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Transitioning from mesophilic to thermophilic anaerobic digestion at VEAS and the influence of co-digesting primary sludge with potato-stillage

Abstract

Anaerobic digestion (AD) is a microbial degradation process in the absence of oxygen by several complex biological reactions where polymeric organic carbon compounds are transformed into biogas and potential biofertilizer. The process is a well-established method for stabilizing solids and biosolids from the treatment of wastes. Traditionally, it has been applied for the treatment and stabilization of municipal sewage sludge (MSS) from wastewater treatment plants (WWTP), animal manure and slurries. As a result of increased environmental awareness, attention to waste management lead to the introduction of organic wastes from industry and municipal waste as biogas feedstock.

This study investigated the feasibility of transitioning from mesophilic anaerobic digestion (MAD) to thermophilic anaerobic digestion (TAD) at VEAS and also examined the effects of co-digestion with potato-stillage in lab-scale reactors. The transition was achieved successfully by a one-step increase in temperature where feeding was completely ceased for 15 days and target organic loading rate (OLR) was achieved after 33 days. Stable TAD was accomplished that matched the mesophilic digesters by biogas and methane yield and volatile solids (VS)-reduction. However, biogas production efficiency was reduced under thermophilic conditions in addition to poorer effluent quality containing large quantities of volatile fatty acids (VFAs). Co-digestion with potato-stillage improved the biogas and methane yield up to 5.7 and 3.3 % respectively. The improvement was most significant at mesophilic conditions. VS-destruction was increase up to 5.1 % and biogas production efficiency was improved under thermophilic conditions. According to the findings of this study, strategies to reduce the VFA-content of the thermophilic digestate should be considered if VEAS chooses to transition. Furthermore, co-digesting with potato-stillage benefitted the process and appears to be a lucrative option.

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Abbreviations

AD	–	Anaerobic digestions
AcoD	–	Anaerobic co-digestion
Bio-LNG	–	Biomethane liquefied natural gas
CSTR	–	Continuously stirred tank reactor
FAN	–	Free ammonia nitrogen (NH_3)
FW	–	Food waste
GC	–	Gas chromatography
HPLC	–	High -performance liquid chromatography
HRT	–	Hydraulic retention time
MAD	–	Mesophilic anaerobic digestion
MAcoD	–	Mesophilic anaerobic co-digestion
MSS	–	Municipal sewage sludge
MW	–	Municipal waste
NIBIO	–	Norsk institutt for bioøkonomi
PoSt	–	Potato Stillage
STP	–	Standard temperature and pressure
TAcoD	–	Thermophilic anaerobic co-digestion
TAD	–	Thermophilic anaerobic digestion
TAN	–	Total ammonia nitrogen ($NH_3 + NH_4^+$)
TKN	–	Total Kjeldahl Nitrogen
TS	–	Total solids
VEAS	–	Vestfjorden avløpsselskap
VFA	–	Volatile fatty acid
VS	–	Volatile solids
WAS	–	Waste activated sludge
WWTP	–	Wastewater treatment plant
UASB	–	Upflow anaerobic sludge blanket

Table of contents

1. Introduction

Anaerobic digestion (AD) is a microbial degradation process in the absence of oxygen by several complex biological reactions where polymeric organic carbon compounds are transformed into biogas and potential biofertilizer [1]. The process is a well-established method for stabilizing solids and biosolids from the treatment of wastes. Traditionally, it has been applied for the treatment and stabilization of MSS from WWTP, animal manure and slurries. As a result of increased environmental awareness, attention to waste management lead to the introduction of organic wastes from industry and municipal waste as biogas feedstock during the 1970s [2]. AD is a cost-effective treatment method that offers many advantages, such as reduction of biomass, recovery of energy by methane production and biofertilizer that as a result prevents potential emissions of methane and carbon dioxide. Hence, it is an attractive waste management option compared to other alternatives like landfill disposal, composting or thermal technologies [1], [2], [3]. The produced biogas is an adaptable renewable fuel that can be used to make electricity and heat in combined heat and power units after removal of sulfur, follows by drying. The gas can also be used to generate heat directly by burning. After upgrading to biomethane, the gas can be utilized in the same applications as natural gas including motor fuel [2].

The increased and improved wastewater treatments have resulted in large volumes of solids that need to be managed. This has shown to be a challenging and costly issue. With the regulations encouraging biosolids reuse, efforts are made to produce solids that are suitable for land application, i.e. fractions with heavy metal and pathogen content below limit values [3]. One method that can be applied to meet the requirements for pathogen content is thermophilic anaerobic digestion (TAD). This advantage, in combination with possibilities of increased digestion rate and biogas production, makes TAD an attractive alternative [4].

Combining various wastes for anaerobic co-digestion (AcoD) offers several advantages compared to mono-digestion of each waste separately, such as many synergistic effects. AcoD of complementary substrates is thought to improve the digester performance by permitting a more significant substrate variability, more differential microbiome from the different wastes and dilute compounds that could eventually inhibit the process [5], [6]. Some anaerobic digesters at WWTP treating MSS are running at low organic loading rate (OLR) and can benefit from co-digestion with other substrates like industrial food waste (IFW) by utilizing excess capacity and increasing biogas production [7]. One potential substrate is stillage which is a non-alcoholic waste product from ethanol production from fermentation of carbohydrate-concentrated materials. It consists of the remains that have not been converted to ethanol [8]. With the increasing numbers of biorefineries and biofuel production facilities like those producing bioethanol, comes large amounts of waste effluents like stillage that needs to be managed [2]. The most common option is to dry it and use it as animal feed, but this is an energy-intensive and costly process depending on the feed and energy price because of the high water content. This characteristic also makes options like composting or landfills challenging. Hence, AD is an appealing treatment option where energy could be recovered from the fractions [2], [9].

Vestfjorden Avløpsselskap (VEAS) is the largest WWTP in Norway, treating wastewater equal to more than 750 000 P.E. from the municipalities Oslo, Asker, Bærum and parts of Røyken and Nesodden. The MSS resulting from the treatment is currently being stabilized by

mesophilic anaerobic digestion (MAD) (37 °C) and sanitized in a subsequent separate step. The resulting biofertilizer is being used for land application, and the biogas has up till now been used internally producing heat and electricity needed for the process. VEAS is now starting the process of upgrading the biogas into liquid biomethane (bio-LNG) that will be commercialized [10]. Therefore, they are looking for strategies to increase their biogas production, and efficiency. Some of the exciting options involves transitioning to TAD and implementing new substrates for AcoD. TAD would possibly eliminate the need for a separate sanitation step and has the potential of increasing the degradation efficiency and biogas yield [4]. AcoD, with its many known potential positive synergistic effects and the possibility of increased biogas production, makes this an attractive opportunity. One interesting alternative is potato-stillage from HOFF potato refinery located in feasible proximity in Gjøvik. It is a by-product from liquor production by fermentation of potatoes that is currently being used as animal feed. This application can be a challenge for HOFF when the production is large in addition to seasonal variations in demand when livestock are grazing outside. Moreover, the number of animals is reduced due to the reduced milk-production in Norway. Besides, there are also practical challenges with feeding construction in modern animal sheds [8], [11]. As a result, alternative waste management methods are being considered. For this reason AD might be an attractive option with no need for pre-treatment and energy recovery [8].

The main objective of this study is to investigate the feasibility for VEAS to convert from MAD to TAD. Furthermore, the study also examines the influence of co-digestion with potato-stillage under both mesophilic and thermophilic conditions.

2. Background/Theory

2.1. AD and production of biogas

During AD biogas is produced by the degradation of biomass without oxygen. The biogas generated consists of mainly of methane (50-75%) and carbon dioxide (25-50%), with traces of other gases like water, oxygen, sulfur, nitrogen and hydrogen sulfide. The energy of the biogas is based on its methane content which has an energy value of 37.78 MJ/m^3. Upgraded biogas, commonly referred to as biomethane, consists of roughly 98% methane and is and is equal to natural gas in areas of application [2].

2.2. Formation of biogas: Microbiology

Biogas is a product of several syntrophic and intricate microbial processes without oxygen where organic material is transformed mainly to the end products methane and carbon dioxide. The main biochemical steps in this process are hydrolysis, acidogenesis, acetogenesis and methanogenesis that are primarily performed by bacteria and archaea. The stability of the process is dependent on these degradation stages and may affect TAD and MAD differently [1], [4]. The hydrolysis stage consists of braking down particulate material into soluble compounds like polymers. Furthermore, hydrolyzing these into smaller compounds, primarily monomers and oligomers like sugars, amino- and fatty acids [3].

Conversion of these compounds into medium and short-chained volatile fatty acids (VFAs) and alcohols is done through the acidogenesis step. This step could be the first step for some industrial wastes containing readily biodegradable compounds like soluble starches or sugars. Through the acetogenesis these products are transformed into mainly acetate, carbon dioxide, hydrogen, propionate and butyrate. The latter two are fermented additionally also to generate acetate, carbon dioxide and hydrogen that hence are the final products of acidogenesis. For propionate and butyrate to be fermented further, low concentration of hydrogen is needed, less than 10^{-4} atm [3], [12].

Finally, methane is produced from acetate or carbon dioxide and hydrogen during methanogenesis. The last step is performed by two main groups of archaea: the acetoclastic methanogens that utilize acetate and the hydrogenotrophic methanogens that use hydrogen and carbon dioxide. The majority of methane is produced from acetate. [3]

The acidogens producing hydrogen, and the methanogens utilizing hydrogen are in a syntrophic relationship where the latter can sustain a low partial pressure of hydrogen that shift the equilibrium of the fermentation reactions to convert more products. Otherwise, the fermentation of propionate and butyrate will be inhibited and lead to the accumulation of VFAs and possibly pH reduction. [3]. Hydrolysis is regarded as the rate-limiting step, while the microbes performing methanogenesis are the most sensitive [4]. The stability and operation of the process are not affected by hydrolysis, but the total conversion of solids is. On the other hand, if the methanogenesis is not working properly, VFA accumulation occurs which thus is a sign of process instability [3].

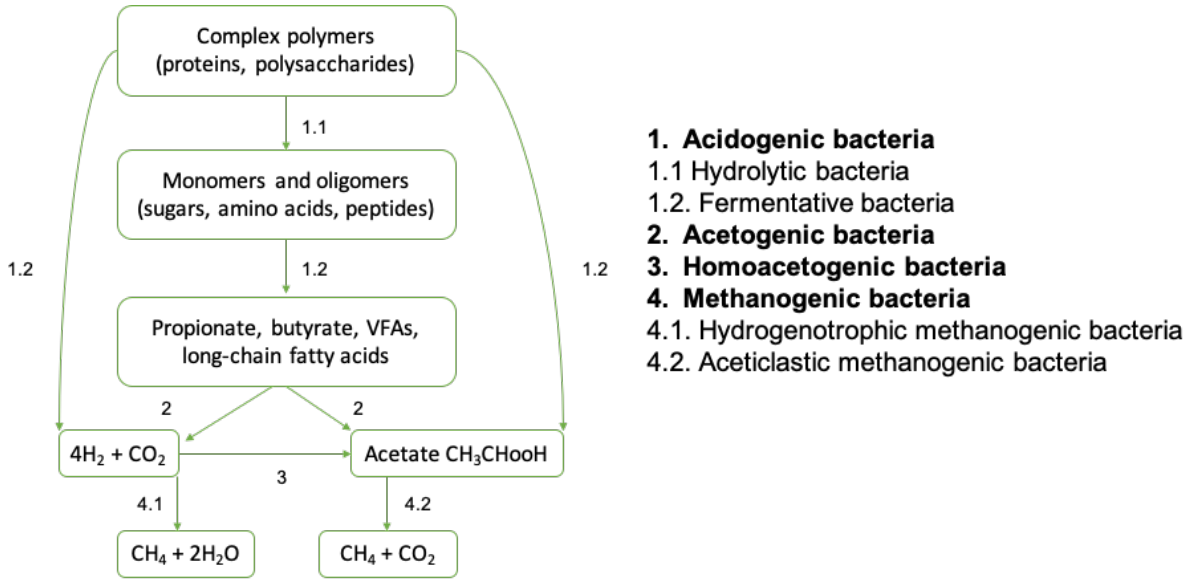


Figure 1: Microbiology of the anaerobic degradation process adapted from *[2]*

2.3. Process parameters

Knowledge and regulation of different process parameters are essential to achieve a stable digestion process. Some potential causes of process instability are organic or hydraulic overload, change of temperature, ammonia or hydrogen sulfide inhibition, unstable feed or other inhibitory compounds like heavy metals [13].

2.3.1. OLR

The mass of volatile solids (VS) fed to the digester is referred to as the OLR and is given be equation 1 below. It frequently lies between 2-3 kg VS/m^3/d for CSTRs, but can be operated successfully as high as 8 kg VS/m^3/d [2], [14]. The net digester volume refers to the volume available to substrates.

$$OLR = \frac{Substrate\ input\ \left(\frac{kg}{d}\right) \times TS(\%) \times VS(\%\ of\ TS)}{Net\ digester\ volume\ (m^3)} \quad (1)$$

Organic overload happens if the degradation capacity is exceeded by feeding the reactor more organic material than the microbes can digest to produce biogas. As a consequence, VFA accumulates because the organic material is not fully degraded to methane. This could lead to a decrease in pH and methane composition of the biogas and could end in acidification, and a complete cease of biogas production [2]. Feeding frequency has shown to have an influence on the OLR capacity of the digesters. Reactors receiving continuous feed has shown to be able to handle larger OLR than discontinuous fed reactors [15]. This has been advocated to be because of the acidification of the digester. The resulting increasing carbon dioxide concentration of the biogas contributes to the acidification. In one study reactors with both continuous and discontinuous feeding ended up at the same pH, but during a period of 24 hours, the pH dropped the 8 first hours after feeding [16]. It has been shown that the microbial communities can handle larger OLR at higher feeding frequency by avoiding shock loadings at low frequencies [15].

2.3.2. HRT

The HRT is the theoretical time the substrates spend in the digesters. It is commonly a mean value with deviations. Shortcuts in a continuous stirred tank reactor (CSTR) will cause differences from this value. HRT is a compromise between digester volume and sufficient substrate degradation where sufficient retention time is needed for all the biochemical reactions. For CSTRs and other systems without recirculation or retention of solids, the HRT is equal to the SRT and is given by equation 2 below [2].

$$HRT(d) = \frac{Net\ digester\ volume\ (m^3)}{Substrate\ input\ \ (m^3/d)} \tag{2}$$

Hydraulic overload can happen if the HRT is too low for the microbes to multiply, resulting in their gradual wash out. Since the growth rate of acidifying microbes generally is higher than that of the methanogens, this will also ultimately lead to VFA accumulation and a decrease in biogas production as this is proportional to microbe concentration [2].

2.3.3. Reactor configurations

AD reactors may be dry or wet, batch or continuous, one-step or multi-step and one-phase or multi-phase. Dry and wet digesters could be defined as 20-40% and >20 % TS of feedstock, respectively. One-stage digestion means that all the microbiological stages take place in one tank, while in a two-stage digestion process the particular stages can be separated. This could mean that hydrolysis and acidification can occur in the first phase and acetogenesis and methanogenesis in the second. This could result in enhancement of the process and hence lead to higher biogas yields but is usually more complex to operate. The difference between batch and continuous digesters is that the former is loaded once and not emptied until complete digestion is done, while in the latter feedstock is continuously or regularly fed and digestate withdrew. Different types of continuous systems are CSTR, plug flow, upflow anaerobic sludge blanket (UASB) and anaerobic filters. There are also so-called high rate digesters where the HRT and SRT are separated by increasing the latter by biomass attachment to carriers. Some examples are anaerobic packed- and fluidized bed reactors, UASB and up-flow anaerobic filters [17]. CSTR is one of the most common reactor types used for AD. It is a continuous wet process, typically being fed substrates between 2-12 % TS. Usually, HRT and SRT are equal in these reactors and process can be operated as a one-step or two-step system [2]. Mixing is an essential factor of this process as it is crucial to circulate microbes, substrates and heat in addition to free gas bubbles to prevent the formation of layers. Mixing is usually done in intervals. The main types of mixing are mechanic, pneumatic and hydraulic. Mechanic mixing us usually paddles or propellers that rotate. Pneumatic mixing is achieved by injecting biogas under pressure at the bottom of the reactor. Vertical agitation is accomplished by rising gas bubbles. By creating a strong hydraulic current, hydraulic mixing is achieved. Thehydraulic current is made by withdrawing and returning digestate with pressure [2], [3].

