

DRY ANAEROBIC DIGESTION OF FOOD WASTE

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Dry anaerobic digestion of food waste at mesophilic and thermophilic temperature

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Foreword

The project has been conducted within the Energiforsk programme Biofuels for Sweden 2030 (Biodrivmedel för Sverige 2030), with the goal to contribute to the development of biofuel production for the transportation sector and a fossil free transportation fleet by 2030.

The programme has been financed by EON Gas Sverige AB, Gasnätet Stockholm AB, Göteborg Energi AB, Neste AB and the Region Skåne.

Within this project dry anaerobic digestion of food waste at mesophilic and thermophilic temperature has been evaluated. The report has been produced by IVL Swedish Environmental Research Institute AB, Swedish University of Agricultural Sciences (SLU), RISE Research Institutes of Sweden AB, Linköping University and the authors are Emelie Persson (IVL), Maria Westerholm (SLU), Anna Schnürer (SLU/ Linköping University), Annika Nordin (SLU), Daniel Tamm (RISE) and Ulf Nordberg (RISE). Emelie Persson (IVL) has led the project. The project has been performed in collaboration with Härnösand Miljö & Energi AB, Gästrike Ekogas AB, Labio Ltd, Västblekinge Miljö AB and Tekniska verken i Linköping AB. This work was also co-financed by the Foundation for IVL Swedish Environmental Research Institute, Swedish University of Agricultural Sciences, Linköping University, Avfall Sverige AB, Hitachi Zosen Inova AG and Thöni Industriebetriebe GMBH.

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These are the results and conclusions of a project, which is part of a research programme run by Energiforsk. The author/authors are responsible for the content.

Sammanfattning

Torrötning i pluggflödesreaktorer är en relativt ny metod för biologisk behandling av matavfall. För att undvika kostsam extern hygienisering vill de flesta svenska torrötningsanläggningarna använda termofila driftsförhållanden. Emellertid har källsorterat matavfall, med sitt höga kväveinnehåll, en ökad risk att orsaka ammoniakinhibering vid rötning vid högre temperaturer.

Detta projekt studerade hur driftstemperaturen påverkar mikrobiell sammansättning, hygienisering, processtabilitet och energieffektivitet vid befintliga torrötningsanläggningar som använder matavfall som huvudsubstrat. En hypotes i projektet var att drift vid hög mesofil temperatur (42–44 °C) skulle kunna bidra till att minska problem med ammoniakinhibering och till att uppnå hög processeffektivitet. Frågan var då om den höga ammoniakkoncentrationen kombinerad med det något högre temperaturintervallet var tillräckligt för att uppnå en bra hygieniseringseffekt.

Processdata och substrat- och biogödselprover från fyra torrötningsanläggningar samlades in och analyserades. Alla anläggningarna använde matavfall som huvudsubstrat, men drevs vid olika temperaturer (38–39, 42 och 54 °C). Den mikrobiella sammansättningen analyserades med DNA-sekvensering. Material från en anläggning användes för att undersöka överlevnad av indikatororganismer vid olika temperaturer och ammoniakkoncentrationer. Inverkan av olika driftstemperaturer på energiförbrukning och kostnader bedömdes, där anläggningarnas egna mätningar kompletterades med teoretiska beräkningar.

Den mikrobiella analysen visade att varje process i den föreliggande studien hade olika mikrobiella profiler och att drifttemperaturen starkt påverkade den mikrobiella strukturen. Resultaten indikerade att olika hydrolytiska grupper var aktivt involverade i det första steget av den anaeroba nedbrytningen. Alla processer drevs också på en ammoniaknivå som är känd för att hämma metanogener som direkt använder acetat för metanbildning. Den mikrobiella sammansättningen stödde detta och visade att en väte-användande metanogen (*Methanoculleus bourgensis*) hade en viktig roll för effektiv metanproduktion i alla processer.

Hygieniseringsanalysen visade att en temperatur >42 och <48 °C kan vara tillräcklig för att nå patogenreduktion enligt ABP-förordningen. Det betyder att vid hög ammoniaknivå kan det vara möjligt att nå tillräcklig hygienisering även vid hög mesofil / låg termofil temperatur. Att kunna sänka processtemperaturen något från 52–55 °C kan ge betydande processfördelar. För att bekräfta resultatet krävs dock fler studier. De teoretiska beräkningarna av värmebehov för olika processtemperaturer visade att om inget pasteuriseringssteg skulle behövas för att uppnå hygienkrav enligt ABP-föreskrifterna för hög mesofil temperatur (42 °C), skulle 16–25 % mindre värmebehov jämfört med mesofil (39 °C) eller termofil (52 °C) rötning behövas.

Höga nivåer av propionat i de termofila processerna uppvisades, vilket starkt indikerar att höga temperaturer i kombination med höga ammoniaknivåer ger ogynnsamma betingelser för propionat-nedbrytande mikroorganismer i de undersökta torrrottningsprocesserna. Metoder för att stödja hydrogenotrofa metanogener och propionat-nedbrytande mikroorganismssamhällen skulle avsevärt förbättra stabiliteten och metanutbytet för termofil rötning. Även om en anläggning är konstruerad och planerad att användas under termofila förhållanden kan det vara lämpligt att förbereda sig för att använda en lägre processtemperatur för att få stabilitet om den visar sig inte fungera. Detta kan medföra att extern efter-pasteurisering krävs.

Anläggning hos Labio, som drivs vid 42 °C, visades vara den mest effektiva processen med avseende på metanutbyte. Vad som är unikt för Labio är processtemperaturen och den stora mängden biogödsel som recirkulerades i rötkammaren. Således indikerar föreliggande studie att dessa faktorer är viktiga för en god processprestanda.

Summary

Dry plug flow anaerobic digestion (AD) is a relatively new technology for the biological treatment of food waste. To avoid costly external hygienisation, most Swedish dry AD plants wish to operate under thermophilic conditions. However, source separated organics (SSO) with high nitrogen content increase the risk for ammonia inhibition at higher temperatures.

This project studied how the operating temperature affects microbial community composition, sanitation, process stability and energy efficiency at existing dry AD plants using food waste as main substrate. One hypothesis within the project was that operation at a high mesophilic temperature (42–44 °C) could help to reduce ammonia inhibition problems and obtain a high process efficiency. The question was then if the high ammonia combined with the slightly higher temperature interval was enough to achieve a good hygienisation effect.

Process data, substrate and digestate samples from four dry AD plants were collected and analyzed. All plants used SSO as their main substrate, but were operated at different temperatures (38–39, 42 and 54 °C). The microbial community structure was analysed using DNA sequencing. Material from one plant was used to investigate the survival of indicator organisms at different temperatures and ammonia concentrations. The influence of different operating temperatures on energy consumption and costs was assessed, where on-site measurements at the plants were complemented with theoretical calculations.

The microbial community analysis demonstrated that each process in the present study had different microbial profiles and that operating temperature strongly influenced the community structure. The results indicated that different hydrolytic groups were actively involved in the first step of the anaerobic degradation. All processes also operated at an ammonia level that is known to inhibit methanogens that directly use acetate for methane formation. The microbial community supported this and showed that a hydrogen-utilizing methanogen (*Methanoculleus bourgensis*) had an important role for efficient methane production in all processes.

The hygienisation analysis indicated that a temperature >42 and <48 °C can be sufficient to reach pathogen reduction according to the ABP regulation. This means that at high ammonia level it might be possible to reach sufficient sanitation even at a high mesophilic/low thermophilic temperature. Being able to lower the process temperature slightly from 52–55 °C can give significant process advantages. However, to confirm this results more studies are needed. The theoretical calculations of heat demand for different process temperatures showed that if no pasteurization step was needed to achieve hygienic standards according to the ABP regulations for high mesophilic temperature (42 °C), 16–25 % less heat demand compared to mesophilic (39 °C) or thermophilic (52 °C) AD was achieved.

High levels of propionate in the thermophilic processes were demonstrated, strongly indicating that high temperatures in combination with high ammonia levels provided unfavorable conditions for propionate degrading microorganisms

in the investigated dry AD processes. Management approaches to support hydrogenotrophic methanogens and propionate degrading communities would substantially improve stability and methane yield for thermophilic AD. Even if a plant is designed and planned to be operated at thermophilic conditions, it might be advisable to prepare for using a lower process temperature to get stability if it does not work. This may require external post-pasteurization.

The Labio plant, operating at 42 °C, was demonstrated to be the most efficient process in regard of methane yield. What is unique for Labio is the process temperature and the large volume of digestate that was recirculated in the digester. Thus, the present study indicates that these factors are important for a good process performance.

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1 Introduction

1.1 BACKGROUND

Over the years, different concepts have been developed for dry digestion. The process can be batch-wise or continuous and can be divided into a number of phases and reactor types. Continuous processes are currently the dominating process design for waste management. During recent years several biogas plants with plug-flow digesters (for dry digestion) have been built in Sweden and several are under planning. The currently operating plants use food waste as main substrate and several of them were initially prospected to operate the process under thermophilic conditions in order to avoid an external hygienisation unit, which would increase the cost and technical complexity. However, in the existing plants, this was proved to be problematic due to the fact that thermophilic digestion of food waste alone involves a risk of ammonia inhibition causing process instability.

Ammonium is formed during protein degradation and is in equilibrium with ammonia, which is inhibitory to microorganisms in the biogas process. High ammonium levels can be problematic for all biogas processes but are particularly difficult for thermophilic processes as the high temperature shifts the equilibrium toward a higher level of the toxic ammonia. However, at the same time an elevated level of ammonia may also have a positive effect in an operation perspective as it can contribute to a sanitation effect on pathogenic microorganisms in the process.

An option to reduce the problem with high ammonia levels during thermophilic operation is to mix the food waste with other substrates such as park and garden waste and/or water, which gives a diluting effect and reduces the ammonia concentration. However, this approach increases the operating cost for the plant and reduces the nutrient concentration in the digestate. Another strategy to circumvent ammonia inhibition is to lower the temperature to mesophilic level, which would require an external hygienisation unit. Moreover, a plant designed for thermophilic operation and changing to mesophilic operation might reduce the digestion capacity and as a consequence the biogas production might be reduced in comparison with forecasted production.

To reduce the risk for inhibition during dry digestion of food waste it is interesting to look at the possibility to operate the process at a lower temperature. Mesophilic digestion is of course an option however might as mentioned result in negative consequences in terms of sanitation and methane yield. Another possibility would be to use a temperature in-between the traditional temperature ranges of mesophilic and thermophilic operation. Operation at this in-between temperature, 42-44 °C, might give a higher degradation rate compared to conventional mesophilic digestion and at the same time reduce the ammonia level as compared with thermophilic digestion. With a relative high ammonia level this temperature might still allow a sufficient hygienisation.

1.2 PURPOSE AND GOAL

The purpose of the project was to investigate the influence by operating temperature on dry digestion of food waste and for hygienisation of the digestate. The hypothesis of the study was that a high mesophilic operating temperature ($>37\text{ }^{\circ}\text{C}$) could reduce the problem with ammonia toxicity, that often is observed at thermophilic temperature, and still provide a sufficient hygienisation effect. The participating plants were studied during a 3 months period.

Following the project's implementation, the following has been done:

- Collect data and evaluate performance of four different full-scale dry digestion plants operating at mesophilic ($37\text{ }^{\circ}\text{C}$), thermophilic ($52\text{ }^{\circ}\text{C}$), or a “high mesophilic temperature level of about $42\text{ }^{\circ}\text{C}$.
- Microbiological analyses were conducted to analyse the influence on the microbial communities by different temperature in the dry digestion plants.
- Evaluate the hygienisation effect at different temperature by investigating the decline time of different indicator organism.
- Estimate energy consumption at the different temperature conditions ($39\text{-}40$, 42 and $52\text{ }^{\circ}\text{C}$) in order to get an indication of the importance of operational temperature for cost efficient biogas production.

2 Theory

2.1 DRY DIGESTION VERSUS WET DIGESTION

Anaerobic digestion can be set up with various configurations depending on substrate and expected outputs. The most commonly used design in Sweden and Europe is the continuously stirred tank reactor (CSTR), used for treatment of various materials including sludge from wastewater treatment plants, slaughterhouse waste, food waste, manure or other industrial waste streams, crops and crop residues (Scarlat et al., 2018). The process is used for materials with dry solids content between a few percent and up to about max 10–15 % and the material is continuously pumped into the reactor or fed in a semi-continuous manner. Dilution is made by addition of water or other liquids with a low content of organic material, such as juices, dairy waste waters etc. An alternative way of handling material with a high TS content (>15 %) is to use dry digestion. Different types of reactor concepts can be applied for this type of digestion and it can be operated in both batch and continuous mode (Kotahari et al 2014). A common reactor design is the plug flow reactors in which the substrate is pushed through a horizontal reactor with a screw or with rotating baffles (Figure 1). The outflow is often de-watered, and the resulting liquid is often recirculated back in order to inoculate new material with microorganisms, improve stirring of the material as well as conditions suitable for microbial growth and nutrient transportation (Li et al., 2014). A water content of at least 65 %, i.e. a maximum of 35 % dry solids, is usually referred to as the limit for maintaining good microbial activity (Kothari et al 2014). Although this type of process is not completely mixed, there are studies that show that both the chemical and microbial composition can be reasonably uniform (Li et al., 2014).

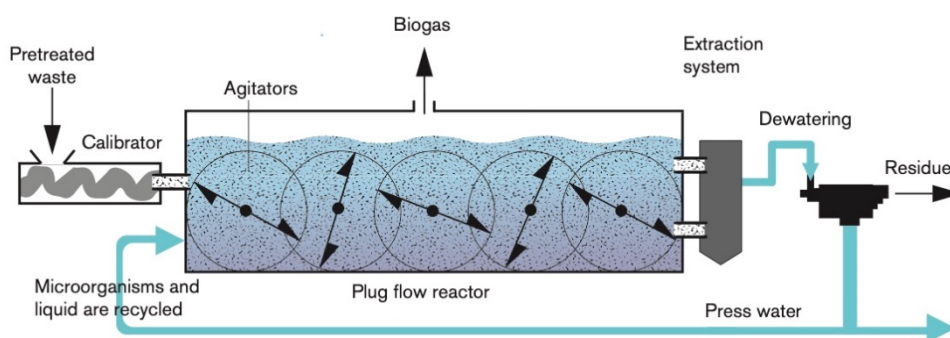


Figure 1. Example of process for a plug flow dry digestion plant. (Source: Schnürer A, Jarvis Å. (2018). Microbial handbook of the biogas process. SLU ISBN 978-91-639-3406-3.)

Many different types of feedstocks have been successfully used for biogas production in the dry digestion process, such as different crops and crop residues, manure and the organic fraction of municipal organic waste. The performance of the process is robust and allows equal or higher loading rates than the CSTR process (Kotahari et al 2014). There are several advantages to this technology over

the wet digestion systems, such as the need for less reactor volume, which reduces the material cost and need for heating (Kothari et al., 2014). Moreover, the digestion residue produced has comparatively lower water content and thus also higher nutrient content per unit wet weight.

The technology is well established in for example Germany, using mainly material from kitchen and yard waste collection (“Grüne Tonne”) containing a high share of structure material. During recent years, dry digestion has becoming more established also in Sweden where source separated organic kitchen waste with a low content of structure material and high levels of nitrogen prevails. Therefore, it is common practice to mix the kitchen waste with shredded yard waste in order to obtain the desired structure. Dry digestion of food waste decreases the need of pre-treatment and post-treatment is instead commonly used to separate unwanted material from the digestate.

2.2 TEMPERATURE AND AMMONIA LEVEL - IMPORTANT PROCESS PARAMETERS

Temperature, together with substrate, is the most strongly determining parameter for stability and process performance. Temperature impacts both strongly on community structure and diversity, as well as on the degradation pathways and rates (Banks et al., 2012; Labatut et al., 2011; Mao et al., 2015; Zhang et al., 2014). Biogas production can proceed at a wide range of temperatures, but the most commonly used ranges are the mesophilic (ca 35–42 °C) and the thermophilic (ca 50–55 °C) temperature range. However, biogas is also produced in processes operating at temperatures between mesophilic and thermophilic conditions (Moestedt et al., 2014; Westerholm et al., 2015). Studies have also shown that it is possible to shift from mesophilic to thermophilic temperature and vice versa (Westerholm et al., 2018). Processes operating at mesophilic temperature are generally considered to be more stable and less sensitive to inhibitory components such as ammonia and LCFA (long chain fatty acid produced during degradation of lipids) (Schnürer et al., 2017). Thermophilic temperatures on the other hand have the potential to give high methane production rates and methane yield, which could allow shorter retention time in the process. However, this is not always the case. Moreover, thermophilic digestion results in comparatively higher reduction of pathogens (Bagge et al., 2005; Sahlström, 2003). Operation at thermophilic temperature is then enough as sole sanitisation method. Disadvantages with higher temperatures include higher risks for ammonia inhibition, lower microbial diversity, with an accompanying risk of a less stable process and less efficient degradation (Schnürer et al., 2017; Schnürer & Jarvis, 2018). Moreover, a higher process temperature might need higher energy input in the form of heating. Thus, when optimising an AD system for net energy production, the additional energy required to raise and maintain feedstock and digester contents at a specific temperature must be taken into account.

Ammonia levels are another strong impact parameter for biogas processes. Ammonium (NH_4^+) is released during degradation of proteins. The level of ammonium-nitrogen in the process depends on the substrate composition and on the degree of mineralisation of the process, i.e. the proportion of organic material

converted to methane. A high content of ammonium provides the process with alkalinity and increases the value of the digestate as a fertilising agent and can also contribute with a hygiensation effect (Ottoson et al., 2008). However, unfortunately high level of ammonium can also cause instability of the process causing accumulation of VFA, decreasing methane yields and sometimes even process failure. The cause of the problem is inhibition of the microorganisms by elevated levels of ammonia (NH_3), in equilibrium with ammonium (NH_4^+). Temperature and pH indirectly affect the level of inhibition, since these parameters shift the equilibrium of ammonium (NH_4^+) and ammonia (NH_3) towards the latter. Consequently, thermophilic processes are more prone to suffer from inhibition than processes operating at a lower temperature. Inhibition has been reported to occur at varying concentrations ranging from 53 to ca 1500 mg NH_3/L , depending on operational conditions (Rajagopal et al., 2013)(Rajagopal et al 2013, esterholm et al 2016). However, if the microbial community is allowed to acclimatise, efficient biogas production can precede at ammonia levels even >1000 mg/L. Other studies of dry digestion processes have for example shown possibilities of stable biogas production at thermophilic conditions and at >1.5 g/L (Goberna et al., 2009; Huang et al., 2017).

