electrode SenTix 41 probe. The probe was immersed into the samples until a constant value was reached. The pH meter was calibrated with standard buffer solutions (pH 4 and pH 7).

3. 4. 2. Conductivity

Conductivity value reflects the ionic content of the samples and is directly linked to the total dissolved solids (TDS). It is a measure of the ability of the sample to conduct an electrical current. The conductivity of all samples was measured before starting any experience. The value served as input for VFAs calculation on TITRA 5 software. In addition, the software calculated the TDS (mg/l) from the value of the conductivity input.

3. 4. 3. COD measurement

Total COD of the samples was measured by the Spectroquant method. A 2 ml of homogenized sample were transferred into a COD vial, then digested in thermos reactor at 150°C for 2 hours. The vial was removed from the reactor and cooled down until room temperature was reached (tubes were swirled a couple of times during cooling). Upon reaching room temperature, COD was measured through a spectrometer (Spectroquant Pharo 300) cell at a defined measuring method. COD measurement were performed before and after the methane potential test in order to estimate the COD removal of all samples. The vial with a range of 100 - 1500 mg/l COD was mostly used. Usually, the samples were diluted 25 to 100 times to match the measuring range of the vial.

3. 4. 4. Total solid (TS) and total volatile solid (VS)

Biomass were characterized by TS and VS determination. Analysis were performed according to the standard method for wastewater characterization SM 2540 B, C, and E (Clesceri et al., 1998). Total solid is defined as all suspended and dissolved, organic and inorganic material that can be found in the sample. The volatile solid characterized the organic compounds of the sample, from which biogas will be generated.

A defined volume of sample was put into a porcelain dish, then heated up to 100°C to remove the water content. The weight obtained is then subtracted by the initial mass of the dish and divided by the initial volume of the sample to get the TS (mg/l), as follows:

$$TS_{(\frac{mg}{l})} = \frac{m_{dish+\,residual} - m_{dish}}{V_{sample}}$$

After finishing the TS measurement, the residual was heated up to 550°C in a muffle oven for one hour, then weighted. The weigh difference between the sample heated after 100°C and 550°C gives the VS content of the sample, as shown below:

$$VS_{(\frac{mg}{l})} = \frac{m_{dish+residual} - m_{dish+ignited\,residual}}{V_{sample}}$$

3. 4. 5. Volatile fatty acids and Alkalinity measurement

VFAs and alkalinity were measured through manual titration of the samples with an acid and computed using the software TITRA 5. VFAs of all samples were analysed based on the five-point titration method.

A defined volume (10-50 ml) of samples were filtered and diluted to 50 ml into a beaker. This was put on a magnetic stirrer at a low, but sufficient rotation (60-100 rpm) to maintain a sufficient mixing of the samples, and to minimise the CO_2 input or loss. At first, conductivity, temperature and pH were measured. Then, samples were titrated to four different pH values: 6.7, 5.9, 5.2 and 4.3. The volumes of acid added upon reaching those pH values were recorded.

A NaOH solution were added into the samples where the initial pH was lower than 6.7. This addition was automatically considered by the program TITRA 5. All data from the titration served as input to the computer program TITRA 5, and result were calculated from it. TITRA calculated the total VFAs expressed as mg acetic acid/l and alkalinity as mg $CaCO_3$/l.

3. 4. 6. Nitrogen and Phosphorus content

Ammonium was measured by pipetting 0.50 ml of the sample into a reaction cell, which is then closed and mixed thoroughly. One dose of reagent NH_4-1K was added and the mixture was shaked vigorously. The cell was left stand for 15 min, then measurement was performed by using a Spectroquant Pharo 300. Similarly, phosphorous as PO_4-P was measured by pipetting 1.0 ml of sample into a reaction cell and mixed vigorously. Then, 5 drops of reagent P2-K was added and mixed, and afterwards 1 dose of reagent P-3K was mixed with the sample. The total was left stand for 5 min and the cell was put into a spectrometer cell (Spectroquant Pharo 300) at a defined range, then concentration was measured and recorded.

4. RESULTS

Data collected from the anaerobic baffled reactor (R2) will be presented in a separate section from the up-flow anaerobic sludge blanket reactor (R1).

4. 1. Solid characterisation of the samples

The samples have various solid concentration varying from 4 450 mg/l to 117 000 mg/l (table 6). For the wastewater samples, the solid content depends on the treatment stage, the type and origin of wastewater treated as well as the load received by the plant at that specific time. COD and pH can be found in Appendix 2.

Table 6: Characteristics of the different substrates used

Plant/Factory	Substrates	TS (mg/l)	VS (mg/l)
SNJ WWTP	Digester sludge	32300	20348
	Primary sludge	48500	35367
	Mixed food waste	57800	43989
Grødaland WWTP	Digester sludge	27000	18400
	Primary/flotation sludge	117000	104500
	Reject Water	4450	1300
Vik WWTP	Biological sludge	63000	51500
	Mixed biological/primary sludge	33600	25000
Hoff	Potato solid waste	72200	57339
	Potato dilute waste	23400	12097
Farm biogas plant	Percolate manure	19100 ± 6984	11795 ± 4487
	Influent R1	9195 ± 4101	4503 ± 2265
	Influent R2	19529 - 31230	11964 - 21200

4. 2. Methane production profile of the samples

The methane profiles presented here are not expressed as the specific methane production for each substrate, thus the gas produced from the inoculum has not been subtracted from the samples.

The methane production profiles are assumed to be proportional to the COD degradation profile and are presented for all experiments in figures 6 to 9. The highest accumulated methane production has been found in the food waste samples, then the primary sludge and less in the biological sludge. The residue of potato slurry from Hoff factory, has a considerable amount of methane, while, the diluted potato slurry produced a lower volume of methane.

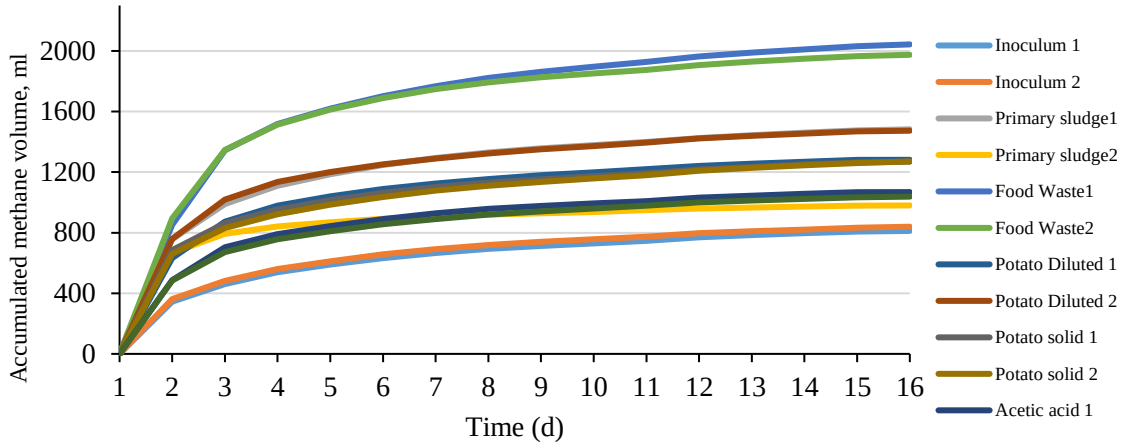

Figure 6: Methane production profile from experiment 1

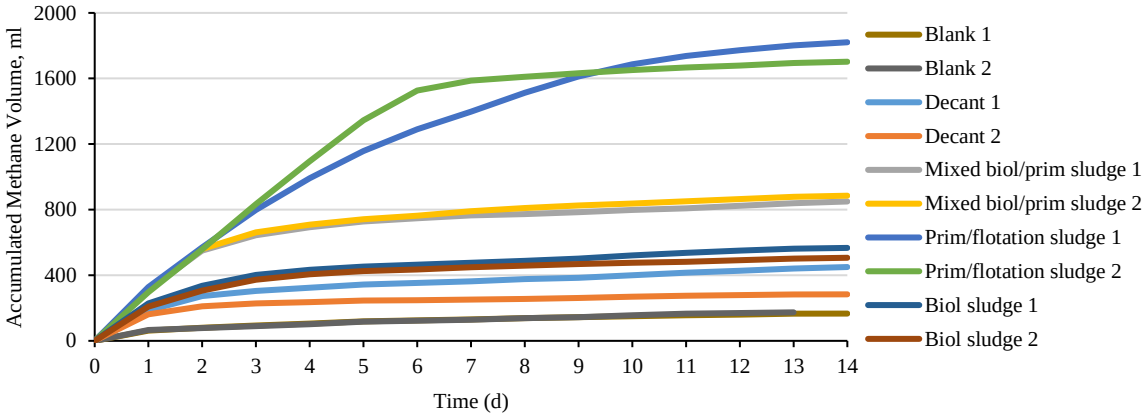

Figure 7: Methane production profile from experiment 2

The manure samples have various methane production profile. The digestion is completed when a plateau is reached on the curve, thus no significant accumulation of methane is expected. For the sludge sample, this plateau was generally reached within 7 days (see figures 6 and 7). However, for the feedstock manure, this plateau is varying from one substrate to another (figures 8 and 9).

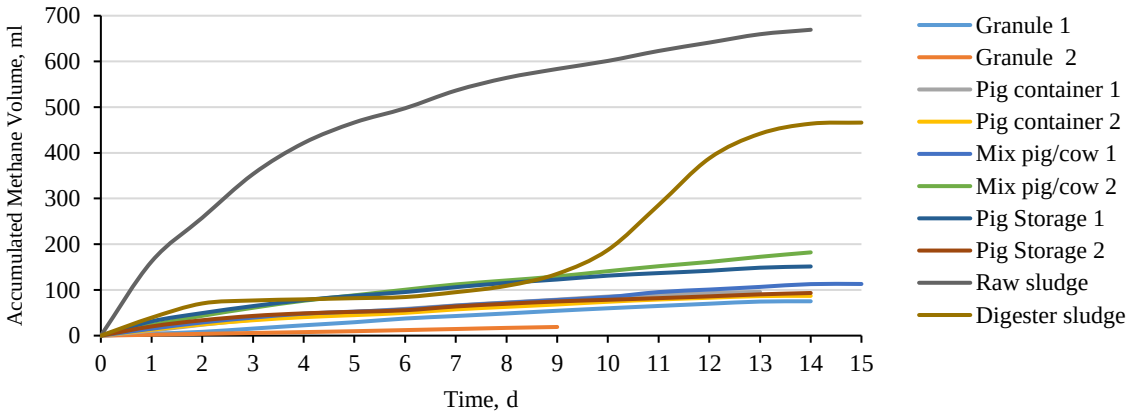

Figure 8: Methane production profile from experiment 3

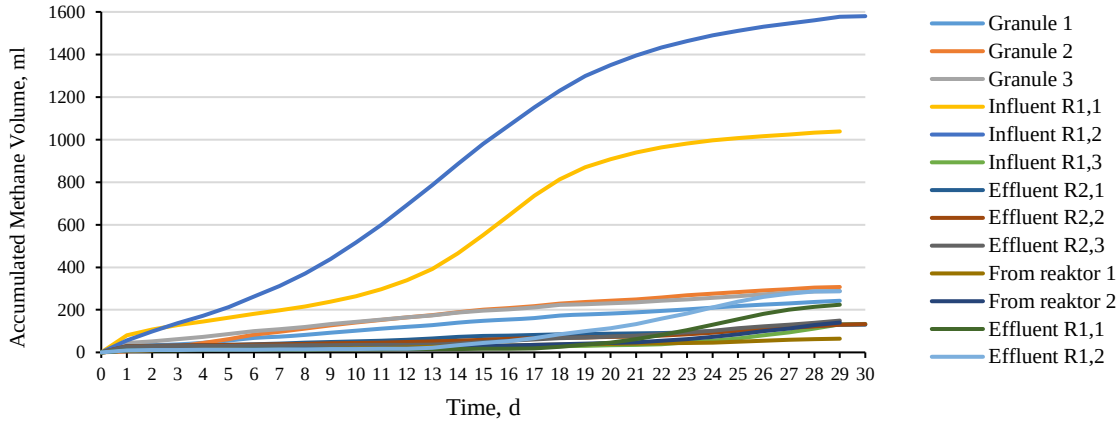

Figure 9: Methane production profile from experiment 4

4. 3. Biochemical Methane Potential

4. 3. 1. Municipal and industrial wastes

The first two experiments focused primarily on the sludge and food waste from different WWTPs and factory. The primary sludge from Grødaland has a very high BMP (0.756 m^3 CH_4/kg VS). Similarly, the reject water has the highest BMP, about 1.514 m^3 CH_4/kg VS. The digester sludge samples (the inoculums) have a lower BMP compared to the primary sludge regardless of the type of WWTP. The diluted potato waste has a higher methane potential (0.570 m^3 CH_4/kg VS) compared to solid potato waste (0.387 m^3 CH_4/kg VS). The food waste has a methane potential of 0.672 m^3/kg VS, which is slightly higher than the BMP of the diluted potato waste (table 7). The unusual high BMP of the reject water will be discussed later on.

Table 7: Biochemical potential of wastewater and food waste samples

Samples	BMP (m^3 CH_4/ kg COD)	BMP (m^3 CH_4/ kg VS)
SNJ Digester sludge	0.083	0.116
SNJ Primary sludge	0.167	0.287
Grodaland Digester Sludge	0.022	0.031
Grødaland Primary/Flotation sludge	-	0.756
Grødaland Reject water	0.164	1.514
Biological sludge Vik	0.252	0.353
Mixed biological/primary sludge	0.372	0.558
SNJ Mixed food waste	0.339	0.672
Diluted Potato waste	0.267	0.570
Solid Potato waste	0.222	0.387

4. 3. 2. Manure

The last part of the methane potential test has been dedicated to the study of mainly manure samples. The highest BMP has been found into the fresh sample coming into the biogas reactor with 0.614 ml CH_4/ mg VS. As a comparative study, digesting the raw sludge from the WWTP with the granule from the farm yielded a higher BMP than the actual manure samples (table 8).

