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Effects of thermal hydrolytic pre-treatment on biogas process efficiency and microbial community structure in industrial- and laboratory-scale digesters

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ABSTRACT

This study examined the impact of thermal hydrolysis process (THP) pre-treatment on anaerobic co-digestion of wastewater sludge and household waste and assessed whether THP was vital to achieve higher process capacity. Performance data were collected for both industrial- and laboratory-scale digesters and response in microbial community structure was evaluated by Illumina sequencing. Implementation of THP at the industrial-scale plant increased methane yield by 15% and enhanced substrate degradability. Possibility to extend the sludge retention time due to a higher solid content of the substrate, sanitisation of the digestate and improved fertiliser quality of the digestate were other industrial-scale benefits of THP installation. Continuously-fed laboratory-scale digesters were fed THP-treated or untreated substrate at an organic loading rate (OLR) of 5 g volatile solid (VS)/L/day, a feeding rate necessary at the corresponding industrial-scale plant to meet the estimated population increase within the municipality. The results indicated that the plant could have increased the capacity with unimpaired stability independently of THP installation, even though the retention time was significantly shortened during operation with untreated substrate. Microbial community analyses revealed increased contribution of the Clostridia class after THP installation in industrial-scale digesters and positive correlation between Firmicutes:Bacteriodetes and methane yield in all digesters. Differentiated profiles in laboratory-scale digesters indicated that a temperature increase from 37 to 42 °C in association with THP installation and altered substrate composition were strong determining factors shaping the microbial community. Overall, these findings can assist industrial-scale plants in choosing management strategies aimed at improving the efficiency of anaerobic digestion processes.
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1. Introduction

Anaerobic digestion (AD) is a well-established waste-to-energy technology that efficiently treats and stabilises sewage sludge (SS) from wastewater treatment plants in many European countries. It is also increasingly being used for converting municipal solid waste (MSW) from households, restaurants and commercial food processing into biogas and, considering the huge amount of MSW generated daily, the potential for further expansion is significant (Ingrao et al., 2018; Karthikeyan et al., 2018). SS and MSW can be used separately as feedstocks for biogas production, but co-digestion of these wastes can be economically and environmentally advantageous, since it often involves increased biogas yield and improved quality of the digestate as fertiliser (Mata-Alvarez et al., 2011; Righi et al., 2013; Silvestre et al., 2015; Sosnowski et al., 2003). Many European waste treatment plants are currently striving to improve their waste collection and treatment systems in order to meet more stringent waste management regulations and cope with estimated population increases (Kelessidis and Stasinakis, 2012). Construction of additional AD chambers is one solution, but involves large investment costs and requires additional land, which may unavailable since many facilities are located in densely populated regions. An alternative for existing biogas plants is to increase the volume of waste that can be treated (degradation capacity) by applying pre-treatments and/or increasing the organic load (Björn et al., 2017; Cano et al., 2015; Westerholm et al., 2018). For MSW, high organic loads can be

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achieved without affecting process efficiency (Westerholm et al., 2018). However, SS generally has low solid content (Björn et al., 2017), which restricts high organic load since the sludge retention time might become too short for microbial degradation. Various pre-treatment approaches for SS have been evaluated at experimental scale and some have been proven to be energetically self-sufficient and are now commercially available for large-scale application (Cano et al., 2015). The thermal hydrolysis process (THP) technology, in which heat is applied to the feedstock for a defined period, is currently the most common pre-treatment approach in wastewater plants (Cano et al., 2015). This treatment improves the accessibility of the material to microbial degradation, but also results in decreased viscosity and increased dewaterability of SS, which permits higher organic loads without loss of efficiency. Moreover, the THP approach reduces the pathogen level to sufficiently low levels to enable direct application of the digestate to agricultural land (Anjum et al., 2016; Barber, 2016). This eliminates the need for an additional sanitisation step and lowers the capital investment and maintenance costs.

The key to successful AD is efficient and balanced microbial activity of the complex multi-step sequence of substrate hydrolysis, fermentation and methanogenesis. In the case of SS, a rigid network structure, low sludge solubilisation and large particle size limit the rate of the initial hydrolytic step, and thus the overall degradation efficiency (Shimizu et al., 1993). Impacts of THP on chemical and physiological properties of SS have been studied in detail (Anjum et al., 2016; Zhang et al., 2018), but the influence of THP on microbial AD communities and how this links to process performance have been less well studied. A more complete understanding on how microbial community responses to pre-treatment is critical in order to optimise AD performance by providing optimal conditions for microbial activity, to reveal long-term effects and to ensure prolonged process stability.

The aim of present study was to investigate the effects on AD process performance and microbial community structure of installing THP in two industrial-scale digesters treating SS and MSW at a wastewater treatment plant (Sundets biogas, Växjö, Sweden). The aim with THP installation at the plant was to increase treatment capacity and integrate a sanitisation step. Sanitisation is currently not required for treatment of SS in biogas plants, but household waste must be sanitised under European Union Regulation (EC) 208/2006 on disposal of animal by-products. Based on estimated future population increase within Växjö municipality, the current feeding rate at the industrial-scale plant of 3 g volatile solid (VS)/L/day must be increased to 5 g VS/L/day, an increase which THP was intended to enable. Installation of THP involved management adjustments in the industrial-scale plant. The experimental set-up in the present study was designed to evaluate the separate or combined effect(s) of these adjustments and the degradability of the substrates in batch trials and in continuous laboratory-scale digesters. The impact of THP installation on the potential to enhance operating capacity was evaluated by gradually increasing the loading rate in the continuous digesters. The processes were evaluated in regard to process performance and stability, including both chemical and microbiological parameters. Furthermore, by constructing taxonomic time series, the effects on microbial community structure of substrate dewatering, THP and increased loading were determined in industrial- and laboratory-scale digesters.

