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Jämförelse av termofil och mesofil slamrötning på Hammarby Sjöstadsverks MBR-pilotanläggning för avloppsvattenrening

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Abstract

Anaerobic digestion is a commonly used method for stabilisation of sewage sludge and production of biogas at municipal wastewater treatment plants (WWTPs). The hydraulic retention time for sludge in the digestion reactors is about 16 days at Henriksdal WWTP in Stockholm. As Henriksdal WWTP is being extended and rebuilt for increased capacity, the hydraulic retention time will be decreased to 14 days. The present study investigated to what extent further decrease in retention time is possible by evaluating data from stress tested anaerobic digestion processes. The study also investigated methods for analysis of volatile fatty acid (VFA) as well as estimation of methane production. The present pilot study, conducted at Hammarby Sjöstadsverk pilot facility, proves that anaerobic digestion can prevail at nine to four days retention time. A retention time of four days resulted, however, in a specific methane production which was 42-48% lower than at Henriksdal WWTP at thermophilic (55 °C) and mesophilic (37 °C) temperatures. The ratio between VFA and alkalinity stayed within normal levels during both stress tests, indicating stable processes. Unexpectedly, VFA did not increase substantially during the stress tests. This might be explained by low organic loading rate and low degradability of the substrate. Alkalinity, however, was one of the first parameters to decrease below normal levels, possibly due to lower nitrogen mineralisation. Although this study shows that it is possible to maintain a viable anaerobic digestion process at nine to four days retention time, the loss of buffering capacity and lower methane generation should discourage long term operation at short retention times. The present study also suggests spectrophotometric VFA analyses for detection of low VFA concentrations as well as methane production estimations based on reduction of fat, protein and carbohydrate.

Popular-scientific summary

Comparison of mesophilic and thermophilic anaerobic sludge digestion at Hammarby Sjöstadsverk MBR pilot plant for wastewater treatment

When wastewater is treated in a wastewater treatment plant, pollutants are continuously being separated from the water. The separated particular pollutants are called sludge. The sludge is rich in biologically degradable material and plant nutrients such as nitrogen and phosphorous. The nutrient rich sludge can be used as a fertiliser, but without treatment it will be bulky and may also start fermenting and smell. To decrease the amount of sludge and minimise the risk of unpleasant odours, the sludge can be stabilised. Stabilisation can be achieved through anaerobic digestion, which implies that organic material is decomposed by microorganisms in an anaerobic environment and turned into biogas. The biogas, which is composed of methane and carbon dioxide, can thereafter be used as a substitute to fossil natural gas.

A multitude of microorganisms have to collaborate to sustain the anaerobic digestion. Larger molecules of proteins, fats and carbohydrates are stepwise degraded to smaller molecules. In the last step of the anaerobic digestion process, a group of microorganisms called methanogens produce methane and carbon dioxide. If the activity of the methanogens is hampered there will be an accumulation of intermediate products such as volatile fatty acids (VFA) in the process. The anaerobic process has a buffering system which neutralises the fatty acids produced. The buffering capacity of the system (measured as alkalinity) will, however, decrease if the load of fatty acids is too high which consequently might result in a decreased pH. This could in turn cause substantial problems with the decomposition in the system. It is therefore important to monitor the amounts of fatty acids through analysis of sludge samples. A cheap, fast and simple method for both VFA and alkalinity analysis is so called titration. VFA can also be assessed through liquid chromatography which demands more expensive equipment and expertise generally only found at larger laboratories. Both VFA and alkalinity can also be measured through spectrophotometric methods which are less costly and can be conducted at smaller laboratories.

The most common kind of methanogen in the anaerobic digestion process at WWTPs has twelve days of generation time. If the sludge is retained in the reactor for a shorter time, the methanogens will not have enough time to reproduce and the population will be flushed out. The amount of time the sludge is retained in the reactor is called retention time. A common retention time for anaerobic digestion of sewage sludge at municipal wastewater treatment plants is 20 days. Methanogens are also sensitive to swift temperature changes and thrive in neutral pH. Anaerobic digestion can be achieved at different temperatures. The two most common temperatures are 37 °C (mesophilic temperature) and 55 °C (thermophilic temperature). Thermophilic temperature can accelerate the digestion and hence more sludge can theoretically be digested during a shorter time span. Higher temperature will, however, also make the process more sensitive to disturbances, such as toxic components and temperature changes. The microbial diversity is furthermore lower at thermophilic temperature in comparison to mesophilic temperature.

In order to treat the wastewater generated in a growing Stockholm, Henriksdal wastewater treatment plant is being extended and rebuilt for increased capacity. To be able to cope with more sludge without an increase in reactor tank size, a transition to thermophilic digestion is planned in combination with a decrease in hydraulic retention time to 14 days. Since municipal wastewater treatment plants in general have 20 days of retention time, it is unknown at what retention time the digestion process will turn ineffective and fail. This study firstly investigated how methane production and other parameters change when the retention time is decreased and secondly which methods of

analysis that are appropriate for monitoring of the anaerobic digestion process. The investigation is based on pilot scale thermophilic and mesophilic anaerobic digestion processes at Hammarby Sjöstadsverk pilot facility.

The present study showed that the methane producing microorganisms can adapt to retention times down to four days. This is substantially shorter than the reproduction time needed by the typical methanogen in an anaerobic sludge digestion process. Thus, the results indicate that other methanogens have taken over the production of biogas. However, at four days retention time the process displayed 42-48% less production of methane per kg incoming organic material and 57-76% lower alkalinity in comparison to Henriksdal wastewater treatment plant, operating at longer retention time. The ratio VFA/alkalinity was the parameter that increased most during the trials, but it never reached levels indicating process instability. Hence, the present study shows that it is possible to maintain the anaerobic process by using retention times of nine to four days. However, due to increased sensitivity to acidic compounds and lower methane production, it would only be recommended to operate the system at such short retention times for limited time periods.

The thermophilic process displayed higher degree of VS degradation and alkalinity than the mesophilic process, which indicates that the thermophilic process stabilises sludge better and has lower sensitivity to acidic substances. Hence, thermophilic anaerobic digestion could be more suitable for short retention times. The results also suggest that spectrophotometry, rather than chromatography or titration, should be used for VFA detection at VFA levels common at Henriksdal WWTP. Estimation of methane production could furthermore be improved if the calculations are based on fat, protein and carbohydrate rather than on volatile solids reduction.

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1. Introduction

Henriksdal wastewater treatment plant (WWTP) receives wastewater from about 860 000 persons in the Stockholm area (SVOA, 2020). In order to ensure appropriate treatment of the wastewater from a growing Stockholm, the capacity of the WWTP has to double. This will be achieved by membrane bioreactors (MBR) replacing the existing secondary sedimentation. At the pilot facility Hammarby Sjöstadsverk, pilot trials are running since 2016 in the same configuration as the future Henriksdal WWTP. Wastewater from the inlet of Henriksdal WWTP is diverted into Hammarby Sjöstadsverk where sludge from the pre-precipitation and membrane tanks is digested in a 5 m^3 reactor for anaerobic digestion (Figure 1).

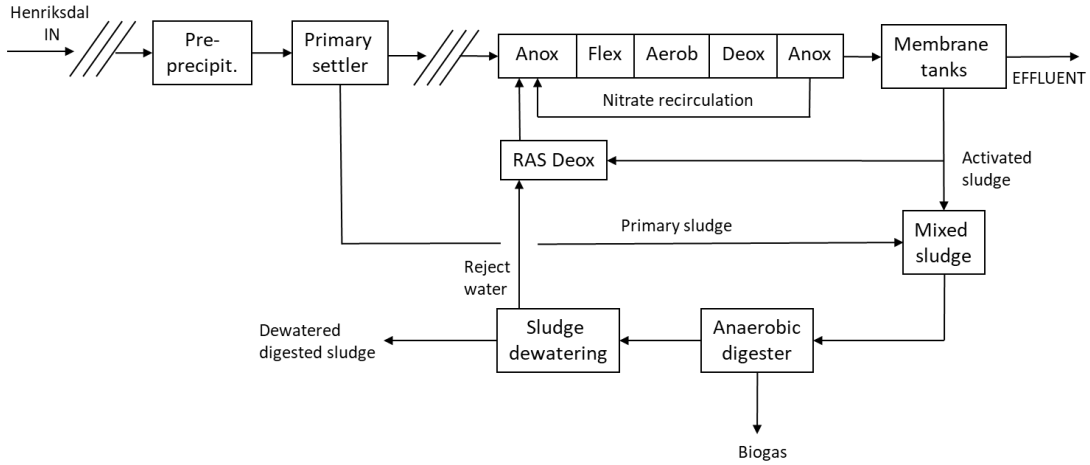

Figure 1. Flow scheme of wastewater and sludge treatment at Hammarby Sjöstadsverk pilot facility (after Andersson et al., 2020).