2.3.4. Temperature

Temperature is an essential factor during AD. It affects the solubility of several compounds that could possibly be beneficial or toxic like ammonia and hydrogen sulfide, the metabolic activity of microbes, gas transfer rates and settling characteristics. In general, the reaction rate increases with temperature. Consequently, the digestion rate is highly affected by temperature, especially hydrolysis and methane formation. Anaerobic microbial enzymes have an optimum activity within mainly three separate temperature modes, and thus AD reactors are usually designed to work at either psychrophilic (<25 °C), mesophilic (30-38 °C) or thermophilic temperature (50-57°C). The microbes usually function optimally at a specific temperature and especially the methanogens are sensitive to temperature variations. Thermophilic microbial communities are generally more sensitive than mesophilic [3], [1]. It has been recommended that the daily variation of temperature is kept less than 1 or 2-3 °C for thermophilic and mesophilic processes, respectively [18]. Temperature also effects the sanitation effect with increasing pathogen inactivation with increased temperature [2].

2.3.5. Ammonia

Wastes having high a content of nitrogen compounds, such as ammonium, proteins and amino acids, might experience problems with ammonia toxicity as these are degraded to ammonia that forms an equilibrium with ammonium. The total ammonia nitrogen (TAN) consists of both ammonium nitrogen and free ammonia nitrogen (FAN). Both are toxic to methanogenic bacteria, but FAN is considered the more toxic form because of its ability to penetrate the cell membrane [19]. In water, FAN acts as a weak base that dissociates to form ammonium. The equilibrium is dependent on pH and temperature [3], [20]. FAN can be a strong inhibitor above threshold levels leading to a decrease in both methane and biogas yield and results in process instability. Simultaneously, VFA accumulation may occur, which may contribute to a decrease in pH and consequently, a decrease in free ammonia contributing to the self-regulation of the system. There have been indications that methanogens can acclimate gradually to higher ammonia concentrations due to a shift in the methanogenic population making it possible for reactors with high concentrations of ammonia to operate successfully [12], [20], [21]. The extent of potential ammonia inhibition is affected by concentrations of nitrogen in the substrate, OLR, temperature, acclimation of inoculum and pH. All these factors make threshold levels of inhibition very variable for different reactors. Inhibition have been reported to start at concentrations as low as 1.5 g/L TAN up to 14 g/L TAN [19], [13], and FAN from 0.215 to 1.45 g/l [20]. High concentrations of TAN can also result in a high buffer capacity, making stable operation possible even though the VFA concentrations are high. The remaining VFA in the digestate might still be an issue [13]. Some of the measures that can be carried out if inhibition from ammonia occurs are modifying the C/N-ratio of the substrate, pre-treatment by ammonia stripping, adjusting the pH of the process, dilution of reactor content or substrates, the addition of activated carbon, glauconite or zeolite [20].

2.3.6. Hydrogen sulfide

In wastes containing high concentrations of oxidized sulfur like sulfite, thiosulfate or sulfate, hydrogen sulfide formation by the sulfate-reducing bacteria can be an issue. At high concentrations, hydrogen sulfide is toxic and a competitor to the methanogenic bacteria in addition to being malodorous and metal corrosive. It is very soluble in water and is more

toxic in the un-ionized form (H_2S) than ionized (HS^-, S^{2-}) and is therefore dependent on pH. Iron is usually added to mitigate this problem by forming iron sulfide precipitate. [3]

2.3.7. Heavy metals and trace elements

Heavy metals and trace elements are essential at low concentrations but become toxic at high concentrations and could also be problematic for end product use. Heavy metals precipitates with carbonate and sulfide, making them less bioavailable. Besides, analyses of digestate quality, including heavy metals, are usually done at treatment plants. Therefore, inhibition of heavy metals is seldom an issue but could be a problem for some biowastes [13]. A lack of trace elements usually occurs during mono-digestion of industrial wastes like stillage. These wastes might not contain enough nutrients like nitrogen, phosphorous and sulfur as a sole substrate. Suggested nutrients concentration for best methanogenic activity is 50, 10 and 5 mg/L of nitrogen, phosphorous and sulfur respectively. Also, some trace metals are especially important like zinc, nickel, cobalt and iron [3].

2.3.8. Kinetics

The reaction rate and type of different reactors can be determined by applying rate expressions and determine reaction rate coefficients. Examples of rate expressions are

$$r = \frac{dC}{dt} = \pm k \qquad (zero - order) \quad (3)$$

$$r = \frac{dC}{dt} = \pm kC \qquad (first - order) \quad (4)$$

Where C is the concentration of remaining organic material and k is the reaction rate coefficient. By integrating equations 3 and 4, they become:

$$C - C_0 = -kt \quad (5)$$

$$ln\frac{C}{C_0} = kt \quad (6)$$

Where C_0 is the concentration of organic material at time 0. By substituting remaining organic material with remaining methane potential, the equations become.

$$(B_{max} - B_t) - B_{max} = -kt \quad (7)$$

$$ln\frac{B_{max} - B_t}{B_{max}} = kt \quad (8)$$

By plotting (B_{max}-B_t) and ln((B_{max}-B_t)/B_{max}) versus t, k can be determined as the negative and positive slope, respectively. In general, bacterial conversion processes like AD are considered first-order reactions that are proportional to the substrate concentration. Zero-order reactions are independent of substrate concentration [3], [22].

2.4. Monitoring parameters

Process monitoring is vital to understand what is happening inside the reactor so that a stable process can be achieved. It is crucial during all phases like start-up, during alterations and steady state of the process to give an overall picture of the process and recognize instabilities that might be dealt with to avoid a crash of the reactor. Some common and important monitoring parameters for monitoring stability and performance are: Gas production and composition, total and individual VFA, alkalinity, TS, VS, pH, TAN and FAN and temperature. VFA is the most frequently used monitoring parameter and can be measured individually, as total VFA or ratio. Individual VFA gives better process information e.g. a ratio of acetic to propionic acid less than 1 and accumulation of long-chained VFAs, particularly branched isomers, are indicators of process issues. The ratio of VFA to alkalinity (VFA/ALK), is also a quick, easy parameter to determine stability. The values vary significantly between different reactors, and stability limits should be determined individually but are generally considered stable at a ratio <0.3. There have been reported stable process up to 0.8 VFA/ALK ratios. Usually, stable digesting processes have an operating pH between 7-8. The pH affects the equilibrium of weak bases and acids in the digesters like FAN, H_2S and VFAs [13]. High alkalinity is needed because of the high carbon dioxide concentration in the biogas. This is brought by digesting proteins and amino acids to make ammonia that is converted to NH_4HCO_3 by combining with H_2O and CO_2. The alkalinity required is typically from 2000-5000 mg/L $CaCO_3$ [3].

2.5. Substrates

Many different types of biomasses are suitable as substrates for AD to produce biogas. Common for most of them is that they can readily be decomposed during AD and often have high concentrations of sugar, fats, proteins or starch. The main categories wastes used as feedstock are organic private or municipal, agricultural or industrial [2]. Agricultural feedstocks include plant crop residues, animal manure and slurries and energy crops. By-products from biofuel and biorefineries industries, and animals are examples of industrial feedstocks. Municipal wastes e.g. MSS and separated organic wastes are common substrates for AD. Aquatic biomass has also gained attention as a feedstock for AD over the last decade [23], [2].

2.5.1. Substrate characterization

The substrates used in AD can be characterized by different parameters to implicate their suitability. Some of these parameters are content of readily digestible organic material, total solids, particle size, methane potential, C/N ratio, pH and content of macro- and micronutrients [2]. Readily digestible materials are low molecular weight compounds like VFAs, carbohydrates, amino acids and alcohols that usually only takes hours to digest. On the other hand, lipids, proteins and hemicelluloses might needs days and cellulose multiple weeks. The digesters can usually handle a wide pH-range in the of substrate because of high buffer capacity in the reactor. But extreme pH-values can result in deviation of the desirable pH range of the digester (pH 7-8). Substrates with high TS values might need to be diluted

with water or other substrates to prevent problems with mixing and other equipment. However, if the TS content is very low, a large digester volume is needed, and nutrients will be diluted. The organic fraction of the TS is often represented by VS, which is important for determination of OLR in the reactor. Volatile solids like VFAs might be a source of error during TS- and VS-determination because these will volatilize and not be included. Hence, COD could be a more suitable method for determining organic matter content. The soluble fraction of the total COD (tCOD) (sCOD) represents the readily biodegradable components like VFAs [2], [13], [3]. The C/N-ratio is the content of total organic carbon (TOC) relative to total nitrogen, usually analyzed as total Kjeldahl nitrogen (TKN). The optimal C/N ratio for AD is around 20-30, but is has been shown that the digestors can be run under a wider range of C/N ratios [24]. In general, too high ratio (>30) could lead to nutrient deficiency and acidification, while a too low ratio (>6) could lead to ammonia inhibition and low concentration of carbon. Both will affect the process negatively [25], [5], [24]. Estimation of the methane potential and degradation rate is usually done by a biochemical methane potential (BMP) test. A continuous fermentation test can give more information about the long-term biogas production of the substrate [13].

2.5.2. MSS

One of the major applications of AD is the stabilization of MSS. It is a by-product of wastewater treatment and commonly a result of different settling processes at a WWTP. Primary sludge is a result of primary settling, which is the first unit operation in the treatment process to generate sludge. Primary settling is sometimes enhanced by adding coagulant and flocculant chemicals [3], [4]. Secondary sludge is a product of secondary sedimentation of waste activated sludge (WAS) consisting of waste biomass from a biological treatment step. The methane potential is commonly more considerable for primary sludge than WAS. The use of MSS as a substrate can sometimes be challenging because of its content of chemical and biological pollutants due to its origin. As a result, standards and national legislations regulate its use as feedstock as well as applications as fertilizer. The regulations contain sanitation requirements for pathogen inactivation in addition to other biological vectors, limit values for the content of persistent organic pollutants and heavy metals [2].

2.5.3. Stillage

During ethanol fermentation from concentrated carbohydrate substrates, stillage is produced, which is a liquid by-product of the process. Estimations show that for every litre of ethanol, 20 L of stillage is generated [26]. Stillage retrieved directly from refineries are usually hot and can be beneficial for energy costs, especially in thermophilic digesters [27]. There have been done several studies on AD of stillage. The concentration of nitrogen and sulfur in stillage can pose problems for the process stability [2], [9]. One study introducing stillage to TAcoD of poultry litter showed an immediate increase in VFA at 20 % stillage, concluding that adaption time was needed. This was attributed to a shift in the microbial community because of change in VFA being fed. They also found that biogas production increased with a larger fraction of stillage up to 80 % when inhibition occurred [27]. A study conducting TAD of mix of primary sludge and WAS and sugar beet pulp stillage in the ratio of 60:40 operated at an OLR of 2.14-3.17 kg VS/m^3/d resulted in a specific methane yield of 357 dm^3/kg VS with a methane composition of nearly 70 %. They did not experience problems

with ammonia or VFA. The stillage was also used in a mono-digestion where acidification and methane production inhibition occurred concluding that is was unfit as sole substrate.

There have been done some studies on AD of potato-stillage that emphasizes the challenge of the low C/N (8-9) ratio of the substrate because of high protein content causing ammonia inhibition. A two-step digestion process has been recommended for the MAD of potato stillage as the only substrate because it enables better process control and the optimization of process conditions for different microbial cultures. At 10 days retention time, 58 % COD removal was accomplished [9]. Another study investigated the mesophilic co-digestion of pig-manure with potato-stillage from glue production of starch [28]. They experienced a gradual accumulation of ammonium-nitrogen in the digesters without any reduction in methane yield. Pig-manure was thought to be the main source of ammonia-nitrogen and that this gave the necessary buffering capacity of the potato waste with low ammonia nitrogen content. However, the stillage from glue production is different than from ethanol-production because of different processes. The substrates were successfully co-digested with a potato waste content of 15-20 % of the feed with a loading rate of 2 kg m^3/d. The thermophilic digestion of sweet potato stillage in a fixed-bed reactor with the addition of trace elements (Ni^{2+} and Co^{2+}) has achieved a successful and stable operation. This substrate might be comparable to regular potato stillage [29]. There have been more studies done on potato wastes and starch, but these are not directly comparable since most of the carbohydrates are removed during the fermentation process to make ethanol.

2.6. AcoD

Combining different substrates for AcoD has some advantages over mono-digestion that include better nutrients availability, substrate variability, product yield, bulk density, lower feed volume, toxicity dilution, synergism and a varied and robust microbiome. AD of IFW alone can suffer from limited macro- and micronutrients, VFA accumulation, inappropriate TS or moisture content and possibly toxic inhibitors. Typically, MSS has a low C/N ratio and high metal content. This can be a positive contributor when co-digesting with organic fractions with high C/N ratios and lack of micro- and macronutrients like metals [30]
MSS generally work at low OLR. AcoD of IFW with MSS can be an opportunity for already existing WWTP to increase their energy production without the need to add facility [1], [24].

For stable and productive AcoD a suitable C/N ratio, stable nutrient supply and sufficient buffering capacity of the digester is essential [1]. MSS generally has a low C/N ratio and low organic load. Substrates like FW with high concentrations of carbohydrates can balance the C/N ratio of the digester, reducing the risk of ammonia inhibition, especially the availability of higher carbon with increasing FW percentage [25], [24]. The mixing of the two substrates can consequently improve the C/N ratio [25], [5], [24]. Co-digestion of WAS with food wastewater (FWW) showed that increasing the fraction of FWW gave increasing methane production and content up to a certain threshold of 75 % volume. Increase in the C/N ratio was thought to be one of the main reasons for the rise [31].

The AcoD of substrates like FW with high hydrolytic potential compared to MSS can result in faster growth of anaerobic microorganisms and improve degradation efficiency and speed up the hydrolysis of MSS which has been described as the rate-limiting step. Hence, higher acidification and methanogenesis potentials are achieved and thereby improving the overall

performance of the digester. Certain carbohydrates and proteins especially have fast conversion rates [24]. The specific OLR needs to be determined for each substrate combination and operating conditions because there is no agreed OLR specified for optimum results [24]. There is also no general agreement on which mixing ratios of MSS and FW is optimal [1]. The co-digestion of various substrates and their synergistic effects are summarized in Table *1*.

Table 1: Co-digestion of various substrates and synergism effects

Substrates	Ratio	Digester	SMP (m^3 CH_4/kg)	Synergism	Ref.
WAS:FW	50:50 (%VS)	Semi-CSTR 35 °C	0.334 (VS)	Balanced macro- and micro-nutrients, improved C/N-ratio and abundant carbohydrates in FW increased methane yield and production rate and VS-removal (53.7%) with increasing FW fraction.	[30]
PS:WAS: IFW	6:14 :80 (%VS)	Semi-CSTR 35 °C	0.402 (VS)	High buffering capacity to high VFA levels but ammonia inhibition of the acetate degrading methanogens.	[7]
PS+WAS: SBPS	60:40	CSTR 35 °C	0.357 (VS)	Prevented acidification of mono-digestion of SBPS by increased buffering capacity and complementing trace elements from MSS	[32]
WAS: FWW	25:75 (v/v)	Semi-CSTR 55°C	0.316 (COD removed)	Improved VS removal (from 43.3 to 77 %), MPR (7.3 times), doubled SMP and methane content (from 54.19 to 68.24 %) compared to WAS alone [a]. Prevented acidification by digestion of easily degradable compound. Increased and balanced archaeal community with increasing co-substrate	[33]
Poultry litter: thin stillage	40:60	CSTR 55.5 °C	0.54 (COD)	Enhanced SMP, COD-reduction, methane content, VFA removal More beneficial C/N-ratio, balanced nutrient content	[27]
PS+WAS: micro-algae	63:37	Batch 37 °C	0.408 (VS)	Addition of micronutrients from microalgae. 23 % higher BMP than MSS alone	[34]
PS+PoSt	75:25	CSTR 37 °C 55 °C	0.444 (VS) 0.43 (VS)	Enhanced SMP (5.7 and 4.8 % improvement at 37 °C and 55 °C respectively), MPR, VS-reduction (4.1 and 5.1 % improvement) compared to mono-digestion. Improvement of C/N-ratio and potentially microbial growth due to the availability of readily degradable substrates.	[b]

[a]Different OLR: 2.83 to 6.88 kg COD/m^3/d

[b]Current study

PS: Primary sludge, FW: Food waste, IFW: Industrial food waste, SBPS: Sugar beet pulp stillage, FWW: Food wastewater, MPR: Methane production rate, SMP: Specific methane production

2.7. MAD and TAD

AD is commonly performed either mesophilic or thermophilic according to the temperature of the digester. The temperatures are according to the optimal temperatures for the microorganisms performing the digestion process. MAD temperature range is thought to be between 30-43 °C with an optimal temperature of 35 °C, while TAD is in the range of 48-60 °C with 55 °C as the optimal temperature [35]. TAD as some advantages over MAD like better efficiency and thereby lower solid retention time (SRT) is needed as a result of the temperature speeding up the biochemical reactions, leading to a higher growth rate of thermophilic bacteria and archaea compared to the mesophilic ones. This can also be problematic due to the simultaneous increased production and accumulation of potential inhibitors, such as ammonia. The higher bacterial growth rate will possibly lead to the need of smaller digester size to treat the same amount of sludge which can lead to an increased capacity of existing reactors and financial savings. In addition, TAD has shown to possibly accomplish higher biogas yields, methane content and pathogen destruction, which is especially important for land application [4]. Land application of biosolids is considered to be a sustainable option for managing biosolids because it permits the reuse of nutrients and organic carbon [36]. This application requires that the biosolids meet the regulatory requirements of quality, including low levels of pathogens and heavy metals that could cause harm to humans, animals, plants and the environment. As a result, WWTP must apply methods for stabilizing sludge that prevents the risk of releasing potentially harmful microorganisms. This makes transition the from MAD to TAD an attractive option because it eliminates the need for subsequent sanitation process [3].