2.3 ANAEROBIC DEGRADATION AND THE MICROBIAL COMMUNITY

The microbial process leading to biogas comprises the main degradation steps hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Figure 2) and this process has to be efficient and balanced in order to obtain successful anaerobic digestion. The first step is performed by hydrolytic bacteria, and possibly also fungi, that convert polymers (polysaccharides, lipids, proteins, etc.) into soluble monomers (long-chain fatty acids, glycerol, amino acids, sugars, etc.) (Kazda et al., 2014; Schnürer, 2016). The hydrolytic reaction is mediated by extracellular enzymes secreted by bacteria and/or attached to their cell wall. In the second step (acidogenesis), soluble monomers are further degraded to intermediate products, such as volatile fatty acids (e.g., acetate, propionate, butyrate, lactate, valerate, and caproate), alcohols, formate, hydrogen (H_2), and carbon dioxide (CO_2) (Kandylis et al., 2016). In the third step (acetogenesis), a group of bacteria called acetogens use the products from hydrolysis/acidogenesis to generate acetate, H_2 , and CO_2 as main products (Drake et al., 2008). In biogas digesters there are two major groups of methanogens, the acetoclastic methanogens that cleave acetate into CH_4 and CO_2 and hydrogenotrophic methanogens using CO_2 and H_2 /formate to form methane. There are also methanogens that convert methylated compounds (e.g. methanol, methylamines, and methylsulfides) to methane (Costa & Leigh, 2014; Liu & Whitman, 2008).

Acetate-utilizing methanogens is believed to contribute with a high proportion of methane production in various biogas systems. However, the acetoclastic methanogens are easily inhibited by intermediates such as ammonia or long-chain fatty acids, formed during AD of protein- and lipid-rich materials. This results in appearance of microbial competitors for acetate and promote the development of syntrophic acetate oxidation (SAO) (Westerholm et al., 2016). The SAO reaction involves oxidation of acetate to H_2 and CO_2 or formate by syntrophic acetate-

oxidizing bacteria, followed by consumption of these products by hydrogenotrophic methanogens for the generation of methane.

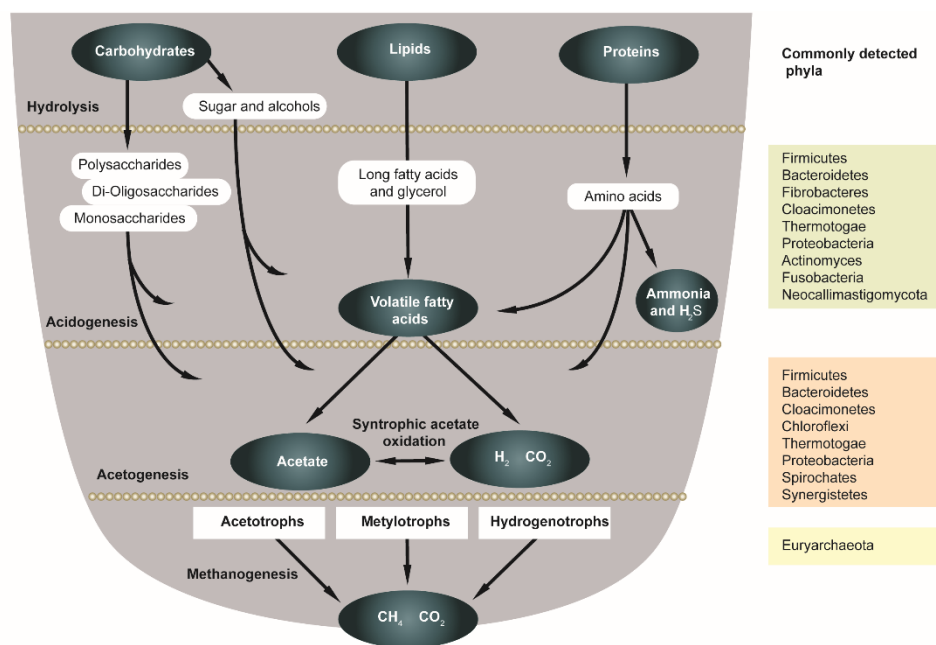


Figure 2. Anaerobic degradation of carbohydrates, lipids and proteins and the phyla commonly reported to be involved in the different steps in biogas digesters. The figure is modified from Westerholm et al (2019).

2.3.1 Analysis of microbial community

The microbial community in biogas digesters is very complex and comprises many different species of microorganism, involved in the different degradation steps described above. The composition of this community is shaped by various parameters, as the ingoing substrate and the operational parameters, such as temperature, organic load and retention time. During the last 10 years many studies have been devoted to the analysis of microbial communities with the aim to understand connection between community structure and function of the biogas processes (Westerholm and Schnürer 2019). Even so, this type of research can be said still to be in its infancy and more research is needed to fully understand the connection between microbiology and function. Different methods can be used to generate knowledge regarding the microbial community in biogas processes, using either cultivation or molecular methods. In present study, the microbial community, both bacteria and archaea, was analyzed in the digesters using a DNA-based technique called Illumina sequencing. In this approach, DNA is extracted from digester samples and a conserved gene that is present in all microorganisms (i.e. the 16S rRNA gene) is sequenced. The order of different nucleotide bases in the gene is mapped and this information is then used to identify different species. The identification is done by comparing the obtained DNA sequences with other sequences in databases. The number of sequences obtained from a certain species is then summed and this value indicates the

abundance of this group relative to the total microbial community in the digester. This procedure makes it possible to identify already known species, but a lot of microorganisms not yet known are also often found. However, if a sequence has been found earlier in other studies a hypothetical function can be available for the microorganism, even though it has not been confirmed in cultivation studies. A more accurate description of the sequencing and identification methods used can be found in some recently published scientific articles (Callahan et al., 2016; Westerholm et al., 2018). If the microorganisms have been discovered and characterized previously their potential role in the process can be anticipated and also provide suggestions of how the digester can be managed in order to improve its performance.

The Illumina analysis was done to show the general development of the microbiological community over time and to analyze differences between the digesters operating at different temperatures. To compare the results obtained from the sequencing of samples from the different reactors, the following analyzes were made:

- Taxonomic barplot. The taxonomic barplots show the structure of the microbial communities in the different digesters at different time points. In these plots the dynamics can be followed, and the relative abundance of different taxonomic groups are shown. The different taxonomic levels used in this study are:

Kingdom – Phylum – Class – Order – Family – Genus

- Principal Component Analysis (PCA): This is a statistical method used in this study to compare the entire microbial community between different sampling points and between different digesters. The analysis shows whether or not there have been changes in the microbial community structure over time and the similarity/divergence of the communities in the different reactors. Two different methods commonly used to estimate variance in community structure are weighted or unweighted UniFrac. Weighted (quantitative) accounts for abundance (relative abundance) of observed organisms and thus examines differences in community structure, whereas unweighted (qualitative) is based on their presence or absence and is more sensitive to differences in low-abundance features. In present study weighted UniFrac was used since this method was anticipated to most accurately show the differences between the reactors.

- Diversity analysis: Diversity can be divided into two parts, richness and evenness. Richness indicates the number of different sequences in a sample. Evenness is the distribution between different species, i.e. if there is a high abundance of a few species the evenness will be low. Diversity can be calculated with different indices and in this report the diversity indices Shannon and Simpson have been used.

3 Participating biogas plants

3.1 SUMMARY OF PARTICIPATING PLANTS

Four dry-digestion plants participated in this study, where one is situated in Finland (Labio) and three are located in Sweden (VMAB, HEMAB and Ekogas). All these plants use organic household waste as their main substrate and have a plug-flow reactor design. At the time for this study, two of them operated at mesophilic temperature (38–39 °C), one of them at a slightly higher mesophilic temperature (42 °C) and one at thermophilic temperature (54 °C) (Table 1). HEMAB, Labio and Ekogas hygienized the substrate/digestate, whereas VMAB had no hygienisation process during the test period (Table 1). The plant operating at a high mesophilic temperature (Labio) used post-composting of the digestate at 70 °C as a sanitation method. The thermophilic plant (Ekogas) used in-situ/internal hygienisation in the digester.

Table 1. Process temperature, method for hygienisation and size of participating dry-digestion plants.

Plant	Process temperature	Method for hygienisation	Size (ton/year)
HEMAB	Mesophilic, 39 °C	1/3 of the digester volume constitutes of a plug-flow digester operated at 60 °C with 20-25 d HRT. (The plant is now rebuilt to use post-pasteurization with hygienisation at 70 °C, 1 hour)	6 000
VMAB	Mesophilic, 38 °C	No hygienisation. (The plant is now rebuilt to use post-pasteurization with hygienisation at 70 °C, 1 hour)	13 000
Labio	High mesophilic, 42 °C	Hygienisation in a separate, last compost unit after digestion and composting, where the temperature is elevated to 70 °C, 1 hour.	26 000
Ekogas	Thermophilic, 53.5 °C	Internal hygienisation in the thermophilic digester.	17 000

3.2 HÄRNÖSAND ENERGI & MILJÖ AB

The biogas plant of Härnösand Energi & Miljö AB (HEMAB) was opened in January 2017 and has the capacity to treat up to 6000 ton of source sorted organic waste per year from the municipality (Figure 3). The produced biogas is upgraded to vehicle fuel quality, the upgrading capacity being approximately 5.2 GWh/a or 375 000 kg vehicle fuel yearly. In the plant food waste is pre-treated by grinding,

followed by anaerobic digestion in two serial digestion units, with the second digester having half the length/volume of the first digester. The produced biodigestate is separated in a liquid and a solid phase in a screw press. The solid phase is used for final covering of the local landfill and the liquid phase is currently being certified (SPCR120) for use by local farmers as fertilizer.

The anaerobic digestion process is operated at mesophilic (39 °C) temperature in the first digester, while the second, smaller digester is used for plug flow hygienisation at 55–60 °C. Currently, the plant is prepared for being extended with a separate post-hygienisation unit for hygienisation at 70 °C for 1 hour. This will free up further capacity for mesophilic anaerobic digestion in the second digester. Supplier of the plant is Eisenmann.

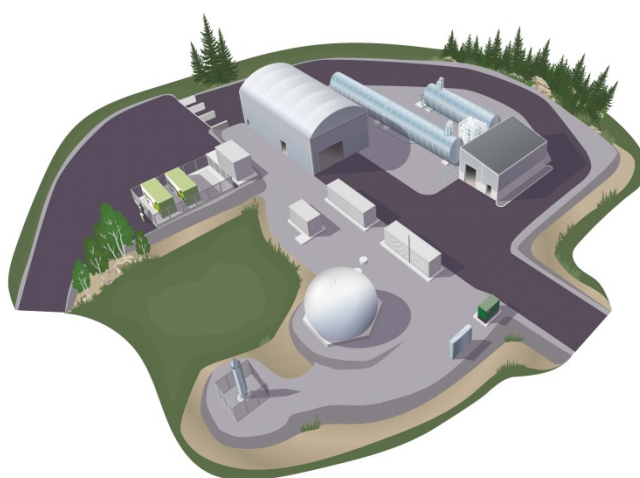


Figure 3. Illustration of the biogas plant of Härnösand Energi & Miljö AB. (Source: Härnösand Energi & Miljö AB)

3.3 VÄSTBLEKINGE MILJÖ AB

The biogas plant in Mörrum at Västblekinge Miljö AB (VMAB) opened in 2013 and was the first of its kind in Scandinavia, using a dry-digestion process to treat the source sorted organic waste from the own and surrounding municipalities. The plant is designed to treat 20 000 ton of organic waste per year and currently treatment reach 13 000 ton of food waste per year. The biogas is upgraded to reach vehicle fuel quality and correspond to around 14–15 GWh. The biodigestate is aimed to replace conventional fertilizers but is at the moment used for covering the local landfill.

At VMAB the source separated organic waste and garden waste pass through a grinder and a star sieve before entering one of the two parallel anaerobic digesters (VMAB1 respectively VMAB2) (Figure 4). In the star sieve unwanted material, such as plastic, foil and bigger pieces, are separated and removed from the organic material. During the test period no post-treatment of the biodigestate was done as this part of the plant was under reconstruction. This means that no separation of the biodigestate in liquid and solid phase, nor hygienisation, was done. However, after this work is finished, separation of the biodigestate into liquid and solid phase will be resumed. The obtained solid fraction will then be sent to incineration

for energy recovery and the liquid fraction will pass a hygienisation step with pasteurization at 70 °C for 1 hour.

The plant has been operated both at thermophilic and mesophilic temperature. During the period of October and November 2017 the operation temperature was changed from thermophilic to mesophilic, due to process instability. The temperature change was made in small steps with lowering the temperature with about 0.5 °C/day. Supplier of the plant is Eisenmann and key processes has been modified and rebuilt by Västblekinge Miljö AB.

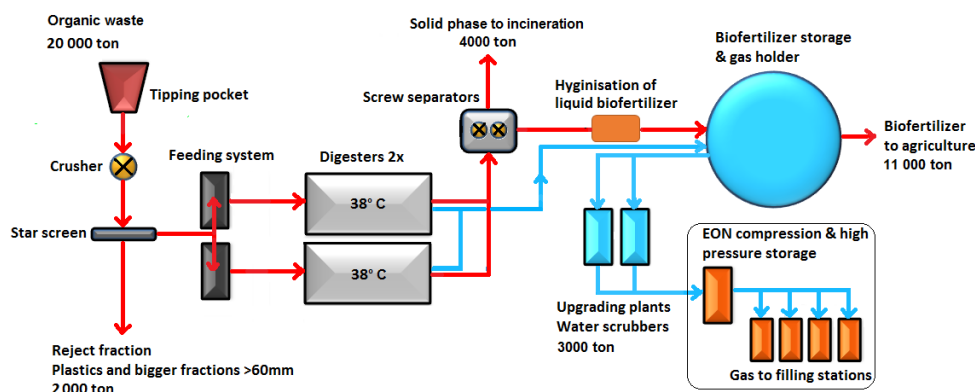


Figure 4. Schematic process diagram for the biogas plant of Västblekinge Miljö. (Source: Västblekinge Miljö AB)

3.4 GÄSTRIKE EKO GAS AB

The biogas plant of Gästrik Ekogas AB (Ekogas) was opened in May 2017 and has the capacity to treat 17 000 ton of organic material, mainly source sorted organic waste from households, per year. The biogas is upgraded to reach vehicle fuel quality. In the plant food waste is pre-treated by a homogenizing mixer and transported with a hydraulic press into the digester.

The produced biodigestate is separated in a liquid and solid phase. The solid phase then passes post-treatment by composting in a tunnel and reprocessing of compost. Both the solid and the liquid phase is certified (SPCR120) for use in agriculture as fertilizer.

The anaerobic digestion process is operated at thermophilic (54 °C) temperature. Supplier of the plant is Thöni.

3.5 LABIO OY

The biogas plant of Labio Oy (Labio) in Lathi, Finland, was commissioned in 2014 and treats 26 000 ton of food waste per year during which about 50 GWh biogas is produced. In the plant food waste is pre-treated by disintegration, mixer screws and spiral sieve before transportation by screws into one of the 4 parallel digesters, each with a volume of 900 m³. In the spiral sieve the material is separated into two fractions, where the fraction < 80 mm is fed into the digesters and the fraction > 80

mm goes directly to the composting facility of the plant. The produced biodigestate is post-treated by composting in tunnels. Hygienisation takes place in a separate, last compost tunnel after digestion and composting, where the temperature is elevated to 70 °C during at least 1 hour. Supplier of the plant is Eisenmann.

4 Methods

4.1 DATA COLLECTION AND CALCULATION OF KPI'S

Operating data from the four participating plants were collected between March 1st and May 31st, 2018. The data were provided by the plant operators taken from their online logging systems (Table 2). The data collected included both digesters at VMAB (VMAB1 and VMAB2) and two out of four digesters at Labio (Labio3 and Labio4).

For VMAB, data was also collected between July and October 2017, to follow the transition from thermophilic to mesophilic operation temperature and from January to February 2018. In the first period, the plant operated at 52.0 °C and the temperature was then gradually decreased to 37.7 °C in end of October. The decrease of temperature was made by turning off the heating system of the digesters, giving a decrease of 0.5–1 °C decrease per day. The process was at this point set to rest by stopping the feeding and no analyses of performance were done during November and December 2017. The feeding and performance analyses were initiated again in January 2018 and a data from a five months period (January to May 2018) were included in this study to investigate the subsequent operation at mesophilic temperature condition.

Table 2: Main parameters included in the collected online log files.

Parameter	HEMAB	VMAB1 VMAB2	Ekogas	Labio3 Labio4
Amount of feed	X	X	X	X
Digester temperature	X	X	X	X
Active volume / level in digester	X	X	X	
CH ₄ content in biogas	X	X	X	X
CO ₂ content in biogas	X	X		X
H ₂ S content in biogas	X	X		
Raw gas production	X	X	X	X
Electricity consumption for digestion and hygienisation units	X ¹	X ³		X ⁴
Heat consumption for digestion and hygienisation units	X ²	X ³	X	X ⁵

1. Includes not only digester and hygienisation units, but also pumps, ventilation, control room, feeding and lightning.
2. Reported separate for digester respectively hygienisation step.
3. Reported as monthly average (for the other plants reported as daily average).
4. Includes not only digester and hygienisation units, but also some auxiliary systems (lightning, reception hall etc). Reported as sum for all 4 digesters. The value for one digester is assumed as ¼ of the reported sum.
5. Reported as sum for all 4 digesters, excluding hygienisation. The value for one digester is assumed as ¼ of the reported sum.

Data collected from the different plants combined with additional analysis of substrate and digestate were used to calculate the below listed parameters (Table 3).

Table 3: Calculated parameters for each plant.