Table 8: BMP of the manure and sludge samples using granular inoculum

Samples	BMP (m^3 CH_4/ kg COD)	BMP (m^3 CH_4/ kg VS)
Granule	0.046 – 0.060	0.030 – 0.077
Pig manure from container	0.080	0.098
Mixed Pig/cow from container	0.243	0.342
Raw sludge	-	0.608
Digester sludge	0.400	0.539
Influent R1	-	0.614
Effluent R1	0.045	0.072
Influent R2 (Pig manure storage)	0.089	0.166
Effluent R2	0.101	0.166

4. 4. Røysland biogass plant

4. 4. 1. Granule characterisation

The granules used for manure digestion in the plant had different shapes, as shown in figures 10.a and 10.b. From here, the granule that came from wood chips waste treatment will be termed as wood chips granule (WCG), and the one transported from the slaughterhouse will be termed slaughterhouse granule (SLG).

Figure 10: Different granule forms

Wood chips granule for manure digestion, (b) round-shaped granule for manure digestion, (c) round-shaped granule into a lab-scale UASB reactor for wastewater treatment (used by Safitri, 2016).

The previous granule used by the plant (WCG) had a VS fraction of 51%, while the new one (SLG) had about 75% VS content. The lower VS fraction in WCG is because of the presence of support material which contain a large percentage of inorganic material compared to SLG. Moreover, the specific methane production is higher in SLG (0.345 m^3 CH_4/ kg VS) than in WCG (0.030 – 0.077 m^3 CH_4/ kg VS) (table 9).

Table 9: Characteristics of the granules

Characteristics	**Wood chips granule (WCG)**	**Slaughterhouse granule (SLG)**
BMP	0.030 – 0.077 m^3 CH_4/ kg VS	0,345 ± 0.1 m^3 CH_4/ kg VS
Assumed immobilisation process	With wood chips as support material	Auto-immobilisation
Shape	Wood chips like shape	Non-ideal round-shaped
Size	Length: 2 – 5 mm, width: 1 – 3 mm	0,5 – 5 mm
Color	Black	Black

As the size of the granule from the auto-immobilisation process (SLG) is not dictated by the size of any support material, the granule has a very wide range of size. WCG with support material is larger and more uniform, due to the presence of the wood chips material. But the latter does not reach the state of round-shape.

4. 4. 2. Substrate characterisations

When reading the figures in this section, three different periods should be considered: (period 1) from 1 to 69 days which shows the data collected by IVAR (period post-starting of the reactor), (period 2) from 85 to 139 days showing the data collected by the author, and (period 3) from 161 days and further, where the granule was changed from WCG to SLG. The period of two weeks between the period 2 and 3 was allocated for the washing and refilling the reactors with new substrates and new granules (figures 11-16). The raw data can be found in appendix 8 and 9.

This section will be mainly allocated to characterize the feed of the UASB reactor, as it was the focus during this study (the ABR reactor will be presented in *section 4.4.5*). Three different substrates are used to feed R1, which can be either a percolate of (1) pig manure or (2) mixture of pig and cow manure or (3) mixture of pig and chicken manure. The use of the mixture has not been specified by the farmer, thus, the fraction of each feedstock types in the percolate (termed *Out substrate container* in the figures) or in the influent R1 is unknown.

The pH of the sample in the reactor varied between 8 and 9.2. No big difference was observed from the influent to the effluent. A pH slightly under the neutral (between 6.7 to 6.9) was observed in the percolate sample (figure 11)

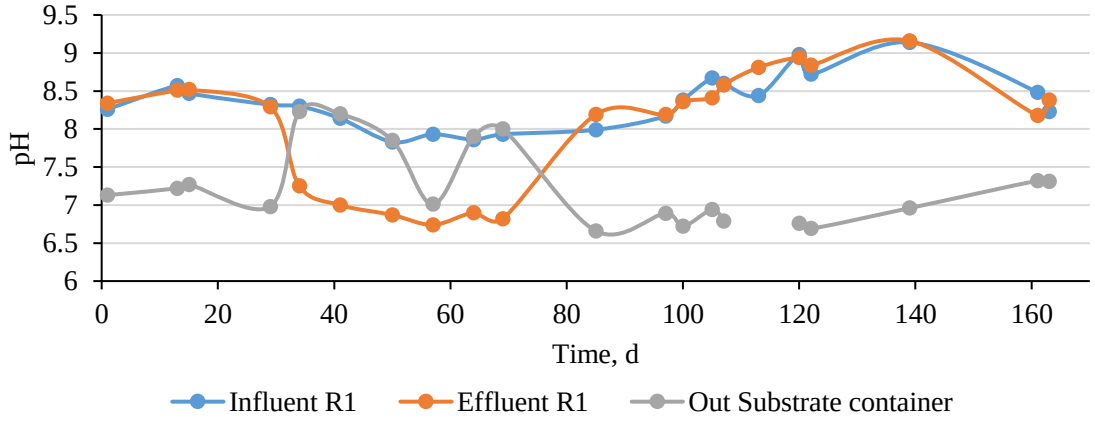

Figure 11: pH profile

An increase in VFA was observed from period 1 to period 2, and decreasing through period 3. In period 2 particularly, the VFA concentration was the highest in the percolate (2657 mg/l to 22 106 mg/l). Influent R1 has a VFA between 295 mg/l to 4 164 mg/l while the effluent has between 347 mg/l to 2984 mg/l, both values are lower compared to the percolate VFA (figure 12).

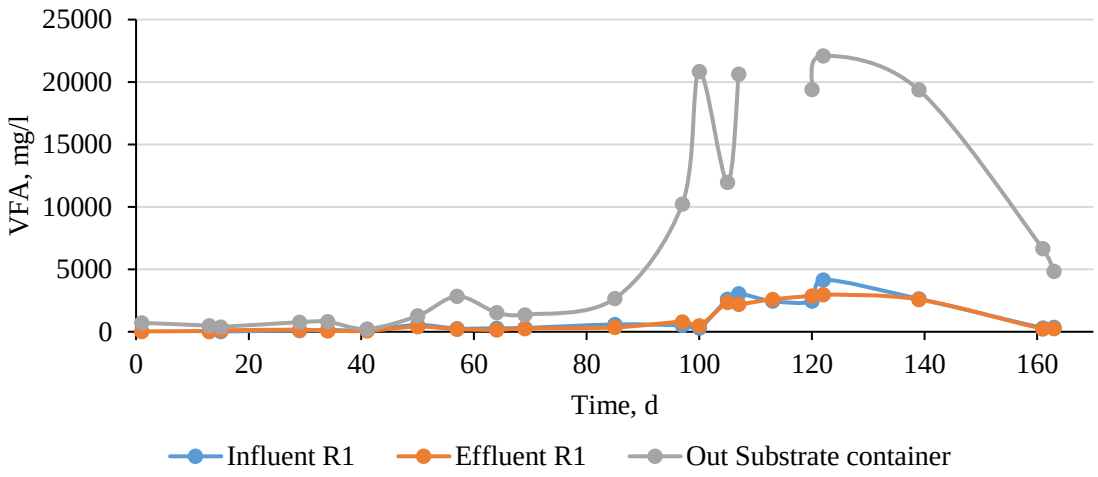

Figure 12: VFA profile

Alkalinity varies from 1740 mg/l to 3830 mg/l in the percolate sample. Influent R1 has an alkalinity between 2563 mg/l and 6261 mg/l, while the effluent has a slightly higher value, ranging from 3384 mg/l to 6410 mg/l (figure 13).

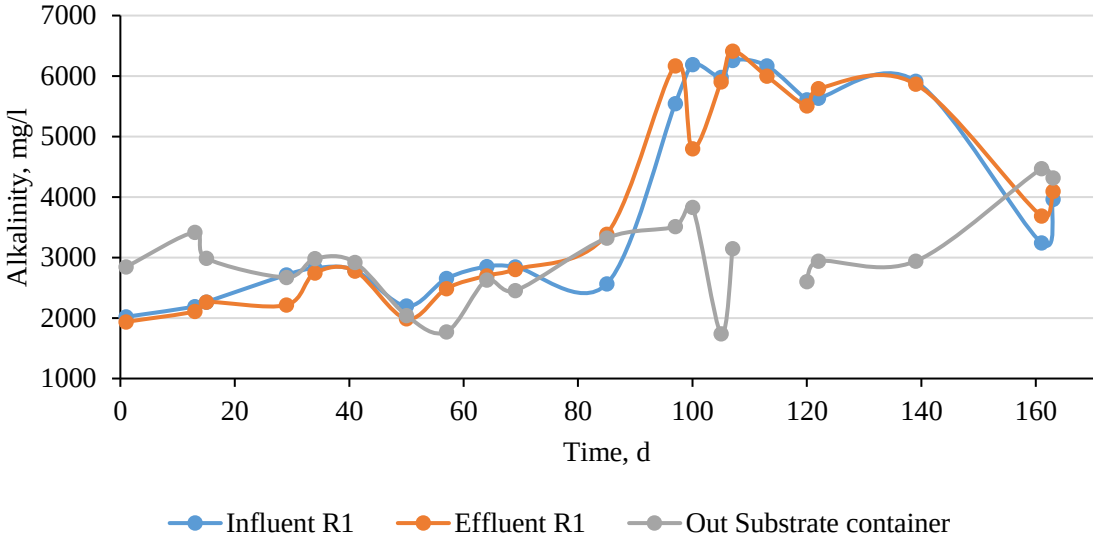

Figure 13: Alkalinity profile

The conductivity value for all samples increases from period 1 to period 2. Influent and effluent R1 have almost the same value throughout the study period (figure 14).

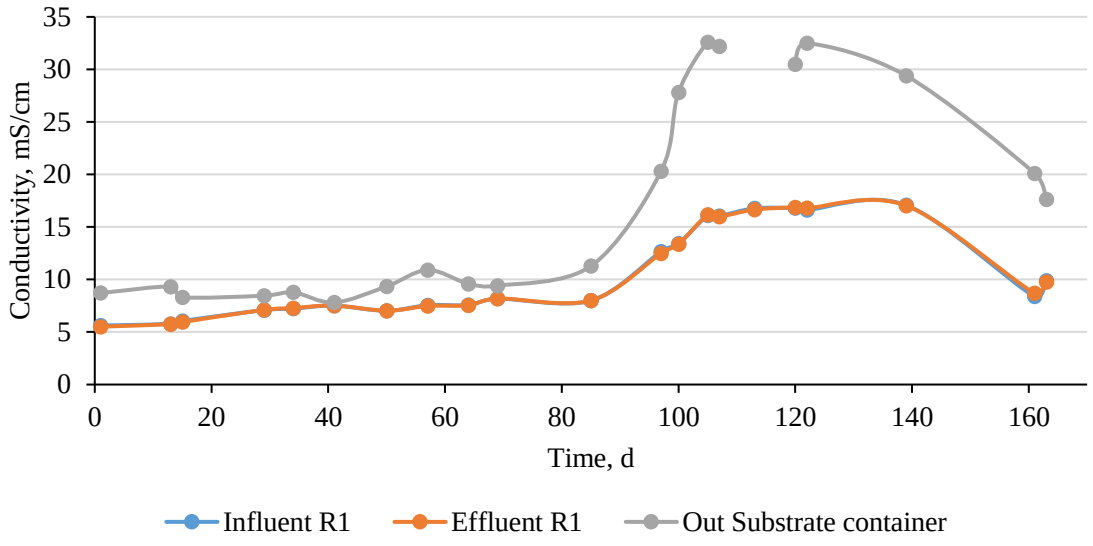

Figure 14: Conductivity profile

4. 4. 3. COD and solid characterizations

COD is almost the same for both influent and effluent. Influent COD varied between 5.3 g/l to 8.2 g/l). Very high COD was found in the percolate sample, ranging between 15 g/l to 63 g/l during period 2 and 3 (figure 15).

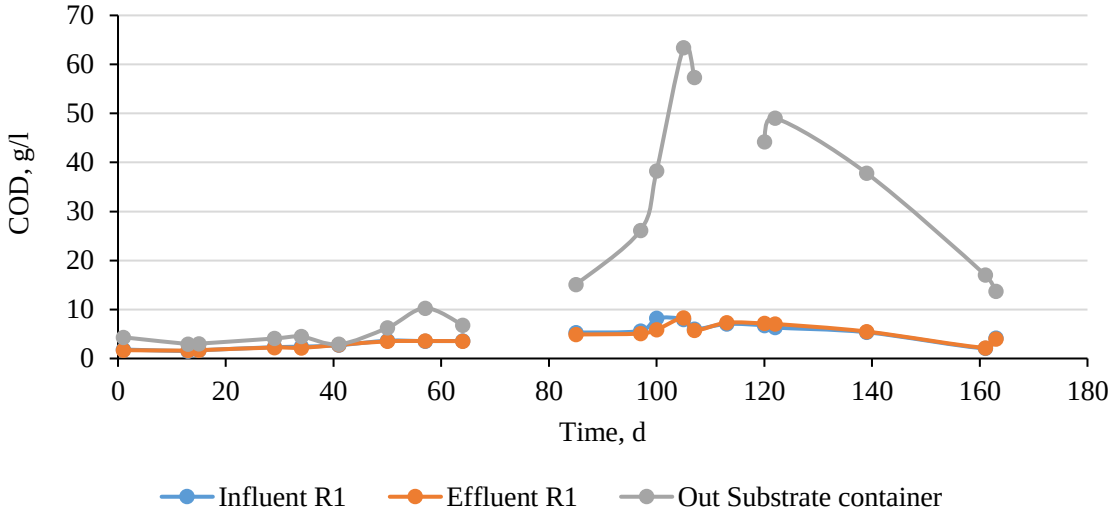

Figure 15: COD profile

A TS concentration between 5.7 g/l and 19.7 g/l, and VS between 2.9 g/l and 10.6 g/l were found in the influent sample. The TS and VS content of the percolate ranged from 11.9 g/l to 29.9 g/l and 7.2 g/l to 19.4 g/l respectively (figures 16 and 17).