Some of the downsides of TAD are higher energy demand, higher sensitivity to temperature fluctuations, possibly worse effluent quality with higher concentrations of VFAs, odor and generally poorer stability [4]. Some microbes, e.g. the thermophilic methanogens are more sensitive to temperature variations than most bacteria [15]. Consequently, TAD requires better temperature control than MAD. It has also been argued that one of the main reasons for instability in the TAD reactors are ammonia accumulation and inhibition due to of the higher amount of free ammonia when temperature is increased.

Several studies have compared TAcoD and MAcoD. Some of the results are increased degradation efficiency and specific biogas yield for thermophilic condition [37], [38], [31], [39], [40]. The thermophilic process has shown to improve the hydrolysis of MSS and FW but also to decrease the conversion efficiency of organic compounds to methane [37], [6]. In full-scale TAD it has been demonstrated greater methane production rate and stability compared to MAD [41], [33]. The bacterial diversity has shown to be lower for TAcoD than MAcoD of MSS and FWW, and also that the diversity decreased with increasing FWW fraction. Additionally, higher density in bacterial and archaeal populations were found under thermophilic conditions compared to mesophilic [31]. Another study showed a stable ratio between bacterial and archaeal cells under TAcoD, while MAcoD showed a more varied ratio suggesting that the thermophilic process is more stable, and moreover, showing that the relative abundance ratio can vary without significantly affecting the biogas production [39].

Increased FAN concentrations have been detected during TAcoD compared to MAcoD under the equal C/N ratio in the substrate [37]. Thermophilic conditions can experience higher FAN and hence ammonia inhibition than during mesophilic conditions with the same substrate [42]. There are few studies specific of the AcoD of primary sludge and mixing primary sludge and WAS is most common approach. Results from a batch tests of chemically enhanced primary treated sludge co-digested with food waste showed high hydrolysis rate but retarded acidogenesis, which led to VFA accumulation for all mixing ratios at thermophilic conditions. As a result, TAD also gave less methane recovery than MAD. The conclusion was that buffer addition would be necessary for this mix to be suitable for TAD [6].

2.8. Start-up and transition from MAD to TAD

The start-up of the thermophilic digester is important to avoid delayed acclimation and ineffective organic matter removal. The success of the transition is dependent on the source and characteristics of inoculum, the OLR during this stage, digester volume and configuration, start-up strategy and the activity and composition of the methanogenic community in the inoculum [35].

Using suitable inoculum is an important element for achieving successful start-up of thermophilic digesters. The occasional unstableness of TAD could be a consequence of the method/procedure in which the thermophilic inoculum has been obtained [35]. Some possible inoculum sources are sludge from TAD or MAD, WAS, municipal solid waste and cow manure. Thermophilic digested sludge is not always readily available in large quantities because of the limited number of thermophilic anaerobic digesters [35], [43]. If available, it can successfully be used as an inoculum for TAD [25]. WAS has proved to be an appropriate inoculum source if easily biodegradable substrates are used in the start-up phase [35]. Mesophilic inoculum is most frequently used as a start-up for TAD.

Since attaining thermophilic inoculum could be difficult, it is usually acquired by increasing the temperature of the MAD reactor. Mesophilic digested sludge can serve as an inoculum because microorganisms growing under thermophilic conditions are present at low concentrations. The sudden increase in temperature selects these populations. Because of the low concentration, the start-up strategy for growing these populations is crucial for success [35], [44]. Different strategies exist for obtaining thermophilic sludge from mesophilic digested sludge: one-step direct increase or gradual increase of temperature. There exist successful cases of the one-step strategy [38], [45] and the gradual increase strategy [31]. The one-step strategy has also been combined with the addition of buffer (sodium bicarbonate) to counteract the rise in VFA at temperature change [44]. The comparison of both approaches concluded that the one-step strategy needed shorter stabilization time [46]. A literature review on start-up strategies concluded that the most favorable option when using mesophilic sludge as an inoculum was an one-step increase in temperature [35]. The argument for this conclusion was that this method selects for the true thermophiles instead of the thermotolerant microbes that would be favored with a slow increase. This is a result of the temperature between 43-50 °C being especially troublesome for an AD process and can lead to an unstable reactor. Following the one-step strategy, the temperature should be increased as fast as possible and the OLR reduced or stopped entirely until a concentration of 500 mg/l VFA has been reached. The OLR could then slowly and gradually be increased while being careful not to overload the system which could lead to

inhibition [35]. One-step increase lets the true thermophiles grow at their optimum temperature from the start-up [44]. The strategy with a gradual increase in temperature with stabilization periods allows the OLR to be maintained but will need a longer time to reach the target temperature and stability [35]. A rapid one step-strategy is limited for a full-scale reactor because of limited heating capacity [47]. After a temperature change, the bacteria and archaea need time to convert cell components like proteins, enzymes, nucleic acids, lipids and other to thermophilic states [48]. This will result in loss of methanogenic activity followed by a recovery because of increasing thermophilic methanogens [49].

2.9. VEAS

The process leading up to the production of primary MSS begins with grit removal of coarse solids and litter, and the removal of fine solids and fats in a ventilated sand trap. The products of these two first steps are disposed of and not collected as sludge. Next, the wastewater is added coagulant and flocculant chemicals on the form of trivalent iron and aluminum, and synthetic polymer that enhances particle aggregation and sedimentation in the primary sedimentation step. The resulting primary sludge of this step is thickened to around 7 % TS and distributed to four 6000 m^3 cylindrical CSTR digesters that are operated at 37 °C with an OLR and HRT of approximately 2.9 kg VS/m^3/d and 20 d respectively. The digesters are operated semi-continuously where effluent is withdrawn, and the substrate is added every 135 min (10 1/d) for 45 min. Mixing is done pneumatically by injecting biogas under pressure at the bottom of the reactor. The influent sludge is heated by mixing it with digester content before entering the digesters. In addition, reactor content is regularly circulated in a heat exchange circuit for heating. The digestate effluent is added lime and pumped to chamber filter presses where it is heated, dried and sanitized. The ammonia rich filtered digestate water is stripped for ammonia in packed stripping towers and is used to produce fertilizer. The dried sludge is used at biofertilizer for land application.

VEAS tried TAD for a year but transitioned back to MAD because of several problems. Some of the issues during the period of TAD included increased odor from the biosolids and process affecting the environment inside and surrounding the treatment plant. There was also received complaints of malodor from the biosolids after land application. Besides, organic material followed the ammonia during the stripping process, worsening the quality of the fertilizer.

2.10. HOFF

Potato liquor is produced from potatoes that are grated, cooked, cooled, added enzyme and yeast assimilable nitrogen (YAN) before it is fermented. After fermentation, the ethanol is distilled. Stillage is the remaining protein-rich liquid containing the leftovers that have not been converted to ethanol. The stillage is kept in storage tanks keeping a high temperature of typically 95 °C [8].

2.11. Identification of knowledge gaps

There are not many studies done on the transition from MAD to TAD and AcoD with specifically primary sludge as a substrate. Commonly a mix of primary and WAS is applied. The studies on AD of potato-stillage are scarce and none on co-digestion with MSS could be discovered. More studies are needed for this particular waste for co-digestion and under thermophilic conditions.

2.12. Objective

The main objective of this study was to investigate the feasibility of VEAS to transition from MAD to TAD and examine the effects on the process stability of co-digestion with potato-stillage. The study was be carried out in lab-scale reactors. A one-step increase in temperature was studied as a transition strategy with mesophilic digested sludge from VEAS as inoculum. The thermophilic digesters were compared to the mesophilic in terms of specific biogas- and methane production, digestion efficiency, digestate quality and stability. The effects of co-digestion with potato-stillage was studied by the same criterions under both mesophilic and thermophilic conditions. Specifically, the potential synergistic effects and how they could improve the process efficiency were considered. The results from this study will be used in a different study at the University of South-Eastern Norway (USN) modifying the ADM1 model to suit the AD process at VEAS. The findings of both studies will lay the foundation to VEAS' the decision of potentially transitioning to TAD and receiving potato-stillage from HOFF for co-digestion.

Research question: Is it feasible is it to convert from mesophilic to thermophilic anaerobic digestion at VEAS, in terms of stability, process efficiency and energy yields, and does the addition of potato-tillage in the substrate mix improve the process?

3. Materials and Methods

3.1. Set-up

Mono- and co-digestion were studied under mesophilic and thermophilic conditions at 37 and 55 °C respectively. The study was conducted in six lab-scale CSTRs consisting of two parallel reactors for each of the thermophilic processes, and one reactor for each of the mesophilic processes that were used as reference when studying the effects of TAD and TAcoD. The reactors performing mono-digestion were fed MSS only, while the reactors performing co-digestion were fed MSS and potato-stillage. The end operating conditions are summarized in Table *5*. The reactors were fed once daily by removing an equal amount of digestate as the amount being added as substrate. The OLR and HRT varied during start-up and transition to TAD as a result of smaller amount of substrate being added. All dilutions were done using tap water. Stirring was kept at 80 rpm throughout the experiment, except during sampling or withdrawal of digestate when stirring was adjusted to 100 rpm in case of any sedimentation to ensure representative sampling. All samples were taken approximately 24 hours after the last feed. The goal was to keep the experimental conditions as similar to the full-scale process as possible in terms of HRT, OLR, substrate and transition strategy.

3.2. Inoculum, substrates and feeding

Inoculum and MSS was collected at the WWTP VEAS in Slemmestad, Norway. The inoculum was obtained from a MAD reactor treating the very primary sludge that was collected as substrate. The MSS substrate is a product of precipitation and thickening with trivalent iron and aluminum and synthetic polymer. Potato-stillage was obtained from a storage tank at HOFF potato refinery in Gjøvik, Norway. Substrates and inoculum were collected and stored in 10 L plastic containers. The inoculum was seeded within one hour of sampling, while the substrates were stored at 4 °C in darkness. During the experiment, MSS was collected twice (day 1 and 50) and potato-stillage once. The characteristics of the inoculum and substrates were analyzed and are summarized in Table *2*Table *3* respectively. Due to the differences in VS-concentration of the two batches of MSS and potato-stillage, the HRT was one day shorter than the target HRT of 20 days when co-digesting with the first batch of MSS. The main priority was to keep the OLR equal in all reactors. The second batch of MSS was more VS-concentrated and as a result was diluted 9 % when fed to the mono-digesters to maintain the HRT of 20 days. Daily fed substrate was withdrawn directly from the containers after thorough mixing and measured by weighing (d=0.1 g) into beakers. The reactors were fed directly from the beakers and rinsed with the corresponding reactor content to ensure no substrate residue.

Table 2: Characterization of inoculum

		Inoculum
TS	**(%)**	3.94 ± 0.02
VS	**(%)**	58.3 ± 0.2
pH		7.72 ± 0.01
VFA[a]	**(g HAc/l)**	0.98 ± 0.03
ALK	**(g $CaCO_3$/l)**	4.0 ± 0.1
VFA/ALK		0.247 ± 0.002

[a]Measured by titration

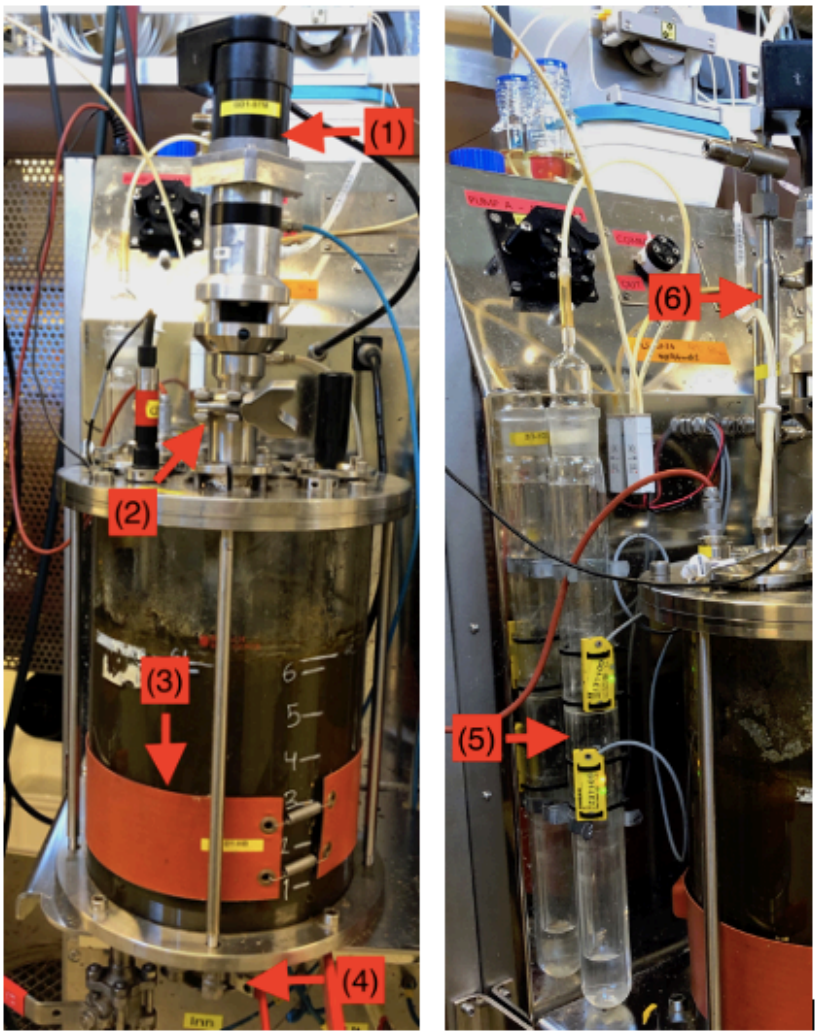
Table 3: Characterization of substrates.

		MSS		PoSt
	Unit	Batch 1	Batch 2	
TS	**(%)**	7.29 ± 0.04	7.88 ± 0.04	5.02 ± 0.03
VS	**(% of TS)**	80.1 ± 0.2	80.5 ± 0.2	86.8 ± 0.3
VS	**(g/L)**	58.4 ± 0.2	63.5 ± 0.2	43.5 ± 0.1
pH		6.34 ± 0.01	6.16 ± 0.01	4.13 ± 0.01
tCOD	**(g/L)**	91 ± 3	109 ± 3	71 ± 2
sCOD	**(g/L)**	9 ± 2	9 ± 2	28 ± 6
kg COD/kg VS		1.5 ± 0.04	1.7 ± 0.05	1.6 ± 0.05
TKN	**(% of TS)**	4.8 ± 0.7	4.5 ± 0.7	3.4 ± 0.5
TAN	**(g/L)**	0.94 ± 0.02	0.72 ± 0.01	0.94 ± 0.02
Tot-C	**(% of TS)**	36 ± 8	36 ± 8	35 ± 8
C/N		7 ± 2	8 ± 2	10 ± 3
Lactic acid	**(g/L)**	0.02 ± 0.01	0.004 ± 0.003	14 ± 10
Formic acid	**(g/L)**	0.015 ± 0.003	0.005 ± 0.001	0.6 ± 0.1
Acetic acid	**(g/L)**	3.22 ± 0.7	3.46 ± 0.7	0.85 ± 0.2
Propionic acid	**(g/L)**	1.18 ± 0.1	1.41 ± 0.1	4.13 ± 0.4
iso-butyric	**(g/L)**	0.12 ± 0.02	0.12 ± 0.02	n.d.
n-butyric acid	**(g/L)**	2.26 ± 0.2	2.53 ± 0.3	n.d.
n-valeric acid	**(g/L)**	0.12 ± 0.03	0.20 ± 0.05	0.04 ± 0.01
iso-valeric acid	**(g/L)**	0.1 ± 0.1	0.06 ± 0.06	0.03 ± 0.03
Tot VFA	**(g/L)**	7.0 ± 2.9	7.8 ± 3.2	19.1 ± 7.8
Tot VFA	**(g HAc/L)**	6.0 ± 2.4	6.6 ± 2.6	14.0 ± 5.6

n.d.: Not detected

3.3. Reactors

The experiment was conducted in six 10 L Dolly© lab-scale CSTRs from "Belach Bioteknik" with dimensions 700 x 1500 x 600 mm (W x H x D) with a 6 L working volume (Figure 1). The reactors are equipped with an automatic top stirrer with a propeller type impeller controlled by an external DC motor and an external bottom heating band and cooling finger for temperature control. Manual feeding and withdrawal of digestate is achieved through the top and bottom valve respectively. The feeding valve consists of a tube going into the reactor content, permitting strictly anaerobic feeding. Produced biogas flows through a condenser on the top, to a water displacement gas counter performing volumetric measurements. Temperature, stirring, gas flow and volume is logged and controlled through the BioPhantom© software.