Parameter	Explanation
VFA _{sum} (mg/l)	Sum of acetic, propionic, n-butyric, isobutyric, valerianic and isovalerianic acids
TS _{comp} (%)	TS content after digester, corrected for VFA with a volatility factor of 98 % [Error! Reference source not found.] : $TS_{comp} = TS + 0.98 \cdot VFA_{sum}$
VS _{comp} (%)	VS content after digester, corrected for VFA: $VS_{comp} = (TS \cdot VS + 0.98 \cdot VFA_{sum}) / TS_{comp}$
TS and VS in feed (ton)	Feed wet weight multiplied with TS and VS.
Active volume (m ³)	Geometrical calculation when log files contained liquid level in digester.
OLR (kg VS/m ³ ·d)	Organic load rate (amount of fed VS per time and active volume).
Specific methane production (Nm ³ CH ₄ /ton VS)	Amount of methane per unit of fed VS.
Volumetric methane production (Nm ³ CH ₄ /m ³ ·d)	Amount of methane per digester volume and time.
Degradation (%)	Share of VS mineralized in the digester: $Degr = 1 - \frac{TS_{comp} \cdot VS_{comp}}{TS_{in} \cdot VS_{in}}$
HRT (d)	Hydraulic retention time, assuming a density of 1000 kg/m ³ . $HRT = \frac{\text{active volume}[m^3]}{\text{feed per day}[ton]}$
Ammonia (g/l)	$NH_3 = \frac{NH_4}{1 + 10^{0.09018 + \frac{2729.92}{T[K]} - pH}}$ (according to (Hansen et al., 1998))

4.1.1 General about calculation of KPI's

The calculation of key performance indicators (KPIs) for the participating plants was made using median values obtained during the 3 months' test period from 1st of March to 31st of May 2018.

If not explicitly stated, the recirculated material of Labio (see chapter 5.1) is not considered in the calculation of KPIs. The same applies for the added percolate (a mix of leachate and rain water from the substrate storage plate) at the plant of Ekogas. The recirculated biodigestate at Labio constitute about 45 % (by wet weight) of the total material fed into their digesters. At Ekogas the added percolate constitutes 8–9 % (by wet weight) of total fed material. In calculation of HRT, for example, added percolate/recirculated biodigestate is considered (Figure 9).

Compensation for loss of VFA in the VS-analysis, VS_{comp} (Table 3), was made for the VS values determined for the digester/in outgoing digestate. No such correction could be done for the substrates as no VFA-analysis was made for the incoming material. This means that for the KPI regarding VS reduction in the digester (Figure 9), VS was compensated for VFA loss for the values regarding VS out of the digester, but not for the values of VS into the digester. For the KPIs regarding methane yield (Figure 6) and OLR (Figure 7) no compensation of VFA for the VS values was made as these KPIs only include VS content of incoming substrate.

4.1.2 KPI's for energy demand

The specific energy consumption regarding electricity and heat per ton substrate treated of the plants was calculated based on the on-line measurements of electricity and heat made at the plants. The data for heat consumption included hygienisation for the plants at HEMAB (separate plug flow reactor) and Ekogas (thermophilic digestion with internal hygienisation in the digester), but not for VMAB (composting as hygienisation step) and Labio (separate hygienisation after composting). For electricity consumption, the scope varied between the plants: HEMAB's data included the pretreatment process, Labio's data included some auxiliary systems such as lightning and reception hall, while the data for the other plants only applied for the digester system. Data of electricity consumption at Ekogas were not available during the experimental period.

4.2 SUBSTRATE AND PROCESS AID

Food waste was the main substrate (77-100 %) at all participating plants. Additionally, VMAB2 received rape husk (5 %) and Ekogas received grease trap sludge (3 %) and yard waste (19 %). Labio added a small amount of wastewater treatment sludge during the first two weeks of the test period, constituting 2 % (by wet weight) of their total substrate mix during the test period. In the reported amounts of food waste from Labio, some industrial organic waste, such as filter cake from vegetable oil refinery, is also included. However, the amount of the industrial organic waste was not measured at the plant and the share is thus unknown. Ekogas added "percolate" (a mix of leachate and rain water from their substrate storage plate), accounting for 8–9 % of total fed volume. This is not

accounted for as substrate because it contributed with negligible VS content but it is included in the calculation of the hydraulic retention time (HRT) for the plant. Labio recirculated part of the digestate, accounting for about 45 % (by wet weight) of the material fed into their digesters. The other plants had no recirculation of digestate during the test period.

All the participating plants, except Labio, are using some process aids in order to improve digestion and/or to abate hydrogen sulphide formation (Table 4).

Table 4: Process aid addition for the participating plants as average values during test period.

Plant	Process aid additions	Purpose	Amount (kg/ton substrate)
HEMAB	Iron hydroxide (dry)	H ₂ S reduction	0.5-2
VMAB1	Kemira BDP100	Trace elements	2
	Schaumann BC TePlex Safe	Trace elements	0.09
	Schaumann BC Atox Scon	H ₂ S reduction	0.4
VMAB2	Schaumann BC TePlex Safe	Trace elements	0.45
	Schaumann BC Atox Scon	H ₂ S reduction	1.8
Ekogas	Biobeta	Trace elements	0.15
	Iron	H ₂ S reduction	1
	Zeolite	NH ₄ reduction	0.3
Labio	No process additions during test period		

4.3 SAMPLING

Digester content and substrate samples for analytical analyses were collected from the plant every second week and stored at -20°C (or on ice during transporting) until further use.

4.3.1 Analytical analyses

Total solids (TS) and volatile solids (VS) were determined according to international standard methods (APHA, 1998). Volatile fatty acids (VFA, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, capronate and isocaproate) concentration were measured high performance liquid chromatography (HPLC) as described elsewhere (Westerholm et al., 2012). Ammonium-nitrogen was determined according to standard methods (Eaton et al., 1995) and concentration of ammonia-nitrogen was calculated as described elsewhere (Angelidaki & Ahring, 1993).

4.3.2 Molecular analyses

Samples from digesters for molecular analyses were selected from the full-scale digesters at 3 (Labio), 5 (Ekogas, HEMAB) or 11 (VMAB) sampling times (Table 5). From HEMAB two samples were also taken from recirculation of digestate. From VMAB sample 1–3 were taken during operation at mesophilic temperature and 4–11 were taken after the shift to thermophilic temperature conditions. All samples were stored at -20 °C until further use.

Table 5. Date of sampling for analysis of microbial community structure

Process	Operating temperature	Date of sampling	Sample no
HEMAB	39 °C	2018-03-08	1
		2018-03-26	2
		2018-04-16	3
		2018-05-07	4
		2018-05-29	5
Recirc HEMAB	-	2018-03-27	R6
		2018-05-07	R7
VMAB1	53 °C	2017-07-18	1
		2017-08-14	2
		2017-09-04	3
	38 °C	2018-01-02	4
		2018-01-23	5
		2018-02-12	6
		2018-03-12	7
		2018-04-03	8
		2018-04-27	9
		2018-05-14	10
		2018-05-22	11
Labio3	42 °C	2018-04-09	1
		2018-04-23	2
		2018-05-14	3
Ekogas	53 °C	2018-03-08	1
		2018-03-29	2
		2018-04-16	3
		2018-05-07	4
		2018-05-28	5

4.4 MOLECULAR ANALYZES

4.4.1 Analysis of total microbial community using Illumina sequencing

DNA extraction, construction of 16S amplicon libraries and Illumina MiSeq sequencing were carried out on triplicate samples from each sampling point as described previously (Müller et al., 2016). The paired end reads were filtered based on quality, trimmed to 300 bp with Cutadapt (Martin, 2011) version 1.13 and further processed with the software package Divisive Amplicon Denoising Algorithm 2 (DADA2) (Callahan et al., 2016) version 1.4, running in a HPC environment in R, version 3.4.0. Sequences were processed according to the DADA2 pipeline tutorial v. 1.4 with modification according to Westerholm et al (2018). Classification was performed using the SILVA reference database v. 128. The *phyloseq* package (McMurdie & Holmes, 2013) was used to organize the data into a single data object and for production of graphics in R Studio v. 1.1.423 (<http://www.r-project.org>, Team RStudio, 2016) as described previously (Westerholm et al., 2018). Principal Coordinates Analysis (PCoA) plots of microbial community profiles were generated using Bray-Curtis weighted UniFrac distance measures. Permutational ANOVA (PERMANOVA) was performed to evaluate the effect of operating parameters on microbial community structure using the Adonis function (vegan) and significant parameters were included in canonical correspondence analysis (CCA) plotting.

4.4.2 Analysis of abundance of methanogens using quantitative PCR

Quantitative PCR (qPCR) was performed to determine 16S rRNA gene copy number for methanogenic archaea, including the orders Methanomicrobiales and Methanobacteriales, the families Methanosarcinaceae, Methanosaetaceae and the species *Methanoculleus bourgensis* and the syntrophic acetate oxidising bacteria *Clostridium ultunense*, *Syntrophaceticus schinkii*, *Tepidanaerobacter acetatoxydans* and *Thermacetogenium phaeum*, using primers and conditions described previously (Westerholm et al., 2011; Yu et al., 2005). Non-template controls were included in each qPCR run to ensure that no contamination had occurred.

4.5 HYGIENISATION STUDY

Digested food waste was collected from Ekogas at two occasions (2018-11-14 and 2019-01-05) and was kept at 37 °C for 24–48 hours before used for the experimental setup. Before inoculation with indicator organisms the material was analysed for pH, total ammonia nitrogen (NH₃/NH₄-N), *Salmonella* spp, *Enterococcus* spp. and *Escherichia coli*. Organisms used in the experiment were: *Salmonella enterica* subspecies enterica serovar Senftenberg (ATCC ATCC 8400), *Enterococcus faecalis* (ATCC 29212), f-RNA phage MS2 (ATCC 15597-B1) and somatic coliphage φx174 (ATCC13706-B1). Phage solutions was propagated according to the manufacturers (American Type Culture Collection) instructions. Bacteria used in the experiment were pre-cultivated overnight in nutrient broth (100 ml per bacterium), followed by centrifugation of the bacterial solution (10 min, 4500 G, 10 °C). The supernatant was discarded and the bacterial pellets re-suspended with the phage solutions (5–6 ml each) to keep the addition of liquid to the digestate at a minimum. The mix of

bacteria and phages was inoculated into a 500 ml portion of the digestate. After stirring, additional aliquots of 500 ml were added, with mixing in-between, until reaching the full volume of digestate (2000 ml). During the mixing some larger debris as wood chips and stones were removed. The inoculated material was aliquoted into 70 ml poly propylene jars with screw caps (Sahrstedth, Sweden), filled with approximately 50 ml digestate in each, leaving approximately 20 ml headspace. A number of the jars was also prepared with 50 ml digestate and bags containing *Ascaris suum* eggs. *Ascaris suum* eggs, retrieved from swine faeces (Excelsior Sentinel Inc), was inserted into nylon bags (Bigman Sefar, Sweden) permeable to fluids (2*100 cm²; mesh, 28 microns) with 2000 eggs per bag. The egg containing bags were washed in physiological saline solution and soaked off on tissue paper before added (one bag/jar) to the jars. During the filling procedure three digestate samples were taken for analysis of start concentrations. The jars were incubated at respective temperature, placed in preheated water baths to allow for fast increase to the targeted temperatures (37, 42, 48, and 52 °C). Temperature was monitored by Tiny tag data loggers (Intab, Sweden). Gas formed in the jars was evacuated after 8 hours, and then after every 24 hours by open the lids slightly to neutralize the pressure.

Sampling were destructive and the frequency varied depending on organism and incubation temperature. At sampling, the nylon bag containing *Ascaris suum* eggs were removed, rinsed in tap water and put in 0.1 N sulfuric acid and incubated for approximately 28 days before viability was checked under the microscope (Arene, 1986). Unfertilized eggs, identified by their incomplete egg shells, were excluded from further counting. Eggs developing to the larval stage were considered viable while pre-larval stages were not. Initial viability of the *Ascaris* eggs was 83 % (CI 95 79–86 %). After removal of *Ascaris* egg bag the digestate in the jar was mixed and aliquoted for the different analyses. For enumeration of bacteria and bacteriophages, 10–25 g of digestate (depending on timing of samples) was diluted tenfold with buffered peptone water (BPW) and tenfold dilution series of this primary dilution was made with buffered NaCl peptone water with surfactant Tween (pH 7) (SVA, Sweden). For analysis of total ammonia nitrogen, ten (10) gram of the digestate was tenfold serial diluted with deionized water. The pH was measured in the remaining, undiluted, digestate (15–25 g) when it had reached room temperature (27 °C).

Enumeration of salmonellae was performed by plating on Xylose Lysine Deoxycholate agar (XLD) (Oxoid) with 1.5 % Novobiocin (24±2 h at 37±0.5 °C). To be able to confirm the absence of salmonella in the non-inoculated digestate, as well as in samples from the later end on the sampling period, the ten-fold primary dilution in BPW was used for pre-enrichment (18 h at 37±1 °C), followed by selective enrichment on Modified Semi- Solid Rappaport-Vassiliadis (MSRV) agar supplemented with 1.0% Novobiocin (41.5±0.5 °C). Suspected salmonellae checked after 24 and 48 h, was further confirmed by triple sugar iron agar tubes and urease broth (SVA, Sweden). *E. coli* was detected on Chromocult Coliform agar (Merck, Germany) (24±2 h at 42±2 °C). *Enterococcus* spp. was detected on Slanetz- Bartley (SlaBa) agar (Oxoid) (48±2 h at 42±2 °C). Phages MS2 and φx174 were detected by standards ISO10705-1:1995 and 10705-2:2000, using the double-layer agar method (17–24 h at 37±1 °C,) with *Salmonella* Typhimurium WG49 (ATCC 700730) and *E.*

coli 13706 (ATCC 13706) used as bacterial host strains for the respective bacteriophages.

Total ammonia nitrogen ($\text{NH}_3 + \text{NH}_4$) was analysed in digestate diluted 100 times and filtered (Filtropur 45 mm, Sarstedt AG & Co, Sweden) by a reagent kit 114544 (Spectroquant Ammonium, Merck, USA) based on the indophenol blue method, at 660 nm on a Genesys 20 spectrophotometer (Thermo Scientific, USA). The pH was analysed with a glass electrode PHC 2011 (Radiometer Analytic, France) connected to a PHM 210 meter (MeterLab, Denmark). The concentration of aqueous ammonia (NH_3) was calculated from total nitrogen concentration, pH and temperature, according to Emerson et al. (1975). Confidence interval for proportions of viable *Ascaris* eggs were derived using the Wilson score interval. Logarithmised bacterial concentration data was linear regression whereas the logarithmised *Ascaris* viability data was fitted against a non-linear inactivation model (Eq. 1-2) by non-linear regression using the Gauss-Newton algorithm.

$$\log_{10} N_t = \log_{10} N_0 [1 - (1 - 10^{-kt})^{10^n}] \quad (\text{Eq. 1})$$

$$\text{Lag phase } l = \frac{n}{|k|} \quad (\text{Eq. 2})$$

All analyses were performed in Minitab 17 (Minitab Inc. US) and alpha levels were ≤ 0.05 , if not indicated other.

4.6 THEORETICAL CALCULATIONS OF ENERGY AND ECONOMY

4.6.1 Heat demand

Calculations of theoretical heat demand for a dry fermentation process were performed using scenarios including and excluding external pasteurization.

Three different fermentation temperatures were assumed, 39, 42 and 52 °C. In the 52 °C case, internal hygienisation was supposed to be applied and no external pasteurization was thus calculated. The heat demand calculation included two parts, heating of substrate and heat losses from the digester. Input data was the Swedish mean outdoor temperature of the year from SMHI and an assumed value of the mean temperature of incoming substrate (see Table 6).

The plant size was set to a volume allowing handling of 15 tons of organic source separated household waste per day (about the size of the plant at HEMAB). Two different calculation scenarios were performed, one with a fixed HRT of 47.5 days, independent of process temperature, and one with reduced reactor size (adapted HRT) assumed to be possible to use when using a higher process temperature. A higher operational temperature was assumed to give an increased gas production rate, which would allow a reduced HRT without reduction of the gas production. The methane yield per ton substrate was assumed to be the same for all calculation cases (see chapter 4.6.3). The heat loss will be lower with a decreased HRT since the reactor surface area is decreased.

The pasteurization was assumed to be performed in pasteurization tanks after the digestion. The pasteurization temperature was set to 71 °C and the holding time to one hour. Heating was assumed to be done with two heat exchangers, where one

recovers heat from a completed pasteurization and the second performs the final heating with the use of hot water from a boiler.

Heat losses from the pasteurization tanks were not calculated but a temperature drop during the holding time was assumed instead. Heat is assumed to be produced in a gas burner, with an efficiency of 92 %.

Two calculations were made to illustrate the influence of outdoor temperature, using one winter and one summer temperature. The base for the calculations was high mesophilic fermentation at 42 °C with external pasteurization. The outdoor temperature has a strong influence on the heat demand. Heat losses are much higher with a low outdoor temperature and household waste is mainly handled outdoors which means that the temperature of the wastes can vary very much. It is not unusual with frozen wastes during wintertime and a high temperature during summer when the degradation of the waste even can have started before feed into the anaerobic digester.

The assumed input values for the calculations of heat demand are summarized in Table 6.

Table 6: Assumed input values for the heat demand calculations.

Process/Parameter	Assumed value	Unit
Fermentation		
<u>Reactor dimensions</u>		
Cylindrical length	60	m
Diameter	4	m
Filling degree	90	%
HRT 39 °C	47.5	d
HRT 42 °C *)	40.0	d
HRT 52 °C *)	35.0	d
*) In the calculations with adjusted HRT, depending on process temperature, the reactor length is changed to adjust the HRT.		
<u>Temperatures</u>		
Incoming substrate, mean value	14	°C
Incoming substrate, winter	5	°C
Incoming substrate, summer	25	°C
Outdoor temperature, mean	7	°C
Outdoor temperature, winter	0	°C
Outdoor temperature, summer	17	°C

Process/Parameter	Assumed value	Unit
<u>Substrate</u>		
Amount	15.0	ton/d
Density	1050	kg/m ³
c _p value	4.1	kJ/kg, °C
<u>Energy</u>		
k (or u) value for the reactor	0.45	W/m ² , °C
Pasteurization		
<u>Tank dimensions</u>		
Cylindrical height	2.25	m
Diameter	1.25	m
<u>Temperatures</u>		
Pasteurization temperature	71.0	°C
Temperature loss, mean	1.0	°C
Temperature loss, winter	1.5	°C
Temperature loss, summer	0.5	°C

4.6.2 Electricity demand

Electricity demand in the calculation cases will vary slightly depending on the HRT in the way that a shorter HRT leads to a diminished electricity need per ton substrate, as the time for the advancing of the material inside the digester is shorter. However, as the advancing of the material inside the digester is a relatively small consumer of electricity at a dry digestion plant, this electricity demand has been neglected in the calculations related to comparison in energy and economy between different process temperatures.