As Henriksdal WWTP will be rebuilt to reach double capacity without an increase in reactor sizes, the digestion of sludge has to be optimised. A possible strategy for optimisation of the anaerobic digestion is increased organic loading in combination with shorter reactor retention time. Since thermophilic (55 °C) digestion normally can manage higher organic loading than mesophilic (37 °C), Henriksdal WWTP plans to transition to thermophilic digestion and a retention time of 14 days. Since municipal WWTPs generally design their reactors for retention times around 20 days, it remains uncertain at what retention time the digestion turns ineffective and fails.

In a pilot project, conducted jointly by IVL Swedish Environmental Research Institute and Stockholm Water and Waste Company, a thermophilic anaerobic digestion process has been stress tested during autumn 2019 and spring 2020 (Andersson et al., 2020). In the test, the hydraulic retention time (HRT) was stepwise decreased from nine to four days while the organic loading rate (OLR) was increased from 2.0 to 4.4 kg. A similar study, but at mesophilic temperature, was initiated during autumn 2020.

In the present study, data from the completed thermophilic stress test was evaluated while the ongoing mesophilic stress test was monitored and analysed. Additionally, different methods for analysis of process efficiency and instability have been investigated. This served to fulfil subsequent goals.

1.1. Goals

These were the goals of the present study:

- Determine the efficiency of anaerobic digestion processes at short retention times.
- Evaluate parameters relevant for indication of process instability.
- Develop guidelines for monitoring of VFA and estimation of methane production, which can be used at Henriksdal WWTP.

2. Background

To reach sustainable development, we have to counteract anthropogenic changes of Earth's biophysical systems. Some of the systems currently under threat of severe, and possibly irreversible, alterations due to human activity are the natural carbon, nitrogen and phosphorous cycles (Rockström et al., 2009). Anaerobic digestion of sewage sludge could help reduce human impact on these systems through production of biogas as well as through the recovery of plant nutrients for agricultural use (SEPA, 2012).

Biogas is produced when organic material is anaerobically decomposed by microorganisms and is mainly made up of methane (CH_4) and carbon dioxide (CO_2). It is a process occurring in natural environments such as wetlands, rice paddies and in the stomach of ruminants – but also in environments created and controlled by humans, such as anaerobic digestion reactors. In such reactors, sludge can be digested and stabilised. Thus, the organic substances in the sludge are being decomposed and volume as well as unpleasant odour is reduced (SVAB, 2010). Biogas emerges as a by-product during anaerobic stabilisation of sludge which, in turn, may be used as a substitute to fossil fuels (SEPA, 2012).

The production of biogas involves several different microorganisms which are dependent on incoming material as well as on collaboration with each other. The sewage sludge entering the reactor is the substrate of which the microorganisms live. Apart from vitamins and trace elements, the substrate also contains organic and inorganic molecules which serve as carbon source, energy source as well as electron acceptors. These components are needed for the organisms to produce energy and carbon structures for growth and cell division.

2.1. Carbon, energy and electrons

The microorganisms in the biogas process use carbon sources that are either organic (carbohydrates, fats and proteins) or inorganic (CO_2), Table 1. As opposed to photosynthesising organisms, the microorganisms in the biogas process always use chemical energy as their energy source. The chemical energy can consist of inorganic compounds such as hydrogen gas (H_2) for so called lithotrophic organisms, or organic compounds such as sugar, fat or protein for organotrophic organisms (Plante et al., 2014). The molecule that is being reduced when it receives the electrons at the end of the electron transport chain is called terminal electron acceptor. Oxygen gas (O_2) is the terminal electron acceptor during aerobic respiration. When oxygen is absent, either anaerobic respiration or fermentation occurs. Anaerobic respiration takes place when the terminal electron acceptor is an inorganic compound e.g. nitrate (NO_3^-), manganese (Mn^{4+}), iron (Fe^{3+}), sulphate (SO_4^{2-}) or carbon dioxide (CO_2). If the terminal electron acceptor is organic, fermentation takes place accompanied by formation of acids, alcohols, hydrogen gas and carbon dioxide.

Table 1. Carbon sources, energy sources and terminal electron acceptors for microorganisms in the biogas process.

Carbon source	Energy source	Term. electron acceptor
‣CO_2 (autotrophy) ‣Organic compounds (heterotrophy)	‣Inorganic compounds: H_2 (lithotrophy) ‣Organic compounds: sugar, fat, protein (organotrophy)	‣O_2 (aerobic respiration) ‣NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2 (anaerobic respiration) ‣Organic compounds (fermentation)

The reduction of electron acceptors generates various amounts of energy to the microorganisms, where O_2 gives the most energy and CO_2 the least (Plante et al., 2014). This enables the microorganisms in the aerobic process to use the surplus energy to produce a lot of biomass, while as much as 90% of the substrate energy in the anaerobic process remains bound to the biogas (Koch et al., 2020). There is an abundance of CO_2 in the biogas process, which favours the methane producing microorganisms (methanogens) that use CO_2 as electron acceptor. The presence of other electron acceptors can reduce the methane production since the methanogens can be outcompeted by other microorganisms using the same substrate.

2.2. The microorganisms

The substrate entering the reactor consists of large molecules of polysaccharides, proteins and fats (Figure 2). These are being decomposed, fermented and oxidised by a number of different bacteria and fungi to smaller components, which the methanogens subsequently can use to produce biogas. The organisms conducting hydrolysis, fermentation and anaerobic oxidation constitute the majority of microorganisms in a biogas process, whereas the methanogens only make up a few percentages of the species (Schnürer et al., 2017). Acidic substances (e.g. fatty acids) are being created during the first two stages of the decomposition of organic compounds. These acidic substances are being converted to methane and carbon dioxide during the subsequent steps.

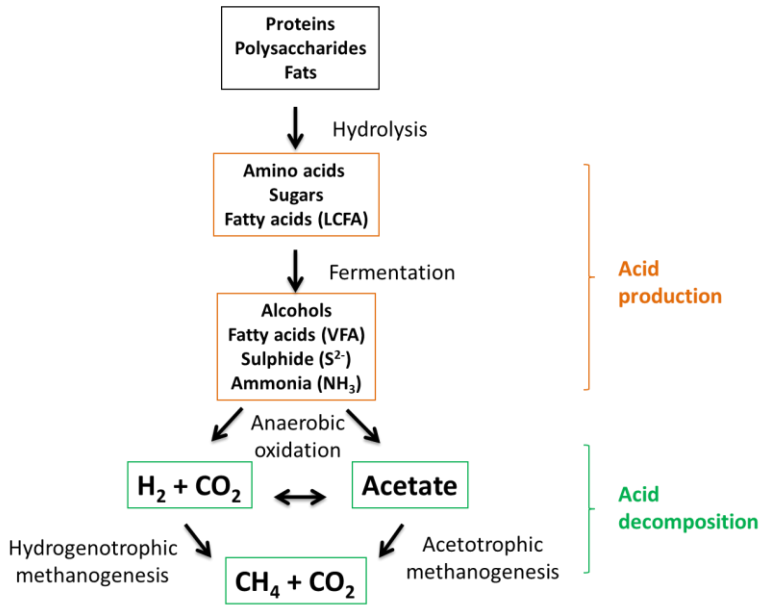

Figure 2. The key process steps of anaerobic digestion, after Schnürer et al. (2017).

Polysaccharides, proteins and fats are decomposed during the hydrolysis to e.g. glucose, amino acids and fatty acids. Sludge from the WWTP normally contains relatively stable compounds that may be difficult to degrade, such as cells from microorganisms that have been active during the aerobic biological treatment steps in the WWTP. The large amount of complex carbohydrates, fats and proteins with low degradability is the reason why the hydrolysis often is the rate-limiting step (Schnürer et al., 2017).

Glucose and amino acids are subsequently fermented to e.g. acids, alcohols, ammonia (NH_3), dihydrogen sulphide (H_2S), CO_2 and H_2 (Figure 2). The next step is the anaerobic oxidation, during which volatile fatty acids (VFA) and alcohols are being oxidised to H_2, CO_2 and acetate. The anaerobic oxidation uses protons (H^+) as electron acceptors, which generates H_2 under the premise that the hydrogen gas concentration in the reactor is low. Since the methanogens consume energy rich H_2 for their production of CH_4, these groups of organisms will collaborate to maintain a low hydrogen gas concentration. Failure of this collaboration, which is called inter species hydrogen transfer (IHT), normally results in accumulation of high amounts of fatty acids and alcohols (Schnürer et al., 2017).