(1): DC stirrer motor
(2): Feeding valve
(3): Heating band
(4): Sampling valve
(5): Gas meter
(6): Biogas condenser

Figure 2: The lab-scale reactors.

3.4. Start-up

Initially, all reactors were treated equally and were seeded with 6 L inoculum, temperature set to 37 °C and stirring to 80 rpm. They were allowed to stabilize for 2 days before start of feeding (day 1) at which they were fed MSS equal to an OLR of 2.5 kg VS/m^3/d for eight days. During this start-up the reactors were checked for errors like leakages, in addition to comparing parameters like gas production, methane composition of biogas, pH, TS, VS and VFA/ALK between the reactors to assess the equality of the starting point. The start-up was limited because of limited time for the experiment. The inoculum used was from the digesters treating the same substrate that was used in the experiment. Hence, start-up time was expected to be less with little to no acclimation period needed, which is in accordance with previous findings [32].

3.5. Transition

Transition to TAD was done on day 9 by a one-step increase in temperature from 37 to 55 °C. The time needed to reach target temperature was approximately 4 hours. Feeding was stopped the next fifteen days while carefully monitoring gas production, methane composition, pH, VFA/ALK, TAN and FAN. On day 22, fourteen days after the temperature raise, the reactors were diluted 1/10 with tap water to accommodate the issue of high

concentration of FAN. The digestate volume was already down 5.5 L due to sampling, thus the reactors were filled with tap water up to 6 L. On day 24, sixteen days after the temperature raise, the reactors were fed with a small amount of substrate corresponding to an OLR of 0.5 kg VS/m^3/d. The feed was slowly increased over the next eighteen days until the same OLR of the mesophilic reactors of 2.92 kg VS/m^3/d, was achieved as illustrated in Figure *3*. From day 29-37 the substrates were diluted with tap water corresponding with an HRT of 20 d (300 ml), to continue to reduce the problem of FAN accumulation. See timeline in Table *4*. The increase in OLR was done under careful monitoring of the parameters pH, VFA/ALK, gas production and methane composition.

Table 4: Timeline

Day	Event
1	First feed
9	Increase of temp. to 55 °C
15	Introduction of potato-stillage
23	Dilution of TAD reactors
24	Feeding of TAD reactors resumed
42	TAD reactors reached equal OLR as MAD reactors
50	New batch of MSS
82	End of experiment

3.6. Introduction of potato-stillage

Potato-stillage was introduced gradually over one week before reaching target VS-ratio of 25 % of total substrate VS. A share of the VS from MSS was replaced by an equal amount of VS from potato stillage, meaning that the total OLR was not modified, compared to the reactors operated on MSS only. The potato-stillage was introduced to one of the mesophilic reactors on day 16 and to the thermophilic reactors at the same day feeding was resumed after temperature increase (day 24).

3.7. Steady state operation and target conditions

From day 42, after transition to TAD and start-up of potato-stillage addition, all reactors were operated at the conditions given in Table *5* below until day 82 when the experiment was ended. In this period all the reactors were fed once daily and kept at an HRT of 20 days by withdrawing 300 mL of digestate and adding 300 mL of substrate.

Table 5: Operation conditions of the reactors

Reactor name	Substrate	Temp. (°C)	Working volume (L)	OLR (kg VS/m^3/d)	HRT (d)	Mixing (rpm)
TAD1	MSS	55	6	2.92	20	80
TAD2	MSS	55	6	2.92	20	80
TAcoD1	MSS+PoSt	55	6	2.92	20	80

TAcoD2	MSS+PoSt	55	6	2.92	20	80
MAcoD	MSS+PoSt	37	6	2.92	20	80
MAD	MSS	37	6	2.92	20	80

PoSt: Potato-stillage

3.8. Analytical procedures

Chemical analyses were carried out at were done at the NIBIO biogas- and chemistry lab in Ås, except the TKN and TOC determinations, which was performed at the VEAS lab in Slemmestad, and an external lab through Eurofins Environment testing Norway AS, respectively. Inoculum, substrate and digestate were characterized by analyzing parameters summarized in Table *6*. Digestate samples were collected and analyzed weekly and even more frequently during critical stages (i.e. transition from MAD to TAD). The different parameters were analyzed according to the methods listed and summarized in Table *6*. Deviations and internal methods are described in more detail in section 3.8.1-3.8.4. The parameters TS, VS, pH, VFA/ALK, TKN and TAN were analyzed immediately after sampling, while tCOD, sCOD and VFA were analyzed in samples preserved at -20 °C. Samples were diluted with type 1 water to achieve concentrations within the range for tCOD, sCOD and TAN analyzing protocols. Before tCOD analysis the diluted samples were homogenized.

3.8.1. Biogas volume and composition

Biogas volume and composition were measured continuously on-line. Biogas volume was detected by a water displacement gas counter with to two sensors, one lower and one upper, attached to a water column registering the water level. The gas volume corresponding to the volume between the two sensors was calibrated using a syringe to push 1 L gas into the column, dividing the volume by the number counts and registering it in the software. The gas counters of all reactors were calibrated right before the experiment started. Before measurement, the biogas is cooled by a condenser on top of the reactor. The temperature of the biogas being measured inside the column was detected by putting a temperature sensor probe into the gas flow pathway and showed to follow temperature of the room for all reactors and would vary accordingly. The biogas volume was converted to dry volume under STP (1 bar, 0 °C) by first removing the contribution of water vapor using a modified Buck equation given by equation 9 [50], following the combined gas law to convert to STP by equation 11. Pressure during measurement was assumed to be 1 atm, and temperature was estimated by an average of 12 measurements performed as previously described.

$$P_w = 611.21 exp\left(\left(18.678 - \frac{T_c}{234.5}\right) * \frac{T_c}{257.14 + T_c}\right) \quad (9)$$

$$P_d = P_{meas} - P_w \quad (10)$$

$$V_{STP} = \frac{P_d T_{ST}}{P_{SP} T} V \quad (11)$$

Where P_w, P_d, P_{meas} and P_{SP} are vapor pressure, dry pressure, ambient pressure and standard pressure respectively given in Pa. T_c and T are ambient temperatures in °C and K respectively, T_{ST} is standard temperature in K. V_{STP} and V are volume at STP and measured volume respectively in m^3. All reported gas volumes were converted by this method.

The composition of the biogas was analyzed by an SRI GC 8610C with a TCD and a 2 m Haysep-D column. The operating conditions of the injector, column and detector were 41, 81 and 153 °C respectively and the carrier gas was helium at 20 mL/min as previously applied in another study [51]. Approximately 12 measurements were performed on each reactor each day and a daily average of these were used.

3.8.2. VFA

The methodology used for VFA-analysis were based on previously described methods with small modifications [51], [52]. The analysis was carried out by centrifuging (EBA 21, Hettich zentrifugen) samples at 15000 rpm (21924 RCF) for 10 min immediately after sampling and transferring 1 mL of the supernatant to a different tube and stored at -20 °C until analysis. The samples where thawed and 10 μL of concentrated sulphuric acid (95 %) added to lower the pH to less than 2.5. The tubes were left to breathe without lids for 2 minutes before mixing. The samples were centrifuged again at 15000 rpm (21924 RCF) for 5 min before the supernatant was transferred to HPLC-tubes. The samples were analyzed using an Aminex® HPX-87H column (300 x 7,8 mm and 9 μm particle size) in a Dionex Ultimate 3000 system with a UV-detector. The column was operated at a flow of 0.6 mL/min and a temperature of 50 °C. An isocratic eluent flow of 4 mM H_2SO_4 was applied and the sample injection volume was 20 μL. The system was calibrated using a dilution series of a reference standard. The individual concentrations of lactic, formic, acetic, propionic, n- and iso-butyric and n- and iso-valeric acid were determined according to the calibration curves.

3.8.3. TKN

Analysis of TKN was performed using a Tecator digester 2520 and Kjeltec 8400 analyzer, FOSS, with automatic distillation, titration and colorimetric detection according to NS-EN 16169:2012 with some adaptions from manufacturer. Fresh samples equal to 1 g TS, were digested in tubes in a heating block for 1 h at 420 °C with 12 mL concentrated sulfuric acid and a catalyst mixture, Kjeltabs, consisting of 0.4 g $CuSO_4$ and 3.5 g K_2SO_4. The digestion transforms all organic-, ammonia-, and ammonium N to NH_4SO_4. After adding 80 mL type 1 water and 50 ml of 32 % NaOH, the samples were distilled using steam converting all NH_4 to NH_3 and transferring it to 30 mL 1 % boric acid solution containing methyl red indicator. The solution was titrated with 0.1 M HCl and the endpoint was determined colorimetrically after 100 mL of distillate had been transferred.

3.8.4. TAN and FAN

TAN was analyzed with the indophenol method according to manufacturer's manual [53]. The TAN reacts with the reagents to form indophenol blue that can be determined photometrically. Fresh samples were centrifuged (EBA 21, Hettich zentrifugen) at 15000 rpm

(21924 RCF) for 10 min and 0.1 mL of the supernatant was transferred to Spectroquant® Ammonium Cell Tests, Merck, together with one dose of NH_4-1K reagent provided by the manufacturer. The cells were mixed and left to react for 15 minutes before measurement with the Spectroquant® Pharo 100 spectrophotometer. FAN was calculated according to equation 12 from the TAN concentration, pH and temperature of the reactors [54].

$$\frac{FAN}{TAN} = \frac{10^{pH}}{10^{pH} + e^{6344/(273+T)}} \quad (12)$$

Table 6: Analytical methods applied

Analyte	Method	Instrument	Pre-treatment
TS	NS-EN 15934:2012 [55], Method A: Drying cabinet at 105 °C	Drying cabinet TS 9000, Termaks	Fresh sample
VS	NS-EN 15935:2012 [56] Ignition in furnace at 550 °C	Thermconcept chamber furnace KS 16/14 with TC505 thermocomputer, Bentrup	Sample dried according to NS-EN 15934:2012
pH	NS-EN 15933:2012 [57]	Orion Star A211 pH meter and green liquid filled pH electrode, Thermo scientific	Fresh sample
VFA/ ALK	Nordmann [58] Two end-point acid titration	TitroLine 6000 titrator and A7780 pH electrode, SI Analytics	Fresh sample
TKN	NS-EN 16169:2012 [59] w/ adaptations. Digestion, distillation and colorimetric determination.	Kjeltec 8400 and Tecator digestor 2520 FOSS	Fresh sample
TAN	Colorimetric [53], Indophenol blue	Spectroquant® Pharo 100 spectro-photometer and Ammonium Cell Test, MERCK	Centrifugation of fresh sample
tCOD	ISO-15705 [60] Sealed tube method with Cr/H_2SO_4 oxidation and Cr^{3+} determination	Spectroquant® TR 620 thermoreactor and Pharo 100 spectro-photometer and COD cell test, MERCK	Homogenization
sCOD	ISO-15705 [60] Sealed tube method with Cr/H_2SO_4 oxidation and $Cr_2O_7^{2-}$ determination	Spectroquant® TR 620 thermoreactor and Pharo 100 spectro-photometer and COD cell test, MERCK	Centrifugation and filtration 0.45 μm mixed cellulose ester filter
VFA	Internal method, HPLC	Dionex Ultimate 3000 w/ Aminex ® HPX-87H column	Centrifugation
TOC[a]	EN 13137 (S30): 2001-12 Combustion	Unknown[a]	EN 15002: 2015-07 Freeze-drying
Gas volume and flow	Internal method, Water replacement gas-meter	On-line gas counter	Cooling
CH_4 and CO_2	Internal method, GC analysis	On-line, SRI GC 8610C, 2 m Haysep-D column	

[a] Performed by Eurofins Umwelt Ost GmbH (Freiberg), Lindenstraße 11, Gewerbegebiet Freiberg Ost, D-09627, Bobritzsch-Hilbersdorf DIN EN ISO/IEC 17025:2005 D-PL-14081-01-00

3.9. Uncertainty

The expanded uncertainty (U) of the analytical methods were estimated by calculating the standard combined uncertainty (u_c) and multiplying with a coverage factor (k). Calculation of u_c was done by combining the standard systematic (u(bias)) and standard random errors (u(R)) according to equation 13 [61]:

$$U = ku_c = k\sqrt{u(bias)^2 + u(R)^2} \quad (13)$$

The systematic errors express the inaccuracy of the method and is a measure of how far the measured value is to the "true" value. The random errors express the imprecision of the method and is a measure of how far the measured values of a homogeneous sample are from each other. Determination of u(R) of the analytical methods was done by collecting one sample of digestate or substrate and dividing it into several sub-samples that were pre-treated and analyzed according to the different methods and determined according to equation 14.

$$u(R) = \frac{S}{\sqrt{n}} \quad (14)$$

Determination of u(bias) was done by analyzing a prepared control sample with a known "true" value and calculate the deviation according to equation 15. This was carried out if a control sample was available

$$Diff = Result - True\ value \quad (15)$$

The samples were assumed to be from a normal distribution, and the uncertainties were calculated applying a Student-t distribution with a confidence interval of 95 %. The k thus become $t_{\alpha/2,\ n-1}$, where α equals a 100x (1-α)% confidence interval, i.e. 0.05, and n-1 is the degrees of freedom where n is the number of results. Equation 13 hence becomes [61], [62]:

$$U = t_{0.025,n-1}\sqrt{Diff^2 + \left(\frac{S}{\sqrt{n}}\right)^2} \quad (16)$$

4. Results

All gas volumes are reported at STP (1 bar, 0 °C) and uncertainties of the different analytical methods and averages of data sets are calculated according to equation 16. The uncertainties of plotted results in figures are given in the figure captions as *U*. When calculating specific biogas and methane production, VS- and tCOD-reduction and acetate/propionate ratio, an average from the period day 62-81 or 58-82 was calculated since it was a little more than one HRT (20 d) after target OLR was reached. To determine if the means of different data sets were significantly different, a t-test was applied with a significance level of p=0.05

4.1. Transition to thermophilic conditions

Transitioning from mesophilic to thermophilic conditions was achieved by a one-step increase in temperature on day 9, followed by 15 days with no feeding as illustrated in Figure 3. This was followed by a careful increase in OLR until target OLR of 2.92 kg VS/m^3/d was reached on day 42, 33 days after the temperature raise.

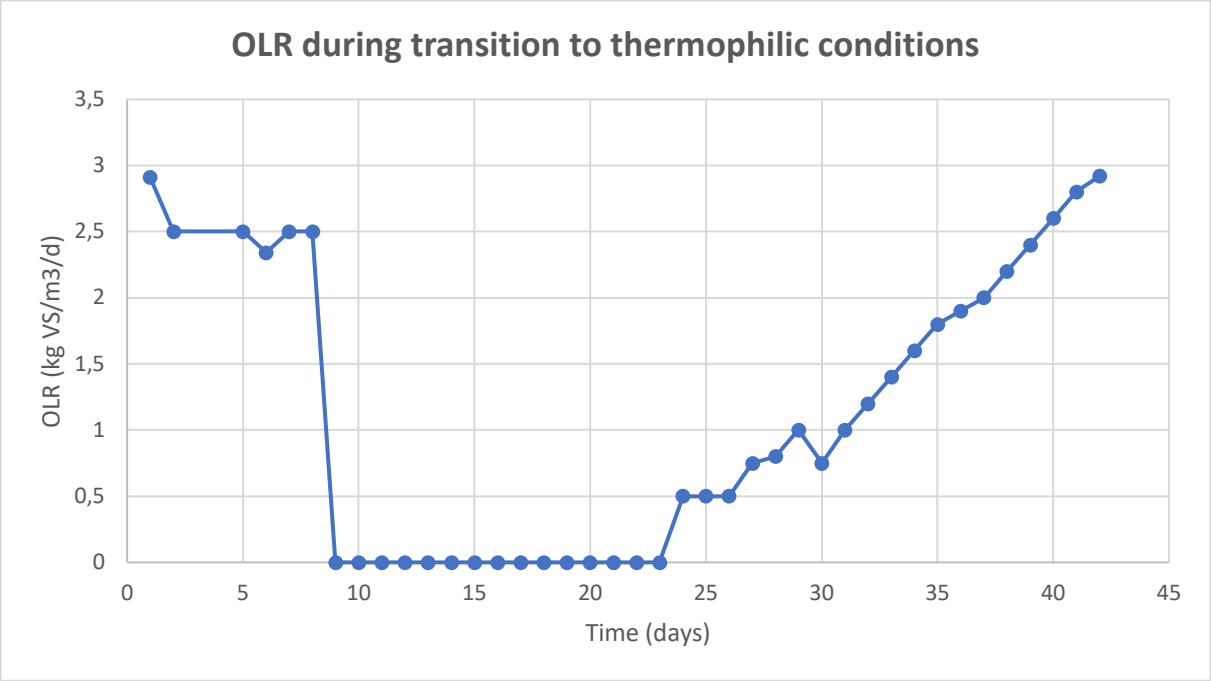


Figure 3: OLR during transition to thermophilic conditions.