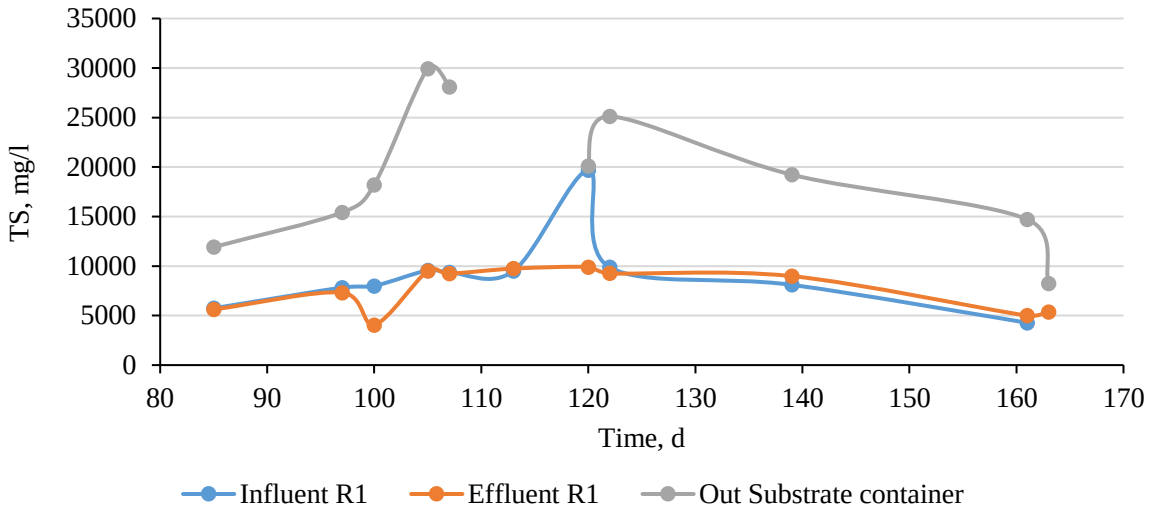

Figure 16: TS profile

The influent and effluent had almost the same VS concentration. In some points, the effluent VS was found to be higher than the influent one (figure 17).

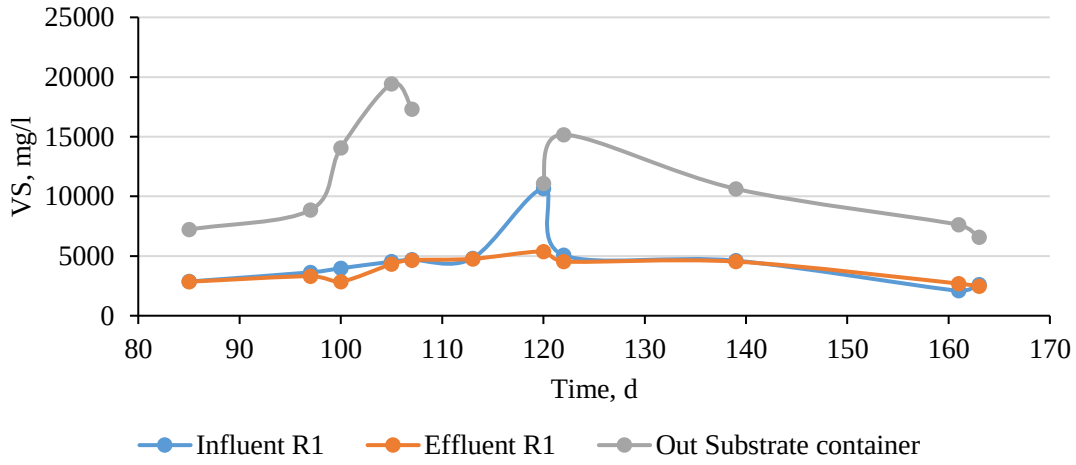

Figure 17: VS profile

A constant correlation was observed between the TS and VS of the sample (figure 18). VS accounted between 46 to 54 % of the influent TS. A bigger VS fraction was found in the percolate, ranging from 55 to 77 % of the TS.

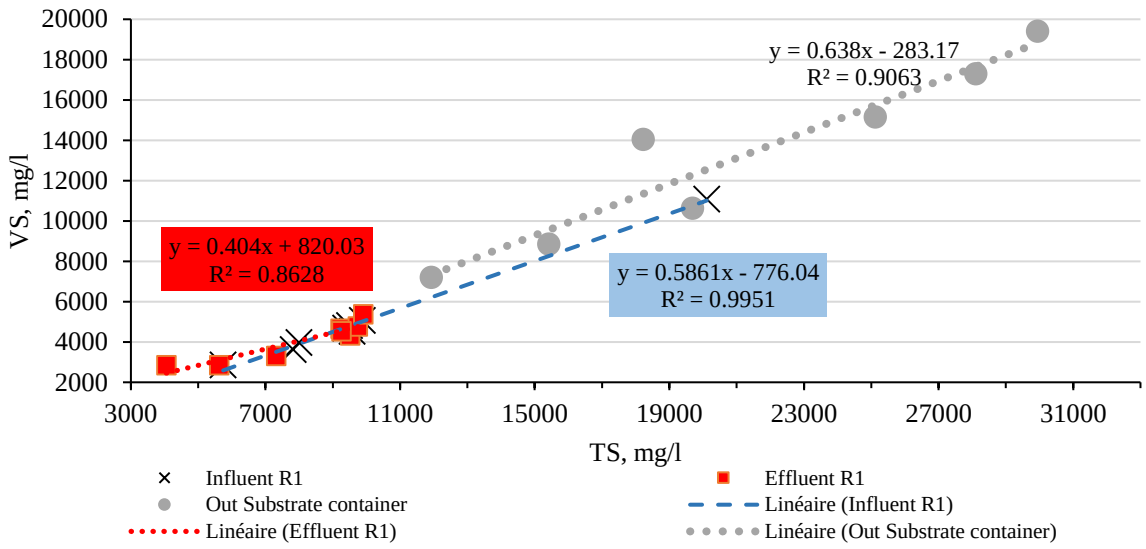

Figure 18: Correlation between total and volatile solid

4. 4. 4. Organic loading rate and COD balance

The HRT was calculated to be 7 h for R1. Depending on the organic matter content of the influent, the organic loading into the UASB reactor varied from 18.2 kg COD/m^3.d to 28.1 kg COD/m^3.d (influent OLR in figure 19). The substrate container OLR presented in the figure below is meant as comparison if the percolate were to be used directly as feed to the UASB reactor without passing through the storage tank for equalization. Its practical importance will be discussed later on.

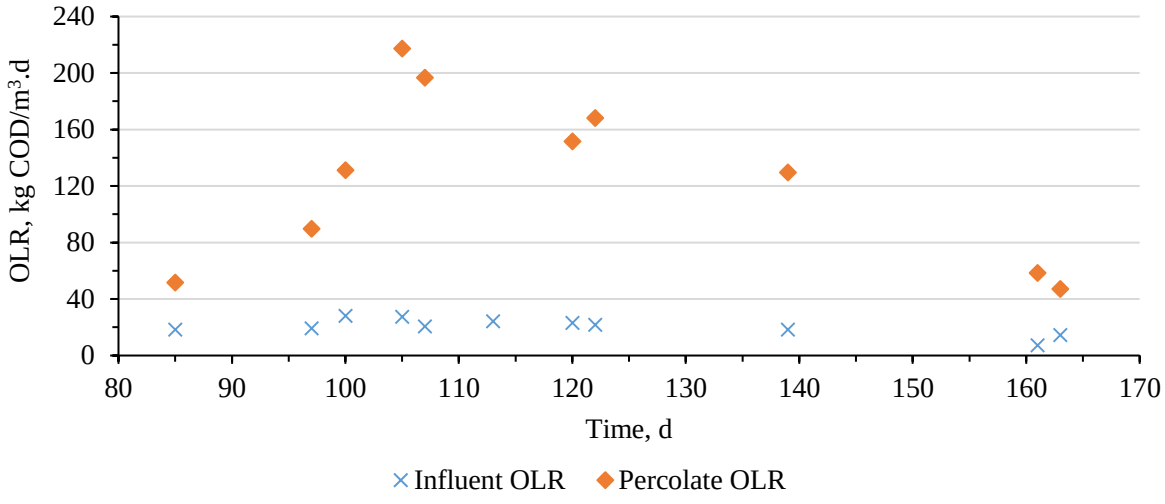

Figure 19: OLR variability between percolate and influent R1

With an average OLR of 20.2 kg COD/m^3.d, the current methane production from R1 was about 3 m^3/d in period 2 and 10 m^3/d from period 3 (personal communication with farm owner).

As shown in figure 16, the influent and effluent R1 had almost the same COD, thus establishing a COD mass balance was not practical, as it will be also discussed later. Therefore, a different approach has been used by replacing the influent R1 COD by the percolate COD. It implies that R1 and ST is considered as one system having the percolate from the substrate container as influent. COD balance was then calculated based on percolate COD (i.e. feed COD), the effluent COD and the theoretical methane yield of 0.40 m^3CH_4/ kg COD (see Appendix 10 for calculation).

During period 2 and 3, the COD reduction ranged from 67 to 90%. The COD balance varied from 11 to 37 % (figure 20).

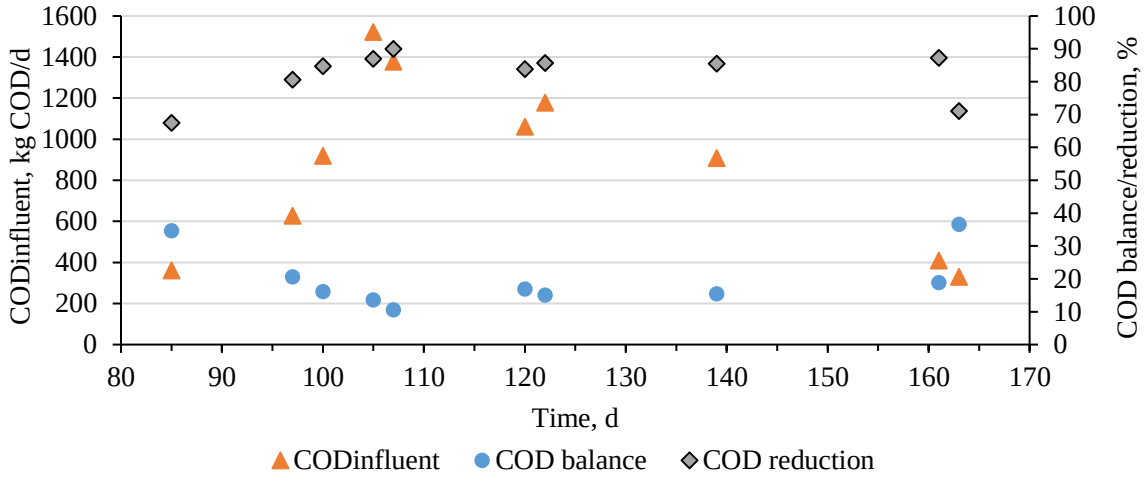

Figure 20: COD balance and COD reduction during period 2 and 3

4. 4. 5. Anaerobic Baffled Reactor Characteristics

The hydraulic retention time was estimated to be 1 d for R2. The TS of the pig manure from the storage ranged from 19.5 g/l to 31.2 g/l. The VS fraction was between 61.3 % to 67.9 %. The influent COD ranged from 10. 3 g/l to 15.1 g/l. While effluent COD varied from 4.6 g/l to 5.5 g/l. One COD test performed in March showed a COD reduction of 52% (COD_{in} = 15.1 g/l and COD_{out} = 7.8 g/l). The other characteristics are presented in table 10.

Table 10: Characteristics of the pig manure from the storage tank

pH	Conductivity (mS/cm)	VFA (mg/l)	Alkalinity (mg/l)	Ortho-P (mg/l)	NH4-N (mg/l)
7.7 – 8.24	19.05 – 25.8	336 – 577.7	6028.5 – 12848.8	390	3090

4. 4. 6. Nutrients

Occasionally, the phosphorous and ammonium nitrogen content of the sample were measured. The values presented in table 11 represent the minimum and maximum observed during the

study. A high phosphorous concentration is found in the filtrate pig manure from the storage (influent R2). Similarly, both influent and effluent of R2 have a high ammonium content (3.09 g/l and 3.16 g/l respectively).

Table 11: Phosphorous and nitrogen content of the manure samples

Substrate types	**Ortho-P, mg/l**	**NH4-N, mg/l**
Pig container	74 - 142	-
Influent R1	40 - 78.5	1840
Effluent R1	40 - 115	600 - 1900
Influent R2 (Pig storage)	120 - 397	3090
Effluent R2	110	3160 - 3480
Out Substrate container	73 - 330	1656 - 3432

5. DISCUSSION

5. 1. Recirculation unit

Although the same dry manure is used as feed for R1, the analytical properties of the manure varies depending on the stage of the process. The difference in pH of the sample coming from the substrate container and the influent of R1 is because the storage tank (ST) acts as an equalization (or diluting) unit. The COD, pH, alkalinity and conductivity are buffered within that unit. The outcome is the appearance of low pH the containers producing percolate manure, and higher pH in the storage tank, the UASB reactor and the digestate recirculating within R1. The analytical results previously presented confirmed that affirmation (see figures 11-14). It showed that the influent and effluent parameters had a similar value compared to the substrate container. Also, the percolate from the container has been found to have a COD value 3 to 9 times higher than the influent COD R1. This ratio is at the lowest in period 1 and highest in period 2 (e.g. in 107 d the percolate has 57.35 g COD/l against 6 g COD/l for the influent R1). As shown in figure 15, an increase in the COD of the manure samples is observed from period 1 to period 2. Furthermore, the influent COD has not varied much compared to the COD percolate from the substrate container, which highlight the importance of the ST as an equalization tank.

The presence of the recirculation unit, when well operated can help to increase the biogas production by regulating the recirculation amount (RA, m^3/d) and the recirculation rate (RR, %), those parameters may dictate the OLR. With an effluent flow rate of 1 m^3/h and a tank volume of 10 m^3, the HRT was estimated to be 10 hours inside ST, whereas HRT for R1 was calculated to be 7 hours (with reactor volume of 21 m^3 and effluent flow rate of 3 m^3/h). With

an influent flowrate of 3 m^3/h and RA equal to of 2 m^3/h, the RR for R1 is equal to 66%. The reason of high RR is because ST was designed to improve hydraulic conditions and prevent channelling and dead spaces inside R1, therefore, maintaining the granules in suspension inside R1. However, a study conducted by Muller (2017) on liquid-manure-based biogas plant showed that a RR of 27% (corresponding to a RA of 5.5 m^3/d) stabilised the fermentation process and lead to significantly higher methane yields (Müller et al., 2017).