Methanogenesis is the last step in the biogas process. This is where the methanogens produce biogas (CH_4 and CO_2). The methanogens can be defined as hydrogenotrophs and methylotrophs. The hydrogenotrophs use mostly H_2 and CO_2 whereas the methylotrophs use different types of methyl groups (e.g. acetate) to create methane. Acetate can also transform into H_2 and CO_2 through bacteria in so called syntrophic acetate oxidation (SAO). The generation time for the methanogens differs but the most common species of methanogen in a sludge process has twelve days generation time. This means that these methanogens face the risk of being flushed out of the process before being able to reproduce if the reactor retention time is shorter than twelve days. The methanogenesis is the most critical step in the biogas process since the methanogens are sensitive to different changes, such as in temperature, oxygen levels, salt, heavy metals, pH and reactor retention time (Table 2). A decreased methanogenesis leads to accumulation of VFA, which makes it an important indicator for process instability.

Table 2. Critical factors related to the different steps of the biogas process, after Schnürer et al. (2017).

Hydrolysis	Fermentation	Oxidation	Methanogenesis
‣High cellulose content decreases the degradation rate ‣A lot of easily degradable material causes fast degradation and accumulation of fatty acids	‣The process survives low pH and high oxygen levels	‣Failed IHT collaboration causes high levels of fatty acids and alcohols	‣Microbes do not survive temperatures above 60 °C ‣Microbes are sensitive to temperature changes, high salt levels, heavy metals and organic pollutants ‣Microbes are strict anaerobes that thrive in neutral pH ‣Microbes have up to twelve days generation time ‣Decreased methanogenesis causes accumulation of fatty acids

2.3. Temperature

The degradation of organic substances is faster at thermophilic than at mesophilic temperature and thermophilic digestion also bring the advantage of a natural hygienisation. However, thermophilic conditions also make the process more sensitive to disturbances. An inadvertent increase in temperature above the thermophilic temperature range can for instance kill off the microbes and the higher degradation rate can cause faster accumulation of toxic components. Ammonia, which is released during degradation of nitrogen rich materials, is in equilibrium with the innocuous species ammonium (NH_4^+). An increase in temperature leads to more of the toxic compound ammonia (Levén et al., 2012). The mesophilic microbial community can also more efficiently degrade some organic pollutants due to its higher microbial diversity in comparison to the thermophilic microbial community.

2.4. Organic loading and retention time

The amount of organic material added to the process per time and volume unit is called organic loading rate (OLR) and is measured as organic substance (volatile solids, VS) per reactor volume (m^3) and day (d). A normal loading rate for a mesophilic process is approximately 2-3 kg VS/(m^3,d) while a thermophilic process often can cope with a higher load (SVAB, 2010; Schnürer et al., 2017). The time needed to replace all material in the reactor is called hydraulic retention time and is normally between 15 and 40 days (Schnürer et al., 2017). The reactors at Henriksdal WWTP have currently an organic loading rate of 2-3 kg VS/(m^3,d) with a retention time of 16 days at mesophilic (37 °C) temperature and a VS level of 2-3%.

2.5. Substrate and biogas potential

The material added to a biogas process is called substrate. The composition of the substrate affects the stability and efficiency of the process as well as the amount and composition of the gas produced. Sludge from WWTPs normally have a methane yield of 0.16 - 0.35 m^3 CH_4 per kg added VS (Schnürer et al., 2017). In a study conducted by Jimenez et al. (2012) the composition of protein, carbohydrate and fat in mixed sludge from larger municipal WWTPs (>1.6 million person equivalents) in Europe were investigated. The average total amount of fat, protein and carbohydrate in mixed sludge amounted to 80 ± 7% of which ca 50% was protein, 40% carbohydrate and 10% fat. Other undefined organic material consisted mainly of humic acids.

The methane potential is the theoretical amount of methane that can be produced from a certain substrate. The maximum theoretical amount of methane produced from anaerobic digestion of fat, protein and carbohydrate can be calculated stoichiometrically through the Buswell equation (Chapter 3, equation 5). When the composition of the substrate is unknown, the general value of 0.9 m^3 biogas per kg decomposed VS can be used as an approximation (Ødegaard et al., 2009). Some factors affecting the actual amount of methane produced from a certain amount of degraded substrate are (i) the amount of energy used by the microbes for production of biomass and heat, (ii) the activity of the non-methane producing microbes and (iii) the composition of the substrate.

2.6. Inhibiting substances and alternative electron acceptors

Materials rich in protein contain nitrogen in form of amine groups (-NH_2) which during decomposition are released as ammonia and ammonium. Ammonium is the parameter often analysed at WWTPs. Indirectly inhibiting levels of ammonium nitrogen have been detected at 2-3 g NH_4^+-N/L, while

inhibiting levels of ammonia nitrogen have been detected at levels as low as 80 mg NH_3-N/L (Schnürer et al., 2017; Westerholm et al., 2016). Decomposition of protein is also releasing sulphide. Sulphide is in equilibrium with H_2S which is toxic to microorganisms at levels above 50 mg H_2S/L or 10 000 ppm H_2S in the gas phase (Haghighatafshar et al., 2012). Levels above 500 ppm H_2S in the biogas can also lead to corrosion of pipes and equipment while levels above 100 ppm are toxic to humans (Choudhury et al., 2019). Many chemical equilibrium states are governed by the pH in the surroundings. The equilibrium between H_2S and hydrogen sulphide (HS^-) will for instance gravitate towards more H_2S and the equilibrium between NH_4^+ and NH_3 shifts towards more NH_4^+ when pH drops.

If the substrate contains high levels of alternative electron acceptors to CO_2, such as NO_3^- or SO_4^{2-}, the risk of methanogens being outcompeted by nitrate and sulphate reducing microorganisms will increase since the microbes use the same energy sources and more energy can be derived from reduction of NO_3^- and SO_4^{2-} than from CO_2. Increasing levels of alternative electron acceptors could hence lead to lower biogas production. When the ratio chemical oxygen demand (COD)/ SO_4^{2-} is below 21, sulphate reducing bacteria have the potential to outcompete methanogens (Moestedt, 2015). Moreover, NO_3 levels above 62 mg/L and COD/NO_3 ratios between 2.0 and 3.7 are advantageous for denitrification bacteria (Schnürer et al., 2017; Sonza et al., 2005). Nitrate can be present in waste activated sludge from the nitrogen removing stage of the WWTP while sulphate is part of the flocculation chemicals used at the WWTPs.

2.7. Buffer systems and alkalinity

The stability of the biogas process is highly dependent on buffer systems resisting sudden changes in pH (Georgacakis, 1982). The buffer systems in a biogas process consist of VFA, bicarbonate and ammonium. Many anaerobic digestion processes have high ammonium concentrations and low VFA concentrations. Such systems are regulated by the bicarbonate buffer system, generating a pH between 6.5 and 8.5 (Georgacakis, 1982). The decomposition of nutrient rich substrates such as proteins increases the buffer capacity since the ammonia, released thorough nitrogen mineralisation, can react with carbon dioxide and create ammonium bicarbonate. Bicarbonate ions alone are measured as bicarbonate alkalinity (BA) while total alkalinity (TA) displays the combined effect of the different buffering systems active in the process.

2.8. Monitoring parameters

The efficiency of the anaerobic digestion process can be determined through the specific methane production as well as the degree of VS degradation. These parameters indicate how much of the incoming organic material that is degraded and how much that is converted to methane by the microbial community. The stability of the process is often determined by VFA and alkalinity. An increase in VFA indicates insufficient degradation of VFA or overloading. VFA accumulation will cause pH decrease and process instability if the alkalinity is low. A VFA/alkalinity ratio between 0.3 and 0.5 indicates some process instability whereas levels higher than 0.5 indicate marked instability (Schnürer et al., 2017). Lower methane and higher dihydrogen sulphide concentration in the biogas could indicate inhibition of methanogens and presence of sulphate reducing bacteria. Furthermore, high concentration of ammonium indicates that the level of ammonia might be inhibiting for methanogens as discussed above (Chapter 2.6).