4.6.3 Methane production

In the calculation of energy balance and economy, the same total methane production was assumed for all investigated process temperature conditions. This is of course not completely true, as the methane production rate is expected to be higher at higher process temperature. In the calculation case with adapted HRT this has been taken into consideration in the way that the reactor size has been assumed to be smaller for higher process temperature for constant methane production. The methane production was assumed to be 500 Nm³/kg VS and with 33 % TS and 85 % VS of TS in the substrate.

4.6.4 Economic analysis

For the economic analyses, an assumed price of produced biogas of 0.5 SEK/kWh was used. The value of the biogas may however differ depending on local conditions and will of course change over time. The calculated energy demand may therefore give a better indication of the resources needed for the different calculation cases.

5 Results

5.1 SUBSTRATE COMPOSITION

Analysis of the substrate composition for the participating plants showed that food waste corresponded to 77–100 % of the substrate during the three month's test period (Figure 5). Additional substrate included yard waste (Ekogas), grease trap sludge (Ekogas), rape husk (VMAB2) and sludge from a waste water treatment plant (Labio). The waste water treatment sludge at Labio was only added during the first two weeks of the 3 months test period. In the reported amounts of food waste from Labio, some industrial organic waste, such as filter cake from vegetable oil refinery, is also included. However, the amount of the industrial organic waste was not measured at the plant and the share is thus unknown.

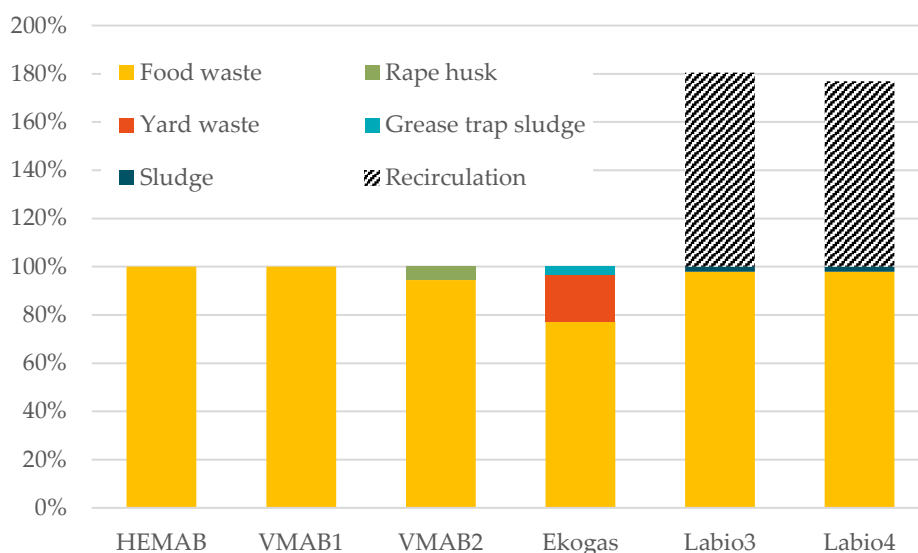


Figure 5: Substrate composition for the participating plants, and added recirculation of digestate, as an average during the test period. 100 % = sum of incoming substrates.

5.2 PROCESS PERFORMANCE

5.2.1 Methane production

The specific methane production was 400–610 Nm³/ton VS fed (no recirculation included) and the volumetric methane production was 2.36–4.98 Nm³/m³ and day for the participating plants (Figure 6). Including recirculation of digestate at the plant of Labio gave about 470 Nm³ CH₄/ton VS fed (Figure 6). Fluctuations in gas production at HEMAB during the three months test period were somewhat more pronounced than at the other plants.

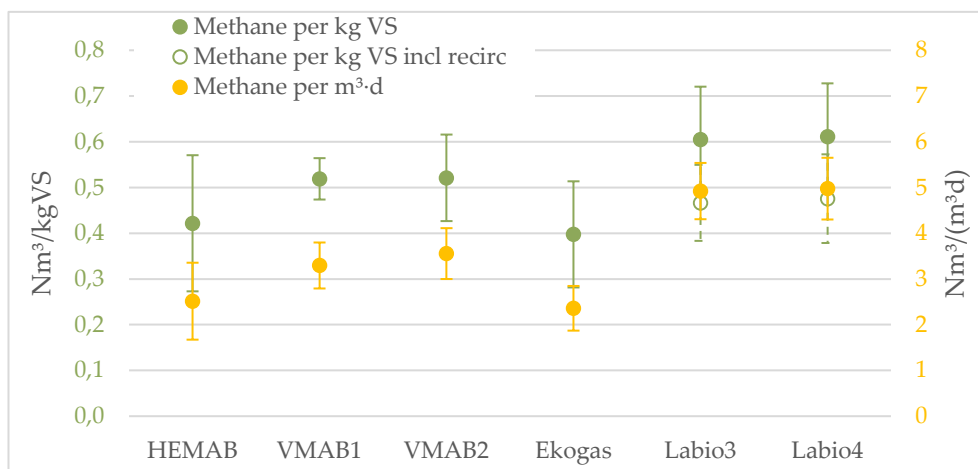


Figure 6: Methane yield and volumetric methane production for the plants. The filled markers (●) only consider the VS of the influent substrate. If recirculation is included in the calculation (hollow markers ○), the methane yield will be lower for Labio3 and Labio4.

5.2.2 OLR and VFA

The organic load rate (OLR) and the sum of volatile fatty acids (VFA) in the digester were calculated/analyzed (Figure 7). The OLR varied between 6 and 8 kg VS/(m³·d) and VFA levels were in most cases below 2 g/l, except for Ekogas. This plant differs from the other processes by the accumulation of high VFA values as well as having larger variations in OLR. The variations in OLR are due to repeatedly reduced feeding on single days throughout the test period, as well as a phase of reduced feeding during a week in the middle of April. As can be seen in Figure 8, mainly the propionate value is unusually high whilst other VFAs are at a normal level. The Labio process operated at a comparably high OLR but showed despite this low VFA levels, indicating a stable process. A relatively high stability and efficiency of the Labio process was further demonstrated by the high specific methane yield (Figure 6) and high degradation efficiency (Figure 9).

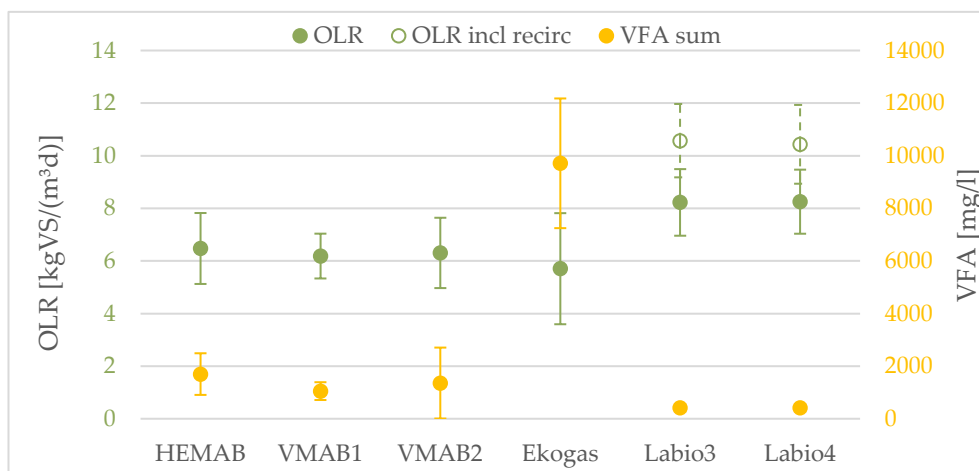


Figure 7: Organic load rate (OLR) from substrates entering the system (recirculation not accounted for, filled markers ●), organic load rate (OLR) including recirculated digestate for Labio3 and Labio4 (hollow markers ○) and sum of volatile organic acids in the digesters (yellow markers). The OLR for Ekogas excludes the percolate for which no VS data was available.

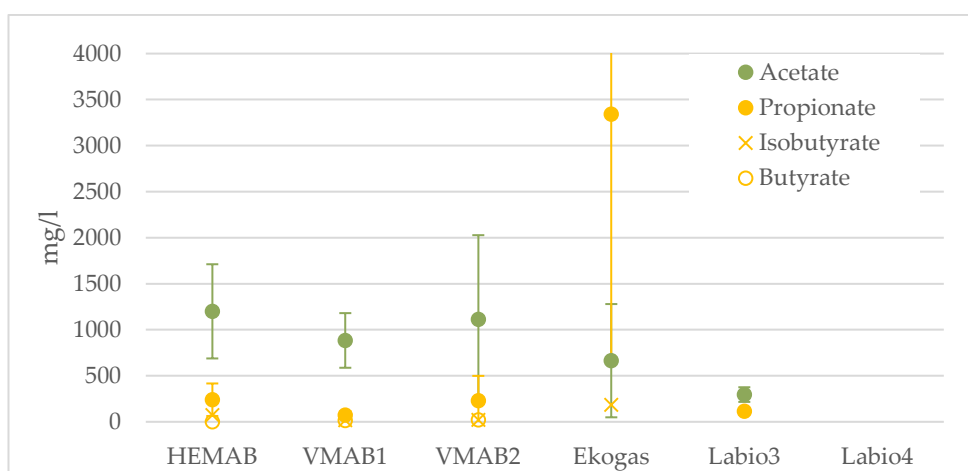


Figure 8: Levels of the different VFAs in the digesters during the test period. No data was available for Labio4.

5.2.3 VS reduction and HRT

The measured reduction of VS spanned between 54–70 % and the hydraulic retention time (HRT) was 36–45 days for the participating plants (Figure 9). No relationship of higher VS reduction at longer HRT can be seen (Figure 9). The plant with the shortest HRT (Labio) shows one of the best decomposition performances. However, a clear difference in VS reduction could be seen for the VMAB1 and VMAB2 reactors, where VMAB2 have 54 % and VMAB1 66 % VS reduction as a median value for the test period.

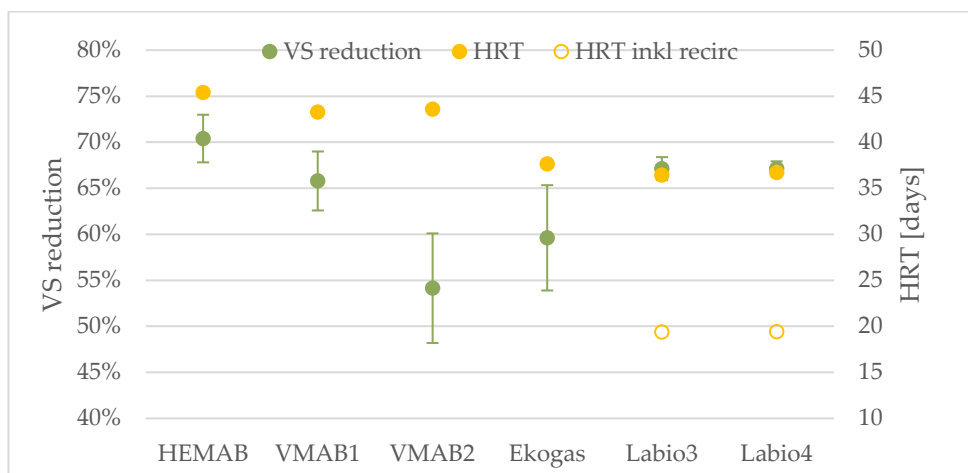


Figure 9: VS reduction during digestion, and hydraulic retention time (HRT). VS reduction values for Ekogas and Labio are based on 5 and 3 samples only, respectively. For Ekogas, the HRT includes the addition of percolate water. For Labio, the HRT gets significantly lower if recirculation is included in the calculation (hollow markers ○).

5.2.4 pH and temperature

Ekogas (53.5 °C) is the only plant operating at a thermophilic temperature, while Labio's reactors (41–42 °C) operates in an intermediate, but near mesophilic temperature range. HEMAB (39 °C) and VMAB (38 °C for their 1st digester) operates at a conventional mesophilic range (Figure 10). Temperature sensors in the 2nd digester at VMAB (VMAB2) are placed too near the heating pipes, which likely explains the observed big variation of measured temperature giving a high standard deviation and an over estimation of the median temperature. The real temperature and deviation are possibly similar to VMAB's 1st digester (VMAB1). This deviation in temperature affects also the (calculated) values for ammonia (see chapter 5.2.5 below). The pH was 7.9–8.4 for all the plants during the test period, with the highest value for Ekogas (Figure 10).

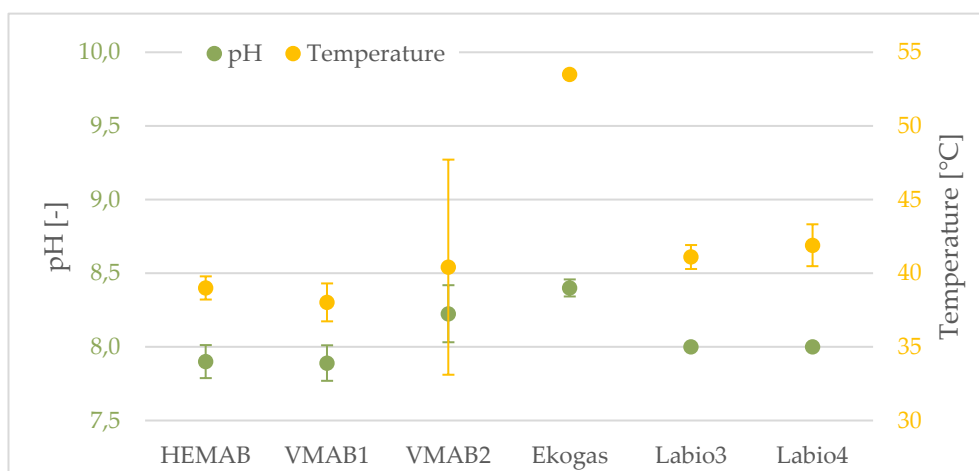


Figure 10: pH and temperature in the digesters. Temperature sensors in VMAB2 are placed too near the heating pipes which lead to a big standard deviation and an over estimation of the median temperature. The real temperature and deviation are likely to be similar to VMAB1.

5.2.5 Ammonium and ammonia

The ammonium (N-NH_4^+) and free ammonia (NH_3) values after digestion were 3.6–5.2 and 0.4–1.7 g/l respectively for the participating plants (Figure 11). Ammonia values were calculated from measured values on ammonium nitrogen, pH and temperature data as described in chapter 4.1 (Table 3). Regarding VMAB2 (which has problems with temperature logging as described in chapter 5.2.4), the ammonia concentration was calculated based on the mean value of the two temperature sensors in the rear part of the digester for the previous, current and next day respectively (i.e. mean value of 6 data points) in order to mitigate the jitter. However, this does not eliminate the systematic error of the sensors being placed too near the heating coils, which leads to systematically too high temperature measurements and hence the calculated ammonia values are too high. Therefore, VMAB2 values are probably incorrect and should not be compared to other values. The ammonium level was about the same in both VMAB reactors. The elevated ammonia concentration in Ekogas (about two times higher than at the other plants) is due to their higher pH and temperature and may explain the relatively lower performance (VS reduction and methane yield) and high VFA values of the plant.

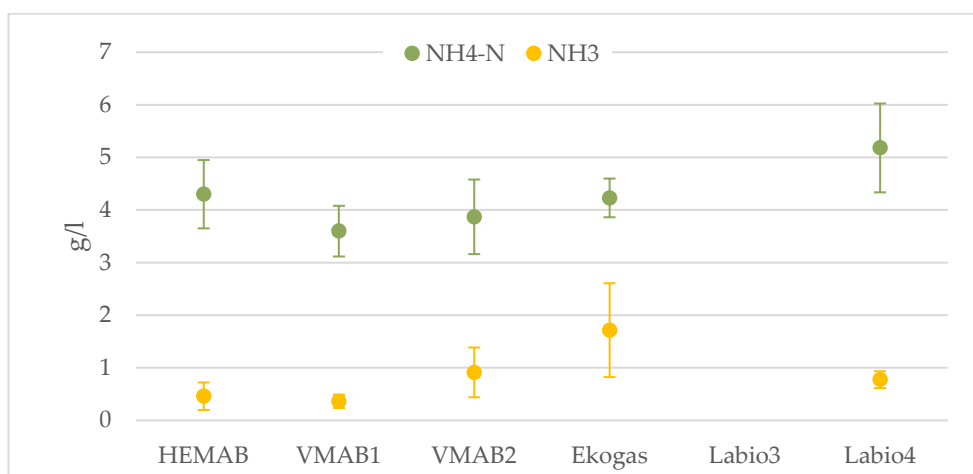


Figure 11: Analyzed ammonium and calculated free ammonia content in the digesters, respectively. The calculated ammonia values for VMAB2 are considered not to be correct, as the temperature logging is not representative in this reactor. No values for Labio3 were available.

5.2.6 Summary of calculated KPIs

The calculated KPIs for the evaluation of the participating dry digestion plants' process performance have been summarized (Table 7).

Table 7. Summary of median values for the 3 month's test period for the participating plants. For Labio, both values with and without recirculation of digestate are presented when applicable. (-) means that no data was available.

	HEMAB	VMAB1	VMAB2	Ekogas	Labio3	Labio3*)	Labio4	Labio4*)
Specific methane production (Nm³ CH₄/kg VS)	0.42	0.52	0.52	0.40	0.60	0.47	0.61	0.49
Volumetric methane production (Nm³ CH₄/(m³, d))	2.51	3.29	3.55	2.36	4.92		4.98	
OLR (kg VS/(m³, d))	6.5	6.2	6.3	5.7	8.2	10.5	8.3	10.4
Sum of VFA (mg/l)	1698	1051	1356	9712	411		411	
VS reduction (%)	70	66	54	60	67		67	
HRT (d)	45	43	44	38	36	21	37	21
pH	7.9	7.9	8.2	8.4	8.0		8.0	
Temperature (°C)	39.0	38.0	40.4	53.5	41.1		41.9	
Ammonium (g NH₄-N/l)	4.3	3.6	3.9	4.2	-		5.2	
Ammonia (g NH₃/l)	0.5	0.4	0.9	1.7	-		0.8	

*) Recirculation of digestate included.