5. 2. Alkalinity, pH and VFA relationship

A specific analysis of the percolate from the container is shown in the figures below. The acidic pH found in the percolate is assumed to have a close relationship with the VFA primarily produced. At increasing VFA concentration the pH is constantly dropping to acidic (figure 21). Also, as shown in figure 22, period 1 with lower VFA concentration has higher pH compared to period 2 with higher VFA content.

As a higher fatty acids concentration induce a decrease in pH, the percolate with a high VFA, has a lower alkalinity compared to the influent R1 which has a low VFA. Also, the digestion process produces ammonium bicarbonate form the breakdown of protein, while carbon dioxide consumes it (Tchobanoglous et al., 2003). The high alkalinity found in the effluent showed that no significant alkalinity consumption occurs inside the UASB reactor. As the digestate is returned into ST, a portion of it is mixed with the percolate coming from the container, thus, a higher alkalinity will also be observed in the influent (day 107 and 122 in figure 13).

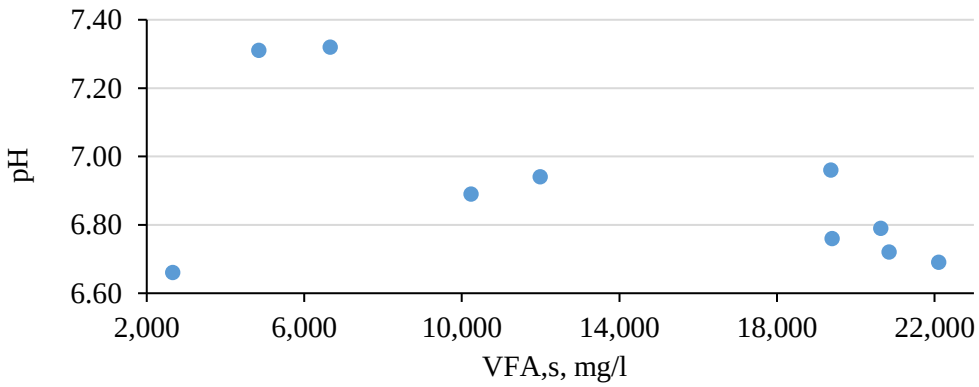

Figure 21: VFA vs pH profiles in percolate

The fact that no drastic pH change occurred with a high VFA concentration is due to the high alkalinity of the manure. Usually, once alkalinity is consumed by the produced acids the pH drops. This leads to the inhibition of the methanogens, which cause the accumulation of non-dissociated VFAs and the subsequent drop of pH (Henze, 2008).

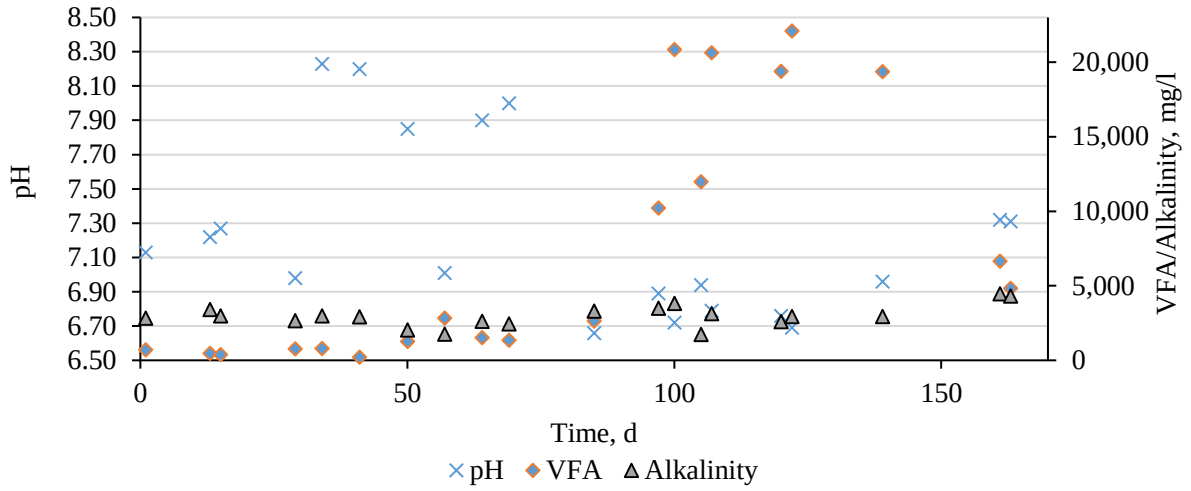

Figure 22: pH, VFA and alkalinity variations in percolate

Considering the VFA content, it is noticeable that the hydrolysis and acidogenesis process occur efficiently. As mentioned by Henze (2008), acidogenesis is the most rapid conversion step in the anaerobic food chain, as the free energy change ($\Delta G°'$) of the acidifying reactions is highest of all anaerobic conversions. Thus souring (i.e. a sudden pH drop) may occur when reactors are overloaded or perturbed by toxic compounds. It is also important to remember that those manure samples already went through a digestion process inside the rumen of the animals, thus a certain VFA amount was already produced.

5. 3. COD and VS reduction

During the first two months of monitoring, no significant reduction of COD has been observed from the UASB reactor R1. The presence of a higher COD in the effluent was assumed to be the effect of overflow from the reactor. The effluent might have carried more solids than what originally came from the influent (the reason behind this will be developed on the next paragraph). Figures 15 and 16 show a good example of this phenomenon, where between 105 and 113 d, the effluent had a higher COD and TS than the influent.

After considering different parameters (high enough VFAs, COD, pH and alkalinity), the original assumption about the factor behind the low performance of the reactor was the insufficient contact between the granule and the manure. A solid deposition on the bottom of the reactor may have restricted the flow of the substrate, which flows unidirectionally, thus, not evenly distributed. This implies: (1) the no-resuspension of the granules, (2) a higher COD of the effluent due to solid overflow (the remaining solid in suspension is carried by the up-flow), (3) a thickened sludge on the bottom of the reactor. Henze (2008) pointed out that if the sludge retained in the reactor remains deprived of substrate, this sludge is of little if any value. Thus,

the low COD reduction observed in R1, due to the factors exposed previously, establishing a COD balance was not practical (the digestion process does not even reach yet any steady state).

At this stage, a reactor failure was assumed as biogas production was merely about 3m^3/d, while the potential from the original design of the plant was about 300 m^3/d. As a solution proposed by the supplier, the granule has been changed by new granule from slaughter house industry (personal communication with farm owner). After the reactor has been stopped for two weeks, it was fed with new granules and new dry manure (drastic change of pH, VFA and alkalinity from 161 d shown in figure 22). The few samplings realized after running the reactor with new granule showed no or little reduction of the COD nor VS. This seems to be normal since the new granule needs to be acclimated with the new substrate. Some of the parameters used by Henze (2008) to classify a good quality granule was its high metabolic activity and a high settleability. Thus, the granular sludge should have a specific methanogenic activity ranging from 0.1 – 2.0 kg COD/kg VSS.d.

5. 4. COD balance

As shown in figure 20 the percolate manure digested into R1 have shown a relatively low COD balance, ranging between 17 and 37 %. Various factors can be considered as reason of this large COD gaps. Primarily, as the sludge COD could not be calculated, a value of 10 % can be considered to fill the COD gap. This proportion has also been used by Safitri (2016) for municipal wastewater. In addition, loss of electrons due to sulphate reduction and denitrification (if nitrate is present) may also affect the COD balance (Henze, 2008). But the extent of this effect is unknown and depend on the local concentration in sulphate and nitrate.

Another reason is the constant value of methane produced used in the COD gas estimation, which could have led to an underestimation. As for period 2 and 3, a methane production of 3 m^3/d and 10 m^3/d respectively were used (personal communication with the farm owner). This implies that the COD gas remains constant during both period, which in reality is not the case.

The large COD gap remaining is assumed to be the effect of the recirculation system. As already explained in section 5.1, the recirculation unit dilutes the percolate manure, which also implied the high COD reduction as shown in figure 20. Thus, the COD originating from the percolate manure is “dissimulated” in the bulk mass of the storage tank.

5. 5. Process loading factor of R1

Considering the variation of organic loading rate coupled with the low biogas production observed during period 2, it was first assumed that the loading was too high to be supported by

the digester. Even if UASB reactor are designed to support a high loading rate, finding the balance of stability between the amount of organic matter loaded, the resuspension and settling of active biomass, and the reduction of COD (thus optimal biogas production) was still problematic. However, the result obtained showed that the alkalinity high enough to prevent from pH drop in the reactor. Thus, the previous assumption is rejected. As mentioned by Henze (2008), a decrease in the methanogenic activity i.e. inhibition will only be observed when unionized VFAs accumulate which lead to a greater pH decreases.

In practice, diluting the manure percolate (which has a very high COD) before entering the digester R1 seems to be an appropriate solution for manure digestion to avoid overload of the reactor in absence of sufficient buffer. However, having a high COD on the effluent might affect the subsequent process, because part of the effluent is pumped back into ST. That recirculation flow has a high VFA content which might cause inhibition to the microorganisms living in the digester if alkalinity is depleted. Also, it is assumed that an up-concentration of the VFA may occur once they are pumped back into the container containing the dry manure. This gives another assumption why the pH was lower in the substrate container. However, a study found out that VFAs, simple sugars, and alcohols degrade quickly within hours, and are converted into CH_4 and CO_2 with traces of hydrogen sulphide and water vapor (NRCS, 2007).

The sudden increase of COD and VS concentrations in period 2 could be considered as the effect of change of feed types in the containers. One of the containers previously containing cow manure has been emptied and filled with chicken manure (personal communication with farm owner). The amount of chicken manure filled into that specific container has not been specified. The use of chicken manure instead of cow manure may have affected the percolate characteristics, as observed in figures 12 to 15. Furthermore, when refilling the container, the amount of dry manure added into the container was not specified. Therefore, a larger amount of dry manure added would imply a higher percolate COD, as experienced in period 2.

Theoretically, with an average OLR of 20.2 kg COD/m^3.d (using an average of influent COD of 5.89 g/l and inflow rate of 3 m^3/h) and an assumed COD conversion of 50%, the theoretical methane production was estimated to be 85 m^3 CH_4/d (see section 2.4. for formula used in this estimation and appendix 3 for calculation). This estimation was far above the current production of the plant which is about 3 m^3/d. Obviously at higher OLR, a higher methane production from the reactor is expected. As mentioned in section 5.1. that the COD of the percolate is much higher than the influent COD of R1 (see also fig. 15 and 19), increasing the OLR can be then easily performed by increasing the flow rate of percolate from the substrate container into ST.

Nevertheless, due to the reasons explained in the previous section concerning the low COD and VS removal of the manure percolate, the current gas produced by the plant cannot cope with the theoretical estimation.

Although numerous studies have proven the effectiveness of sludge bed anaerobic digestion in treating manure slurry in lab scale (high process capacity, stability and robustness to high and changing loads), the current application in Røysland biogas plant has shown some process performance issues. This is due to the scale and time unit factors, as for full scale biogas plant, noticing the effect of parameter changes might take a long time. Another issue is the absence of inline sampler unit to carry out analysis on time. This has been pointed out also by many researchers, where industries lack equipment to measure pH, alkalinity and VFA on-line. These parameters constitute the control strategy of the process, which can have a disastrous impact, and may lead to a reactor failure.

5. 6. Methane potential

Ward et al (2008) claimed that a direct comparison of biogas production from different feedstocks is difficult as performance data for specific types are often produced under a wide variety of experimental conditions (e.g. mixing regime, temperature, total solids, volatile solids, and hydraulic retention time). Also, in order to get a better result, it was suggested to compare feedstocks by their ultimate methane yield (Ward et al., 2008), determined by BMP assay (Owen et al., 1979). The BMP assay provides information on the potential extent and rate of methane production available from a specific feedstock (Ward et al., 2008).

When using the digester sludge as inoculum (Table 7), the BMP is the highest for Grødaland Primary sludge with 0. 756 m^3 CH_4/kg VS, followed by the mixed food waste with 0.677 m^3 CH_4/kg VS (the unusual high BMP of Grødaland reject water will be reviewed separately later). However, using the granular inoculum from the farm plant showed that the raw sludge has the highest BMP with 0.608 m^3 CH_4/kg VS, followed by the digester sludge with 0.539 m^3 CH_4/kg VS (table 8). A study performed in Korea found a methane yield of 0.472 m^3/kg VS for mixed food waste sample (Cho et al., 1995), which is lower yield compared to the mixed food waste from SNJ which is 0.672 m^3/kg VS (table 7). The difference can be attributed to the composition of the food waste and to the extent of the fat fraction of the substrates. A substrate with a large portion of fat will yield a higher COD per gram VS than if it was mainly composed of carbohydrates and proteins, thus a higher methane yield per VS. Also, a food mixture with abundance of easily-degraded material will have a higher yield than if it was composed mainly of lignocellulosic materials (due to the presence of recalcitrant materials).

High methane yield was found in the primary sludge of Grødaland with 0.756 m^3 CH_4/kg VS, and SNJ with 0.287 m^3 CH_4/kg VS compared to their respective digester sludges which are 0.031 m^3 CH_4/kg VS and 0.116 m^3 CH_4/kg VS for. As previously mentioned the high methane yield found in the primary sludge from Grødaland is due to its high fat fraction. A study also found a methane yield of 0.590 m^3/kg VS for primary sludge (Chynoweth et al., 1993), which is lower than what was found in Grødaland and higher than the one from SNJ. In addition to the macromolecular composition of the substrate, the high methane yield found in the sewage sludge is generally due to its property to be easily-degraded, thus a higher fraction of the organic matter is available for anaerobic decomposition (Ward et al., 2008).