2.9. Stress test

Stress tests of biogas processes can be used to determine how a process reacts to shorter retention times. In a study conducted by Moen et al. (2003) a mesophilic (35 °C) and a thermophilic (55 °C) biogas process were stress tested in completely mixed reactors (4 L). The reactors were fed with sludge from a municipal WWTP in Seattle, USA, and the solid retention time (SRT) was decreased from ten to six and four days, respectively. The organic loading rate increased from 2.0 to 5.3 kg VS/(m^3,d) in the thermophilic process and from 2.0 to 3.7 kg VS/(m^3,d) in the mesophilic process. The thermophilic process failed at four days SRT whereas the mesophilic process failed at six days SRT. Both failures were characterised by absence of steady-state conditions in combination with accumulation of VFA and decreased pH and methane concentration. The results indicate that methanogens can survive at shorter retention times in both thermophilic and mesophilic processes.

During the shortening of the SRT, the VS degradation, methane concentration, pH, alkalinity and ammonia decreased in both processes (Table 3). VFA did not increase until four days SRT in the thermophilic process. The VS degradation was higher in the thermophilic process than in the mesophilic, which could explain the higher ammonia, alkalinity and VFA in the thermophilic process. The higher pH in the thermophilic process could partly be explained by higher temperature decreasing the solubility of CO_2 and hence preventing formation of carbonic acid (Moen et al., 2003).

Table 3. Process parameters during SRT 10, 6 and 4 and difference between highest and lowest SRT at thermophilic and mesophilic temperatures, respectively (from Moen et al., 2003).

Parameter	**Thermophilic (55 °C) digestion**				**Mesophilic (35 °C) digestion**		
	SRT10	SRT6	SRT4	Diff. SRT10-SRT4 (%)	SRT10	SRT6	Diff. SRT10-SRT6 (%)
VS degradation (%)	57	56	49	-14	56	53	-5
Spec. methane prod. (m^3/kg red. COD)	0.52	0.46	0.43	-17	0.44	0.6	+36
pH	7.7	7.6	7.3	-5	7.4	7.2	-3
VFA/TA	0.14	0.14	0.41	+189	0.015	0.004	-74
- VFA (mg/L)	1300	1100	2700	+108	130	30	-77
- TA (mg/L)	9080	8130	6530	-28	8430	7480	-11
Methane conc. (%)	61	61	58	-5	61	58	-5
NH_3-N (g/L)	2.7	2.2	1.5	-43	2.4	2.1	-13

3. Process parameters

Specific organic loading rate (*Spec. OLR*) is the amount of volatile solids (*VS*) fed into the reactor per cubic meter and day, equation 1. Assuming a substrate density of 1 kg/L the *Spec. OLR* can be calculated according to:

$$Spec.\,OLR\ \left(\frac{kgVS}{m^3,d}\right) = \frac{VSin(\%)\times Qin\left(\frac{L}{d}\right)}{100\times RKvol(m^3)} \quad \text{[eq. 1]}$$

where *VSin* and *Qin* are influent VS and flow rate to the reactor, respectively, and *RKvol* is the volume of the reactor tank.

Hydraulic retention time (*HRT*) is equal to SRT for all single chamber reactors. *HRT* is calculated according to:

$$HRT(d) = \frac{RKvol(L)}{Qmean\left(\frac{L}{d}\right)} \quad \text{[eq. 2]}$$

where *Qmean* is the average of the flow in and out of the reactor.

The *Degree of VS degradation* shows how efficiently the substrate has been degraded in the reactor by comparing VS from incoming and outgoing sludge according to:

$$Degree\ of\ VS\ degradation\ (\%) = \left(\frac{Qin\times VSin - Qout\times VSout}{Qin\times VSin}\right)\times 100 \quad \text{[eq. 3]}$$

where *VSout* and *Qout* are effluent VS and flow rate out of the reactor, respectively.

The methane production from sewage sludge (*Estimated methane prod.*) based on VS reduction can roughly be estimated as follows:

$$Estimated\ methane\ prod.\left(\frac{m^3}{d}\right) = reduced\ VS\ \left(\frac{kg}{d}\right)\times 0.9\left(\frac{m^3}{kgVSr}\right)\times [CH_4] \quad \text{[eq. 4]}$$

where *reduced VS* is the amount of degraded VS per day, *0.9* is the volume of biogas that typically is produced per kg reduced VS during anaerobic digestion of municipal wastewater sludge (Ødegaard et al., 2009) and *[CH_4]* is the methane concentration.

The Buswell equation (equation 5) gives the theoretical specific methane yield for fat, carbohydrate, protein and COD in accordance with Table 4 (Angelidaki et al., 2011).

$$C_cH_hO_oN_n + \left(\frac{4c-h-2o-3n}{4}\right)H_2O \rightarrow \left(\frac{4c-h+2o+3n}{8}\right)CO_2 + \left(\frac{4c+h-2o-3n}{8}\right)CH_4 \quad \text{[eq. 5]}$$

Table 4. Theoretical specific methane yields from different substrates (after Angelidaki et al., 2011).

Substrate (S)	Theoretical specific methane yield (m^3/kg reduced substrate)
Fat ($C_{57}H_{104}O_6$)	1.014
Protein ($C_5H_7O_2N$)	0.496
Carbohydrate ($C_6H_{12}O_6$)	0.374
COD	0.35

The estimated methane production (*Estimated methane prod.*) based on reduced fat, protein, carbohydrate or COD is calculated according to:

$$Estimated\ methane\ prod.\left(\frac{m^3}{d}\right) = Sr\left(\frac{kg}{L}\right)\times Qin\left(\frac{L}{d}\right)\times Th.sp.methane\ yield\left(\frac{m^3}{kgSr}\right)\times 0.9 \quad \text{[eq. 6]}$$

where *Sr* is the amount reduced substrate, *Th. sp. methane yield* is the theoretical specific methane yield according to Table 4 and 0.9 is the methane production efficiency, i.e. the fraction of the reduced material used for methane production (Koch et al., 2020).

4. Methods

In the present study, stress test is defined as a test during which the retention time of the anaerobic digestion system is shortened whereas the organic loading rate is increased. This was achieved by increasing the influx of wastewater in the anaerobic digestion reactor. Wastewater from the inlet of Henriksdal WWTP was pre-precipitated in Hammarby Sjöstadsverk pilot plant to create primary sludge. Activated sludge originated from the membrane tanks substituting the secondary sedimentation. Characteristics of the mixed sludge, consisting of 60% primary sludge and 40% activated sludge, as well as the volume of the continuous stirred tank reactor (CSTR) used in the stress tests are presented in Table 5.

Table 5. Properties of mixed sludge and the rector volume.

TS mixed sludge (%)	**VS mixed sludge (% av TS)**	**Reactor volume (m^3)**
2.4	66.2	5.0

Mixed sludge was continuously fed into the reactor at thermophilic (55 °C) and mesophilic (37 °C) temperature, respectively. The retention time was stepwise decreased from nine to four days while the organic loading rate increased (Table 6). For each HRT regime, the reactor was operated for at least three full retention times to ensure that steady state was reached.

Table 6. Operating schedule for thermophilic and mesophilic stress test, including dates and weeks during which the respective HRT regime was in operation.

Thermophilic stress test			**Mesophilic stress test**		
Date	Week	HRT(d)	Date	Week	HRT(d)
Jul-Aug 2019	1-6	9	Jul-Sep 2020	1-7	9
Aug-Oct 2019	7-14	8	Sep-Oct 2020	8-11	8
Okt-Dec 2019	15-22	7	Oct 2020	12-14	7
Dec-Feb 2020	23-31	6	Nov 2020	15-17	6
Feb-Mar 2020	32-35	5	Dec 2020	18-20	5
Mar 2020	36-39	4	Jan 2021	21-24	4

4.1. Sampling stress test

Grab samples of incoming and outgoing sludge from the stress tests were collected from point P1 (mixed sludge) and P2 (reactor sludge) while gas composition was analysed at point P3 (gas) at Hammarby Sjöstadsverk pilot plant (Figure 3). Grab samples were collected two times every week and sludge as well as gas composition was analysed according to Table 7. 1 L of mixed sludge and reactor sludge, respectively, were additionally stored in freezer (-18 °C) as backup samples and for external analyses.