5.3 PROCESS DURING TEMPERATURE TRANSITION AT VMAB

In order to further investigate the relation between temperature, process performance and microbiology the VMAB process was studied during a period, in which the process changed the operating temperature (Figure 12).

The operation at 52 °C caused severe process disturbance in the VMAB process resulting in extremely high acetate and propionate levels and decreased methane yield (Figure 13). The decrease in temperature did not have a direct positive effect on stability and both acetate and propionate levels were extremely high at this point (in total about 16 g/L, Figure 13). At this point the plant decided to stop the feeding of the process. The two months of acclimatization without feeding stabilized the process and the VFAs were maintained at low levels over the course of the study period of the mesophilic operation (<1 g/L, Figure 13).

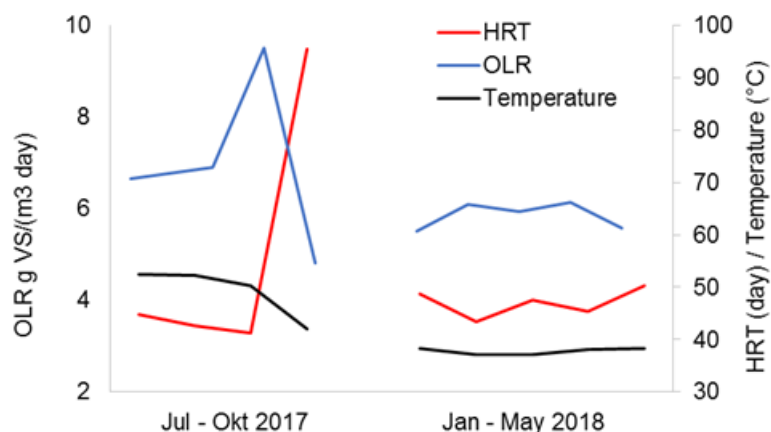


Figure 12. HRT, OLR and temperature during the time of temperature change (July-October 2017) and during established mesophilic operation (January-May 2018).

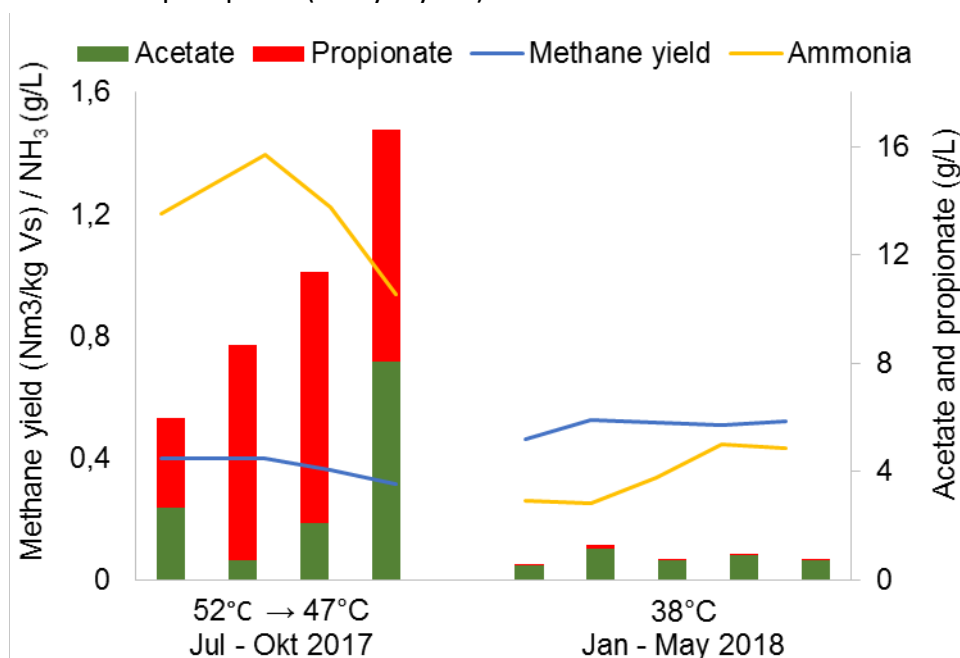


Figure 13. Methane yield, ammonia, acetate and propionate concentration during the time of temperature change (July-October 2017) and during established mesophilic operation (January-May 2018).

5.4 MICROBIAL COMMUNITY

Statistical analysis of the sequencing of the total microbial community showed strong correlation between community structure and temperature (Figure 14). The phyla Halanerobiateota, Synergistetes and Thermotogae were more abundant at higher temperature, whereas phyla such as Cloacimonetes and Bacteroidetes correlated with mesophilic temperature. However, even though samples were separated based on temperature, mesophilic and thermophilic samples still diverged considerably. Strong temporal dynamic in the VMAB microbial community was illustrated by the positioning of VMAB samples 1–3 in similar direction as Ekogas, whereas when VMAB had changed operating temperature to

mesophilic condition (samples 4–8), the community was highly similar to HEMAB, but thereafter (samples 9–11) drifted towards higher similarity to Labio.

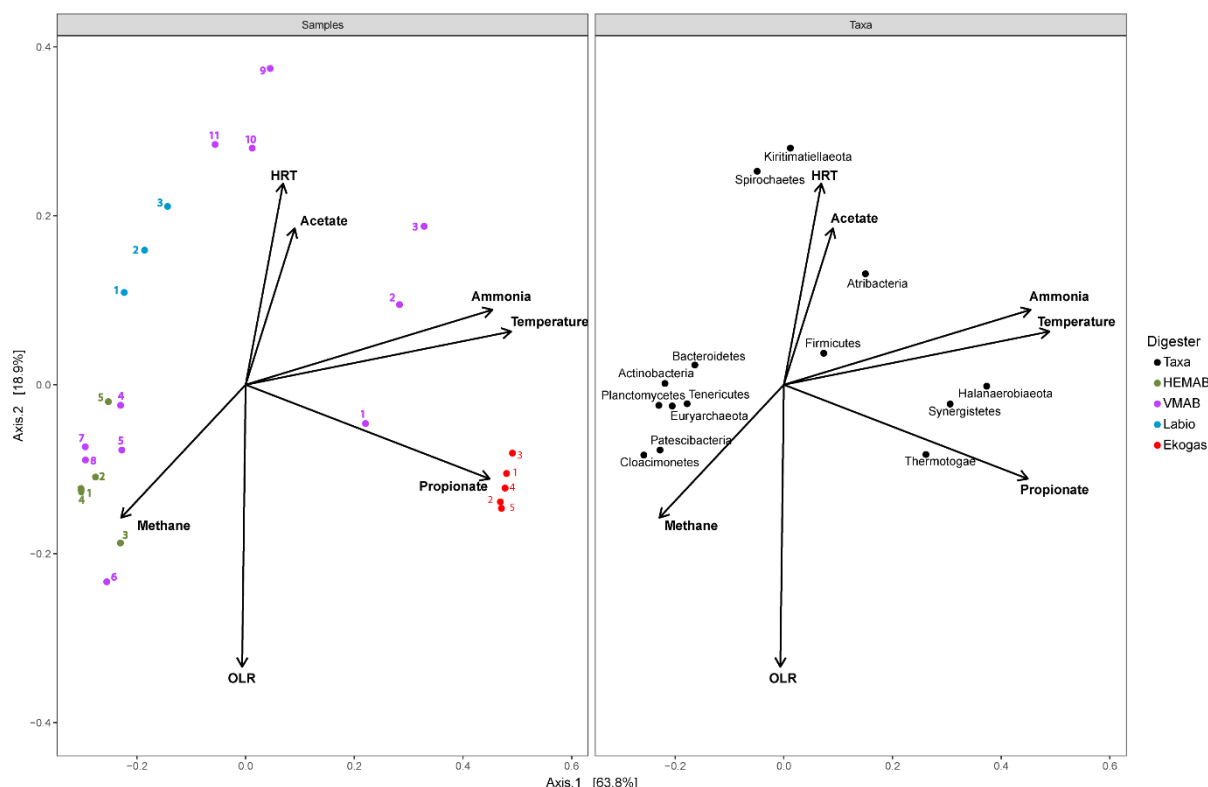


Figure 14. Principal Coordinates Analysis (PCoA) plot of the microbial community structure using weighted UniFrac. Digester parameters significantly associated with changes in microbial community structure are plotted as vectors, where the length and direction indicate the contributions of the variable to the principal components.

In the present dry digesters, relatively similar levels in microbial richness and evenness were obtained in all processes (data not shown). The sequencing approach also revealed core populations that were present at high relative abundance in all digesters, including uncultured *Clostridia* group MBA03 (0.6–50.9 % of total community) and *Syntrophaceticus* (0.3–4.1 %) both belonging to phylum Firmicutes and the genus *Deftuviitoga* (0.1–33.3 %, not detected in Labio sample 3) belonging to phylum Thermotogae (Figure 15). Except for these core populations, all processes had distinct community profiles, which are described more in detail below.

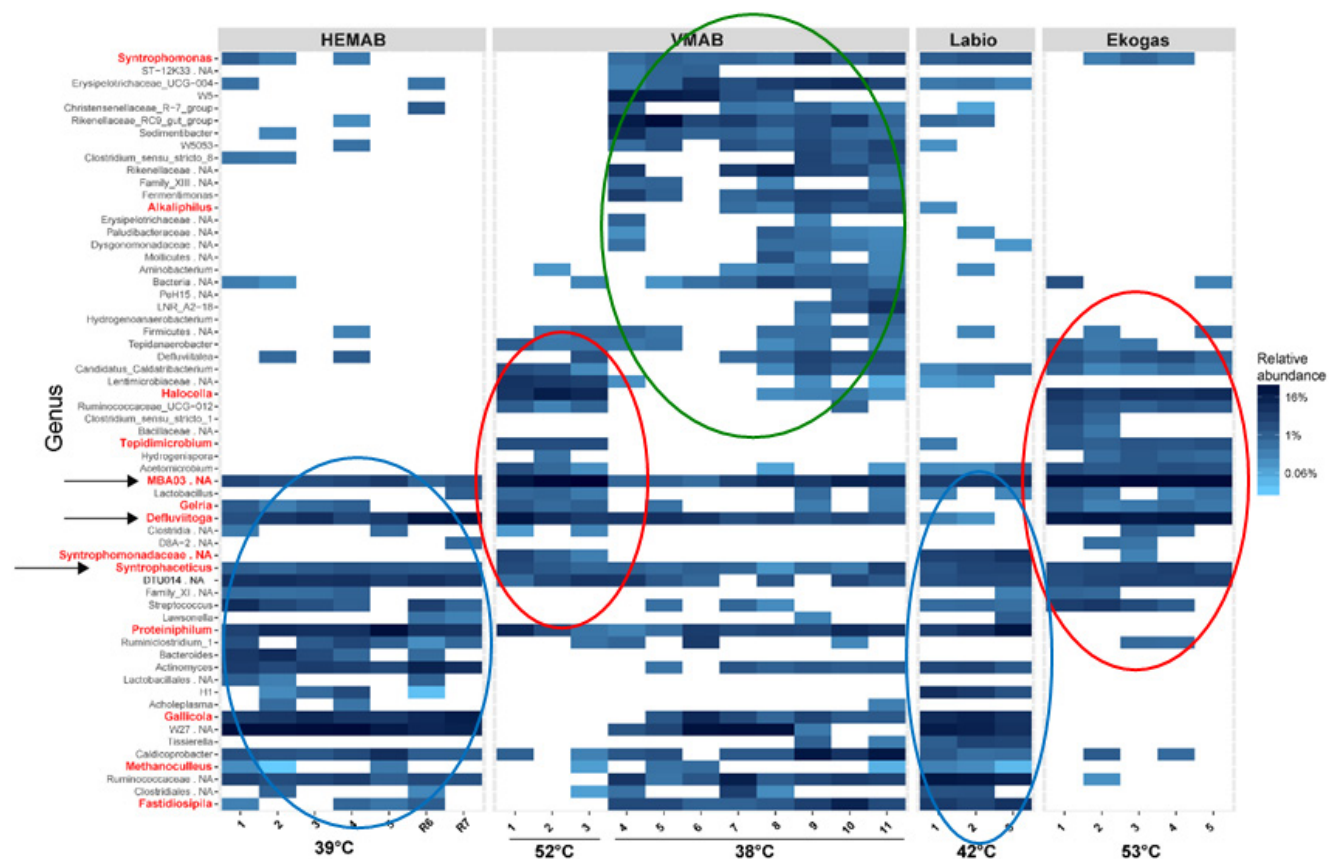


Figure 15. Heatmap showing relative abundance (dark blue – high, light blue – low, white – not detected, based on total bacterial and archaeal sequences) of genera in mesophilic (38-42 °C, HEMAB, VMAB sample 4-11, Labio) and thermophilic digesters (52 °C, VMAB sample 1-3, Ekogas). The temperature at the point of sampling is given on the x-axis. Genera discussed in this report are labelled red. Circles indicate genera typically present in the mesophilic HEMAB and Labio processes (blue circles), the thermophilic Ekogas and VMAB processes (red circles) and after transition to mesophilic conditions in VMAB (green circle). Arrows indicate core populations that were present in all digesters.

The qPCR analysis of methanogenic abundance demonstrated presence of the hydrogenotrophic methanogens Methanobacteriales and Methanomicrobiales and the mixotrophic (both acetoclastic and hydrogenotrophic) methanogens Methanosarcinaceae in all digesters, with some dynamics over time (Figure 16). Illumina sequencing revealed high relative abundance of the hydrogenotrophic *Methanoculleus bourgensis* in all digesters.

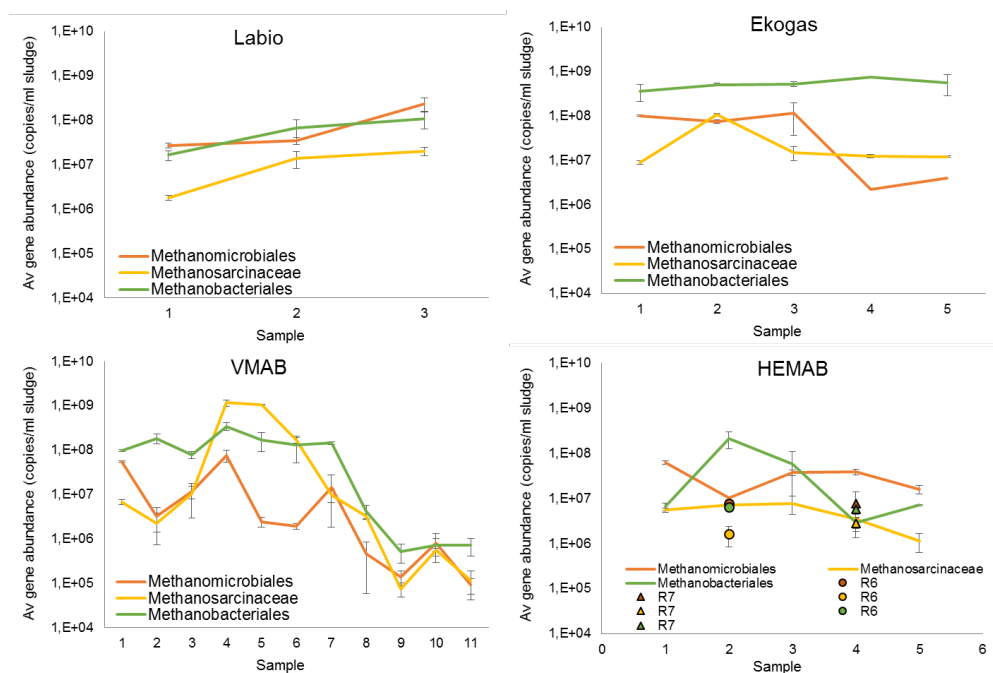


Figure 16. Abundance of methanogenic groups belonging to the hydrogenotrophic Methanobacteriales and Methanomicrobiales and the mixotrophic (both acetoclastic and hydrogenotrophic) Methanosarcinaceae. Scatter points (R6 and R7) in HEMAB plot are dewatered digester content (methanogenic group of these points is indicated by coloring).

HEMAB

The community in the mesophilic HEMAB digester was dominated by the phyla Firmicutes (24–39 % of the total community), candidate Cloacimonetes (42–47 %) and lower levels of Bacteroidetes (10–21 %), except for the last sample (no 5, Figure 17) in which Cloacimonetes was comparably lower (23 %) and Bacteroidetes was higher (41 %). In addition to the core populations mentioned above, high abundant genera included *Gallicola* (Firmicutes), candidate W27 (Cloacimonetes) and *Proteiniphilum* (Bacteroidetes). The molecular community in the dewatered digestate, later used for recirculation in HEMAB, had a higher proportion of the genera *Defluviitoga* (29–33 %) and *Actinomyces* (phylum Actinobacteria 8–16 %) compared with the digesters content (3–9 % and 3–5 %, respectively). The analysis of the methanogenic community demonstrated variation of Methanobacteriales over time but there was no clear increasing or decreasing trend. Methanomicrobiales and Methanosarcinaceae were both stable in abundance throughout the study period.

VMAB

At thermophilic conditions (52 °C, sample 1-3), VMAB microbial community had a distinct dominance of phylum Firmicutes (42–79 %) and lower levels of Thermotogae (5–28 %), Bacteroidetes (4–20 %) and Halanaerobiaeota (6–20 %) (Figure 17). After decreasing temperature to 38 °C, strong dynamics in microbial community structure were observed with dominating phyla changing from Bacteroidetes (34–49 %) in sample 4–5 to Cloacimonetes (40–53 %) in samples 6–8 and finally to Firmicutes (68–77 %) in samples 9–11. At lower rank, the most distinct impacts by the change from thermophilic to mesophilic temperature were decline of the genera *Acetomicrobium*, *Tepidimicrobium* and members of the family Syntrophomonadaceae. Instead, the genera *Fastidiosipila*, *Gallicola*, *Alkaliphilus*, *Syntrophomonas* and members of the family Ruminococcaceae increased in relative abundance. In addition to the core populations mentioned above, the genus *Proteiniphilum* (Bacteroidetes) was present both at mesophilic and thermophilic conditions (Figure 17). Analysis of methanogenic abundances in VMAB demonstrated higher levels of Methanobacteriales and lower levels of Methanosarcinaceae and Methanomicrobiales. Directly after changing to mesophilic level, the mixotrophic Methanosarcinaceae increased (sample 4–5) but thereafter decreased below the initial values. Similar decrease of both Methanobacteriales and Methanomicrobiales was observed in sample 7–11.