As mentioned above, testing the BMP of the raw sludge with a granular sludge rather than the regular digester sludge yielded a higher methane yield which is 0.608 m^3 CH_4/ kg VS compared to the SNJ primary sludge with 0.287 m^3 CH_4/ kg VS, and lower compared to Grødaland primary sludge with 0.756 m^3 CH_4/ kg VS. All inoculums had a lower methane yield compared to the samples tested: SNJ digester sludge yielded 0.116 m^3 CH_4/ kg VS, Grødaland digester sludge yielded 0.031 m^3 CH_4/ kg VS and SLG with a methane yield between 0.030 and 0.077 m^3 CH_4/ kg VS. It can be assumed that the low methane yield is a special trait of inoculums, because inoculums are primarily composed of active biomass, and normally without external substrate. Thus, respiration of the biomass will be the main process for the methane production. Even with a considerable VS/TS fraction, for instance 68% for Grødaland digester sludge, the methane yield will remain low in the absence of external substrate.

The study conducted by Ward et al (2008) showed that the different biomass from agriculture wastes have a high methane potential, but they may require a pre-treatment. The solid potato slurry tested had a methane yield of 0.387 m^3 CH_4/ kg VS (sample was about 7% TS). A maximum methane yield of 0.321 m^3 CH_4/ kg VS was obtained by Parawira et al (2004) at 40% TS and an inoculum-to-substrate ratio (ISR) of 1.5 (Parawira et al., 2004). A potato peel tested by Gunaseelan (2004) had a BMP of 0.267 m^3 CH_4/ kg VS. For comparison, the potato solid slurry sample has a higher BMP compared to a potato peel (0.267 m^3 CH_4/ kg VS), a banana peel (0.277 m^3 CH_4/ kg VS), and a rotten tomato (0.298 m^3 CH_4/ kg VS), but lower BMP compared to an onion outer peel (0.400 m^3 CH_4/ kg VS), a lemon pressings (0.473 m^3 CH_4/ kg VS) or a garden pea pods (0.390 m^3 CH_4/ kg VS) (Gunaseelan, 2004).

Pig manure alone had a very low methane yield either for the percolate from the container which is 0.098 m^3 CH_4/ kg VS or the filtrate from the storage which is 0.166 m^3 CH_4/ kg VS. When pig manure was mixed with the cow manure into the substrate container, an increase in methane

yield was observed, which is 0.342 m^3 CH_4/ kg VS. A separate analysis showed a higher methane yield for pig manure with 0.356 m^3 CH_4/ kg VS, and decreasing potential with sow manure (0.275 m^3 CH_4/ kg VS) and dairy cattle (0.148 m^3 CH_4/ kg VS) (Møller et al., 2004). Usually, manure has lower methane yield than municipal sludge (see BMP with COD unit in tables 7 and 8). Therefore, the unusual high BMP found in influent R1which is 0.614 m^3 CH_4/ kg VS can be due to overestimation of the methane produced from the sample.

Using the effluent of the reactor as substrate resulted in a lower gas production compared to the fresh substrate from the substrate container. Testing the used substrates with the same inoculum was aimed to provide information whether the remaining COD contained on those substrates can be further utilized to produce methane. The BMP of the effluent of R1 and R2 has been estimated to be 0.045 m^3 CH_4/kg COD and 0.101 m^3 CH_4/kg COD respectively. Generally, recirculation (as in R1) is used mainly to maintain a certain pressure inside the reactor system to allow resuspension and re-settling of the granule successfully, thus increasing the contact between the substrates and the granules (Henze, 2008). Considering the amount of COD that comes with the effluent, suggesting a second reactor for a full digestion of the organic material might be appropriate.

Furthermore, this study showed that different samples (manure and sludge) having the same COD concentrations can have different BMP. The BMP is somewhat independent of the COD and TS concentrations. The main parameters that play an important role on the methane potential are: (1) the availability of substrates (dissolved COD are more assimilated by the microbial cell than particulate COD), (2) the optimal growth parameters (growth rate, pH, temperature, inhibitory substances etc…) and (3) the composition of the substrate i.e. the extent of the different fractions (fat, carbohydrate and protein). As seen from table 7, the reject water sample of the sludge from Grødaland WWTP (which has a low suspended solid, thus low particulate COD) has a higher BMP (even with a lower COD) compared to the other samples (SNJ and Grødaland digester sludges). However, surprisingly, in term of VS, the reject water has the highest BMP with 1.514 m3 CH4/kg VS. Supporting the previous assumption is the fact that the diluted potato slurry had a higher BMP with 0.570 m^3 CH_4/kg VS than the solid potato slurry with 0.222 m^3 CH_4/kg VS. This can be associated to the positive effect of dissolved COD and the negative influence of the particulate COD on the biogas production. That unusual high BMP might also be due to underestimation of the VS value of the reject water and diluted potato slurry substrates during analysis. As the total solid analysis might have evaporated a large portion of the volatile fraction. Thus, leaving a low VS content, which then caused the large

BMP values. Similar case has also been observed at IVAR (personal communication with responsible at IVAR). Also, another possible reason could be that the high biogas production reduced the adsorption of CO_2 into the CO_2-fixing unit, which would obviously lead to an overestimation of the CH_4 production.

From the different results obtained, the methane potential can highly vary from one substrate to another. However, the fact that the methane potential test has been performed into a batch reactor implies that no considerable application in estimating the potential of a full-scale biogas plant can be considered (and particularly for UASB and ABR reactors used in this study). Because this might lead to an overestimation of the actual potential of a plant, thus, BMP must be used with caution. Also, the HRT and SRT, as well as the recirculation system should match the actual plant system for the BMP test to be valuable. The HRT of the batch test was between 15 to 29 days, while the manure digester R1 and R2 has a HRT of 7 hours and 1 day respectively. A typical HRT and SRT values between 20 to 24 d for treating cow manure can be performed by using a plug-flow covered lagoon process (Tchobanoglous et al., 2003). The latter will put in good use the BMP results from the tests. Also, for UASB reactor with a reactor temperature above 26°C, an average HRT between 6 and 8 hours is appropriate (Tchobanoglous et al., 2003).

One of the issues observed from this study is the difficulty to efficiently separate the methane potential of each substrate like pig, cow and chicken manure. The assessment was based on estimating the methane yield of the filtrate mixture (except for the pig manure from the storage used in R2) of unknown manure fraction. The design of the plant did not permit to efficiently sample from each substrate container (as there was no sampling port available).

5. 7. Nutrient recovery

One of the advantages of using livestock manure as feed for biogas production is the possibility to recover nutrients such as phosphorus and nitrogen, which are important for the use in agriculture industry. Occasionally, the phosphorous and ammonium nitrogen content of the sample were measured. The values presented in table 10 represent the minimum and maximum observed during the study. A high phosphorous concentration is found in the filtrate pig manure from the storage (influent R2) which is between 120 and 397 mg P/l. Similarly, both influent and effluent of R2 have a high ammonium content of 3.09 g/l and 3.16 g/l respectively. No significant reduction of both phosphorous and nitrogen was observed from the influent to the effluent for both R1 and R2. NRCS (2007) pointed out that anaerobic digestion has little effect

on phosphorous, other than moving some of the dissolved portion into the bodies of bacteria that carry out anaerobic digestion process.

The most common and important forms of nitrogen in the water/soil environment are ammonia, ammonium, nitrogen gas, nitrite ion and nitrate ion. Organic nitrogen can be converted easily to ammonium through the action of microorganisms in that environment (Tchobanoglous et al., 2014). When the manure digestate is returned to an aerobic environment, the ammonia nitrogen (NH_3-N and NH_4-N) is being converted to nitrites and nitrates. Therefore, the high fertilizer value of manure lies on the fact that the nutrients will be available for crops growth, because nitrate is needed for making protein.

The driving forces behind the need for phosphorous recovery from wastes are the unsustainable nature of phosphorous mining and the potential future shortage. Compared to municipal wastewaters which typically contains between 3.7 mg/l to 11 mg/l of phosphorous as P, the percolate manure samples contain a very high orthophosphate (PO_4-P) ranging from 40 mg/l to 115 mg/l at the effluents of both reactors R1 and R2 (see table 10). Similarly to nitrogen, the importance of orthophosphate lies on the fact that it is readily available for biological metabolism without further breakdown (Tchobanoglous et al., 2014). Thus, nutrients recovery is a great advantage for agricultural purposes.

5. 8. Performance of the AMPTS II

It is important to mention that even though the basic knowledge for conducting a BMP experiment is the same for any test, the non-uniformity of the results obtained and the absence of a standard method for performing the test is still problematic. Several studies were dedicated to the BMP methodology adding to the increase in the number of publications concerning the BMP test of different organic materials (Cabrita et al., 2016). However, the AMPTS II already defined a standard procedure for conducting a methane potential test for organic materials. It considers the inoculum-to-substrate ratio and overestimation, and uses an advanced technology to simplify control of the most of its components. It also offers an online measurements of low methane production of degradable substrate. On the contrary, other techniques performed in batch reactor cannot give the highest accuracy when it comes to measuring the biogas produced while sampling is needed for rate calculation, VS reduction and other parameters.

As presented in section 4.2. regarding the methane production profile, instead of presenting the specific methane profile expressed as volume CH_4 per gram VS added, the methane production expressed as accumulated methane volume was presented (figures 6 to 9). The reason behind

this is because some samples (mainly the manure samples in figures 8 and 9) had a lower methane production than the inoculum itself. Thus, subtracting the inoculum CH_4 production from the sample CH_4 production would obviously lead to a negative specific methane production profile. Also, as mentioned in section 5.5, the inoculum should have a lower methane production than if inoculum and substrate is digested together. Several assumptions can then be made: (1) the presence of inhibitory substances in the samples used, (2) the non-adaptation of the substrate tested to the inoculum used, (3) the equipment/setting defects. The first two assumptions are straightforward (e.g. VFA inhibition, feed not adapted to inoculum, etc…), while equipment defect can be referred to the motor stop spinning due to loss of power within the motor cable from the first cell to the last cell (sometimes the stir inside the cell/reactor was not rotating for many hours). The latter was corrected by increasing the speed adjustment (the rpm). However, this gave another effect, the cells from 1 to 10 had a similar stirring pace, while the stirring speed significantly decreased from cell 11 to cell 15. This brings about questions about the relationship or effect that the speed adjustment may have on the BMP value obtained, as no reference was provided by BPC on that point.

6. CONCLUSION

The biochemical methane potential varies within the substrates tested. It is not only dependent on the organic content of the samples, but also on the specific methanogenic activity of the inoculum used (digester sludge or granular sludge), as they contain the active biomass responsible for the digestion of the organic matter. The extent of the BMP (VS based) is highly linked to the fraction of fat contained in the substrate, as it implies a higher COD/VS ratio compared to protein and carbohydrate.

High BMPs were found in Grødaland primary sludge with 0.756 m^3 CH_4/kg VS, SNJ mixed food waste with 0.677 m^3 CH_4/kg VS, the raw sludge with 0.608 m^3 CH_4/ kg VS which were relatively higher compared similar studies. The high methane yield observed is probably due to the high fat fraction of the substrates. Significant BMPs were found in solid potato slurry with 0.387 m^3 CH_4/ kg VS, and mixed pig/cow percolate manure with 0.342 m^3 CH_4/ kg VS. Between all the substrates (i.e. excluding inoculums and effluents samples), the lowest BMP has been found in the percolate of pig manure from the container which is between 0.030 and 0.077 m^3 CH_4/ kg VS. A slightly higher value was found in influent R2 with 0.166 m^3 CH_4/ kg VS. In fact, the methane yield of the pig manure is very low, and is probably slowly degradable.

The performance of anaerobic digestion in the UASB reactor was quantified in terms of methane yield, maximum OLR and COD removal, three of the most important economic factors when considering the feasibility of an anaerobic digestion process. The fact that the methane yield was relatively high in the batch test, along with the high VFA concentration (which indicates a successful acidogenesis step while providing enough substrates for the acetogenesis and methanogenesis processes to proceed) found in the influent R1 as well as the percolate from the substrate container, imply the suitability of filtrate manure as feed for anaerobic digestion. Due to the inhibition effect, too high VFA concentration should be avoided, however a sufficient amount is needed for the process to be effective. The high alkalinity indicated process stability, which lowers the risk of reactor souring due to the high VFA concentration, thus pH stability.

The digestion process is not very effective, as indicated by the low COD reduction, which is far beyond the normal acceptable level. In both a stable condition reactor and feed characteristics, the VFAs concentration can be used as indicator of the process stability. Measuring the pH can give an information about the VFA concentration inside the reactor, and can be used to avoid from overloading. Even with a high OLR (average 20.2 kg COD/m^3.d in period 2) found in R1,

due to sufficient alkalinity concentration, no significant pH drop was observed. The main reasons behind the low methane production could be (1) the low methanogenic activity of the granules, thus bad granules, and (2) the poor hydraulics. It is assumed that part of the reactor was ineffective and channelling of the substrate through the reactor may have happened.

Setting up the COD balance from the percolate manure instead of influent R1 has shown a significantly low COD balance which is between 11 to 37 %, and 67 to 90 % COD reduction. This results in a huge COD gap, which is assumed to be directly linked to the effect of the recirculation system. In addition, the ST acts as a diluting unit for VFAs, alkalinity, and COD parameters.

The highest nitrogen and phosphorus concentration that can be recovered from manure are 3.5 g NH_4-N/l and 0.397 g Ortho-P/l respectively. High nutrient content of the manure is important since digestate is used as fertilizer. This is another great advantage of having manure as feed for digestion process.

The results obtained during this study were within the expectations. Also, new data about the manure potential has been produced, among others the possibility to use the effluent for further digestion into a second digester. Manures are still a valuable source of organic material for their use as feedstock in anaerobic digesters. Furthermore, from the quality of results obtained, the AMPTS II instrument seems to be a better tool to test the methane potential of various substrates. However, given with the lack of some standard references, suggesting a study of the effect of mixing intensity inside the reactor cell on the biogas production would be interesting as it might influence the degradation rate of the substrate, thus may permit to estimate the maximum methane potential.

Lastly, investigating the manure potential of the Røysland biogas plant as well as monitoring various parameters provided a good introduction to what might have caused the upset of the process. Since the plant has been implemented for several months only, and no previous study has been performed on it, one part of this thesis can be labelled as “preliminary study of the performance of the farm scale Røysland biogas AS”. The data collected can be used to improve the capacity and stability of the UASB and ABR reactors to produce more biogas and recover more nutrients. A deeper analysis should be performed on the influence of the recirculation rate on the biogas production in order to establish a stable process performance.