The impact of the temperature increase to thermophilic and recovery is also illustrated well by the biogas production in Figure *4* and its methane composition in Figure *5* in the time period day 9-42. Immediately after the temperature raise, biogas production fell to approximately 2-3 % of the original production, the methane composition went from 60-65 % to 30-40 %. The first sign of recovery was an increase in methane composition on day 19, 10 days after the was temperature changed. The biogas production varied considerably in the beginning because of a varied feeding routine where the digesters were not fed every day. A drop in the methane composition of all reactors can also be observed around day 53 which corresponds time where a new batch of MSS was put into use on day 50. The concentration of methane varied between 55-65 % in al reactors after steady state was reached.

The daily biogas production in Figure *4* also shows how one reactor, TAD1, deviated from the rest of the reactors by a lower biogas production throughout the experiment. Several measures were taken to identify the reason for the deviation e.g. searching for leakages, changing instrument parts, re-calibration and replacement of volumetric gas sensors without improvement. The same deviations could not be seen on any other monitoring parameter and this particular reactor had a history similar issues in earlier experiments. These factors lead to the decision to use the results from this reactor but evaluate the probability of the biogas production results being valid compared to its duplicate reactor.

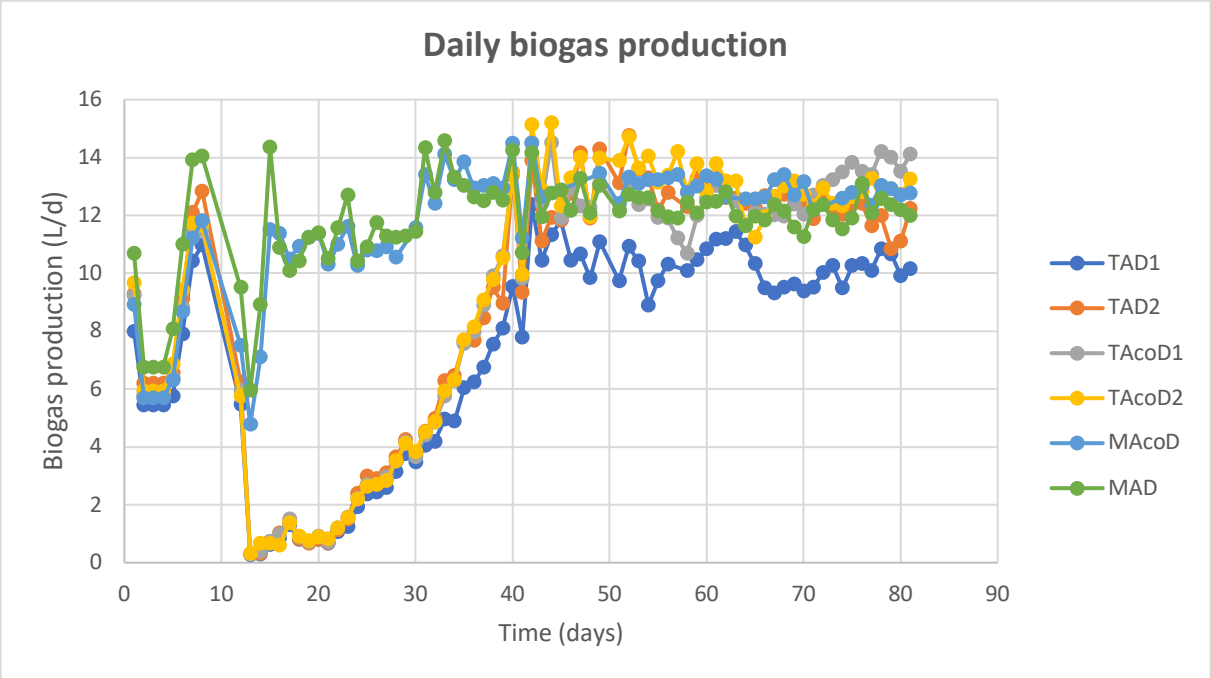


Figure 4: Daily biogas production (U = ±13 %).

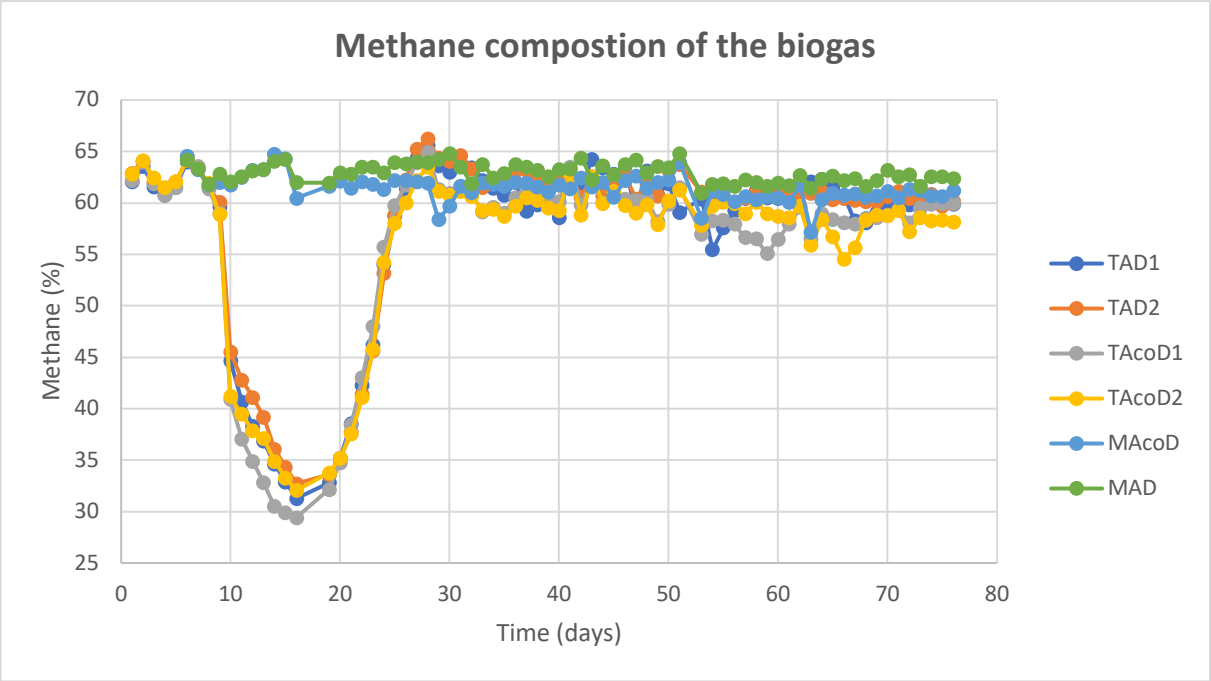


Figure 5: Methane composition of the biogas (U = ±3 %).

After the temperature increase, VFA/ALK-ratio increased by 55 % to 0.6-0.7 (Figure *6*) and VFA increased to more than 3 g HAc/l (Figure *7* and Figure *16*). The recovery was evident from these parameters from approximately day 23. This was partly due to the dilution done on day 23 that was motivated by the high TAN and FAN concentrations. The VFA, alkalinity and VFA/ALK-ratio level of all reactors seemed stabilized toward the end of the experiment. All the thermophilic reactors stabilized at a higher concentration of VFA and VFA/ALK-ratio than the mesophilic, and the MAD reactor a little higher than MAcoD reactor. Interestingly, the VFA-results of titration and HPLC corresponded well at high concentrations, down to approximately 1.5 g HAc/L. At lower levels, the VFA-results of titration appear to be overestimated. According to total VFA analyzed with HPLC, the mesophilic reactors also had a small peak after start-up before reaching a stable low level on day 37.

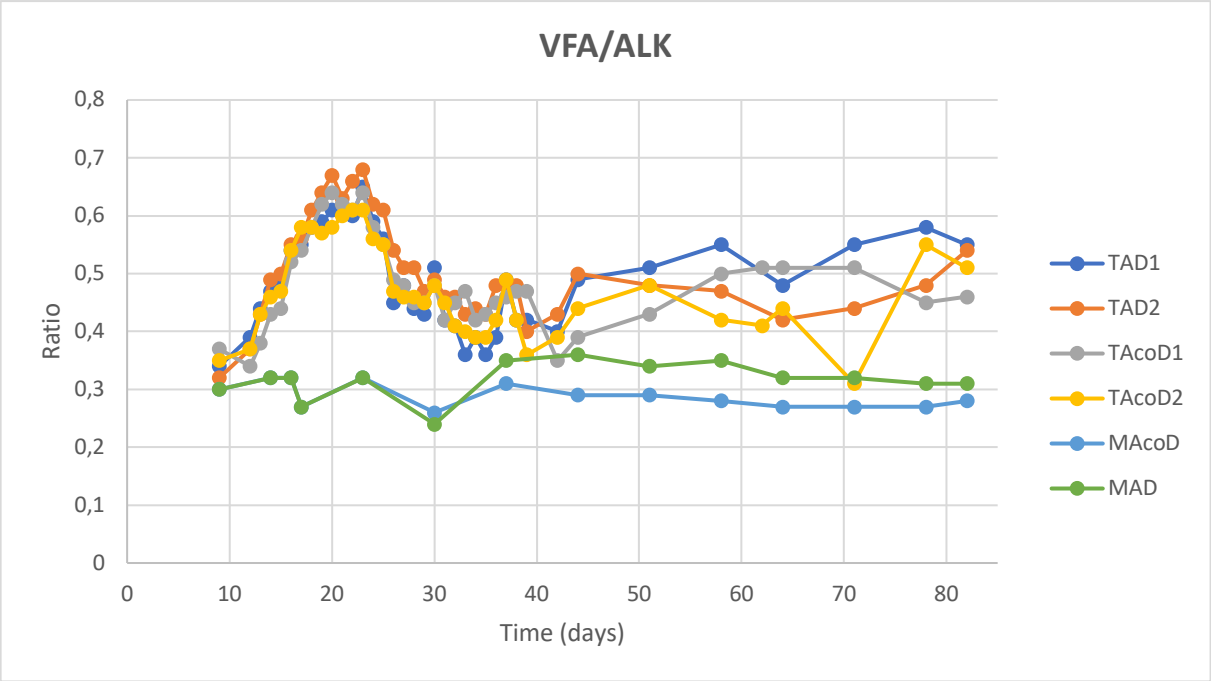


Figure 6: VFA/ALK of digestate (U = ±0.8%).

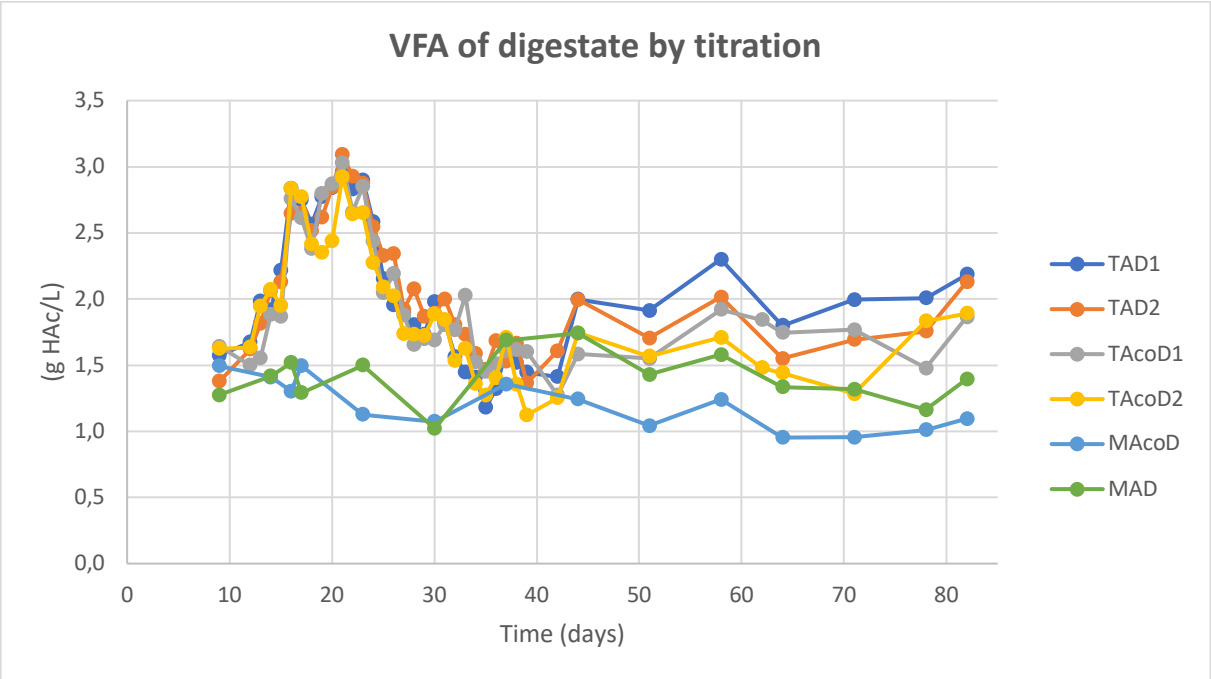


Figure 7: VFA of digestate analyzed by titration (U = ±3 %).

The alkalinity of the digesters are illustrated in Figure *8* and it appear to be higher during the transition period of the thermophilic digesters where no new substrate was added, before stabilizing at a lower lever together with the mesophilic reactors. The alkalinity of the MAD reactor seems to be generally higher than the others under apparent stable conditions.

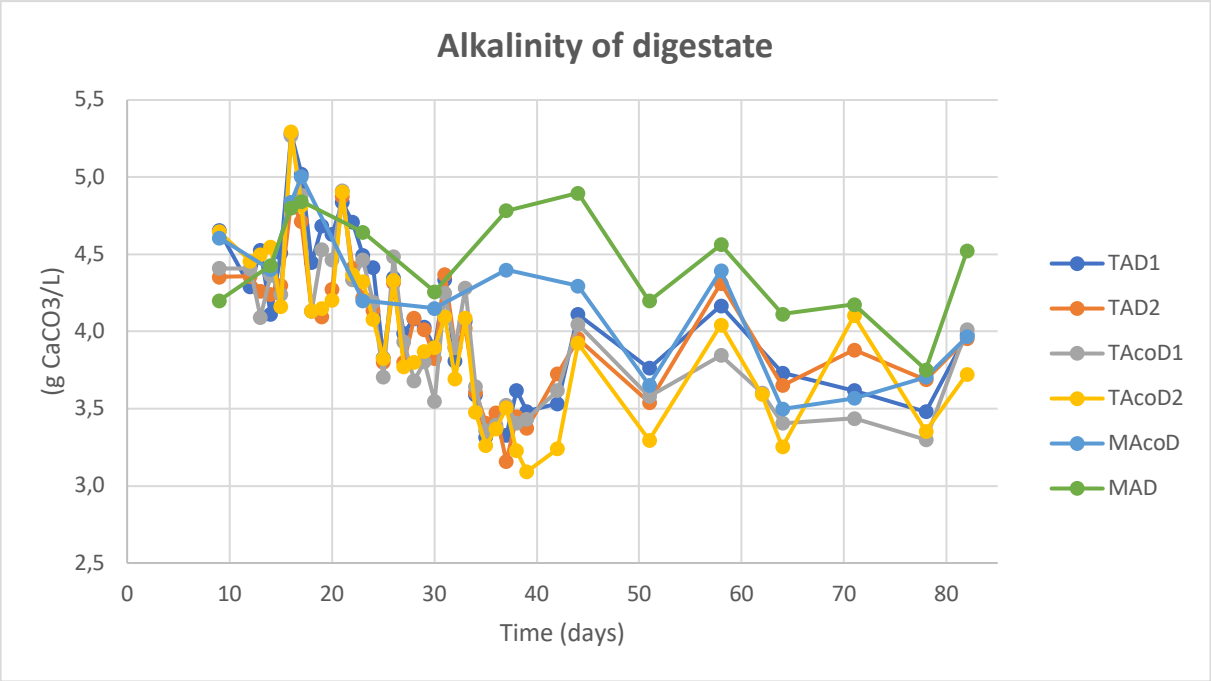


Figure 8: Alkalinity of digestate (U = ±3 %).