Labio

In the Labio process operating at 39–42 °C, Firmicutes dominated with 57–64 %. The other highly abundant phyla, Cloacimonetes and Bacteroidetes, varied between 8–21 % and 14–31 %, respectively. In addition to the core populations, the genera *Syntrophomonas*, *Acetomicrobium*, *Proteiniphilum*, *Fastidiosipila*, *Gallicola* and members of Syntrophomonadaceae were abundant at persistent levels (Figure 17). The methanogenic community analysis revealed high levels of the hydrogenotrophic Methanobacteriales and Methanomicrobiales, whereas the mixotrophic Methanosarcinaceae was slightly lower (Figure 16).

Ekogas

The microbial community in Ekogas, operating at thermophilic temperature (52 °C) was highly stable during time and had a distinct dominance of the phyla Firmicutes (60–69 %), Thermotogae (21–29 %) and Halanaerobiaeota (4–10 %) (Figure 17). Clostridia uncultivated MBA03 was, as mentioned above, present in all digesters, but this group was considerably highly abundant in Ekogas, representing 42–51 % of the total community at order level. In addition to high abundance of the core populations mentioned above, the genera *Tepidimicrobium* and *Halocella* (both belonging to Firmicutes) were highly abundant. Compared with the mesophilic processes, Ekogas had higher levels of Methanobacteriales, whereas Methanosarcinaceae and Methanomicrobiales were present at lower levels (4–5, Figure 16). Similar methanogenic structure was found in the VMAB process during operation at thermophilic conditions.

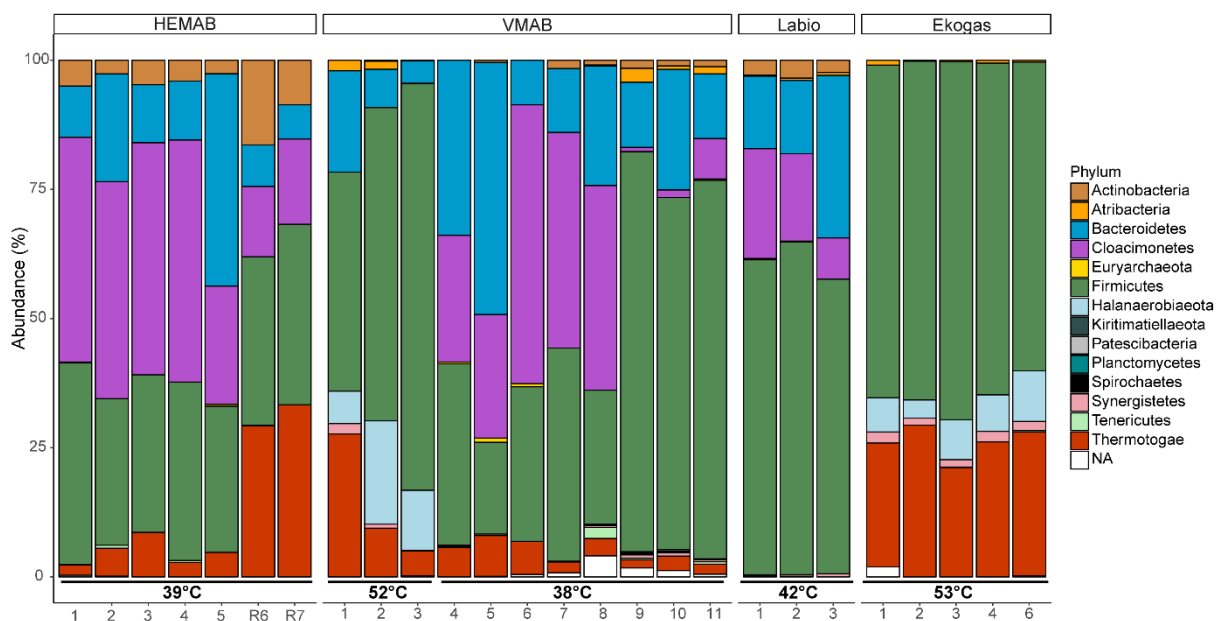


Figure 17. Relative abundance of microbial phyla (based on total bacterial and archaeal sequences) in thermophilic (52 °C, VMAB sample 1–3, Ekogas) and mesophilic (38–42 °C, HEMAB, VMAB sample 4–11, Labio) digesters. Samples taken from dewatered digestate aimed to later be used for recirculation in HEMAB are labelled R6 and R7. The temperature at the point of sampling are given in bold on the x-axis.

5.5 HYGIENISATION EFFECT

Before the addition of microorganisms to the digestate, the material was analyzed for presence of somatic and RNA-phages and *Salmonella* spp, *E. coli* and *Enterococcus* spp. Only *Enterococcus* spp. was detected in the material at concentrations of 1.5 cfu g⁻¹. The two batches of material from Ekogas had an initial pH of 8.20 and 8.26 and initial NH₃/4-N concentrations of 4.7 and 6.1 g L⁻¹ respectively. The inoculation with organisms resulted in start concentrations of 6.6–7.6 log₁₀ g⁻¹ for bacteria and 4.5–6.7 log₁₀ g⁻¹ for the phages. The addition of organisms did not change the pH or the total ammonia concentrations. For the experiment performed with the first batch of digestate the pH increased slightly during the incubation at respective temperature, with a higher increase at the higher the temperature (Table 8). For the second batch of material a slight decrease in pH over time was observed for all temperatures. At the higher temperatures more gas was formed in the jars indicating a higher degradation rate at the higher temperatures. The differences in pH resulted in NH₃ concentrations that overlapped between the closest studied temperatures but in general the higher the temperature the higher the NH₃ concentrations (Table 8).

By mistake the experiment with the first batch of material had temperatures that partly deviated from target temperatures (48 and 52 °C) which resulted in that the sampling frequencies did not catch the inactivation and thus the experiment was repeated. Results from both batches are however included in the report (Table 8). The bacteriophage analyses performed with the first batch had disturbances and here only data for the second batch is reported (Table 8). The inactivation of the investigated bacteria followed a log-linear inactivation trend (Figure 18) and the

inactivation could be summarized by the inactivation rate constant k ($-\log_{10} \text{ h}^{-1}$) (Table 8). For some temperature and organisms the inactivation of bacteria or phages was so fast that only the start value and detection limits was used for assessment of inactivation rates and such inactivation kinetics is reported as censored, “less than”, values (Table 8) and is in Figure 20 marked with an arrow. These censored values indicate that the inactivation was faster than the given rate and differences in censored values in Table 8 is sometimes dependent only on different start concentrations e.g. for the two phages at 52 °C.

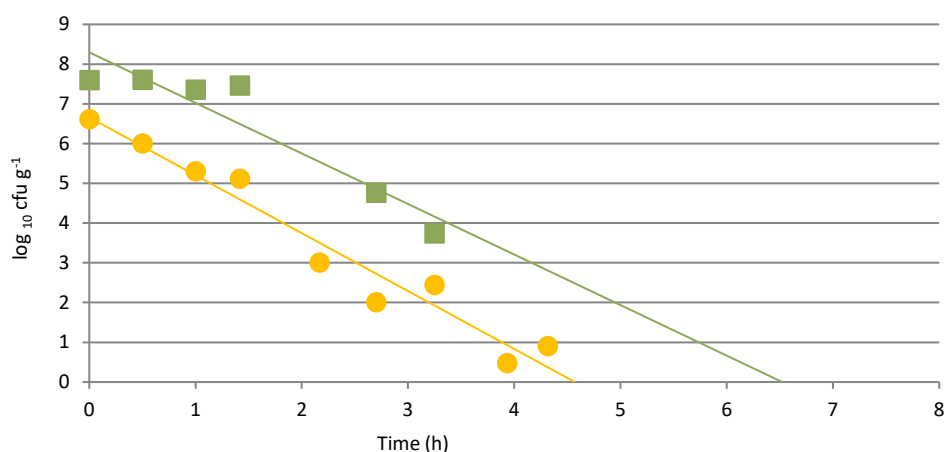


Figure 18. Decrease in $\log_{10}$ concentration over time at 48°C for *Salmonella* Senftenberg (●) and *Enterococcus faecalis* (■) showing the regression from which inactivation rate constants k were derived.

For the bacteria and bacteriophages, the inactivation was in general a magnitude faster at temperatures ≥ 48 °C with the exception of the MS2 phage that was inactivated fast at all temperatures except 37 °C (Table 8).

Table 8. The inactivation of the studied organisms given as the reduction rate constant k ($\log_{10} \text{ h}^{-1}$) / the time (h) for a 5 $\log_{10}$ reduction (bacteria) or 3 $\log_{10}$ reduction (bacteriophages). Ranges gives the outermost values measured for the parameter pH and NH_3 concentrations where the percentages for the latter give how much of total ammonia nitrogen that was present as NH_3 .

Parameter		Temperature (°C)			
		37	42	48	52
pH	-	8.05-8.25	8.10-8.28	8.15-8.31	8.13-8.38
$\text{NH}_3\text{-N}$	(g L ⁻¹)	0.8-0.9	0.9-1.2	1.6-1.8	1.6-2.1
	(%)	13-19	18-25	27-34	31-44
<i>Salmonella</i> Senftenberg	(k/ 5 $\log_{10}$ red)	-0.063 / 79	<-0.063 / <79	-1.27* / 3.9	-
		-0.099 / 51	-	-	<-1.65 / <3.0
<i>Enterococcus faecalis</i>	(k/ 5 $\log_{10}$ red)	<-0.068 / <74	-0.125 / 40	-	-
		-0.093 / 54	-0.122 / 41	-0.47 / 11	-0.63 / 7.9
ϕ x174 phage	(k/ 3 $\log_{10}$ red)	-	-	-	-
		-0.0255 / 118	-0.0346 / 87	-0.63 / 4.7	<-1.9 / <1.6
MS2 phage	(k/ 3 $\log_{10}$ red)	-	-	-	-
		-0.057 / 53	<-0.98 / <3.1	-	<-2.0 / <1.5

*in reality higher temperature than the targeted 48°C

The inactivation of *Ascaris suum* eggs followed a biphasic inactivation pattern for all the temperatures except at 48 °C where the sampling period of 7 hours was too short to cover the full inactivation and did only catch the initial lag-phase (Figure 19a). At 52 °C no viable eggs were detected after 2.5 hours (588 counted) indicating the effect of even small temperature differences at this temperature range.

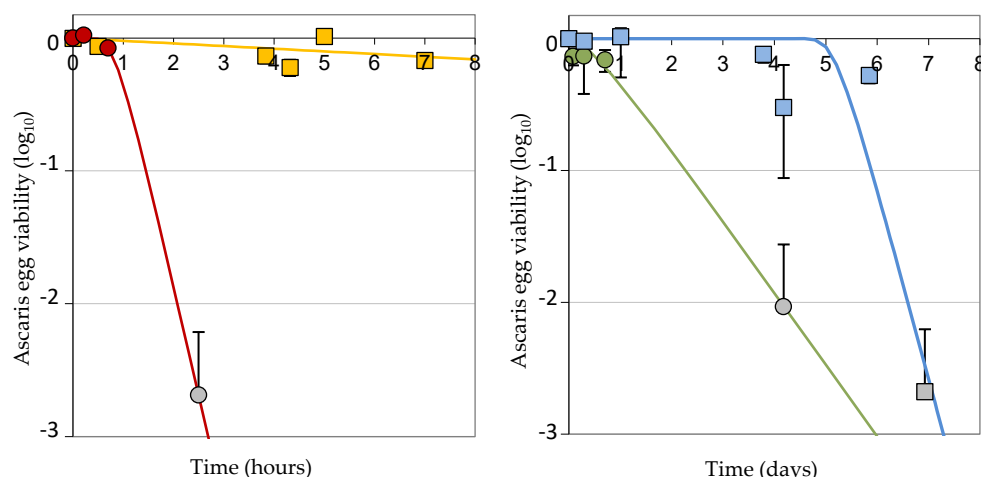


Figure 19 a) Reduction in ascaris egg viability over time in digestate kept at a) 52 °C (red) and 48 °C (yellow) and b) at 42 °C (green) and 37 °C (blue) with the biphasic model used to derive inactivation kinetics as a line. When no viable eggs were detected the detection limit (depending on number of eggs counted) is shown by shaded symbols. Error bars show the 95 % confidence interval for the proportion of viable eggs. Note the different time scale in figure a and b.

Since the inactivation after the initial lag phase was fast the detection limit (when no viable eggs were found) was used to fit the inactivation model. This means that the inactivation time (Table 9) is an over estimation and that the inactivation could have been faster, especially at 42 °C (Figure 19b). However, the overall trend shows the impact of temperature and that an increase from 42 °C to 52 °C changes the inactivation time from days to hours. For all temperatures where the full egg inactivation was studied the initial lag-phase constituted a substantial part of the inactivation time (Table 9).

Table 9. Inactivation kinetics for *Ascaris* eggs derived with Eq. 1-2, given as n , lag phase and k and time for a 3 $\log_{10}$ reduction of egg viability.

	Temperature (°C)			
	37	42	48	52
n	13.3	0.244	0	1.40
Lag (h)	139	10.8	0	0.855
k ($\log_{10} h^{-1}$)	-0.0953	-0.0226	-0.0200*	-1.63
3 $\log_{10}$ red (h)	<175	<143	na*	<2.7

*With the current sampling interval probably only the initial lag phase were detected and extrapolation to derive time for $\log_3$ reduction not applicable.

Food waste from households, restaurants and catering facilities is according to EU law classified as animal by products (ABP) of category 3. Commission Regulation (EU) No 142/2011 of 25 February 2011 implementing Regulation (EC) No 1069/2009

of the European Parliament and of the Council laying down health rules as regards animal by-products, propose approved treatments but also that alternative treatments can be validated by a 5 log₁₀ reduction of *Salmonella* Senftenberg or *Enterococcus faecalis*. When viruses are identified as a hazard the antiviral effect can be validated by a 3 log₁₀ reduction of e.g. parvo viruses. A 3 log₁₀ reduction of viable Ascaris eggs is also required to validate treatments, though only for chemical treatments.

The time for a 5 log₁₀ reduction of bacteria, which according to ABP regulations are the organisms that should be used to validate a treatment such as anaerobic digestion, decreased from 50–80 hours at 37 °C to around 3–8 hours at 52 °C. Considering all the organisms the greatest shift in inactivation was by the 6-degree increase from 42 to 48 °C (Figure 19). The Ascaris eggs and the φx174 phage would require the far longest treatment time (Figure 20a) at temperatures 37 and 42 °C. For digestate kept at 48 and 52 °C (orange and red bars) the time for a 5 log₁₀ inactivation of *E. faecalis* would require more than twice the treatment time compared the same inactivation of *S. Senftenberg*. However, even the shorter time for a 5 log₁₀ reduction of *S. Senftenberg* corresponded to a 3 log reduction of Ascaris eggs and the more persistent of the bacteriophages, the φx174 (Figure 20b).

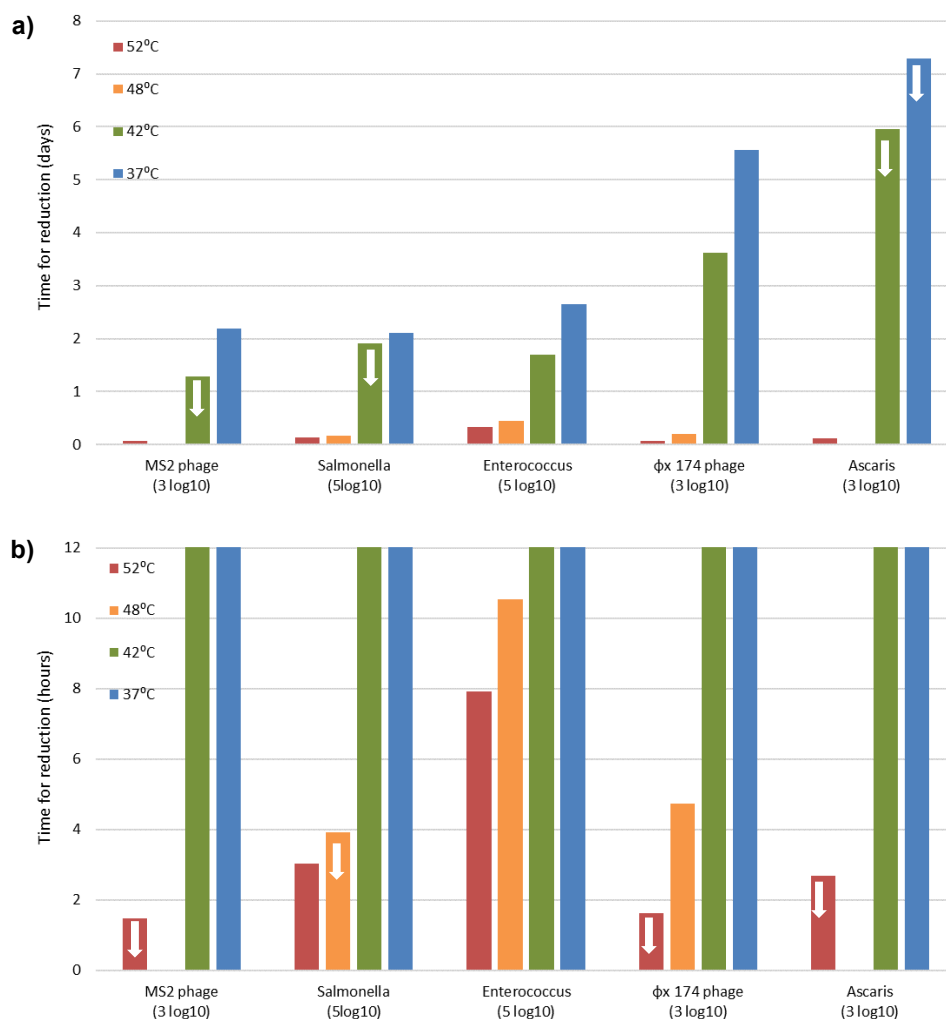


Figure 20. Time for a 5 log₁₀ reduction of *Salmonella Senftenberg* and *Enterococcus faecalis* and for a 3 log₁₀ reduction of bacteriophages MS2 and φx 174 and Ascaris eggs in digestate kept at 37-52 °C with the time given as a) days and b) hours.