7. REFERENCES

Ahring, B., Sandberg, M., & Angelidaki, I. (1995). Volatile fatty acids as indicators of process imbalance in anaerobic digestors. *Applied Microbiology and Biotechnology, 43*(3), 559-565. doi:10.1007/BF00218466

Andersson, J., & Björnsson, L. (2002). Evaluation of straw as a biofilm carrier in the methanogenic stage of two-stage anaerobic digestion of crop residues. *Bioresource Technology, 85*(1), 51-56. doi:10.1016/S0960-8524(02)00071-8

Batstone, D. J. (2002). *Anaerobic Digestion Model No.1 (ADM1)* (Vol. v.13). London: IWA Publishing.

Cabrita, T. M., Santos, M. T., & Barreiros, A. M. (2016). Biochemical methane potential applied to solid wastes - review. 21.

Cho, J. K., Park, S. C., & Chang, H. N. (1995). Biochemical methane potential and solid state anaerobic digestion of Korean food wastes. *Bioresource Technology, 52*(3), 245-253. doi:http://dx.doi.org/10.1016/0960-8524(95)00031-9

Chynoweth, D. P., Turick, C. E., Owens, J. M., Jerger, D. E., & Peck, M. W. (1993). Biochemical methane potential of biomass and waste feedstocks. *Biomass and Bioenergy, 5*(1), 95-111. doi:http://dx.doi.org/10.1016/0961-9534(93)90010-2

Clesceri, L. S., Greenberg, A. E., Eaton, A. D., American Public Health, A., Water Environment, F., & American Water Works, A. (1998). *Standard methods for the examination of water and wastewater* (20th ed. ed.). Washington: American Public Health Association.

Fang, H. H. P., & C., C. D. W. (1999). Anaerobic treatment of proteinaceous wastewater under mesophilic and thermophilic conditions. *Water Science and Technology, 40*(1999), 77-84.

Grady, C. P. L., Daigger, G. T., & Lim, H. C. (2011). *Biological wastewater treatment* (3rd ed. ed.). London: IWA Publishing.

Gunaseelan, V. N. (1995). Effect of inoculum/substrate ratio and pretreatments on methane yield from Parthenium. *Biomass and Bioenergy, 8*(1), 39-44. doi:http://dx.doi.org/10.1016/0961-9534(94)00086-9

Gunaseelan, V. N. (2004). Biochemical methane potential of fruits and vegetable solid waste feedstocks. *Biomass and Bioenergy, 26*(4), 389-399. doi:10.1016/j.biombioe.2003.08.006

Guwy, A. J., Hawkes, F. R., Wilcox, S. J., & Hawkes, D. L. (1997). Neural network and on-off control of bicarbonate alkalinity in a fluidised-bed anaerobic digester. *Water Research, 31*(8), 2019-2025. doi:http://dx.doi.org/10.1016/S0043-1354(97)00016-X

Hazen, T. C., Looney, B. B., Fliermans, C. B., Eddy-Dilek, C. A., Lombard, K. H., Enzien, M. V., . . . Wear, J. (1994). *Summary of in-situ bioremediation demonstration (methane biostimulation) via horizontal wells at the Savannah River Site Integrated Demonstration Project*: Battelle Press, Columbus, OH (United States).

Hegde, G., & Pullammmanappallil, P. (2007). Comparison of thermophilic and mesophilic one-stage, batch, high-solids anaerobic digestion. *Environmental Technology, 28*(2007), 361-369.

Henze, M. (2008). *Biological wastewater treatment : principles, modelling and design*. London: IWA Publ.

Kim, J., Park, C., H., K. T., Lee, M., Kim, S., Kim, S. W., & Lee, J. (2003a). Effects of various pretreatments for enhanced anaerobic digestion with a waste activated sludge. *Journal of Bioscience and Bioenginnering, 95*(2003), 271-275.

Lahav O., & R., L. (2000). Measurement of VFA in anaerobic digestion: The five-point titration method revisited. *Water S.A*, 4.

Lettinga, G. (2010). *The Route of Anaerobic Waste (Water) Treatment toward Global Acceptance*: Imperial College Press.

Lettinga G., H. P. L. W. (1991). UASB process design for various types of wastewater. *Wat.Sco.TEcj, 24*(8), 87-107.

Madigan, M. T., Martinko, J. M., Stahl, D. A., Clark, D. P., & Brock, T. D. (2012). *Brock biology of microorganisms* (13th ed. ed.). Boston, Mass: Pearson.

Miljødirektoratet. (2013). *Underlagsmaterial til tverrsektoriell biogass-strategi*. (TA 3020). Norway: Miljødirektoratet Retrieved from http://www.miljodirektoratet.no/old/klif/publikasjoner/3020/ta3020.pdf.

Møller, H. B., Sommer, S. G., & Ahring, B. K. (2004). Methane productivity of manure, straw and solid fractions of manure. *Biomass and Bioenergy, 26*(5), 485-495. doi:http://dx.doi.org/10.1016/j.biombioe.2003.08.008

Müller, F., Maack, G.-C., & Buescher, W. (2017). Effects of Biogas Substrate Recirculation on Methane Yield and Efficiency of a Liquid-Manure-Based Biogas Plant. *Energies, 10*(3), 325.

NRCS. (2007). Manure Chemistry - Nitrogen, Phosphorus and CArbon. *National Resources Conservation Service*, 4.

Owen, W. F., Stuckey, D. C., Healy, J. B., Young, L. Y., & McCarty, P. L. (1979). Bioassay for monitoring biochemical methane potential and anaerobic toxicity. *Water Research, 13*(6), 485-492. doi:http://dx.doi.org/10.1016/0043-1354(79)90043-5

Parawira, W., Murto, M., Read, J. S., & Mattiasson, B. (2007). A Study of Two-Stage Anaerobic Digestion of Solid Potato Waste using Reactors under Mesophilic and Thermophilic Conditions. *Environmental Technology, 28*(11), 1205-1216. doi:10.1080/09593332808618881

Parawira, W., Murto, M., Zvauya, R., & Mattiasson, B. (2004). Anaerobic batch digestion of solid potato waste alone and in combination with sugar beet leaves. *Renewable Energy, 29*(11), 1811-1823. doi:10.1016/j.renene.2004.02.005

Razafimanantsoa, V. A. (2010). *Improving BOD removal at SNJ wastewater treatment plant by biological treatment at low temperature* Masteroppgave / UIS-TN-IMN, Vol. 2010.

Reis, M. A. M., Almeida, J. S., Lemos, P. C., & Carrondo, M. J. T. (1992). Effect of hydrogen sulfide on growth of sulfate reducing bacteria. *Biotechnology and Bioengineering, 40*(5), 593-600. doi:10.1002/bit.260400506

Safitri, A. S. (2016). Biogas potential of high strength municipal wastewater treatment in laboratory scale up-flow anaerobic slugde blanket (UASB) reactors: University of Stavanger, Norway.

Siegert, I., & Banks, C. (2005). The effect of volatile fatty acid additions on the anaerobic digestion of cellulose and glucose in batch reactors. *Process Biochemistry, 40*(11), 3412-3418. doi:http://dx.doi.org/10.1016/j.procbio.2005.01.025

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

Tchobanoglous, G., Metcalf, Eddy, & Aecom. (2014). *Wastewater engineering : treatment and resource recovery : Volume 1* (5th international ed. ed. Vol. Volume 1). New York: McGraw-Hill.

Wang, Q., Kuninobu, M., Ogawa, H. I., & Kato, Y. (1999). Degradation of volatile fatty acids in highly efficient anaerobic digestion. *Biomass and Bioenergy, 16*(6), 407-416. doi:http://dx.doi.org/10.1016/S0961-9534(99)00016-1

Ward, A. J., Hobbs, P. J., Holliman, P. J., & Jones, D. L. (2008). Optimisation of the anaerobic digestion of agricultural resources. *Bioresource Technology, 99*(17), 7928-7940. doi:10.1016/j.biortech.2008.02.044

Yu, H. Q., & Fang, H. H. P. (2002). Acidogenesis of dairy wastewater at various pH levels. *Water Science and Technology, 45*(2002), 201-206.

Appendix 1: Experiment scenarios

Experiment 1			
Cell	**Name**	**Inoculum (sludge)**	**Substrate**
1	Inoculum 1	350 ml	0
2	Inoculum 2	350 ml	0
3	Primary sludge 1	350 ml	40 ml
4	Primary sludge 2	350 ml	40 ml
5	Food Waste 1	350 ml	40 ml
6	Food Waste 2	350 ml	40 ml
7	Potato diluted 1	350 ml	80 ml
8	Potato diluted 2	350 ml	80ml
9	Potato solid 1	350 ml	20 ml/20 g
10	Potato solid 2	350 ml	20 ml/20 g
11	Acetic acid 1	350 ml	0.9 ml
12	Acetic acid 2	350 ml	0.9 ml
Experiment 2			
1	Blank 1	300 ml	0
2	Blank 2	300 ml	0
3	Decant 1	300 ml	100 ml
4	Decant 2	300 ml	100 ml
5	Mixed biol/primary sludge 1	300 ml	50 ml
6	Mixed biol/primary sludge 2	300 ml	50 ml
7	Prim/flotation sludge 1	300 ml	20.08 g
8	Prim/flotation sludge 2	300 ml	20.20 g
9	Biological sludge 1	300 ml	20.31
10	Biological sludge 2	300 ml	20.05
Experiment 3			
1	Granule 1	300 ml	0
2	Granule 2	300 ml	0
3	Pig container 1	300 ml	50 ml
4	Pig container 2	300 ml	50 ml
5	Mix pig/cow container 1	300 ml	50 ml
6	Mix pig/cow container 2	300 ml	50 ml
7	Influent R2,1	300 ml	50 ml
8	Influent R2,2	300 ml	50 ml
9	Raw sludge Grødaland	300 ml	50 ml
10	Digester sludge Grødaland	300 ml	50 ml
Experiment 4			
1	Granule 1	400 ml	0
2	Granule 2	400 ml	0
3	Granule 3	400 ml	0
4	Influent R1,1	95.16 ml	304.84 ml
5	Influent R1,2	95.16 ml	304.84 ml
6	Influent R1,3	95.16 ml	304.84 ml
7	From R1,1	82.47 ml	295.08 ml
8	From R1,2	82.47 ml	295.08 ml
9	Effluent R1,1	94.41 ml	305.59 ml
10	Effluent R1,2	94.41 ml	305.59 ml

Appendix 2: Characteristics of the wastes tested

Plant/Factory	Substrates	pH	TS (mg/l)	VS (mg/l)	COD (g/)
SNJ WWTP	Digester sludge	7.3	32,300	20348	28.5
	Primary sludge	6.2	48,500	35367	60.6
Grødaland WWTP	Digester sludge	7.75	27,000	18400	
	Primary/flotation sludge	-	117,000	104500	-
	Reject Water	5.57	4,450	1300	12
	Mixed food waste	4.2	57,800	43989	87.2
Vik WWTP	Biological sludge	-	63,000	51500	72.1
	Mixed biological/primary sludge	5.62	33,600	25000	37.5
Hoff	Potato solid slurry	5.5	72,200	57339	100
	Potato dilute slurry	6	23,400	12097	25.9

Appendix 3: Estimation of the theoretical methane production

The methane production can be estimated as follow:

$$V_{CH_4} = COD_{in} \times Q_{in} \times COD_{conversion} \times Y_{CH_4}$$

Where: COD_{in}= 5.89 g COD/l

Q_{in}= 3 m^3/h

Assumed $COD_{conversion}$ = 50%

Theoretical methane yield, Y_{CH4} (1 atm, 35°C) = 0.40 m^3 CH_4/ kg COD

Thus,

$$V_{CH_4} = 5.89 \frac{kg\ COD}{m^3} \times 3 \frac{m^3}{h} \times 24 \frac{h}{d} \times 0.5 \times 0.40 \frac{m^3 CH_4}{kg\ COD}$$

$$V_{CH_4} = 84.816\ \frac{m^3 CH_4}{d}$$

Appendix 4: Raw data for experiment 1

Accumulated methane volume, ml

Day	Inoculum 1	Inoculum 2	Primary sludge1	Primary sludge2	Food Waste1	Food Waste2	Potato Diluted 1	Potato Diluted 2	Potato solid 1	Potato solid 2	Acetic acid 1	Acetic acid 2
0	0	0	0	0	0	0	0	0	0	0	0	0
1	345.2	361.6	757.5	665.6	852.6	893.4	631.7	759.6	685.5	660.1	486.6	484.7
2	460.7	482.5	989.4	792	1343.8	1346.6	873.2	1019.5	862.3	831.6	704.3	672.5
3	539.1	560.8	1108.9	841.3	1517.1	1512.8	979.1	1134.8	954.2	921.4	789.9	756.2
4	590.2	611.9	1186.4	868.3	1620	1611.9	1040.5	1199.9	1013.9	984.4	843.7	809.5
5	632.3	657.1	1247.3	891.8	1701.8	1688.3	1088.4	1250.9	1063	1036.6	890.5	855.4
6	665.2	691.1	1292.9	907.6	1768	1748.2	1124.2	1289.5	1100.5	1077.5	927.1	889.7
7	692.4	718.2	1329.3	920.3	1822.2	1793.4	1154.4	1322.6	1131	1110.5	957	919.6
8	712.4	739.2	1356.1	929	1863	1826.1	1178.5	1350.2	1153	1135.4	977.3	941.4
9	728.7	756.3	1379	937	1896.5	1852.2	1197.9	1372.1	1172.5	1158.6	993.4	959.1
10	746	773.4	1399.4	948.1	1927	1874.9	1219	1395.3	1192.6	1180.1	1008.6	978.1
11	770.3	796.4	1425.5	959.5	1962.7	1907.1	1241.4	1423.2	1218.2	1208.8	1031.6	999.7
12	784.3	809.9	1443.9	966.2	1988.7	1929	1255.8	1440.3	1235.2	1228.7	1045.1	1012
13	796.4	821.2	1460.6	972.5	2011.1	1948.7	1267.8	1454.8	1250.3	1244.6	1055.9	1023.4
14	807.7	832.2	1476.2	979.4	2030.5	1964.7	1280.1	1469.6	1264.3	1259.8	1067.3	1034.6
15	811.8	839.5	1482.3	979.9	2043.3	1974.6	1281.3	1473.3	1269.3	1270.4	1068	1038