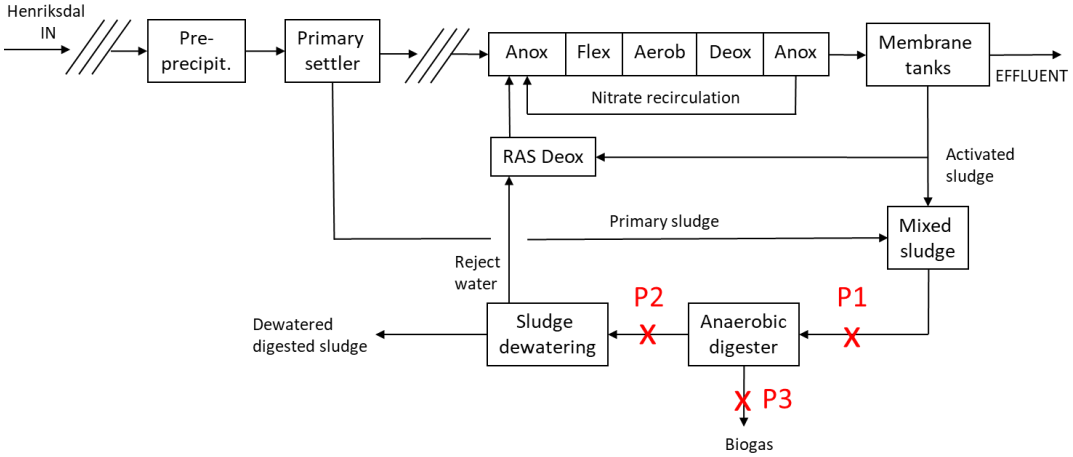

Figure 3. Grab sampling points for mixed sludge (P1), reactor sludge (P2) and biogas (P3) at Hammarby Sjöstadsverk pilot plant. The anaerobic reactor was used during two stress tests with varying retention times conducted at mesophilic and thermophilic temperature.

4.2. Analyses stress test

The thermophilic and mesophilic stress tests were analysed in accordance with Table 7, unit 1-6. Sludge from the reactor was analysed for VFA, TA and NH_4^+ at Hammarby Sjöstadsverk laboratory two times per week. The sample was centrifuged in Eppendorf® Centrifuge 5804 (Eppendorf AG, Hamburg, Germany) at 9700 rpm for 11 min, after which the liquid was filtered through 0.45 µm Ministart® filter (Sartorius AG, Göttingen, Germay). The sample was prepared according to Spectroquant® cell test (Merck Millipore, Burlington, United States) for VFA, TA and NH_4^+ and analysed in spectrophotometer WTW photoLab® 6600 UV-VIS (Xylem Analytics LLC, College Station, United States). Temperature and pH were analysed with pH meter WTW® pH 3110 (Xylem Analytics LLC, College Station, United States) on unfiltered sample immediately after sampling. Biogas production (m^3/d), methane (%), carbon dioxide (%), oxygen gas (%) and dihydrogen sulphide (ppm) was measured daily through gas meter Multitec® 540 (Sewerin GmbH, Gütersloh, Germany). TS on sludge from the reactor and mixed sludge tank was analysed two times per week through evaporation of water at 105 °C for 20 hours while VS was calculated after combustion at 550 °C for 2 hours. Ammonium in reactor sludge from Henriksdal WWTP was also analysed once at accredited laboratory (Eurofins Environment Testing Sweden AB) to get a reference value for ammonium at Henriksdal WWTP.

Estimated methane production. The estimated methane production during HRT 8, 7 and 5 in the mesophilic process was calculated based on (i) VS reduction, (ii) protein, fat and carbohydrate (PFC) and (iii) COD. VS reduction was analysed three times per retention time at Hammarby Sjöstadsverk laboratory according to Table 7, unit 3. PFC and COD were analysed three times per retention time (Table 7, unit 7) at accredited laboratory (Eurofins Environment Testing Sweden AB). Estimated methane production based on VS, PFC and COD were calculated according to equations 4 and 6 (Chapter 3). The estimated methane production was then divided by the actual methane production during HRT 8, 7 and 5 in the mesophilic process and presented as mean values.

Alternative electron acceptors. Sludge from the reactor and mixed sludge tank was analysed for COD, NO_3^- and SO_4^{2-} with Spectroquant® cell test at Hammarby Sjöstadsverk laboratory at four occasions during HRT 8 (Table 7 unit 8). The tests were conducted in order to determine potential presence of alternative electron acceptors in the sludge. NO_3^- as well as the ratios COD/ SO_4^{2-} and COD/NO_3^- were determined and compared to intervals within which alternative electron acceptors normally do not

compete with CO_2 as an electron acceptor. These levels are COD/ SO_4^{2-} ratios above 21, NO_3^- levels below 62 mg/L and COD/NO_3^- ratios above 3.7 (Moestedt, 2015; Schnürer et al., 2017).

Table 7. Analysis plan for mesophilic anaerobic digestion. Sampling point, frequency and method/protocol are presented for each analysed parameter. All analyses were made in singles.

Unit	Parameter	Sampling point (Fig. 3)	Frequency	Method/Protocol
1	pH, temp	P2	2 times/w	pH meter WTW® pH 3110
2	CH_4, CO_2, H_2S, O_2	P3	daily	Gas meter Multitec® 540
3	TS, VS	P1. P2	2 times/w	105 °C for 20 hours + 550 °C for 2 hours
4	VFA	P2	2 times/w	Volatile organic Acids Cell Test 50 - 3000 mg/L Spectroquant® (all VFA presented as acetate)
5	Soluble NH_4^+	P2	2 times/w	Ammonium Cell Test 4.0 - 80.0 mg/L NH_4-N Spectroquant®
6	TA	P2	2 times/w	Acid Capacity Cell Test to pH 4.3 (total alkalinity) 20 - 400 mg/L $CaCO_3$ Spectroquant®
7	Fat, protein, carbohydrate, COD	P1. P2	3 samples per retention time	Frozen samples were sent for analyses to Eurofins Environment Testing Sweden AB
8	Soluble COD, NO_3^-, SO_4^{2-}	P1. P2	4 samples during HRT 8	COD Cell Test 10 - 150 mg/L Spectroquant® Nitrate Cell Test 0.5 – 18.0 mg/L NO_3-N Spectroquant® Sulphate Cell Test 5 – 250 mg/L SO_4^{2-} Spectroquant®

4.3. Statistics

The investigated process parameters for each retention time are presented as mean values. Standard deviations of the parameters are presented in Appendix A2. A paired, one-tailed Student's t-test with a 95% confidence interval was used to determine statistically significant difference between the process parameters of the thermophilic and mesophilic processes at different retention times (Appendix A2).

4.4. Additional analyses VFA

Different VFA and TA analysis methods were investigated and compared. An accuracy test was also conducted for spectrophotometric VFA analysis.

VFA accuracy test spectrophotometer. The accuracy of Spectroquant® VFA cell test was investigated through reference samples of 80, 200, 800 and 1600 mg/L acetic and butyric acid. The reference samples were created by dilution of 96% acetic acid and 99% butyric acid (Merck Millipore, Burlington, United States). The samples were prepared according to the test method *Spectroquant® Volatile organic Acids Cell Test 50 - 3000 mg/L* and analysed in spectrophotometer WTW photoLab® 6600 UV-VIS.

VFA titration. Sludge from the mesophilic process was analysed through titration, whereby sludge was centrifuged at 9700 rpm for 11 min, after which the supernatant was filtered through Munktell Ahlstrom® filter paper 1002, 6-10 µm (Ahlstrom-Munksjö, Helsinki, Finland). 60-80 mL liquid was analysed for conductivity with WTW Portable Conductivity Meter ProfiLine® Cond 3110 (Xylem Analytics LLC, College Station, United States). Sludge was also analysed for pH and temperature. The

titrant (0.05 M HCl) was added using volumetric burette and the volume used titrant was registered at five pre-defined pH steps (pH 6.7, 5.9, 5.2 and 4.3). Ammonium, phosphate and sulphate were analysed at Hammarby Sjöstadsverk laboratory with Spectroquant® cell test (*Ammonium Cell Test 4.0 - 80.0 mg/L NH_4-N; Phosphate Cell Test 0.2 - 15.3 mg/L PO_4^{3-}; Sulphate Cell Test 5 – 250 mg/L SO_4^{2-}*) and used for calculation of VFA through an MS Excel version of the titration program TITRA5 (Vannecke, 2015).

VFA chromatography. Saved frozen sludge samples from the thermophilic stress test (three from each HRT) were analysed for VFA with high-performance liquid chromatograph Agilent® 1100 HPLC (Marshall Scientific, Hampton, United States) at SLU in Uppsala. Sludge was centrifuged in Eppendorf® Centrifuge (Eppendorf AG, Hamburg, Germany) at 12 000 rpm for 5 min after which 700 mL supernatant was mixed with 70 µL 36% sulphuric acid (H_2SO_4) and filtered through 0.2 µm filter. The filtrate was analysed in HPLC for VFA C2-C6 according to Westerholm et al. (2012).