4.2. Comparing different processes

4.2.1. Biogas production

The calculated specific biogas and methane production of all reactors and the relative differences of the different processes are listed in Table *7* and Table *8* respectively. They are calculated relative to the amount of VS or tCOD added. The specific biogas and methane production exhibit a small increase and decrease respectively when comparing thermophilic to mesophilic digesters for both mono- and co-digestion. The differences were too small to be statistically significant (p=0.05), except the decrease in specific methane production when comparing TAcoD to MAcoD. However, when comparing co-digestion to mono-digestion both specific biogas and methane production increased. The increase was more considerable under mesophilic conditions. As previously mentioned, the biogas volume of reactor TAD1 deviated distinctly from the others and had a history of similar issues. The specific biogas and methane production of TAD1 was calculated to be significantly different from its parallel reactor (p=0.05), TAD2. The biogas and methane production results of this reactor were therefore excluded when the relative differences were calculated in Table *8*.

Table 7: Specific biogas and methane production. Average from day 62-81.

Reactor	Specific biogas production		Specific methane production	
	m^3 biogas/kg VS	m^3 biogas/kg COD	m^3 CH4/kg VS	m^3 CH4/kgCOD
TAD1	0.58 ± 0.02	0.34 ± 0.01	0.35 ± 0.01	0.203 ± 0.007
TAD2	0.70 ± 0.01	0.406 ± 0.008	0.42 ± 0.01	0.244 ± 0.006
TAcoD1	0.74 ± 0.02	0.43 ± 0.01	0.43 ± 0.01	0.256 ± 0.008
TAcoD2	0.72 ± 0.01	0.425 ± 0.009	0.42 ± 0.01	0.247 ± 0.006
MAcoD	0.729 ± 0.009	0.429 ± 0.005	0.444 ± 0.006	0.261 ± 0.003
MAD	0.69 ± 0.01	0.402 ± 0.007	0.429 ± 0.007	0.250 ± 0.004

Table 8: Relative differences of specific biogas and methane production of different processes. Average from day 62-81.

	Relative differences			
	Specific biogas production		Specific methane production	
Processes being compared	**VS^{-1} (%)**	**COD^{-1} (%)**	**VS^{-1} (%)**	**COD^{-1} (%)**
TAD relative to MAD	0.9[a]	0.9[a]	-2.4[a]	-2.4[a]
TAcoD relative to MAcoD	0.1[a]	0.1[a]	-3.9	-3.9
TAcoD relative to TAD	4.8	5.8	1.8[a]	2.9[a]
MAcoD relative to MAD	5.7	6.8	3.3	4.4

[a]Not statistically significantly different (p=0.05)

The gas flow of the thermophilic and mesophilic reactors evolved differently as illustrated in Figure *9*. After feeding, the mesophilic reactors responded with a sharp peak that decreased the next 15 hours where the gas flow curve started to flatten with no apparent difference between mono- and co-digestion. The thermophilic reactors performing mono-digestion instead had more even and low gas flow throughout the day after feeding and did not respond with a sharp peak. The TAcoD digesters however, demonstrated a flow pattern a bit more similar to the mesophilic ones and looks like something in between thermophilic mono-digestion and mesophilic.

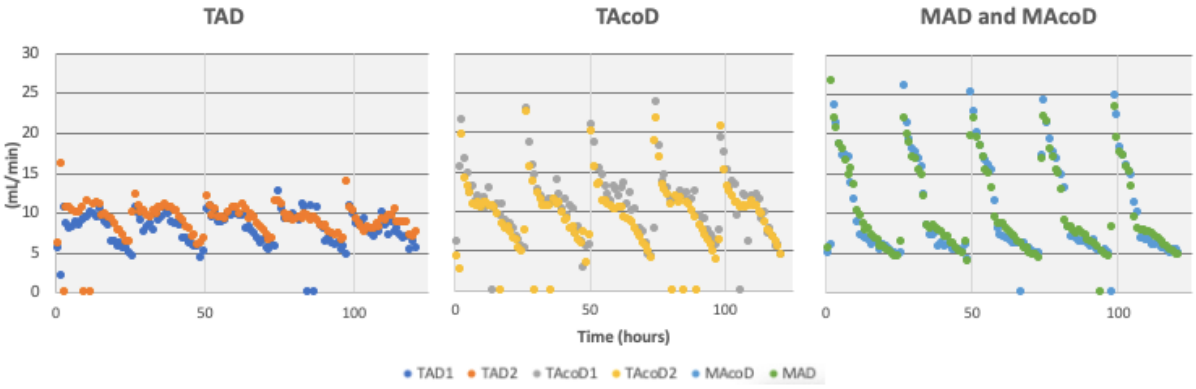


Figure 9: Gas flow patterns of all reactors from day 76-81 (U = ±13 %).

Equations 7 and 8 were applied to determine which reaction rate order fitted best and determine rate coefficients (k). From the results presented in Table *9* it is apparent from the correlation coefficients that a first-order reaction model fitted the mesophilic best, while a zero-order reaction model fitted better for the thermophilic processes. Furthermore, the TAcoD digesters corresponded better with the first-order model than the TAD reactors. This made it difficult to compare the different k-values

Table 9: Kinetics – Correlation and rate coefficients (k) of applied zero- and first-order kinetics equations.

Process	Zero-order		First-order	
	Correlation	k (mL/d)	Correlation	k (1/d)
TAD1	0.996	292	0.986	0.059
TAD2	0.997	337	0.966	0.077
TAcoD1	0.988	375	0.924	0.120
TAcoD2	0.987	344	0.977	0.087
MAcoD	0.941	324	0.987	0.089
MAD	0.949	338	0.975	0.106

4.2.2. Digestate

The pH of the digestates are illustrated in Figure *10*, and clearly display the higher pH of the thermophilic reactors compared to the mesophilic which ended at pH 8.2 and 7.7, respectively. The thermophilic reactors also experienced an increase in pH after the temperature raise where it was as high as 8.5. There were some variations in the pH, where the peak at day 64 was especially noticeable. The result and instrument were checked, and a new sample was taken to be certain. No explanation for this peak could be found as it dropped to the same level as before afterwards.

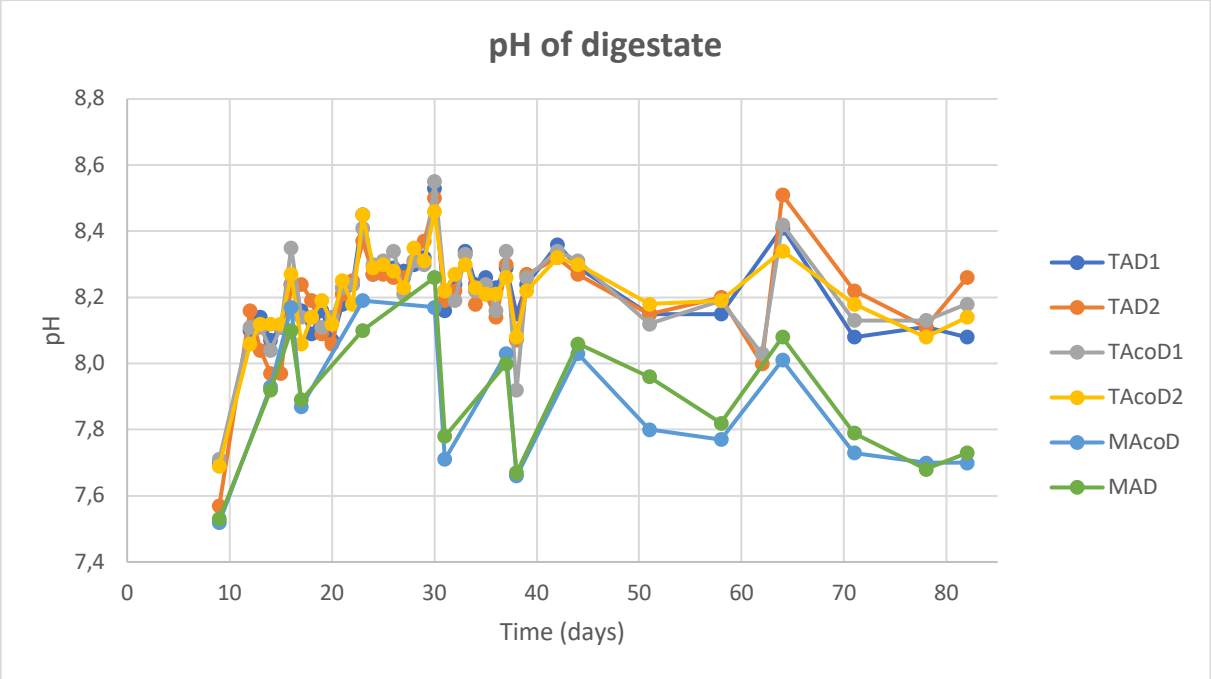


Figure 10: pH of digestate (U = ±0.1 %).

The TAN and FAN of the digestate are illustrated in Figure *11* and it shows the accumulation of TAN during the transition to thermophilic conditions as no new feed was added. This

together with the high pH lead the dilution on day 22. It was obvious that the mono-digesters had a higher TAN content than the co-digesters because of the lower nitrogen-content of the potato-stillage. FAN was calculated using equation 12. The variations in FAN towards the end was mainly due to the fluctuations in pH as the TAN was very stable. The end TAN concentrations (day 82) of TAD, TAcoD, MAcoD and MAD were 2.07, 1.83, 1.65 and 2.01 g/L, respectively. The FAN concentrations for the same reactors were 0.76, 0.66, 0.1 and 0.13 g/L, respectively.

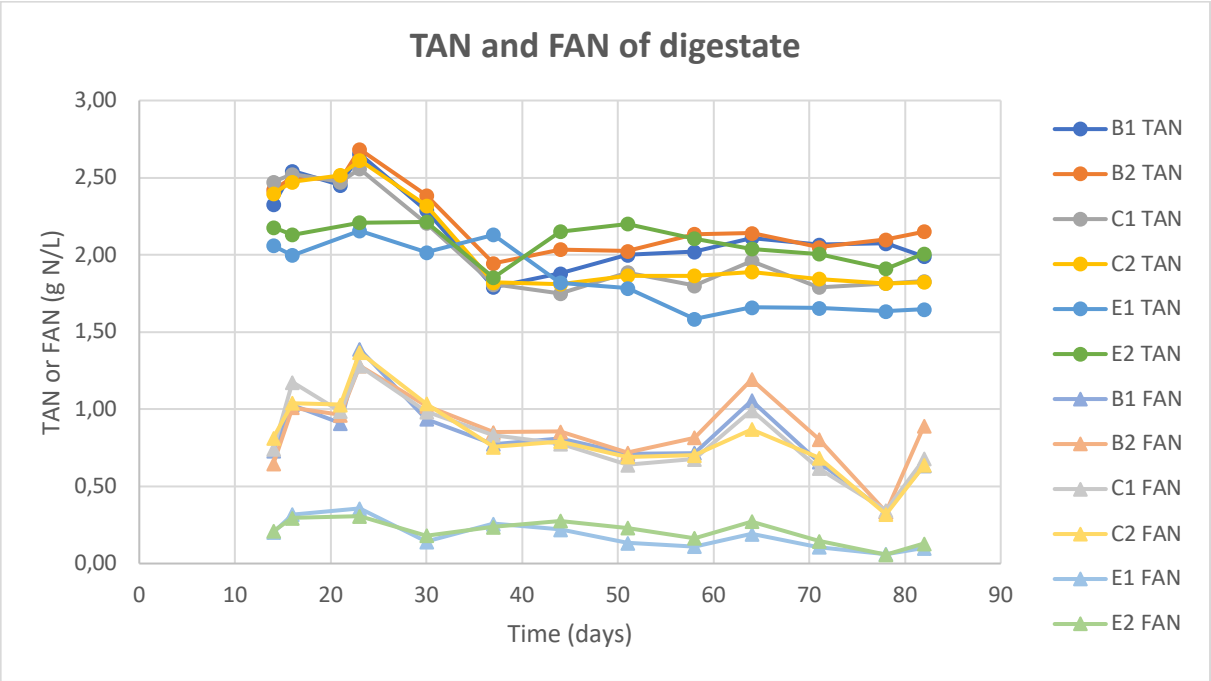


Figure 11: TAN (U = ±2 %) and FAN (U = ±2 %) of digestate.

The development of TS, VS and tCOD in the digestate are illustrated in Figure *12*,Figure *13* andFigure *14*, respectively, and the transition to thermophilic was evident from day 9 where no new substrate was added and the reactors were diluted on day 22. Additionally, there was a difference in TS between mono-and co-digesters where the latter displayed lower values because of the lower TS content of the potato-stillage than the MSS. The TS content of all reactors appeared to be stabilized towards the end of the experiment. The VS of TS of all the digesters are illustrated in Figure *13* and showed an increase towards the end of the experiment. The increase of the mesophilic reactors appeared to happen after day 51 which corresponded with the time of a new batch MSS. It is not clear that the VS of TS stabilized towards the end of the experiment. The same trend could only partly be observed for the tCOD in as illustrated in Figure *14*.

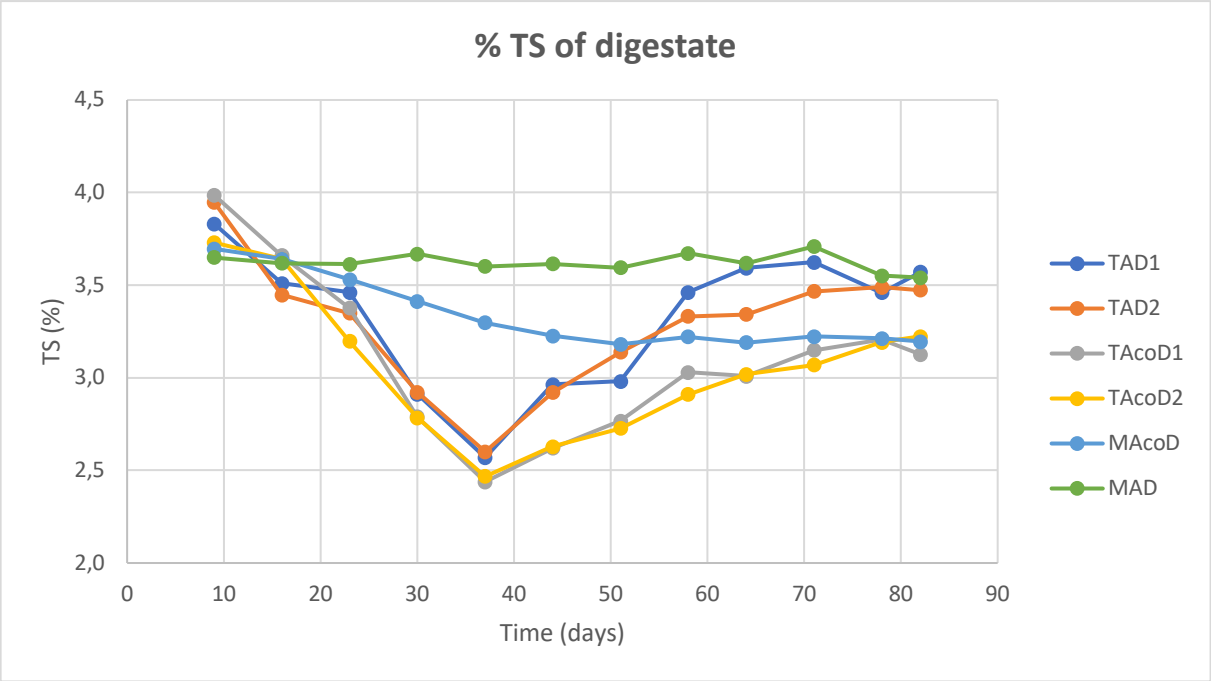


Figure 12: % TS of digestate (U = ±0.5 %).