5.6 ENERGY AND ECONOMY

5.6.1 Measurements at the plants

The measured specific heat consumption for the anaerobic digestion was about 40 kWh/ton treated substrate for HEMAB, Ekogas and Labio (Figure 21). If the second plug flow reactor (operated at 60 °C) at HEMAB is included in the calculation, the value was 86 kWh/ton for that plant (Figure 21).

For VMAB, the measured heat consumption was only 13–14 kWh/ton (Figure 21), which is a low value in relation to theoretical values. The value was expected to be at least the double according to theoretical calculations (see chapter 5.6.2).

The electricity consumption was 105 kWh/ton for HEMAB and 19 kWh/ton for the reactors at VMAB and Labio (Figure 21). For HEMAB, electricity consumption also

includes the pretreatment at the plant, for Labio some auxiliary systems such as lightning and reception hall is included, whereas the consumption of electricity from VMAB only includes the anaerobic digestion. No electricity data was available for Ekogas.

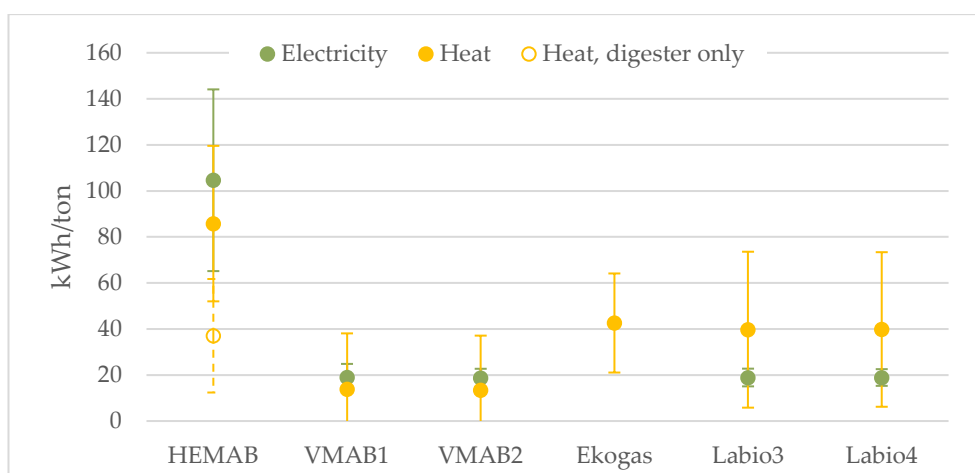


Figure 21: Specific energy consumption for the anaerobic digestion process relative to the amount of substrates treated for the participating dry digestion plants. For HEMAB and Ekogas, hygienisation is included in the total heat consumption, and pre-treatment is included in the electricity consumption for HEMAB. No data of electricity consumption was available for Ekogas.

The specific heat consumption for the anaerobic digestion relative to the produced biogas varied from 0.02 kWh per kWh produced biogas for VMAB and Labio to 0.03 and 0.04 for HEMAB and Ekogas, respectively (Figure 22). The electricity consumption was 0.01 kWh per kWh produced biogas for VMAB and Labio and 0.09 for HEMAB, respectively.

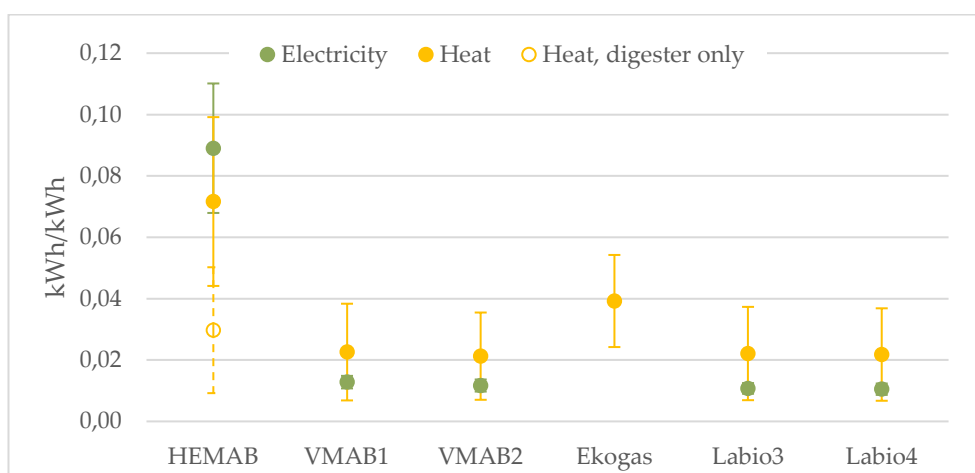


Figure 22: Specific energy consumption for the anaerobic digestion relative to biogas production at the participating dry digestion plants. For HEMAB and Ekogas, hygienisation is included in the total heat consumption, and pre-treatment is included in the electricity consumption for HEMAB. No data of electricity consumption was available for Ekogas.

5.6.2 Theoretical calculations of heat demand

Theoretical calculations of heat demand according to the description in chapter 4.6 and with fixed HRT (see chapter 4.6) resulted in a heat demand of 50.5 and 67.0 kWh/ton at mesophilic temperature (39 °C) without and with a pasteurization step, respectively. For high mesophilic (42 °C) and thermophilic (52 °C) process temperature without external hygienisation and fixed HRT, a heat demand of 56.0 and 74.5 kWh/ton was calculated, respectively. If a pasteurization step was added to the high mesophilic process, the heat demand with fixed HRT was estimated to 70.8 kWh/ton (Table 10).

For the calculation cases with adapted HRT (Table 6), taking into account that a shorter HRT potentially could be used at higher temperature due to higher methane production rate, a heat demand of 50.5, 52.7 and 67.5 kWh/ton was obtained, without an external hygienisation step during operation at 39, 42 and 52 °C, respectively. If a pasteurization step was added to the same calculation cases, 67.0 and 67.5 kWh/ton were obtained for 39 and 42 °C, respectively, thus giving almost equal heat demand per ton substrate (Table 10).

To illustrate the influence of outdoor temperature on the theoretical calculations of heat demand, the annual mean outdoor temperature was complemented with a summer and winter mean temperature as well for the high mesophilic process with pasteurization step. The heat demand obtained in the case with adapted HRT was 48.2, 67.5 and 82.9 kWh/ton for summer, annual and winter mean temperatures respectively (Table 10).

The share of the total methane production needed for the heat demand of the plant was calculated for the calculation cases with adapted HRT, meaning that a shorter HRT was assumed for higher process temperature. For these calculations, the methane production has been assumed to be 500 Nm³/ton VS (independent of process temperature) and with a TS of 33 % and a VS of 85 % (of TS) in the substrate. The calculation then indicated that between 3.6 and 4.8 % of methane production at the plants would be needed for heating at annual mean outdoor temperature (Table 10, Figure 23).

The theoretical calculations of heat demand indicate that, if a pasteurization step is required for mesophilic as well as for high mesophilic process temperature to achieve hygienic standard according to the ABP-regulation, there might be no clear difference in heat demand between dry digestion plants operated at mesophilic, high mesophilic and thermophilic process.

Table 10. Theoretical calculations of heat demand and share of methane production needed for heating to different process temperatures, with/without pasteurization, different outdoor temperature and with fixed or adapted HRT respectively.

Calculation cases	Outdoor temperature ¹⁾	Fixed HRT ²⁾ (kWh/ton)	Adapted HRT ³⁾ (kWh/ton)	Share of CH ₄ -production at adapted HRT ⁴⁾ (%)
Mesophilic, 39 °C	Annual mean	50.5	50.5	3.6
Mesophilic, 39 °C with pasteurization	Annual mean	67.0	67.0	4.8
High mesophilic, 42 °C	Annual mean	56.0	52.7	3.8
High mesophilic, 42 °C with pasteurization	Annual mean	70.8	67.5	4.8
High mesophilic, 42 °C with pasteurization	Summer	50.5	48.2	3.4
High mesophilic, 42 °C with pasteurization	Winter	86.8	82.9	5.9
Thermophilic, 52 °C with internal hygienisation	Annual mean	74.5	67.5	4.8

¹⁾ Swedish average outdoor temperature set to 7, 17 and 0 °C for annual mean, summer and winter temperatures respectively.

²⁾ HRT set to 47.5 days independent of process temperature.

³⁾ HRT set to 47.5, 40 and 35 days for mesophilic, high mesophilic and thermophilic process temperature respectively.

⁴⁾ Assuming the following conditions of the substrate: 500 Nm³ CH₄/ton VS, 33 % TS and 85 % VS of TS.

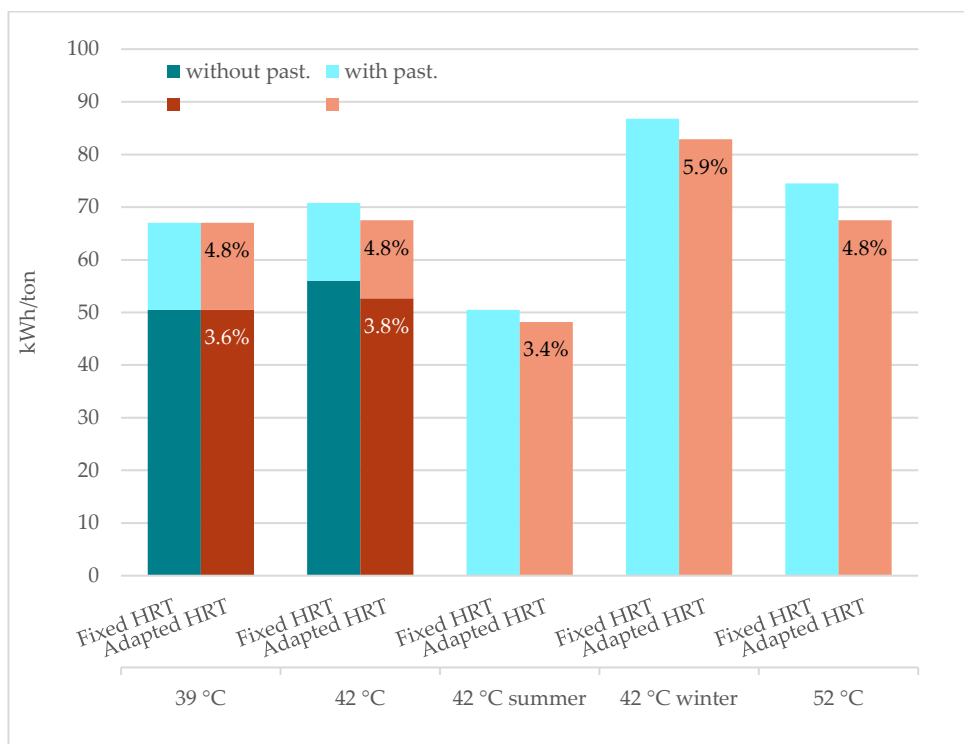


Figure 23: Heat demand. Dark stacks refer to systems without pasteurization, light stacks to systems with pasteurization. Percent numbers indicate the share of the energy in the produced biogas the heating demand corresponds to. "Fixed HRT" means HRT is set to 47.5 days independent of process temperature. "Adapted HRT" means HRT is set to 47.5, 40 and 35 days for mesophilic, high mesophilic and thermophilic process temperature, respectively.

5.6.3 Economic analysis

The cost for heating for the different calculation cases, assuming a biogas price of 0.5 SEK/kWh, gives a cost of 25-37 SEK/ton substrate (Table 11).

Table 11: Cost for heating at different process temperatures and with and without a pasteurization step at annual mean outdoor temperature of Sweden. Cost calculated both for fixed and adapted HRT.

Calculation cases	Cost at fixed HRT ¹⁾ (SEK/ton)	Cost at adapted HRT ²⁾ (SEK/ton)
Mesophilic, 39 °C	25	25
Mesophilic, 39 °C with pasteurization	33	33
High mesophilic, 42 °C	28	26
High mesophilic, 42 °C with pasteurization	35	34
Thermophilic, 52 °C with internal hygienisation	37	34

¹⁾ Assumed biogas price 0.5 SEK/kWh. HRT set to 47.5 days independent of process temperature.

²⁾ Assumed biogas price 0.5 SEK/kWh. HRT set to 47.5, 40 and 35 days for mesophilic, high mesophilic and thermophilic process temperature respectively.

6 Discussion

6.1 PROCESS PERFORMANCE

6.1.1 Methane yield

Based on BMP tests of food waste in previous studies, a specific methane production of approx. 500 Nm³/ton VS could be expected (Carlsson & Uldal, 2009). The methane yield of the investigated plants was in this range but with some differences between the plants. The specific methane production at Labio was comparably high and also VMAB, during operation at mesophilic condition, showed slightly higher values than reported in literature values. On the other hand, HEMAB and Ekogas showed values about 20 % lower than the theoretical yield. If recirculation of digestate is not accounted for, the values from Labio are 20 % above that level. However, if recirculation is included in the calculations of the specific methane production from Labio, the methane yield coincides with what is expected (Figure 6). As the VS of the recirculated material could be viewed as “counted twice” when the recirculation is included in the calculation of the methane yield, achieved methane yield at Labio still is unexpectedly high. As a share of the reported added food waste was organic waste from industrial origin, such as filter cake from vegetable oil refinery, this is probably a contributing factor to the high methane yield achieved at the plant. As the amount of industrial waste was not measured at the plant, the significance of this addition is difficult to estimate.

The VS measurements of the incoming substrates were not corrected for loss of VFA during the analysis (no VFA analysis was made for the substrates at the plants). This might give an over-estimation of the calculated specific methane production for the plants, especially as food waste is typically partly hydrolysed when arriving to the plant.

6.1.2 Recirculation of digestate

Recirculation is common during dry digestion, but it is not obvious how to adjust parameters, such as OLR and HRT, when recycling is used. The recycled fraction contains organic matter that can be seen as an increased load of to the reactor. However, the organic compounds in the recirculate are already accounted for when initially added via the substrate. Also, recirculation of digestate may reduce the plug flow effect in the reactor since TS can get too low. On the positive side, recirculation of biodigestate may significantly contribute with reinoculation of important microorganisms. How recirculation of digestate and the importance of maintaining a plug flow should be weighted to each other for a good process performance needs to be further investigated.

6.1.3 Addition of process aids

All the participating plants, except Labio, were using some process aids during the test period in order to improve digestion and/or to abate hydrogen sulphide formation. It is well known that biogas processes operating with food waste can

benefit from addition of trace metals such as iron (Bragulia et al., 2018; Wei et al., 2014; Xu et al., 2018). The sewage sludge added at Labio before the start of the test period and at the beginning of the test period was very rich in iron sulfate according to the operator of the plant and might explain why this plant had good performance without any additives. Moreover, the addition of sewage sludge might also have contributed with addition of trace elements. After the test period ended, Labio again started to add some sewage sludge into the digester.

6.1.4 Calculation of HRT

No consideration of the mass reduction in the digester was made when calculating the HRT. In practice, the mass reduction means that the calculated HRT stated in the report is underestimated, i.e. HRT is somewhat longer than stated in the present study.

6.1.5 VMAB1 and VMAB2

There is a difference in process performance between VMAB1 and VMAB2, especially pronounced in the VS reduction capacity (based on 12 measurements of weekly samples of outgoing digestate). The difference might partly be due to the addition of rape husk at VMAB2 and to different addition of trace elements. As no microbiological analysis has been made for VMAB2, no guidance to the difference can be found there.

6.2 MICROBIOLOGY AND PROCESS

6.2.1 The bacterial community

The microbial community analysis demonstrated that each process in the present study had an individual microbial profile and that operating temperature strongly influenced the community structure. However, the analysis still identified three core populations (the genera *Deftuviitoga*, *Syntrophaceticus* and the uncultured Clostridia group MBA03) that were present in all digesters. The group MBA03 even represented half of the communities in the thermophilic Ekogas and VMAB sample 1-3, indicating an important role in these processes. This group has previously been found in large quantities in AD processes fed with agricultural residues, where it correlated with high TS load and high ammonium levels (Calusinska et al., 2018). On the contrary, in another mesophilic AD processes fed with animal manure, the abundance of this group correlated with increased levels of starch and lower levels of ammonia. Group MBA03 has also been suggested to perform syntrophic degradation reactions with *Methanosarcina* (Calusinska et al., 2018; Rago et al., 2018). Consequently, based on the large variation of proposed functions of this group, it is difficult to fully anticipate its role in the processes of the present study and further research is required to establish linkages to operating parameters.

6.2.2 Hydrolysis and acid formation

Another group of the core populations, *Defluviitoga*, is a hydrolytic bacterium involved in the first steps of the biogas producing chain and using various complex polysaccharides, including cellulose, chitin and xylan for growth. This is a hydrogen producing bacterium but other products such as ethanol, acetate and carbon dioxide are also formed (Ben Hania et al., 2012; Maus et al., 2016). Another hydrolytic bacterium found in the present study, *Halocella*, is known to degrade cellulose, starch and a broad spectrum of carbohydrates. In conversion of cellulose, it produced acetate, ethanol, lactate, hydrogen and carbon dioxide (Simankova et al., 1993). Consequently, the high relative abundance of *Defluviitoga* and *Halocella* at thermophilic conditions indicates a high production rate of hydrogen at Ekogas and VMAB (sample 1–3). This requires a high activity of hydrogen-utilizing methanogens in order to keep the hydrogen partial pressure low. However, the considerably high propionate level in the thermophilic process is a strong indication that the high hydrogen pressure could be an inhibiting factor at Ekogas and VMAB (sample 1–3, further discussed below). Furthermore, both *Defluviitoga* and *Halocella* have previously been shown to be halophilic, i.e. tolerate high or moderate salt concentrations (Ben Hania et al., 2012; Simankova et al., 1993), and their presence in the high-ammonia, thermophilic digesters of the current study demonstrates their members to be ammonia-tolerant.