5. Results och discussion

5.1. Stress test

Changes in HRT and OLR during the thermophilic stress test are displayed in Figure 4a. HRT successively decreased from nine to four days whereas the OLR increased from 2.0 to 4.4 kg VS/(d,m^3). Figure 4b displays the changes in HRT and OLR during the mesophilic stress test where HRT decreased from nine to four days and the OLR increased from 1.4 to 3.6 kg VS/(d,m^3). Each data point represents the average HRT or OLR for the corresponding week. The peaks in the data series emerge from calibration errors or operative errors of the pump system. Such weeks are exempted from subsequent calculations.

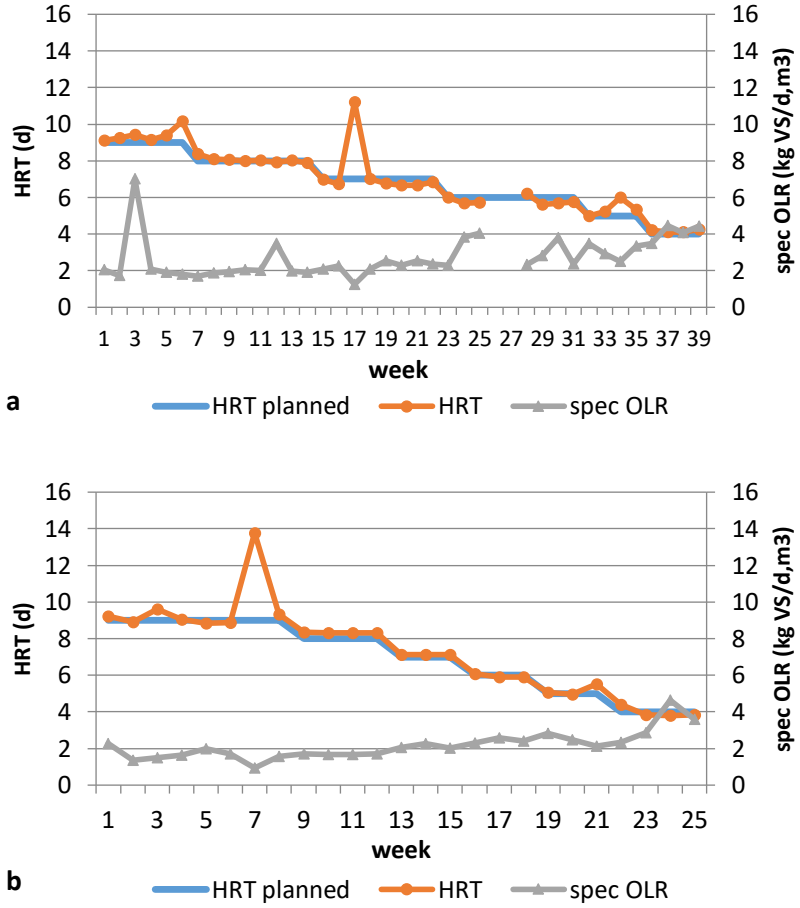

Figure 4. The blue lines represent the planned HRT during the (a) thermophilic and (b) mesophilic stress test. The orange lines represent actual HRT for each week during the tests whereas the grey lines show specific OLR for each week. HRT and OLR during week 26 and 27 in the thermophilic stress test are exempted due to pump failure.

Process parameters as averages for each retention time are displayed in Table 8. Standard deviation and statistical significance is presented in Appendix A2. The process parameters are compared to corresponding parameters at Henriksdal WWTP and normal intervals for anaerobic digestion of wastewater sludge. Normal intervals are defined as levels associated with stable processes at WWTPs (Schnürer et al., 2017; Bachman, 2015; Cioabla, 2012; Choudhury et al., 2019; Nègre & Jonsson, 2010). All measured parameters in both processes were suboptimal from a stability and efficiency perspective at four days HRT compared to Henriksdal WWTP. Alkalinity, methane concentration, pH, dihydrogen sulphide concentration and VS degradation did furthermore deviate from normal intervals during the stress tests.

Table 8. Process parameters during HRT 9 to 4 of the thermophilic and mesophilic stress tests, corresponding parameters at Henriksdal WWTP (HRT 16, yearly average), normal intervals for WWTPs and the difference between HRT 4 of the stress tests and Henriksdal WWTP. Number of measurements (N) varied between six and ten. Values for thermophilic specific methane production during HRT 9 and 8 are missing due to equipment failure.

Process parameters thermophilic digestion	Henriksdal WWTP HRT16	Normal interval	HRT9 N=8	HRT8 N=10	HRT7 N=10	HRT6 N=10	HRT5 N=6	HRT4 N=6	Difference HRT4-Henriksdal(%)
VS degradation (%)	50[4]	45-55[2]	52	48	32	46	42	49	-6
Spec. CH_4 prod. (m^3/kg added VS)	0.31[4]	0.16-0.35[1]	-	-	0.21	0.22	0.16	0.16	-48
pH	7.2[4]	6.8-7.2[3]	6.84	6.74	6.75	6.90	6.71	6.58	-7
VFA/TA	<0.03[4]	0-0.3[1]	0.09	0.06	0.08	0.06	0.08	0.08	+166
- VFA (mg/L)	<100[4]	50-500[6]	146	107	118	129	104	110	+10
- TA (mg/L)	3400[4]	3000-5000[6]	1779	1598	1486	2161	1257	1454	-57
CH_4 (%)	66[4]	63-67[2]	61	60	59	59	57	54	-18
H_2S (ppm)	-	0-100[5]	49	126	232	113	190	190	-
NH_4^+-N (mg/L)	900	0-2000[1]	281	322	313	435	316	253	-72
Process parameters mesophilic digestion	Henriksdal WWTP HRT16	Normal interval	HRT9 N=8	HRT8 N=8	HRT7 N=6	HRT6 N=6	HRT5 N=6	HRT4 N=6	Difference HRT4-Henriksdal(%)
VS degradation (%)	50[4]	45-55[2]	41	36	41	29	28	32	-35
Spec. CH_4 prod. (m^3/kg added VS)	0.31[4]	0.16-0.35[1]	0.22	0.22	0.20	0.21	0.20	0.18	-42
pH	7.2[4]	6.8-7.2[3]	6.63	6.52	6.46	6.48	6.52	6.56	-9
VFA/TA	<0.03[4]	0-0.3[1]	0.06	0.06	0.07	0.07	0.12	0.12	+313
- VFA (mg/L)	<100[4]	50-500[6]	72	70	60	55	78	102	+2
- TA (mg/L)	3400[4]	3000-5000[6]	1233	1225	890	825	662	822	-76
CH_4 (%)	66[4]	63-67[2]	58	58	54	57	60	59	-10
H_2S (ppm)	-	0-100[5]	101	166	179	133	98	79	-
NH_4^+-N (mg/L)	900	0-2000[1]	185	190	231	172	170	139	-85

Ref.: [1]Schnürer et al., 2017 [2]Bachman, 2015 [3]Cioabla, 2012 [4]Hellström et al., 2009 [5]Choudhury et al., 2019 [6]Nègre & Jonsson, 2010

● equivalent to Henriksdal WWTP ● within normal interval ● deviating from normal interval

Adaptation and efficiency. Both processes produced CH_4 at down to four days retention time (Table 8). This proves that the microbial community could adapt to the shorter retention time and that viable methanogens can have a generation time of four days. Previous studies (Fernandez-Rodriguez et al., 2014; Moen et al., 2003; Nges & Liu, 2010) have shown that anaerobic digestion of municipal wastewater sludge and organic material is possible at four days retention time under thermophilic conditions. The present study, however, proved that it also is possible for a mesophilic process to survive a retention time of four days. The survival of the mesophilic microbial community could be explained by the lower organic loading rate in the present study compared to the previous studies, which brings lower risk of VFA accumulation and pH decrease (Peces et al., 2020). In comparison to Henriksdal WWTP, the thermophilic and mesophilic processes displayed 42-48% lower specific CH_4 production. Although there was a substantial decrease in specific CH_4 production, and hence efficiency, the levels stayed within normal intervals for biogas processes at WWTPs.