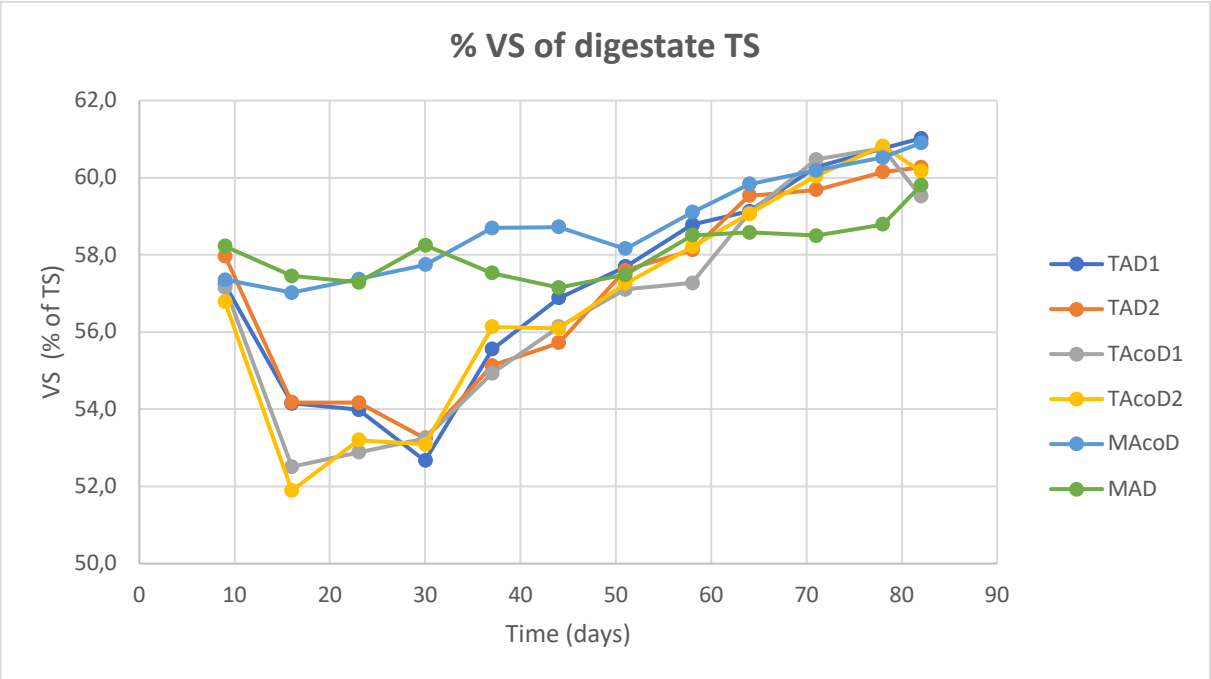


Figure 13: % VS of digestate TS (U = ±0.3 %).

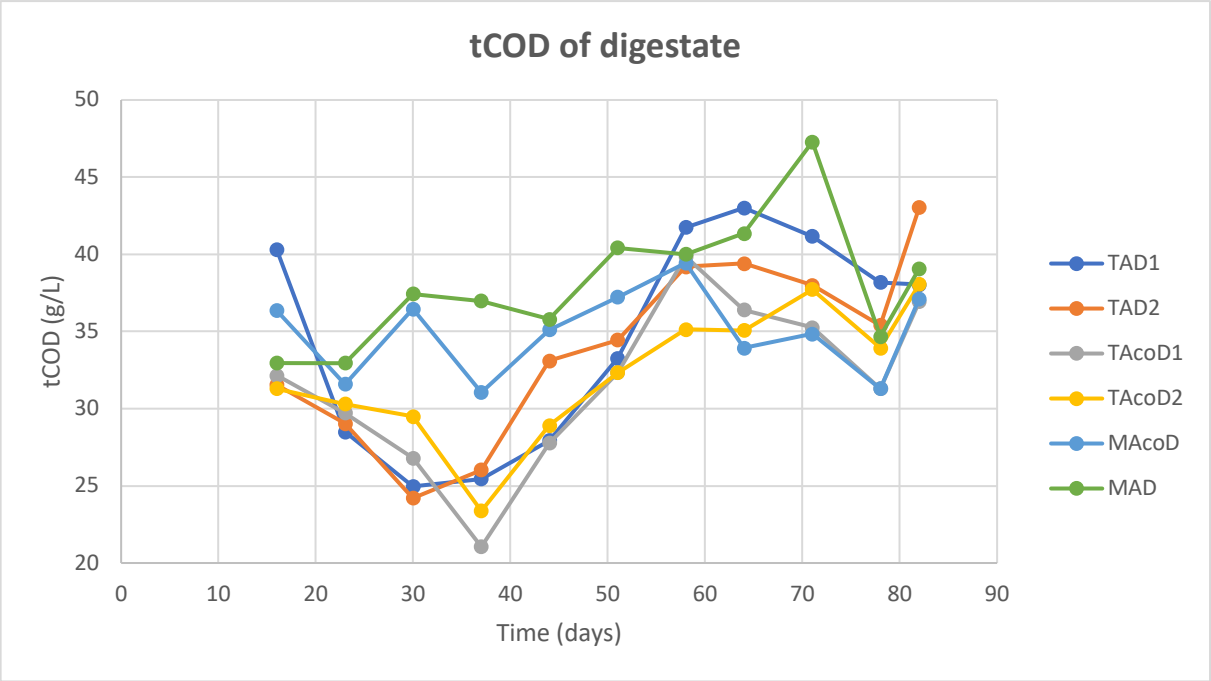


Figure 14: tCOD of digestate (U = ±3 %).

The calculated VS- and tCOD-reduction and the relative differences of the particular processes are presented in Table *10* andTable *11* respectively. The most notable difference was the increase in both VS- and COD reduction of co-digestion compared to mono-digestion at both thermophilic and mesophilic conditions. The differences between mesophilic and thermophilic digesters were minor and none of them were statistically significant, except the increase in VS-reduction of TAcoD compared to MAcoD.

Table 10: VS- and tCOD-reduction of all processes from day 58-82.

Process	**VS-reduction (%)**	**tCOD reduction (%)**
TAD	64 ± 1	60 ± 2
TAcoD	68 ± 1	63 ± 2
MAcoD	66.1 ± 0.4	63 ± 4
MAD	63.5 ± 0.7	60 ± 6

Table 11: Relative differences in VS- and tCOD-reduction from day 58-82.

Processes being compared	**Relative differences**	
	VS-reduction (%)	**tCOD-reduction (%)**
TAD relative to MAD	1.3[a]	1.3[a]
TAcoD relative to MAcoD	2.3	-1.0[a]
TAcoD relative to TAD	5.1	4.0

MAcoD relative to MAD	4.1	6.4

[a]Not statistically significant (p=0.05).

There was a large difference in sCOD content of the digestates of the mesophilic and thermophilic reactors and is illustrated in Figure *15*. The average of the thermophilic reactors at the end on day 82 was 7.9 g/L sCOD which was more than twice as much as the mesophilic at 3.3 g/L sCOD. This trend is consistent with the total VFA results in **Feil! Fant i kke referansekilden.**.

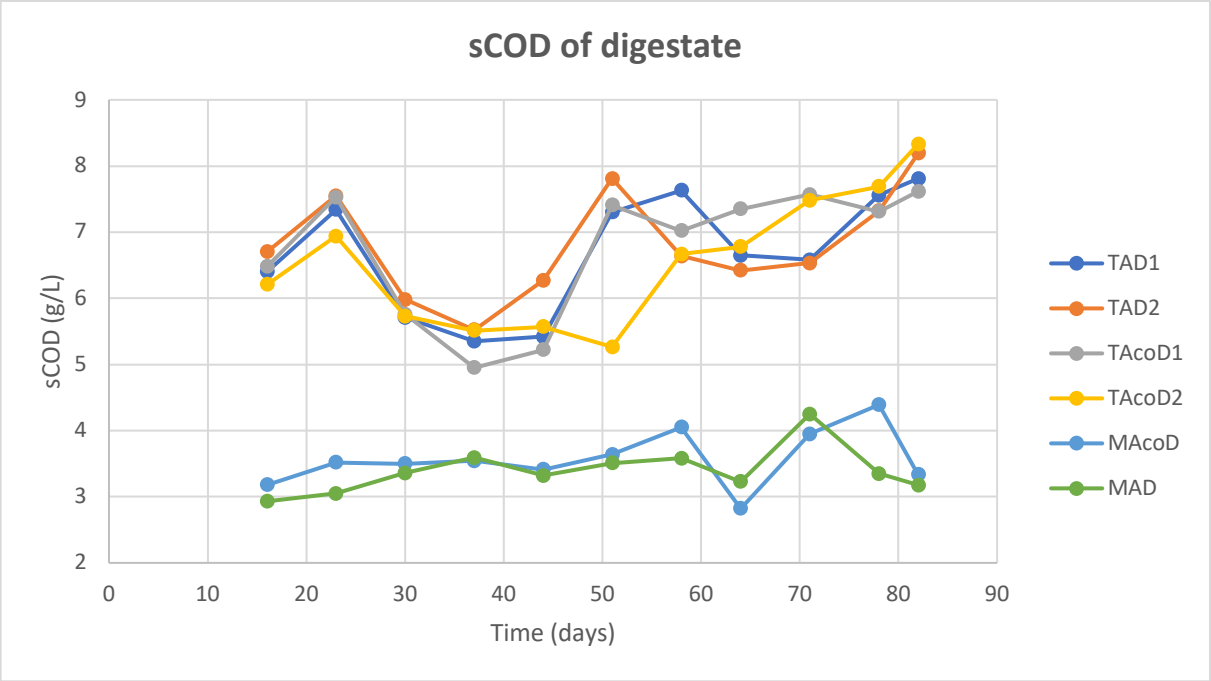


Figure 15: sCOD of digestate (U = ±21 %).

Several VFAs were measured as listed in 3.8.2 and the most prevalent ones are illustrated in Figure *16* andFigure *17* below. Formic, lactic and iso-valeric acid were measured but were either not detected or only detected in insignificantly small amounts and are therefore not displayed. The concentrations of total VFAs, acetic and propionic acid are illustrated in Figure *16*. Total VFas demonstrated a rapid increase and recovery during transition to thermophilic where all the digesters reached concentrations of approximately 3500 mg HAc/L where acetic acid was the most dominating VFA as illustrated in Figure *16*. After the recovery, the concentrations of total VFAs and acetic acid slowly decreased until the end of the experiment but were consistently higher than the mesophilic digesters that also had a small increase in the first 30 days. The TAD digesters acetic acid concentrations were a little higher than the TAcoD digesters. The propionic acid concentrations were clearly higher in the thermophilic digesters compared to the mesophilic. It appeared to be steady increasing after the transition and possibly showed signs of stabilization towards the end. The TAcoD digesters had the highest content the last 30 days but decreased at the last measurement.

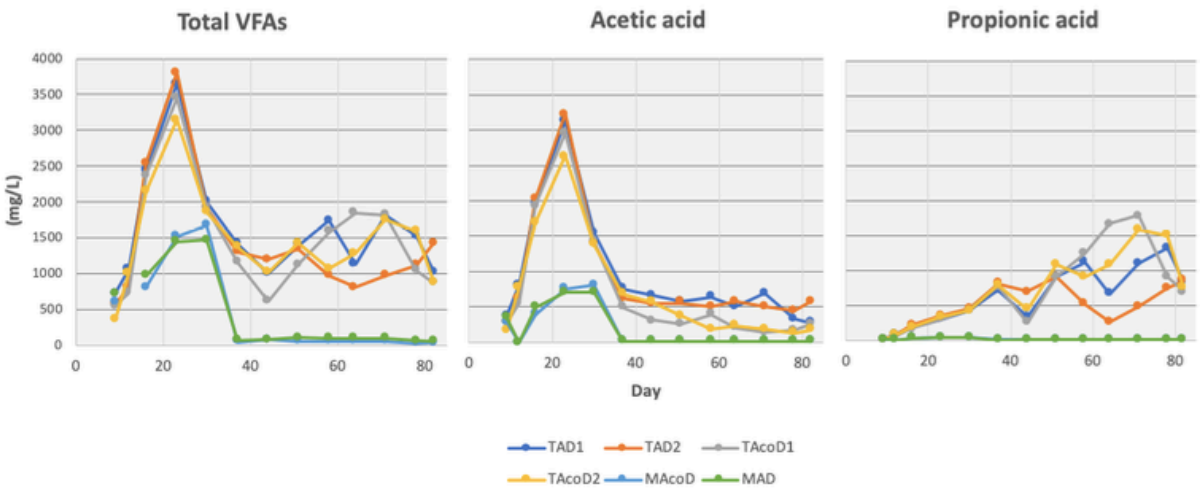


Figure 16: Total VFAs (U = ±40 %), acetic (U = ±21 %) and propionic acid (U = ±10 %) in digestate analyzed with HPLC.

The average acetic/propionic acid ratios of the digestates of the reactors were lower for the thermophilic compared to the mesophilic, and co-digestion compared to mono-digestion as presented in Table *12*.

Table 12: Acetic/propionic acid ratio of digestate from day 58-82.

Acetic/propionic acid ratio			
TAD	**TAcoD**	**MAcoD**	**MAD**
1 ± 0.5	0.24 ± 0.08	2 ± 1	6 ± 4

The VFAs n- and iso-butyric and n-valeric acid showed many similar patterns as illustrated in Figure *17*. They all had an increase followed by a recovery during the transition phase and the thermophilic digesters were generally higher than the mesophilic. Most notable were the relatively high concentrations of n-butyric acid of the TAcoD digesters, the peak of n-valeric acid during to first 30 days of the mesophilic digesters and that these VFAs exhibited low values the last 1 or 2 measurements from day 78 and 82.

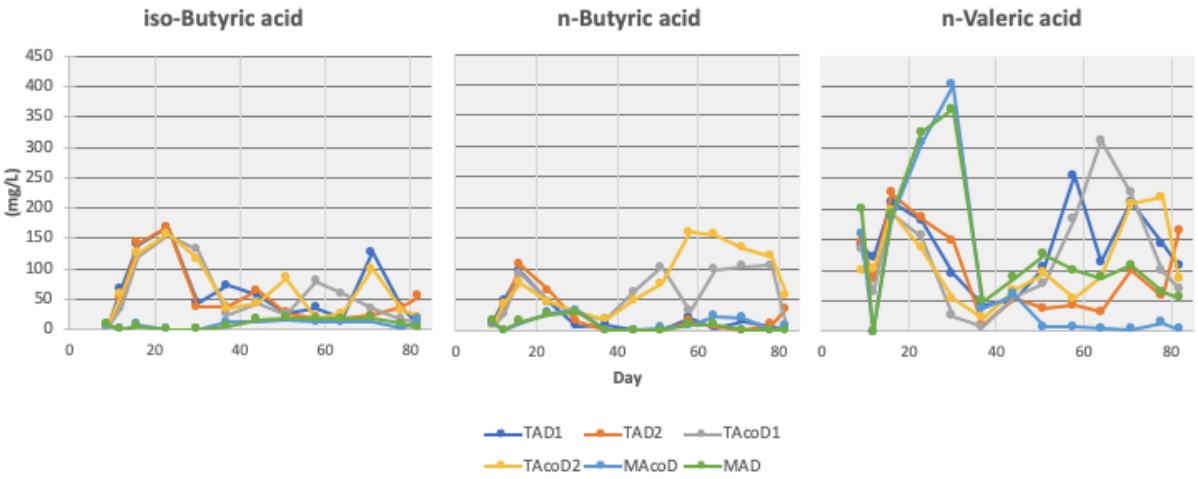


Figure 17: iso- and n-Butyric and n-valeric acid in digestate (U = ±10, 20 and 26 %, respectively.

The change of methane concentration of the biogas during one feeding cycle, i.e. from on feed to the next, is illustrated in Figure *18*. All of the reactors show a decrease immediately after feeding. The variations were most prominent for the co-digesters, and least for the thermophilic mono-digesters.