Methane Flow rate, Nml/d

Day	Inoculum 1	Inoculum 2	Primary sludge1	Primary sludge2	Food Waste 1	Food Waste 2	Potato Diluted 1	Potato Diluted 2	Potato solid 1	Potato solid 2	Acetic acid 1	Acetic acid 2
0	0	0	0	0	0	0	0	0	0	0	0	0
1	345.2	361.6	757.5	665.6	852.6	893.4	631.7	759.6	685.5	660.1	486.6	484.7
2	115.5	120.9	231.9	126.4	491.3	453.3	241.4	259.9	176.9	171.6	217.6	187.9
3	78.4	78.3	119.5	49.2	173.3	166.2	105.9	115.3	91.8	89.7	85.7	83.6
4	51.2	51.1	77.5	27	102.9	99.1	61.4	65.2	59.7	63	53.8	53.4
5	42.1	45.2	61	23.5	81.8	76.4	47.8	51	49.1	52.3	46.8	45.9
6	32.9	34	45.6	15.8	66.2	59.9	35.8	38.6	37.5	40.9	36.6	34.3
7	27.2	27.1	36.3	12.7	54.1	45.2	30.2	33.1	30.6	33	29.9	29.9
8	20	21.1	26.9	8.6	40.8	32.7	24.1	27.5	22	24.9	20.3	21.8
9	16.2	17.1	22.9	8.1	33.5	26	19.4	21.9	19.5	23.1	16.1	17.7
10	17.3	17.1	20.5	11.1	30.5	22.7	21.1	23.2	20.2	21.6	15.2	18.9
11	24.3	22.9	26.1	11.4	35.7	32.2	22.5	27.9	25.6	28.6	23	21.6
12	14	13.5	18.3	6.6	26	21.9	14.4	17.1	17	19.9	13.5	12.3
13	12.1	11.3	16.8	6.3	22.4	19.7	12	14.6	15.1	15.8	10.8	11.4
14	11.3	11	15.5	6.9	19.4	16	12.3	14.8	14	15.2	11.4	11.2
15	11	8.8	12.7	6.9	15.3	15.1	12.6	14	11.8	12.8	11.7	10.1

Appendix 5: Raw data for experiment 2

Accumulated methane volume, ml

Day	Blank 1	Blank 2	Decant 1	Decant 2	Mixed biol/primary sludge 1	Mixed biol/primary sludge 2	Primary/flotation sludge 1	Primary/flotation sludge 2	Biol sludge 1	Biol sludge 2
0	0	0	0	0	0	0	0	0	0	0
1	62.3	66.6	185.4	161.5	310.3	309.9	327.7	297.2	228.3	208.2
2	79.5	78.2	273.2	210.3	549.6	559.9	569.7	558.1	335.9	306.1
3	94.1	89.4	304.4	227.7	644.5	662.3	798.5	832.9	401.9	373.7
4	105.8	101.5	324	235.8	692.4	707.9	991.3	1096	434.2	405.9
5	118.8	117	344.1	245.2	727.5	741.6	1156.8	1345.1	453.6	425.1
6	124.7	122.6	352.7	248.8	747	764.2	1291	1526.5	464.9	435.4
7	131.6	129.7	363.8	251.8	765	791.2	1397.6	1586.6	475.6	449.3
8	138.7	137.7	377.2	254.8	773.4	810	1513.7	1610	487.6	458.7
9	144.2	144.7	385.2	260.9	785	825.4	1612.8	1632.1	502.4	468.9
10	149.8	155.8	401	268.8	797.9	837	1685.8	1650.9	520.2	475.9
11	155.5	165.7	415.6	275.5	808.9	851.4	1736.6	1667.4	536.5	483
12	160.4	170.5	426.9	278.9	823.1	864.6	1772	1679.7	549.7	491.1
13	165.2	173.8	440.3	282.2	840.3	878.8	1802.3	1694.2	562	500.8
14	166.3		450.2	283.6	849.9	885.3	1820.9	1702.4	566.9	506.6

Methane Flow rate, Nml/d

Day	Blank 1	Blank 2	Decant 1	Decant 2	Mixed biol/prim sludge 1	Mixed biol/prim sludge 2	Prim/flotation sludge 1	Prim/flotation sludge 2	Biol sludge 1	Biol sludge 2
0	0	0	0	0	0	0	0	0	0	0
1	62.3	66.6	185.4	161.5	310.3	309.9	327.7	297.2	228.3	208.2
2	17.2	11.5	87.7	48.8	239.3	250	242	260.8	107.6	97.9
3	14.6	11.2	31.3	17.4	94.9	102.4	228.8	274.8	66	67.6
4	11.7	12.1	19.6	8.1	47.9	45.6	192.8	263.2	32.3	32.1
5	13	15.5	20.1	9.4	35.1	33.7	165.5	249.1	19.4	19.3
6	5.9	5.7	8.5	3.5	19.5	22.6	134.3	181.3	11.3	10.3
7	6.9	7	11.2	3	18	27	106.6	60.1	10.7	14
8	7.1	8.1	13.3	3	8.4	18.8	116.1	23.4	12	9.4
9	5.5	6.9	8.1	6.1	11.5	15.3	99.1	22.1	14.8	10.1
10	5.6	11.1	15.7	7.9	12.9	11.6	72.9	18.8	17.8	7
11	5.7	10	14.6	6.7	11	14.4	50.9	16.5	16.4	7.2
12	5	4.8	11.3	3.3	14.2	13.2	35.4	12.3	13.2	8.1
13	4.8	4.8	13.4	3.3	17.2	14.2	30.2	14.5	12.3	9.7
14	4.8		11	3.3	11.4	9.9	24.2	11.7	13.5	9.9

Appendix 6: Raw data for experiment 3

Accumulated methane volume, ml

Day	Granule 1	Granule 2	Pig container 1	Pig container 2	Mix pig/cow 1	Mix pig/cow 2	Influent R2,1	Influent R2,2	Raw sludge Grødaland	Digester sludge Grødaland
0	0	0	0	0	0	0	0	0	0	0
1	4.1	1.9	10.9	12.9	15	23.5	31.2	20.1	162.1	39.3
2	8.3	3.9	23.9	24.5	28.8	43.3	49.3	33.6	257.9	70.4
3	15.4	5.8	34.8	33.6	39.4	60.9	64.6	43.1	353.4	76.9
4	22.6	7.7	44.4	40.4	48	76.3	77.7	48.7	421.5	79.4
5	29.3	9.7	51.9	44.8	52.5	88.2	87.2	52.1	466.3	81.9
6	37.4	12.1	58.4	49.8	57.2	100.4	95.3	55.5	497.5	84.4
7	42.9	14.5	66.1	57.4	65.5	112	106	63.8	536.2	95
8	48.5	16.9	72.5	62.9	71.8	120.7	115.3	69.7	564	108.9
9	54.4	18.9	78.7	68.2	78	129.6	122.8	74.8	583.5	135.2
10	59.8		85.4	74.1	84.3	141	131.2	78.4	601.1	187.5
11	64.9		89.3	79	95.2	151.9	136.8	82	622.9	285.8
12	69.8		93	82.8	100.8	161.2	141.9	85.9	641.2	388
13	74.5		95.7	86.5	106.6	172.5	148.6	90.3	659.5	441.8
14	75.1			86.9	112.7	182.1	151.3	93.3	669.2	463.7
15					113					466.1

Methane Flow rate, Nml/d

Day	Granule 1	Granule 2	Pig container 1	Pig container 2	Mix pig/cow 1	Mix pig/cow 2	Influent R2,1	Influent R2,2	Raw sludge Grødaland	Digester sludge Grødaland
0	0	0	0	0	0	0	0	0	0	0
1	4.1	1.9	10.9	12.9	15	23.5	31.2	20.1	162.1	39.3
2	4.1	1.9	12.9	11.5	13.9	19.8	18	13.5	95.8	31
3	7.1	1.9	10.9	9.1	10.6	17.6	15.3	9.4	95.6	6.6
4	7.2	1.9	9.6	6.8	8.6	15.4	13.1	5.7	68.1	2.5
5	6.7	2	7.5	4.4	4.5	11.9	9.5	3.4	44.8	2.5
6	8.1	2.4	6.5	5	4.7	12.2	8.1	3.4	31.3	2.5
7	5.6	2.4	7.7	7.7	8.3	11.7	10.7	8.3	38.6	10.6
8	5.6	2.4	6.4	5.5	6.3	8.7	9.3	5.9	27.8	13.9
9	5.9	2.4	6.3	5.3	6.2	8.9	7.4	5.1	19.5	26.3
10	5.4		6.6	5.8	6.3	11.3	8.4	3.6	17.6	52.3
11	5.1		4	4.9	10.9	10.9	5.6	3.6	21.8	98.3
12	4.8		3.6	3.8	5.6	9.4	5.1	3.9	18.3	102.2
13	4.8		3.6	3.8	5.8	11.3	6.6	4.4	18.3	53.8
14	4.8			3.8	6.1	10	6.6	4.4	13.6	21.9
15					6.1					18.4

Appendix 7: Raw data for experiment 4

Accumulated methane volume, ml

Day	Granule 1	Granule 2	Granule 3	Influent R1,1	Influent R1,2	Influent R1,3	Effluent R2,1	Effluent R2,2	Effluent R2,3	From R1,1	From R1,2	Effluent R1,1	Effluent R1,2
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	30.5	4.7	44.5	80.7	56.6	10	28.8	29.3	28.2	9.9	9.8	9.3	9.8
2	34.1	9.3	51.4	107.5	97.8	10.7	30.5	30.8	28.9	10.8	10.6	9.9	10.5
3	37.7	27.6	61.6	128.7	137.5	11.4	32.2	32.3	29.6	11.7	11.3	10.4	11.2
4	45.1	44	72.6	145.5	172.8	12.1	33.9	33.8	30.3	12.6	12	11	12
5	55.4	62.4	86.1	163.2	213.2	12.8	35.5	35.3	31	13.5	12.8	11.5	12.7
6	68.1	82.8	99.9	181.7	262.3	13.5	37.8	36.8	31.7	14.4	13.5	12	13.4
7	73.9	97.2	108.4	197.2	312.6	14.2	41.7	38.5	32.4	15.3	14.2	12.6	14.2
8	82.2	111.5	119.9	215.9	370.7	14.8	45.6	40.3	33.1	16.2	15	13.1	14.9
9	92.5	126.6	132.4	238.4	438.9	15.5	48.8	42.1	33.8	17.1	15.7	13.7	15.6
10	101.9	139.9	142.8	264.1	516.3	16.2	51.9	43.9	34.5	18.1	16.4	14.2	16.4
11	111.6	152.2	154.2	296.7	599.3	16.9	55	45.8	35.2	20.2	17.2	14.7	17.1
12	120.1	165.1	164.1	338.7	692.5	17.6	59.7	48.2	35.9	22.9	17.9	15.3	17.8
13	128.1	174.7	174	392	785.8	18.3	64.9	51.9	36.6	25.7	19.4	15.8	21.8
14	139.7	188.6	186.6	465.1	885	19.4	72.8	55.5	41.9	28.3	25.3	16.4	33.8
15	148.4	200.3	196.1	551.8	980.4	21.8	76.6	59.3	48.9	30.6	29.5	16.9	44.9
16	154.6	208.1	202.7	643.4	1066.3	24.3	79.4	63.1	54.4	32.9	32.6	17.4	53.2
17	161.2	216.7	210.7	736	1150.8	26.7	82.3	66.3	60.9	35.2	35.7	18	66.9
18	173	229.4	222.9	813.6	1230.3	29.1	84.5	68.4	66.3	37.2	38.5	25.7	84.2
19	178.2	236.1	226.6	869.6	1298.3	31.2	86.2	70.5	70.3	38.6	41.2	37	99.3
20	182.5	242.5	230.4	908.2	1350.4	33.4	88	72.6	74.3	40.1	43.8	46.5	113.3

21	188.2	248.9	235.8	938.9	1395.3	35.6	89.8	74.7	79.6	41.5	46.8	61.8	133.7
22	194.9	258.5	242.6	963.2	1432.8	37.8	91.5	79.6	85.7	43	54.1	82.4	158.4
23	202.1	267.8	248.6	981.9	1463.2	46.7	94	85.6	93.7	44.4	62.9	105.1	183
24	210.2	276	255.7	996.2	1489.3	54.9	99.2	92.3	101.9	45.9	73.1	129.9	210.6
25	217.8	283.8	263.9	1007.3	1511	65.5	105.3	100.7	112.9	50.3	84.6	155.7	238.3
26	224.5	290.9	272.5	1016.2	1530.3	80.5	112.4	108.3	121.9	55	99.2	181.5	260.6
27	230.2	297.1	279.7	1024	1545.7	93.6	117.9	114.9	130.5	58.8	111.8	201	275.9
28	236.9	305	287	1032.2	1560.3	111.4	123.6	122.1	139.3	62.6	127.8	214.1	286.2
29	242.6	307.3	288.6	1038.3	1577.6	131.3	129.5	130.4	149.4	64.4	140	224	287
30					1580	133.7	130.2	132.7					

Methane Flow rate, Nml/d

Day	Granule 1	Granule 2	Granule 3	Influent R1,1	Influent R1,2	Influent R1,3	Effluent R2,1	Effluent R2,2	Effluent R2,3	From R1,1	From R1,2	Effluent R1,1	Effluent R1,2
0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	30.5	4.7	44.5	80.7	56.6	10	28.8	29.3	28.2	9.9	9.8	9.3	9.8
2	3.6	4.7	6.9	26.8	41.2	0.7	1.7	1.5	0.7	0.9	0.7	0.5	0.7
3	3.6	18.3	10.1	21.2	39.7	0.7	1.7	1.5	0.7	0.9	0.7	0.5	0.7
4	7.4	16.4	11.1	16.8	35.3	0.7	1.7	1.5	0.7	0.9	0.7	0.5	0.7
5	10.3	18.4	13.4	17.6	40.3	0.7	1.7	1.5	0.7	0.9	0.7	0.5	0.7
6	12.7	20.4	13.8	18.6	49.2	0.7	2.3	1.5	0.7	0.9	0.7	0.5	0.7
7	5.8	14.3	8.5	15.5	50.3	0.7	3.9	1.7	0.7	0.9	0.7	0.5	0.7
8	8.3	14.4	11.5	18.7	58	0.7	3.9	1.8	0.7	0.9	0.7	0.5	0.7
9	10.3	15	12.6	22.5	68.2	0.7	3.2	1.8	0.7	0.9	0.7	0.5	0.7
10	9.4	13.4	10.4	25.7	77.4	0.7	3.1	1.8	0.7	0.9	0.7	0.5	0.7