Instability indicators. Alkalinity was among the first parameters in both processes to deviate from normal intervals (Table 8). At four days retention time the alkalinity was 57-76% lower than at Henriksdal WWTP. The decrease in alkalinity could probably be attributed to less degradation of nitrogen rich compounds which leads to low NH_4^+ levels in the processes and hence lower alkalinity in terms of ammonium bicarbonate. As a consequence of the decreased alkalinity, the ratio VFA/TA was 166-313% higher at four days retention time in comparison to Henriksdal WWTP. The ratio did nonetheless stay below 0.3, indicating absence of process instability (Schnürer et al., 2017). VFA stayed at low levels during the stress tests (Table 8). The low and rather stable VFA concentrations were probably a consequence of the low degradability of the substrate and the relatively low organic loading rate.

Inhibition. CH_4 concentration was in both processes below normal levels at HRT 9. The lower CH_4 concentration could indicate inhibition of the methanogens. Alternatively, it could also be a result of declined pH which decreases the concentration of CH_4 in the gas phase (Schnürer et al., 2017). The increased level of H_2S could indicate increased activity of sulphate reducing microorganisms at the expense of methanogenic activity. The high levels of H_2S during the stress tests could also be a consequence of decreased pH, which shifts the equilibrium between H_2S and HS^- towards more H_2S. The NH_4^+ levels were moreover within normal intervals (Table 8) in the thermophilic and mesophilic process, respectively, which leaves out inhibitory effects from NH_3 on the microbial community.

Thermophilic vs mesophilic process. In accordance with the study of Moen et al. (2003), the thermophilic as compared to the mesophilic process displayed higher degree of VS degradation, TA and pH. No significant difference could be seen for specific CH_4 production (Table A5, Appendix A2). The higher degree of VS degradation might be attributed to the higher overall rate of degradation which is a result of higher temperature (Moen et al., 2003). The higher degree of VS degradation in the thermophilic process ensures more stabilised sludge and lower risk of fermentation and odours in comparison to the mesophilic process. More degradation of nitrogen rich substances results in higher NH_4^+ levels. The higher NH_4^+ levels contributed to higher TA and pH in the thermophilic process. Another contributing factor to the higher pH is higher temperature decreasing the solubility of CO_2 and subsequent carbonic acid formation (Moen et al., 2003). In accordance with a previous study by Nges & Liu (2010), the mesophilic process displayed higher CH_4 concentration than the thermophilic process at short retention times. The higher CH_4 concentration in the mesophilic process might be caused by (i) better adaptability to short retention times in the mesophilic compared to the thermophilic community, (ii) lower organic loading rate and thus reduced risk of overloading in the mesophilic process or (iii) lower temperature in the mesophilic process which increases the solubility of CO_2 and hence increases the CH_4 concentration in the gas phase.

Alternative electron acceptors. Investigation of alternative electron acceptors in the mixed sludge (MS) tank and the reactor in the mesophilic process revealed that none of the investigated parameters (soluble NO_3^-, COD/NO_3^- and COD/SO_4^{2-}) were beyond the intervals recommended to avoid competition from nitrate and sulphate reducing microorganisms (Schnürer et al., 2017; Moestedt, 2015), Table 9. The presence of nitrate and sulphate did, however, show that nitrate and sulphate reducing microorganisms could exist in the process. Among the investigated parameters the ratio soluble COD/SO_4^{2-} in the reactor was the parameter closest to exceed the recommended intervals.

Table 9. Levels of alternative electron acceptors in the mixed sludge (MS) tank and the reactor during the mesophilic process (standard deviations in parentheses).

Parameter	Rec. interval	MS tank	Reactor
NO_3^- (mg/L)	<62[1]	0.8 (0.2)	1.1 (0.1)
COD/NO_3^-	>3.7[1]	598 (136)	294 (62)
COD/SO_4^{2-}	>21[2]	45 (4)	27 (5)

[1]Schnürer et al., 2017 [2]Moestedt, 2015

5.2. Method comparison

Estimated methane production. Estimations of methane production based on VS in the mesophilic process were calculated using the standard value 0.9 m^3 biogas per kg reduced VS (Ødegaard et al., 2009). Estimations based on protein, fat and carbohydrate (PFC) and COD were furthermore calculated using theoretical methane yields for PFC and COD (Chapter 3, Table 4) and a methane production efficiency of 90% (Koch et al., 2020). Figure 5 shows that the ratios between estimated and measured methane production were on average 87±17% for calculations based on VS, 102±9% for PFC and 122±23% for COD. Hence, estimations based on PFC were closest to the measured methane production (+2%) while estimations based on VS were 13% lower and estimations based on COD were 22% higher than the measured methane production. The reason why estimations based on COD differed a lot from the measured value could be that measurements of COD in substrate rich materials such as sludge is more difficult than e.g. water and wastewater since the sludge requires homogenisation and dilution before COD measurements can be conducted (Raposo et al., 2011). The analyses are based on one grab sample per week. More frequent sampling would be necessary to ensure a representative result. The benefits of increased accuracy in estimation of methane production based on PFC must be balanced against the high analysis costs and time consuming sampling regime.

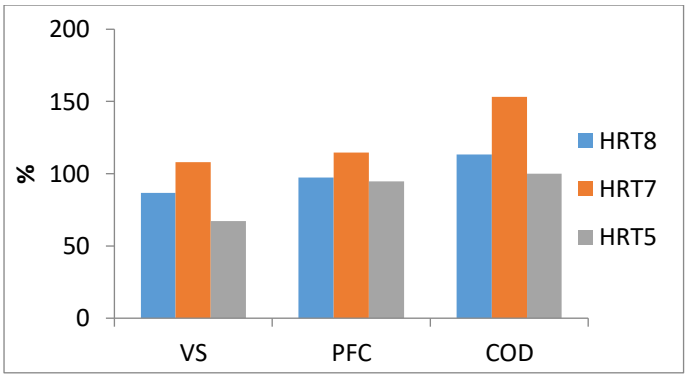

Figure 5. The bars represent average (N=3) estimated methane production (m^3/d) for each HRT based on VS, PFC (protein, fat and carbohydrate) and COD as percentage of measured methane production on corresponding dates during HRT 8, 7 and 5 in the mesophilic process.

VFA monitoring. An investigation of the spectrophotometric method showed that it detected acetate with high accuracy (101%) at concentrations 80, 200, 800 and 1600 mg/L, Table 10. However, the spectrophotometric method did only detect 66% of the propionate at the same concentrations. Since the spectrophotometric method presents all VFAs as acetate, this might be a result of differences in molar weight between acetate and propionate (Eastman and Ferguson, 1981).

Table 10. Accuracy of spectrophotometric measurements (N=4) of the VFAs acetate and propionate (standard deviations in parentheses).

VFA	Accuracy (%)
Acetate	101 (0.8)
Propionate	66 (1.5)

Results from HPLC and corresponding spectrophotometric measurements of VFA are compared at four different occasions during the thermophilic stress test (Figure 6a). Based on the accuracy data in Table 10 and species distribution obtained from the HPLC analysis, the spectrophotometric results could be

adjusted to correct for the inaccuracy. These results suggest that the spectrophotometric method is more accurate at low VFA levels (below ca 200 mg/L) and that HPLC is more accurate at VFA concentrations above ca 200 mg/L. Results from titration measurements and corresponding spectrophotometric measurements are compared at 26 different occasions during the mesophilic stress test (Figure 6b). The titration measurements show a low correlation (r^2=0.004) with the spectrophotometric measurements at VFA concentrations below ca 100 mg/L, whereas the correlation is higher (r^2=0.88) at VFA concentrations higher than ca 100 mg/L. The spectrophotometric method, rather than HPLC or titration, is hence preferable at Henriksdal WWTP which normally has VFA levels below 100 mg/L.

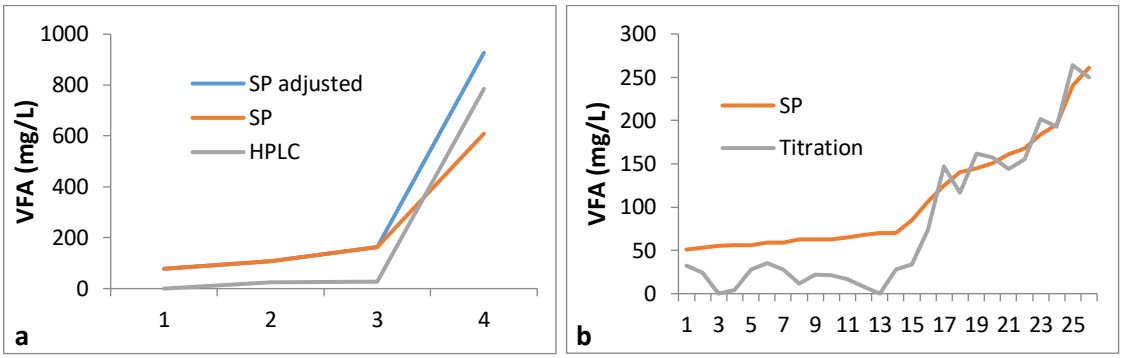

Figure 6. VFA concentrations detected with (a) spectrophotometer (SP) and HPLC at four different occasions (N=1). Adjusted VFA concentrations from the spectrophotometric analyses (SP adjusted) are based on accuracy data from Table 10 and species distribution from the HPLC analyses. VFA concentrations detected with (b) spectrophotometer (SP) and titration at 26 different occasions (N=1).