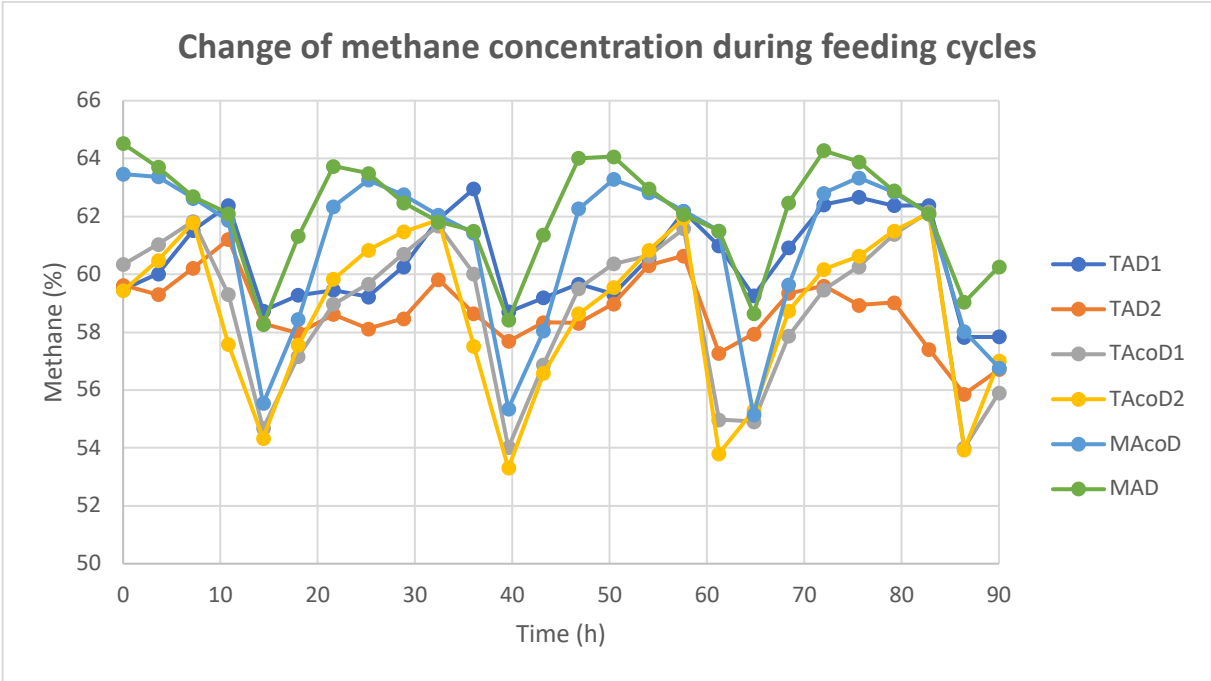


Figure 18: Change of methane concentrations during feeding cycles

5. Discussion

5.1. Inoculum and substrates

The characteristics of the final MAD reactors were similar to the initial inoculums Table *2*. This is as expected since this reactor would simulate the digester that the inoculum was retrieved from. The results from the characterization of the substrates presented in Table *3* show that the MSS and potato-stillage were mainly similar in terms of TS, total organic content (VS, tCOD and TOC) and low C/N-ratio. However, there was more sCOD in the

potato-stillage which was also consistent with the higher VFA content where lactic acid was the most prominent one. The results indicate that the potato-stillage had a higher content of readily biodegradable compounds expressed by the sCOD and VFA, which are expected to have instant high conversion rates. Both substrates types had low C/N-ratios, significantly below the recommended value of 20. The ratio in the potato-stillage was slightly higher but did not raise the overall C/N-ratio of the co-digestion substrate mix significantly (Table *3*).

5.2. Start-up and transition to TAD

The transition from MAD to TAD was achieved successfully by the one-step increase in temperature where feeding was completely ceased for 15 days and the reactors were running at target OLR (2.92 kg VS/m^3/d) 33 days after the temperature shift. The time required is slightly longer than that reported in other studies using the same strategy of 20 and 28 days [46], [63]. After the temperature increase, a decrease in methanogenic activity was observed (i.e. lower concentration of methane in the biogas) (Figure *5*) and as result, VFAs accumulated, primarily acetic acid (Figure *16*). When the feeding was resumed, the concentration of VFA was above 3 g HAc/L which is higher than recommended concentrations, suggested by literature with concentrations less than 0.5 g HAc/L [35]. Because of high buffer capacity due to the high TAN concentrations (Figure *11*), the pH was still high, 8.2-8.3 (Figure *10*), which assumingly prevented acidification. The high TAN concentration (>1 g/L) was however possibly a critical inhibitor. The increase of TAN was assessed to be a consequence of the high HRT because no new substrate was added, nor any digestate withdrawn which caused the TAN to accumulate as organic material was converted to biogas and more protein and amino acids were potentially converted to TAN and FAN. Combining this with high temperature and pH resulted in calculated concentrations of FAN to be 1.2-1-4 g/L. The strategy to avoid reactor failure from TAN inhibition was dilution with water. In addition, to reduce the TAN concentration, the dilution would also possibly have a positive effect on the negative impact from elevated VFA concentrations, and thus overall aid the transition. It was decided to resume feeding despite high VFA concentrations due to an observed recovery of methanogenic activity i.e. increase in methane concentration in the biogas and also increased total biogas production (Figure *4*Figure *5*) in addition to the high buffer capacity (Figure *8*). Also, since the pH was relatively high (Figure *10*), it was assumed that new substrate, with lower pH level than the reactor content, could help lower the pH in the process.

The combination of dilution and resume of feeding proved to be a successful strategy as the methane concentration continued to increase rapidly (Figure *5*), pH lowered (Figure *10*) and VFA- and TAN concentrations decreased (Figure *16*Figure *11*, respectively). Dilution could therefore be a useful measure when possibly facing similar issues in full scale. Although, there is a possibility that the recovery would be similar also without dilution. When target OLR was reached, the concentration of VFA was still high at 1 g HAc/L. From the VFA-results, it seems that also the mesophilic reactors needed time to acclimate by the peak concentrations of acetic and n-valeric acid (Figure *16*Figure *17*). This could suggest that the inoculum was not completely acclimated at the time of transition.

5.3. Comparing MAD and TAD

5.3.1. Biogas production

From the results of specific biogas and methane production in Table *7*Table *8*, it can not be concluded that the thermophilic digestion had any significant effect on specific biogas or methane production. There was a significant decrease in specific methane production of TAcoD compared to MAcoD. The change of methane concentration during feeding cycles (Figure *18*) shows that the TAcoD reactors dropped to the lowest level of methane concentration compared to the other processes immediately after feeding. This coincides with previous studies that have proposed that this is due to acidification whit discontinuous feeding [16]. Therefore, the drop in methane concentration could be partly due to the discontinuous daily feeding frequency and could be causing the lower methane yield

5.3.2. Digestion efficiency

When comparing the comparing the gas flow patterns of the thermophilic to the mesophilic reactors in Figure *9*, they appear contradictory to the general argument that the thermophilic conditions improve reaction rates [4] as they need longer time to produce the same amount of biogas. But within in an HRT of 20 days they end up at the same specific biogas production. From these results it is not evident that the HRT retention time could be reduced under thermophilic conditions and still achieve the same biogas yield. In addition, regarding the kinetics, it is apparent that the thermophilic reactors correlate best with zero-order kinetics and that it evolves independent of substrate concentration, while the mesophilic correlates with first-order kinetics that is dependent on substrate concentration. This was also found to be the case in a study doing temperature phased AD of a primary sludge and WAS mix [64]. This could possibly be a sign of inhibition.

There were no significant differences in VS- or tCOD-reduction when comparing thermophilic to mesophilic digesters. The only exception was a 2.3 % higher VS-reduction of the TAcoD compared to MAcoD, but the difference was not significant when comparing tCOD reduction. Since there was mainly no difference in VS-reduction when comparing thermophilic to mesophilic digestion, it is an indication that the thermophilic conditions did not have a significant improvement of hydrolysis with the given HRT. Insufficient hydrolysis is thought to be indicated by lower VS-reduction [3]. The reactors achieved VS-reduction in the range of 63.5±0.7 % and 68±1 %, and tCOD-reduction between 60±6 % and 63±4 % which is similar to what has been reported in previous studies [12].

5.3.3. Digestate quality

The higher pH of the thermophilic digestate, 8.2, to that of the mesophilic (Figure *10*) was outside what is generally recommended between pH 7-8. The resulting high FAN concentrations illustrated in Figure *11*, did not occur to inhibit the methanogenic activity when looking at the specific methane production listed in Table *7* Table *8*. Although, it could be a contributing factor to the gas flow pattern as illustrated in **Feil! Fant ikke r eferansekilden. Feil! Fant ikke referansekilden.** where the gas flow production seem somewhat lower compared to the mesophilic in **Feil! Fant ikke referansekilden.**. There have b een reported inhibition at and below these concentrations in other studies [20]. The mesophilic digested sludge from VEAS that was used as inoculum, was already acclimated to the TAN and FAN concentrations of the MSS under mesophilic conditions which is reported

to be an advantage [20]. This might also have been an advantage in the present experiment, even though the FAN concentration increased under thermophilic conditions.

The high content of sCOD and VFAs of the thermophilic digesters compared to the mesophilic, demonstrates poorer degradation and hence a worse effluent quality. This is in accordance to previous findings [4], [65]. Of the VFAs, propionic and acetic acid were the main contributors. The ratio of acetic to propionic acid was 1:1 or below, for the thermophilic digesters, which have been suggested as a sign of an unstable digester [13]. There were also generally higher concentrations of, n- and iso-butyric and n-valeric acid. These could be signs of hydrogen inhibition of the acidogens that are not able to convert these VFAs to acetate [3], [12]. An elevated content of hydrogen could mean that the hydrogenotrophic methanogens are inhibited. It has been reported that these are not the most ammonia sensitive of the microbes which makes ammonia inhibition a less plausible explanation [20]. The high concentration of VFA could explain an issue occurring later in the process at VEAS when the effluent is stripped for ammonia by also being stripped together with the ammonia because of its volatile nature. According to previous findings, the elevated concentrations of VFAs under thermophilic compared to mesophilic conditions were considered to be a result of high hydrolysis rate together with a poor capability of the methanogens to convert the produced VFAs [65].

The thermophilic digestate was considered to be more malodorous. Since iron is used as coagulant to generate the MSS, it is unlikely that H_2S was responsible for the odor as it would precipitate out. Other possible contributors that stood out in the thermophilic digestate were n- and iso-butyric and n-valeric acid and FAN. The higher temperature of the digestate could also make the odor appear more intense.

5.4. The influence of co-digestion with potato-stillage.

5.4.1. Biogas production

The co-digestion with potato-stillage had positive effects on specific biogas and methane production under both mesophilic and thermophilic conditions (Table *8*) which is coherent with other studies comparing co-digestion to mono-digestion of MSS [33], [34]. The positive effects were most significant under mesophilic conditions with a 3.3 and 4.4 % increase in specific methane production relative to VS and COD added respectively. The increase is less than reported by the other studies partially due to the OLR being held constant and the low ratio of potato-stillage of this study. The largest increase was seen in biogas yield, while the methane yield was less improved. Some of the explanation could be that the overall lower methane concentration of the biogas was due to the varying concentrations during one feeding cycle as illustrated in Figure *18*. The digestion of MSS with 40 % sugar beet pulp stillage obtained 0.357 m^3 CH_4/kg VS which is less than achieved in this study of 419-444 m^3 CH_4/kg VS

5.4.2. Digestion efficiency

When looking at the gas flow patterns of the thermophilic reactors (Figure *9*), it is obvious that the potato-stillage improved the biogas production rate at the beginning of the feeding cycle. Because of the higher amount of sCOD and VFAs in the potato-stillage compared to MSS, it was expected that these would be readily biodegradable to produce biogas. It has been reported that the co-digestion of more readily biodegradable substrates like food

waste [30] and food wastewater [33] have improved methane production rate. The potato-stillage also increased the VS- and tCOD-reduction of the process compared to mono-digestion. These improvements were generally more significant under mesophilic conditions (Table *11*). Even though the MSS and potato-stillage were similar in organic content measured in VS and tCOD, the potato-stillage had more easily degradable compounds like carbohydrate residues from the fermentation process, added enzymes and proteins and VFAs. As a result, it had a positive effect on the degradability of the feedstock when mixed with MSS. One study found that the addition of easily degradable food wastewater enhanced VS and tCOD removal by increasing microbial growth and hydrolytic enzyme activities and found increasing methanogenic archaea populations with increasing food wastewater fraction [33]

5.4.3. Digestate quality

The slightly higher C/N ratio in the potato-stillage due to the lower nitrogen-content, was visible by the lower TAN concentration of the digestate of the co-digesters compared to the mono-digesters. Hence, the potato-stillage had a positive effect by improving the C/N-ratio of the feedstock and a result decreasing the risk of ammonia inhibition. Enhanced C/N-ratio has been presented as one of the common synergistic effects of co-digestion [30], [7], [32], [33], [27]. The mono-digestion of stillage has also shown problems with rapid acidification and low buffer capacity [32], making co-digestion with substrates with high buffer capacity like MSS beneficial. The mesophilic co-digesters also had a lower VFA content in the digestate compared to the mono-digester. Although, this was not the case for the thermophilic digesters. Due to the higher VS- and tCOD-reduction of the co-digesters, the resulting digestate also had lower content of these parameters.

5.5. Comparison to full-scale

There were several differences between the lab-scale and full-scale reactors including different techniques of mixing and heating as previously described, but perhaps more importantly the difference in feeding frequency. From previous studies it has been demonstrated that the feeding frequency influences the capacity of the digester and that less frequent feeding increases the risk of shock loadings and acidification. From Figure *18* it is apparent that the methane concentration of the biogas dropped immediately after feeding. It has been shown that this happens to much smaller extent during continuous feeding [16]. Therefore, the feeding strategy during the experiment could have resulted in less favorable results than what would have been the case in full-scale. When considering the transition strategy, dilution was chosen as a measure to reduce the high FAN concentration. In full-scale, the dilution would correspond to a huge amount of water that would also need to be heated, which is more challenging and demanding in full-scale. Also, the heating of the reactors from 37 to 55 °C would be more time consuming in full-scale. I addition, only two batches of MSS was applied in the experiment and the OLR was kept constant at stable conditions. In full-scale, more substrate and loading variations would occur.

5.6. Limitations and error analysis

It has been suggested that the digesters need three HRT before proper representative conditions are achieved [2]. Only two HRT was carried during this experiment due to limited time available. Hence, stable conditions might not have been sufficiently achieved to

conclude properly. On the last day, the VFA-concentration of all the reactors appeared to decrease, which may imply a stable process. Nevertheless, the VFAs concentrations could possibly have decreased further if given time.

Two batches of MSS was used during the experiment, in which the last batch was more VS-concentrated than the first batch. The properties of the second batch did possibly result in some alterations in methane concentration and VS compared to the first batch at previously mentioned. The digesters were fed the second batch for 32 days and could have needed more time to stabilize. Also, when co-digesting with the first batch of MSS the HRT was slightly shorter, 19 days, because of the lower VS-concentration of this first batch and the potato-stillage.

The biogas production of reactor TAD1 deviated significantly from the other reactors as previously mentioned. The reactor was troubleshooted almost throughout the whole experiment, including the majority of possible improvements (change transitions and gaskets, search for leakages, calibrate gas meter, etc.) except opening up the reactor. Since no other deviations could be seen from any of the other parameters being monitored and because of this reactor's history of similar problems, it was considered to most likely be caused by something instrumental. It was decided to continue the experiment without opening up the reactor and exclude the biogas results but still use the results of the digestate. However, there is still uncertainty to what caused this issue.

5.7. Future perspective

According to the findings of this study, several of the same issues encountered in full-scale with elevated VFAs in the effluent and odor of the digestate was experienced. Since these issues could possibly cause problems later in processes with malodourous biosolids and complications in the ammonia stripping process, it could be beneficial to look for solutions to improve the digestate quality by reducing the VFA-concentrations. A solution that has been suggested is to apply a two-stage temperature phased anaerobic digestion configuration where the benefits of sanitation and enhanced hydrolysis rate are combined with the lower VFA content in the effluent. AcoD with potato-stillage had positive effects on the overall digestion efficiency and biogas-production rate and yield. Hence, this option appears to be beneficial on the process and should be considered. Also, other substrates with readily biodegradable compounds or a higher C/N-ratio could be considered to improve the process, since these synergistic factors were believed to be the ones improving the process in this study.

This study focused on keeping a stable VS-fraction of potato-stillage as feedstock and constant OLR. In the future, larger fractions and higher OLR could be studied to see the effect on the process and if it could possibly improve the process further.

6. Conclusion

This study investigated the feasibility of transitioning from MAD to TAD at VEAS and also examined the effects of co-digestion with potato-stillage in lab-scale reactors.
The transition was achieved successfully by a one-step increase in temperature where feeding was completely ceased for 15 days and target OLR was achieved after 33 days.

Stable TAD was accomplished that matched the mesophilic digesters by biogas and methane yield and VS-reduction. However, biogas production efficiency was reduced under thermophilic conditions in addition to poorer effluent quality containing large quantities of VFAs. Co-digestion with potato-stillage improved the biogas and methane yield up to 5.7 and 3.3 % respectively. The improvement was most significant at mesophilic conditions. VS-destruction was increase up to 5.1 % and biogas production efficiency was improved under thermophilic conditions. According to the findings of this study, strategies to reduce the VFA-content of the thermophilic digestate should be considered if VEAS chooses to transition. Furthermore, co-digesting with potato-stillage benefitted the process and appears to be a lucrative option.

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