11	9.7	12.3	11.4	32.6	83	0.7	3.1	1.8	0.7	2.1	0.7	0.5	0.7
12	8.5	12.9	10	42	93.2	0.7	4.7	2.5	0.7	2.8	0.7	0.5	0.7
13	8	9.6	9.9	53.3	93.3	0.7	5.1	3.6	0.7	2.8	1.5	0.5	4
14	11.6	13.8	12.6	73	99.2	1.1	7.9	3.6	5.2	2.6	6	0.5	12
15	8.7	11.8	9.5	86.7	95.4	2.4	3.8	3.7	7	2.3	4.2	0.5	11
16	6.2	7.7	6.6	91.6	85.9	2.4	2.9	3.8	5.5	2.3	3.1	0.5	8.4
17	6.6	8.7	8	92.6	84.5	2.4	2.9	3.3	6.5	2.3	3.1	0.5	13.7
18	11.8	12.7	12.2	77.6	79.5	2.3	2.2	2.1	5.5	2	2.8	7.8	17.2
19	5.2	6.7	3.8	56	67.9	2.2	1.8	2.1	3.9	1.4	2.7	11.3	15.1
20	4.3	6.4	3.8	38.7	52.2	2.2	1.8	2.1	4	1.4	2.7	9.6	14
21	5.7	6.4	5.4	30.6	44.8	2.2	1.8	2.1	5.4	1.4	2.9	15.2	20.4
22	6.6	9.6	6.8	24.3	37.5	2.2	1.8	4.8	6.1	1.4	7.3	20.6	24.7
23	7.2	9.3	6.1	18.7	30.5	8.9	2.4	6	8	1.4	8.8	22.7	24.7
24	8.1	8.2	7	14.3	26.1	8.2	5.2	6.8	8.2	1.4	10.2	24.7	27.6
25	7.6	7.8	8.3	11.1	21.6	10.6	6.1	8.4	11	4.4	11.5	25.8	27.6
26	6.7	7.1	8.6	9	19.3	15	7.2	7.6	9	4.7	14.5	25.8	22.3
27	5.7	6.3	7.2	7.8	15.4	13.2	5.5	6.6	8.6	3.8	12.6	19.6	15.3
28	6.7	7.8	7.2	8.2	14.6	17.7	5.7	7.2	8.8	3.8	16	13.1	10.3
29	7.7	8	7.2	10.2	17.3	19.9	5.9	8.3	10.3	3.8	18.2	13.3	9.9
30					16.7	18.4	5.9	8.6					

Appendix 8: Raw data obtained from manure samples analysis

Date	Parameters									
	Samples	TS mg/l	TVS mg/l	pH	Conductivity mS/cm	VFA mg/l	Alkalinity mg/l	COD mg/l	Ortho-P mg/l	NH4-N mg/l
17/01/2017	Mix cow/pig percolate manure 1	11,400	3,600	7.50	9.70	1,825	2,446	5,766		
	Mix cow/pig percolate manure 2	7,600	4,240	7.33	9.24	1,709	1,884	5,957		
	Influent R2	18,000	5,700	8.45	24.50	602	6,968	10,588		
	Pig percolate	6,000	3,300	7.38	8.53	2,304	2,748	4,060		
	WCG 1 meter over bottom	4,667	2,350	7.99	8.05	107	3,750	4,200		
	WCG bottom	6,333	3,267	7.99	5.80	1,102	1,937	5,489		
	Grødaland Digester sludge	25,667	14,500	7.69	10.76	2,926	3,972	19,562		
	Grødaland Raw sludge	25,300	19,533	6.55	8.54	2,453	7,055	19,587		
24/01/2017	Pig percolate	8,270	4,700	7.33	8.93	923	1,450	6,992	142	
	Influent R2	31,230	21,200	7.70	19.05	336	6,029	10,293	4	
30/01/2017	Pig percolate	6,114	3,246	7.40	8.39	789	4,486	6,075	74	
	Mix pig/chicken percolate	5,909	3,115	7.47	8.44	798	3,442	6,850	73	
01/02/2017	Influent R1	5,741	2,867	7.99	7.99	585	2,563	5,300		
	Out Substrate container	11,919	7,219	6.66	11.29	2,657	3,322	15,050		
	Effluent R1	5,626	2,851	8.19	8.00	347	3,384	4,900		
13/02/2017	Influent R1	7,824	3,634	8.17	12.65	505	5,547	5,625		
	Out Substrate container	15,415	8,862	6.89	20.30	10,235	3,514	26,125		
	Effluent R1	7,320	3,310	8.19	12.49	801	6,171	5,075		
16/02/2017	Influent R1	7,993	3,979	8.38	13.41	295	6,191	8,200		
	Out Substrate container	18,216	14,062	6.72	27.80	20,848	3,830	38,250		
	Effluent R1	4,055	2,855	8.36	13.36	489	4,797	5,850		

Date	Parameters									
	Samples	TS mg/l	TVS mg/l	pH	Conductivity mS/cm	VFA mg/l	Alkalinity mg/l	COD mg/l	Ortho-P mg/l	NH4-N mg/l
21/02/2017	Influent R1	9,575	4,528	8.67	16.11	2,611	5,977	7,950	79	
	Out Substrate container	29,942	19,421	6.94	32.60	11,984	1,740	63,400	277	
	Effluent R1	9,516	4,326	8.41	16.18	2,355	5,904	8,300	57	
23/02/2017	Influent R1	9,386	4,689	8.60	16.05	3,057	6,261	6,000		
	Out Substrate container	28,101	17,308	6.79	32.20	20,634	3,147	57,350		
	Effluent R1	9,233	4,656	8.58	15.98	2,205	6,410	5,750		
01/03/2017	Influent R1	9,493	4,816	8.44	16.77	2,452	6,168	7,050	50	1,840
	From reactor	8,443	4,013	8.56	16.67	755	18,853	5,750	30	1,580
	Effluent R1	9,746	4,766	8.81	16.66	2,599	5,997	7,300	115	1,900
	Effluent R2	10,716	5,490	7.30	3.15	768	12,456	4,645	110	3,160
	WCG	44,242	23,157	8.17	27.20	273	1,113	11,600	30	200
08/03/2017	Influent R1	20,110	11,091	8.98	16.80	4,164	5,609	6,750	50	1,740
	Out Substrate container	19,683	10,642	6.76	30.50	19,399	2,603	44,200	325	3,320
	Effluent R1	9,907	5,383	8.97	16.86	2,874	5,507	7,150	50	1,760
10/03/2017	Influent R1	9,877	5,076	8.72	16.63	2,856	5,636	6,300	40	1,700
	Out Substrate container	25,112	15,169	6.69	32.50	22,106	2,941	49,050	330	3,576
	Effluent R1	9,262	4,541	8.84	16.80	2,984	5,792	7,050	40	1,680
27/03/2017	Influent R1	8,103	4,609	9.14	17.07	2,637	5,916	5,350	45	
	Out Substrate container	19,229	10,634	6.96	29.40	19,369	2,940	37,800	280	
	Effluent R1	8,985	4,537	9.16	17.03	2,589	5,867	5,500	30	
	Effluent R2	11,573	5,940	8.26	27.40	99	13,387	<10000	90	

Date	Parameters									
	Samples	TS mg/l	TVS mg/l	pH	Conductivity mS/cm	VFA mg/l	Alkalinity mg/l	COD mg/l	Ortho-P mg/l	NH4-N mg/l
30/03/2017	Influent R2	19,529	11,964	8.24	25.80	578	12,849	15,100	390	
	Effluent R2	11,433	5,580	8.37	27.30	222	13,173	7,800	110	
01/04/2017	SLG	26,657	18,914	7.09	3.85	34	1,998	2,650	35	
18/04/2017	Influent R1	4,275	2,088	8.48	8.39	305	3,241	2,075	35	
	Out Substrate container	14,722	7,619	7.32	20.10	6,653	4,468	17,050	120	
	Effluent R1	4,997	2,693	8.18	8.69	227	3,685	2,175	45	
	SLG	107,800	86,051	7.39	2.33	19	1,201	2,750	215	
20/04/2017	Influent R1	82,492	2,603	8.23	9.90	360	3,962	4,200		
	Out Substrate container	8,231	6,568	7.31	17.63	4,845	4,318	13,700		
	Effluent R1	5,370	2,478	8.38	9.78	269	4,094	3,970		

Appendix 9: Raw data from analysis performed by IVAR

Date	Samples	pH	Conductivity µS/cm	Alkalinity mg/l	VFA mg/l	Dissolved COD g/l	Total COD g/l	NH_4 mg/l	Orto-P mg /l
	Influent R1	8.3	5,620	2,021	0	1.36	1.83	460	39
08/11/2016	Out Substrate container	7.1	8,720	2,848	716	3.88	4.33	665	71
	Effluent R1	8.3	5,510	1,937	28	1.34	1.70	440	22
	Influent R1	8.6	5,770	2,189	12	1.31	1.51		
21/11/2016	Out Substrate container	7.2	9,320	3,415	486	2.58	2.95		
	Effluent R1	8.5	5,750	2,110	63	1.31	1.60		
	Influent R1	8.5	6,050	2,264	25	1.35	1.67		
23/11/2016	Out Substrate container	7.3	8,310	2,987	392	2.67	3.02		
	Effluent R1	8.5	5,950	2,265	115	1.35	1.69		
	Influent R1	8.3	7,090	2,713	100	1.95	2.31		
07/12/2016	Out Substrate container	7.0	8,460	2,673	768	3.33	4.08		
	Effluent R1	8.3	7,110	2,214	165	1.86	2.24		
	Influent R1	8.2	7,230	2,826	143	2.01	2.41		
12/12/2016	Out Substrate container	7.3	8,780	2,981	808	4.46	4.48		
	Effluent R1	8.3	7,280	2,744	80	1.73	2.17		
	Influent R1	8.1	7,510	2,789	117	2.18	2.72	595	
19/12/2016	Out Substrate container	7.0	7,810	2,919	225	2.54	2.90	595	
	Effluent R1	8.2	7,520	2,779	74	2.09	2.78	575	
	Influent R1	7.9	7,020	2,198	541	2.99	3.62		
28/12/2016	Out Substrate container	6.9	9,340	2,043	1,271	5.45	6.25		
	Effluent R1	7.8	7,030	1,986	408	2.75	3.50		

Date	Samples	pH	Conductivity µS/cm	Alkalinity mg/l	VFA mg/l	Dissolved COD g/l	Total COD g/l	NH_4 mg/l	Orto-P mg /l
	Influent R1	7.9	7,550	2,653	255	3.03	3.55		
04/01/2017	Out Substrate container	6.7	10,900	1,772	2,835	8.92	10.26		
	Effluent R1	7.9	7,480	2,487	207	2.77	3.57		
	Influent R1	7.9	7,570	2,852	283	2.93	3.58		
11/01/2017	Out Substrate container	6.9	9,590	2,631	1,538	5.98	6.81		
	Effluent R1	7.9	7,550	2,700	149	2.77	3.54		
	Influent R1	7.9	8,170	2,848	304				
16/01/2017	Out Substrate container	6.8	9,410	2,452	1,367				
	Effluent R1	8.0	8,190	2,803	264				

Appendix 10: COD mass balance calculation

The COD mass balance can be written as:

$COD_{influent} = COD_{gas} + COD_{sludge} + COD_{effluent}$ (a)

Each COD types (except for the sludge) can be estimated as following:

- $COD_{influent} = COD_{percolate} \times Q_{percolate}$
- $COD_{gas} = \frac{Q_{gas}}{Y_{\frac{l\ CH_4}{g\ COD}}}$
- $COD_{effluent} = COD_{effluent\ R1} \times Q_{effluent}$

With $Q_{percolate}$= 24 m^3/d, $Q_{effluent}$ = 24 m^3/d, Q_{gas}= 3 m^3 CH_4/d (personal communication with farm owner) and $Y_{\frac{l\ CH_4}{g\ COD}}$= 0.40 m^3 CH_4/ kg COD (at 35°C)

Then, (a) can be written as following:

$$COD_{percolate} \times Q_{percolate} = \frac{Q_{gas}}{Y_{\frac{l\ CH_4}{g\ COD}}} + COD_{effluent} \times Q_{effluent}$$

The COD reduction (%) is the difference between $COD_{influent}$ and $COD_{effluent}$

$$COD_{reduction} = \frac{COD_{influent} - COD_{effluent}}{COD_{influent}} \times 100$$

The COD balance (%) is calculated as follow:

$$COD_{balance} = \frac{\frac{Q_{gas}}{Y_{\frac{l\ CH_4}{g\ COD}}} + COD_{effluent} \times Q_{effluent}}{COD_{percolate} \times Q_{percolate}} \times 100\%$$

Time	$COD_{percolate}$	$CODe_{ffluent\ R1}$	$COD_{influent}$	$COD_{effluent}$	COD_{gas}	$COD_{reduction}$	$COD_{balance}$
d	kg COD/m^3	kg COD/m^3	kg COD/d	kg COD/d	kg COD/d	(%)	(%)
85	15.1	4.9	361.2	117.6	7.5	67	35
97	26.1	5.1	627	121.8	7.5	81	21
100	38.3	5.9	918	140.4	7.5	85	16
105	63.4	8.3	1521.6	199.2	7.5	87	14
107	57.4	5.8	1376.4	138	7.5	90	11
120	44.2	7.2	1060.8	171.6	7.5	84	17
122	49.1	7.1	1177.2	169.2	7.5	86	15
139	37.8	5.5	907.2	132	7.5	85	15
161	17.1	2.18	409.2	52.2	25	87	19
163	13.7	4.0	328.8	95.28	25	71	37