6. Conclusion and recommendations

The present study shows that the mesophilic and thermophilic process both survived four days of retention time. This suggests that the microbial community in the biogas process can adapt to very short retention times. The successful microbial adaptation to short retention times is likely partly attributed to the high amounts of degradation resistant material in wastewater sludge and the relatively low organic loading rate, which averted overloading of the system and accumulation of VFA. The stepwise decrease of the retention time did also give the microbial community time to adapt to the new environment. Under similar circumstances, it could hence be possible for Henriksdal WWTP to run the anaerobic digestion system at down to four days retention time without system failure.

The shorter retention time did, however, reduce the specific methane production with 42-48% and alkalinity with 57-76% during the thermophilic and mesophilic stress tests as compared to full-scale Henriksdal WWTP. Processes operated at very short retention times will therefore be more sensitive to acidic substances and less methane will be produced per unit substrate. In order to avoid potential process failure and economic loss, it would not be recommended to stay at such short retention times for longer time periods. This knowledge could be useful when planning for maintenance of reactors. Further studies are needed to determine how fast it is possible to reduce the retention time without process failure as well as how the processes react to a subsequent increase in retention time.

The thermophilic and mesophilic processes showed no significant difference in specific methane production at short retention times. However, the thermophilic process displayed higher degree of VS degradation and alkalinity than the mesophilic process, which indicates that the thermophilic process stabilises sludge better and has lower sensitivity to acidic substances. Hence, thermophilic anaerobic digestion could be more suitable for short retention times. Monitoring of the alternative electron acceptor sulphate is furthermore recommended since high levels were detected in the reactor during the present study. Future studies with DNA analysis for microbial community profiling could determine the actual abundance of different microorganisms in the digestion processes.

The results from the present study also suggest that (i) VFA should be analysed with spectrophotometry rather than liquid chromatography or titration at VFA levels common in digested sludge at Henriksdal WWTP and (ii) estimation of methane production is more accurate if based on fat, protein and carbohydrate rather than on volatile solids reduction.

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Appendix

A1. Methane potential

Decomposition of PFC (protein, fat and carbohydrate) and COD as well as calculated methane potential based on the Buswell equation (equation A1) are presented in Table A1-2. Decomposition of VS and calculated methane potential based on 0.9 m^3 biogas per kg reduced VS are presented in Table A3.

$$C_cH_hO_oN_n + \left(\frac{4c-h-2o-3n}{4}\right)H_2O \rightarrow \left(\frac{4c-h+2o+3n}{8}\right)CO_2 + \left(\frac{4c+h-2o-3n}{8}\right)CH_4 \quad \text{[eq. A1]}$$

Table A1. Decomposition of protein, carbohydrate and fat as well as potential and actual methane production.

	Decomposition (kg/d)				**CH_4 (m^3/d)**	
HRT	protein	carbohydrate	fat	total	potential	actual
8	0.7	3.4	0.4	4.5	2.0	1.9
7	1.0	4.6	0.2	5.8	2.4	1.9
5	0.7	1.5	1.3	3.5	2.2	2.1

Table A2. Decomposition of COD and potential and actual methane production.

		CH_4 (m^3/d)	
HRT	decomposition (kg COD/d)	potential	actual
8	6.8	2.4	1.9
7	9.1	3.2	1.9
5	6.7	2.3	2.1

Table A3. Decomposition of VS and potential and actual methane production.

		CH_4 (m^3/d)	
HRT	decomposition (kg VS/d)	potential	actual
8	3.1	1.6	1.8
7	4.3	2.2	2.1
5	4.4	1.7	2.5

A2. Standard deviations and statistical significance

Standard deviations of the results from the stress tests are presented in parentheses in Table A4.

Table A4. Process parameters during thermophilic and mesophilic stress tests (mean values with standard deviations in parentheses, N is the number of analyses).

Process parameters thermophilic digestion	HRT9 N=8	HRT8 N=10	HRT7 N=10	HRT6 N=10	HRT5 N=6	HRT4 N=6
VS degradation (%)	52 (5)	48 (10)	32 (11)	46 (10)	42 (7)	49 (11)
Spec. CH_4 prod. (m^3/kg added VS)	-	-	0.21 (0.03)	0.22 (0.03)	0.16 (0.04)	0.16 (0.02)
pH	6.84 (0.01)	6.74 (0.04)	6.75 (0.05)	6.90 (0.04)	6.71 (0.05)	6.58 (0.08)
VFA/TA	0.09 (0.02)	0.06 (0.02)	0.08 (0.01)	0.06 (0.00)	0.08 (0.00)	0.08 (0.01)
- VFA (mg/L)	146 (29)	107 (9)	118 (17)	129 (18)	104 (17)	110 (4)
- TA (mg/L)	1779 (79)	1598 (191)	1486 (210)	2161 (292)	1257 (111)	1454 (155)
CH_4 (%)	61 (0.3)	60 (1.1)	59 (0.6)	59 (1.5)	57 (0.6)	54 (0.2)
H_2S (ppm)	49 (5)	126 (25)	232 (103)	113 (9)	190 (23)	190 (25)
NH_4^+-N (mg/L)	281 (2)	322 (39)	313 (45)	435 (29)	316 (93)	253 (24)
Process parameters mesophilic digestion	HRT9 N=8	HRT8 N=8	HRT7 N=6	HRT6 N=6	HRT5 N=6	HRT4 N=6
VS degradation (%)	41 (14)	36 (1)	41 (1)	29 (6)	28 (11)	32 (16)
Spec. CH_4 prod. (m^3/kg added VS)	0.22 (0.05)	0.22 (0.01)	0.20 (0.00)	0.21 (0.01)	0.20 (0.01)	0.18 (0.03)
pH	6.63 (0.04)	6.52 (0.03)	6.46 (0.03)	6.48 (0.02)	6.52 (0.03)	6.56 (0.01)
VFA/TA	0.06 (0.02)	0.06 (0.00)	0.07 (0.01)	0.07 (0.01)	0.12 (0.03)	0.12 (0.01)
- VFA (mg/L)	72 (19)	70 (9)	60 (8)	55 (6)	78 (19)	102 (9)
- TA (mg/L)	1233 (124)	1225 (99)	890 (33)	825 (25)	662 (28)	822 (32)
CH_4 (%)	58 (1.3)	58 (0.3)	54 (5.5)	57 (3.1)	60 (0.6)	59 (0.2)
H_2S (ppm)	101 (23)	166 (8)	179 (12)	133 (12)	98 (5)	79 (11)
NH_4^+-N (mg/L)	185 (16)	190 (9)	231 (37)	172 (4)	170 (7)	139 (7)

A paired, one-tailed Student's t-test with a 95% confidence interval was used to determine if there was a statistically significant difference between the process parameters of the thermophilic and mesophilic processes (Table A5).

Table A5. P-values derived from a paired, one-tailed Student's t-test comparing mesophilic and thermophilic stress tests at HRT 9-4. Black values indicate statistically significant difference (α=0.05).

Process parameters	p-value HRT9	p-value HRT8	p-value HRT7	p-value HRT6	p-value HRT5	p-value HRT4
VS degradation (%)	0.18	0.07	0.25	0.05	0.20	0.04
Spec. CH_4 prod. (m^3/kg added VS)	-	-	0.19	0.44	0.12	0.29
pH	0.01	0.01	0.01	0.00	0.04	0.35
VFA/TA	0.08	0.36	0.02	0.10	0.09	0.03
- VFA (mg/L)	0.02	0.02	0.01	0.00	0.19	0.12
- TA (mg/L)	0.03	0.13	0.00	0.00	0.01	0.01
CH_4 (%)	0.05	0.10	0.15	0.28	0.04	0.00
H_2S (ppm)	0.05	0.00	0.20	0.18	0.02	0.01
NH_4^+-N (mg/L)	0.00	0.02	0.26	0.00	0.09	0.01