All mesophilic digesters had relatively high abundance of the genera *Proteiniphilum*. This bacterium breaks down proteins to acetate, carbon dioxide and ammonia (Chen & Dong, 2005), indicating an important role for conversions of proteins at HEMAB, Labio and VMAB (sample 4–11). In contrast to the thermophilic Ekogas, *Proteiniphilum* was highly abundant at VMAB also during operation at thermophilic conditions. It could be hypothesized that a high abundance of *Proteiniphilum* led to an efficient protein conversion, which is furthermore supported by the high ammonium level in this process. However, further studies are required to establish such relation.

High abundance of phylum Cloacimonetes was a distinct feature of the mesophilic HEMAB, Labio and VMAB (but only in samples 4–8). This is a candidate phylum and its members have thus not been isolated so far. However, genomic studies and suggested involvement of Cloacimonetes in degradation of cellulose (Limam et al., 2014) or amino acid to acetate, CO₂ and H₂. The activity of Cloacimonetes is thus likely dependent on syntrophic cooperation with hydrogen-consuming and/or acetate-utilizing partners (Stolze et al., 2017; Stolze et al., 2016). Cloacimonetes has also been suggested to perform syntrophic propionate oxidation (Nubo et al., 2015).

6.2.3 Acid degradation

Syntrophomonas, foremost found in Labio and the mesophilic period of VMAB, is commonly enriched in mesophilic digestion of lipid-rich materials (Amha et al., 2017; Baserba et al., 2012; Kougias et al., 2016; Ning et al., 2018; Shigematsu et al., 2006; Sousa et al., 2007; Yekta et al., 2017; Ziels et al., 2015; Ziels et al., 2017). These bacteria degrade long chain fatty acids (LCFAs) to hydrogen acetate/propionate (Ziels et al., 2018) and depend on hydrogen-using methanogens for an efficient

degradation. The conversion of LCFA can represent a rate-limiting step (Cirne et al., 2007; Cuetos et al., 2008) and high abundance of *Syntrophomonas* thus indicates that the Labio and mesophilic VMAB had capacity for efficient lipid degradation. Both *Methanoculleus* and *Methanosarcina* could have been the hydrogen-utilizing partners for the LCFA-degrading bacteria (Amha et al., 2017; Treu et al., 2016).

Presence of the fourth core population, *Syntrophaceticus*, in all digesters indicates a strong dependence on syntrophic acetate oxidation for efficient methane production in the processes. The only characterized species in *Syntrophaceticus* is a mesophilic bacterium that tolerates high ammonia levels (Westerholm et al., 2010). This is a slow growing bacterium and the relatively long retention time in the dry digester processes seems to support its presence compared with other digester types. However, members of this genus have also been detected in various biogas systems operating at high ammonia conditions (Westerholm et al., 2016). The variation and occasionally slightly higher acetate levels in the mesophilic HEMAB and VMAB (sample 4–11) and the thermophilic Ekogas (0.2–2.1 g/L) indicate a potential to optimize the activity of syntrophic acetate oxidizing bacteria in these processes.

The extremely high propionate levels in the thermophilic digesters at VMAB (sample 1–3) and Ekogas point to propionate degradation being a major limiting step at elevated temperatures. Propionate is a common intermediate in AD, which is degraded via syntrophic interactions between syntrophic propionate degrading bacteria and hydrogen and acetate utilizing microorganisms. Knowledge of propionate degradation at high ammonia levels is currently lacking (Westerholm et al., 2015). However, ammonia-tolerant propionate degrading bacteria from biogas digesters have been identified (data in preparation) but these groups were not found in present processes.

6.2.4 Methanogenesis

M. bourgensis was highly abundant in all reactors, particularly at Labio, and can potentially be a key factor to the high performance of this process. *M. bourgensis* has high ammonia tolerance and a high affinity for hydrogen. These two features make this methanogen a good partner to syntrophic bacteria. Due to its high hydrogen affinity, the hydrogen partial pressure can be lowered, which facilitates syntrophic reactions, such as fatty acids degradation (Westerholm, 2012). *M. bourgensis* has been suggested to be supported by trace element addition and was suggested as a resilient promoter for lower hydrogen partial pressure and enhanced performance of high-ammonia processes (Westerholm et al., 2015). Since low hydrogen level is of particular importance in anaerobic processes with syntrophic acetate degradation, a well-functioning hydrogen-utilizing community structure will increase acid conversion rates and likely have a major impact for improved process stability and efficiency. Syntrophic acetate oxidation with *M. bourgensis* as hydrogen partner and *Syntrophaceticus* as syntrophic bacteria has shown optimal methane production rates at 42 °C (Westerholm et al., 2019). Another insight obtained in growth studies of syntrophic microorganisms is that they are slow-growers. Syntrophic acetate degraders can therefore easily be washed out and increased HRT, immobilization of microorganisms and

recirculation of process material are therefore suitable actions to maintain syntrophic microorganisms in biogas processes and to prevent ammonia inhibition (Moestedt et al., 2013; Rajagopal et al., 2013; Westerholm et al., 2016). Consequently, the strong dependence of SAO in all processes in the present study indicates that the operation at SAO-optimal temperature and the recirculation of process material in Labio could have contributed to the higher performance of this process.

6.2.5 Impact by ammonia, temperature and recirculation on microbiology

Analyses of operating parameters demonstrated that all processes operated at ammonia levels above the anticipated threshold, above which methane is mainly formed through syntrophic acetate oxidation instead of acetoclastic methanogenesis (Westerholm et al., 2016). This was furthermore supported in the present study by high abundance of known syntrophic microorganisms in all processes. This insight is important for process operation. Through cultivation studies we know that syntrophic acetate degrading microorganisms grow slowly and can be washed out if HRT is too short. As hypothesized above, recirculation of process material can be an effective way to maintain high activity of these microorganisms in the process. Operation at temperatures between 42 and 45 °C are other approaches demonstrated to improve methane formation in laboratory cultures (Westerholm et al., 2019) or performance of biogas processes with syntrophic acetate degradation as dominant methane production pathway (Moestedt et al., 2014).

In the present study, microbial analyses on the liquid phase after dewatering of digester content were conducted for the HEMAB process. This liquid was occasionally used for recirculation in the process. Dewatering will likely remove microorganisms attached to solid particles, whereas microorganisms occurring in liquid phase will increase in abundance. The analysis of the dewatered samples in HEMAB (sample 6, 7) revealed higher levels of *Defluviitoga* and *Actinomyces*. As mentioned above, *Defluviitoga* degrades complex polysaccharides and also *Actinomyces* has been suggested to be involved in the hydrolytic step and degradation of cellulose (Wang et al., 2016). However, since the recirculation of digestate was made occasionally (and not at all during the test period) at HEMAB, it is difficult to interpret whether this recirculation had an impact on the overall process performance.

Higher temperature has in many studies been reported to increase diversity (number and distribution of different species) in anaerobic CSTRs (Levén et al., 2007; Westerholm et al., 2018). However, this was not observed in the present dry digesters, in which relatively similar levels were obtained in all processes. It is not clear how microbial diversity impacts on process performance, but a high diversity and even distribution of different species have been suggested to contribute to high process stability and flexibility (Carballa et al., 2011; De Vrieze et al., 2018; De Vrieze et al., 2013). However, this likely depends on the process and cannot be applied as a general rule.

6.3 HYGIENISATION EFFECT

The total ammonia nitrogen in the first batch of digestate ($4.7 \text{ g NH}_{3/4}\text{-N L}^{-1}$) was similar to concentrations measured at the Ekogas plant whereas the second batch ($6.1 \text{ g NH}_{3/4}\text{-N L}^{-1}$) can be considered high compared to what was measured at the plant (Figure 11). The pH measured during this bench scale study, in average 8.2, was a bit lower than the average pH of 8.4 measured at the plant (Figure 10) and probably due to the disturbance when handling the material at the start-up of the study, i.e. stirring and mixing. The bench scale study evaluated the hygienic effect at different temperatures where 37°C is close to operation temperatures by HEMAB and VMAB1, 42°C to operation temperatures at Labio 3 and 4 and the 55°C fairly close to the operating temperature of Ekogas. Since the digestate used in the bench scale study originated from Ekogas it had higher pH than the other plants (Figure 10), and this higher pH was reflected in the calculated NH_3 concentrations. The NH_3 concentrations at 52°C in the present study was in line with what was measured at Ekogas, but especially for 37°C the NH_3 concentrations were slightly higher than what was measured at the plants operating at mesophilic temperatures (Figure 11 and Table 8).

Studies where the investigated organisms have been held at similar temperatures in ammonia free pH buffers confirm that it is a combination of temperature and ammonia that are contributing the observed inactivation (Nordin et al., 2009a; Pecson et al., 2007). The effect of ammonia has been confirmed for all the organisms in this study but most often at higher ammonia concentrations but lower temperatures (Fidjeland et al., 2015; Nordin et al., 2009b; Pecson et al., 2007). These studies do however confirm the difference in sensitivity to ammonia, i.e. that the MS2 phage appear to be more sensitive than the ϕx174 phage (Decrey et al., 2016; Vinnerås et al., 2008). The difference in sensitivity is however less pronounced at the higher temperatures in this study ($\geq 48^\circ\text{C}$) where the temperature effect starts to overshadow the effect of ammonia, e.g. for the *Ascaris* eggs (Figure 19a).

The calculated NH_3 values ranged from $0.8\text{--}2.1 \text{ g N L}^{-1}$ (Table 8). In a study of sanitization by anaerobic digestion performed at 35 and 55°C , however at lower NH_3 concentrations than in the present study, *Salmonella* spp. was not detected after 13 and 3 days respectively, whereas a $5 \log_{10}$ reduction of fecal coliform bacteria required 60 days at both temperatures (Scaglia et al., 2014). Sahlström et al. (2008) who studied inactivation in digestate at 55°C found that 15 minutes were enough to inactivate *Ascaris suum* eggs whereas in this study viable eggs were observed after 42 minutes at 42°C (Figure 19a). Sahlström et al. (2008) also found that *E. coli* was inactivated after 1 hour whereas *Enterococcus* spp. sustained. Such differences can be due to the higher dry matter in the present study offering some protection to the pathogens but may also just depend on the three-degree difference in temperature.

The pH and ammonia measured at Ekogas was rather confined indicating that the hygienisation will be rather predictable and stable. Even though it was a marked difference in inactivation rate between 42 and 48°C , the time for a $5 \log_{10}$ reduction of the bacteria was for all organisms still shorter than the digester retention time (30–40 days, Figure 9). Thus, under conditions of real plug flow also the

lower temperatures could sanitise the material according to the ABP related legislations during the digestion, given that the NH_3 concentrations resulting also from pH and total ammonia nitrogen will be around $1 \text{ g NH}_{3/4}\text{-N L}^{-1}$ (Table 8).

6.4 ENERGY AND ECONOMY

6.4.1 Assumptions for the theoretical calculations

There have been too little data, in this study as well as in literature, to say anything about different total methane production in relation to different process temperatures in the calculation cases regarding energy and economy. Therefore, the same specific methane production has been assumed in all calculation cases. In the calculation cases with adapted HRT, a higher methane production rate has been taken into consideration in the way that smaller digesters have been assumed for the higher process temperatures.

In all calculations of heat demand, the resulting temperatures after both anaerobic digestion and pasteurization are rather high. It is possible to recover more heat with an additional heat exchanger, but the temperature levels are too low to be useful in a boiler. To utilize more recovered heat, an additional heating circuit needs to be installed in the inlet part of the digester.

For the economic analyses, an assumed price of produced biogas of 0.5 SEK/kWh was used. The value of the biogas may however differ depending on local conditions and will of course change over time. The calculated energy demand may therefore give a better indication of the resources needed for the different calculation cases.

6.4.2 Theoretical calculations versus measurements at the plants

The theoretical calculations of heat demand per ton incoming substrate are all higher than the heat measurements at the plants. For HEMAB, Ekogas and Labio, the measured heat demand is about 24–36 % lower than the theoretical calculations. If the actual HRT for the plant is used in the theoretical model, together with a somewhat warmer outdoor and substrate temperature (3°C higher) than of the assumed annual mean temperature (due to the test period being in spring), the measured respectively theoretical calculated heat consumption for HEMAB and Labio is similar (theoretical calculation at HEMAB gives 36.4 kWh/ton and at Labio 42.9 kWh/ton, and the corresponding measured values at the plants were 37 and 40 kWh/ton, respectively). For Ekogas, the same calculation experiment still gives about 28 % higher heat demand in the theoretical model compared to the measured value (theoretical calculation gives 59.8 kWh/ton, and the corresponding measured value was 43 kWh/ton), indicating that the measurement at the plant underestimates the heat consumption. In the case of VMAB, the measured heat consumption seems to be unrealistically low, as for example the theoretical calculations show that a temperature of about 30°C would be needed in the incoming substrate and negligible heat losses would be a prerequisite to achieve the measured value.

7 Conclusions

7.1 PROCESS PERFORMANCE

- The plant at Labio shows the best process performance in comparison with the other plants. It has the shortest HRT and highest OLR and still showed the highest VS reduction, specific methane yield (recirculation not included) and volumetric methane production and at the same time the lowest VFA and the highest ammonium concentration in its digestate. What is unique for Labio compared with the other plants in this study is the temperature (high mesophilic) and the recirculation of digestate (45 % by wet weight). The background to the positive effect of these factors needs to be further investigated. Additional factors that may have contributed positively to Labio's good process performance are that a proportion of the added food waste was industrial organic waste, contributing to the high methane yield, and that iron rich sewage sludge was added before the start of the test period.
- The participating dry digestion plants were operated at higher OLR than most corresponding CSTR plants treating food waste. Despite high ammonia concentrations in the dry digestion plants, they had good process performance at mesophilic and high mesophilic temperature, where trace elements and iron additives may have been positively contributing factors. Previous studies of other dry digestion processes have shown stable operation at similar high ammonia levels as in the present study, and also at thermophilic conditions (Goberna et al., 2009; Huang et al., 2017).
- The high levels of propionate in the thermophilic processes (Ekogas and VMAB 2017) clearly show that high temperatures, in combination with high ammonia levels, give unfavorable conditions for degradation of propionate in dry digestion processes.
- The number of plants included in the study is too low to be able to clearly decide which operating temperature is most optimal. However, it can be said that a thermophilic plug flow dry digestion process with food waste as principal substrate is more difficult to operate in a stable manner and with high methane yield compared to likewise mesophilic operation. How this should be weighed against not having a sufficient in situ pasteurization has to be assessed in each case. If a plant is designed and planned to be operated thermophilically, it is wise to secure good surveillance and keep in mind that the temperature might need to be decreased. A decrease to a lower thermophilic temperature might be sufficient to reduce the risks of ammonia inhibition, alternatively a mesophilic temperature might be needed, requiring external post-pasteurization.

7.2 MICROBIAL COMMUNITY ANALYSIS

- Relative abundance of bacteria performing hydrolysis of proteins, carbohydrates and lipids clearly diverged in mesophilic and thermophilic processes indicating that the hydrolytic efficiency varied between the plants. Further studies will be conducted to analyse this difference.

- All processes are operated above the ammonia threshold shown to direct methane formation to syntrophic acetate oxidation instead of aceticlastic methanogenesis. This was furthermore supported in the present study by high abundance of known syntrophic microorganisms in all processes.
- The relatively higher levels of *M. bourgensis* at Labio than in the other processes indicate that a slightly higher temperature (42 °C) and recirculation at Labio supported the hydrogenotrophic methanogenic activity. Accordingly, the considerably high levels of propionate and absence of potential syntrophic propionate oxidisers in thermophilic digesters indicate that this degradation step was a bottleneck in these processes and could be strongly connected to high hydrogen levels. Management approaches to support hydrogenotrophic methanogens and propionate degrading communities would substantially improve stability and methane yield in the thermophilic processes.

7.3 HYGIENISATION EFFECT

- Considering the 5 log₁₀ reduction of *Enterococcus faecalis* and *Salmonella Senftenberg* and 3 log₁₀ reduction of *Ascaris* eggs suggested by ABP regulations for validation of sanitising treatment, temperatures of 48 °C and above could achieve the required reduction in 8–10 hours which is favorable if the minimum retention time of the digesters is short. At temperatures of 37–42 °C, the 7 days needed for hygienisation may exceed the minimum retention time unless conditions for real plug flow are achieved. It should be noted that the digestate used in the study had higher pH and total ammonia nitrogen concentration than the mesophilic digester contents and the results should be validated using mesophilic sludge.
- The study showed that the time for inactivation of pathogens and indicators can markedly be reduced by increasing temperature from 42 to 48 °C. At temperatures between 37 °C and 42 °C it was mainly the reduction of *Ascaris* eggs and bacteriophages that was slow.
- Efficient killing of the different indicator organism was likely caused by a combined effect of the high temperature and high ammonia concentrations, as shown before (Lloret et al., 2013) (Scaglia et al, Lloret et al 2013). The study indicates that with the ammonia concentrations in the material it might be possible to reach sufficient sanitation even at a high mesophilic/low thermophilic temperature. Being able to lower the process temperature slightly from 52–55 °C can give significant process advantages. However, to confirm these results, more studies are needed.

7.4 ENERGY AND ECONOMY

- The theoretical calculations of heat demand indicate that if a pasteurization step is required for mesophilic as well as for high mesophilic process temperature to achieve hygienic standards according to the ABP-regulation, there might be no difference in heat demand between dry digestion plants operated at mesophilic, high mesophilic and thermophilic temperature.
- If no pasteurization step was needed to achieve hygienic standards according to the ABP regulation for a high mesophilic temperature process (42 °C), the theoretical calculations indicate that 16–21 % less heat would be needed compared to mesophilic process temperature (39 °C) with pasteurization. Accordingly, compared with thermophilic processes (52 °C), the heat demand would be 22–25 % lower for the high mesophilic temperature process (42 °C) if a pasteurization step could be excluded.

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DRY ANAEROBIC DIGESTION OF FOOD WASTE

Dry plug flow anaerobic digestion (AD) is a relatively new technology for the biological treatment of food waste. To avoid costly external hygienisation, most Swedish dry AD plants wish to operate under thermophilic conditions. However, source separated organics (SSO) with high nitrogen content increase the risk for ammonia inhibition at higher temperatures.

The hygienisation analysis indicated that a temperature >42 and <48 °C can be sufficient to reach pathogen reduction according to the ABP regulation. This means that at high ammonia level it might be possible to reach sufficient sanitation even at a high mesophilic/low thermophilic temperature. Being able to lower the process temperature slightly from 52–55 °C can give significant process advantages.

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