2. Material and methods

2.1. Industrial-scale biogas plant

Two large-scale (1700 m^3) anaerobic digesters, hereafter referred to as P1 and P2 (Sundets biogas plant, Växjö, Sweden), were used in the analysis. Sundets biogas plant treats sewage sludge from a wastewater treatment plant (Växjö, Sweden) and a source-sorted organic fraction of MSW from households, restaurants and industries. In the reference period (December 2013–February 2014), the operating temperature was 37 °C and the waste treated consisted of 57% SS and 43% MSW (total solids (TS) basis). P1 and P2 operated in semi-serial mode during this period, in which P1 was fed with the SS:MSW mixture and P2 received 50% of the SS:MSW mixture plus the effluent from P1 (Table 1). The SS was dewatered via belt presses prior to being fed into the digesters and the organic loading rate (OLR) was on average 3.3 and 4.3 g volatile solids (VS)/L/day for P1 and P2, respectively. The hydraulic retention time (HRT) was 19 days for P1 and 10 days for P2.

To intensify treatment capacity and integrate a sanitisation step, THP (CambiTHP™) technology was installed in early 2015. The CambiTHP process consists of three main units: pulper, THP-reactor and a flash tank (Fig. 1). To improve energy efficiency the influent material to the THP-system should have TS between 14 and 20% in order to minimize the volume that needs to be heated and to obtain suitable viscosity for the pumping system used at the plant. SS was thus thickened and dewatered (from 6 to 16% TS) through a centrifugation step before entering the pulp tank. MWS had ~14% TS content and could be introduced directly into the pulp tank. In the pulp tank, centrifuged SS and MWS were homogenized and preheated to ~97 °C by recycled steam (see below). The preheated material subsequently entered the THP-reactor for treatment by high-pressure steam at 165 °C and 6 bar for 20 min. When the material thereafter was introduced into the flash tank the pressure rapidly dropped to atmospheric pressure with the aim to cause a vapour explosion that disrupt cell structures within the biomass. The material was finally cooled to 107–110 °C using heat changers (to recover heat for use in other plant facilities) before entering the AD digesters. The steam produced in the THP-reactor was returned to the pulper tank to heat the material to avoid losses of organic compounds vaporised during treatment. The load of material to the AD reactor was maintained at the same level as before the treatment, e g OLR about 3.7 g VS/L/day. As the substrate after the treatment had a higher TS level the substrate volume fed to the digesters was reduced, with the effect that HRT increased to 20–23 days (Table 1). As a consequence of the high temperature of the ingoing substrate, digester operating temperature was unexpectedly raised from 37 °C to 39–42 °C. Furthermore, the SS:MSW ratio was also changed to 76:24 (TS basis), due to changes in substrate availability. The feeding strategy varied for maintenance reasons during this period, with both serial and parallel operation of the two digesters. Daily process data for the THP period were collected between February 2015 and February 2016.

2.2. Batch degradation assays

Batch trials were conducted in order to determine the effect of THP on substrate degradability and biomethane potential. Triplicate cultures were prepared in 1000-mL serum bottles, each containing 3 gVS/L untreated or THP-treated SS, MSW and SS:MSW (TS ratio 67:33) and 9 g VS/L digestate (inoculum) taken from the industrial-scale plant during the reference period or during the THP period (3 HRT after THP installation). The samples were placed in a shaking incubator and the incubation temperature was set to mimic the conditions used in the industrial plant, i.e. 37 °C (reference period) and 42 °C (THP period). Further details regarding preparation and monitoring of the batch assays can be found in Westerholm et al. (2015). Triplicate batch trials with cellulose powder (Sigma-Aldrich), egg albumin powder (Källbergs Industries, Sweden), triolein (Alfa Aesar) or wheat straw (Agroväst) were conducted to evaluate degradability of polysaccharide, protein, fat

Table 1
Operating conditions and digester performance of production-scale digesters (P1 and P2) in the reference and thermal hydrolysis process (THP) period. Average values and standard error of mean were calculated from monthly values.

Digester	Temp (°C)	Fraction SS:MWS[1] in feedstock	OLR (g VS/L/day)	HRT (days)	pH	Alkalinity ($CaCO_3$ g/L) $CaCO_3$/mL)	Total volatile fatty acids (g/L)	NH_4^+-N (g/L)	Degree of degradation (%)[2]	Volumetric methane production (NmL/L/day)	Specific methane production (Nml CH_4/g VS)
P1 reference	37	57:43	3.0	19	7.3	6.9	0.12 ± 0.02	0.82 ± 0.02	47	3535	351
P2 reference	37	57:43	4.3	10	7.4		0.14 ± 0.003	0.87 ± 0.02	43		
P1 THP	40–42	76:24	3.9	23	7.7	9.5	0.7 ± 0.2	1.42 ± 0.05	48	3943	403
P2 THP	39	76:24	3.5	20	7.7		0.2 ± 0.02	1.46 ± 0.07	49		

[1] SS = sewage sludge; MSW = source sorted municipal solid waste from households, restaurants and industries. Total solids basis.
[2] Volatile solid basis.

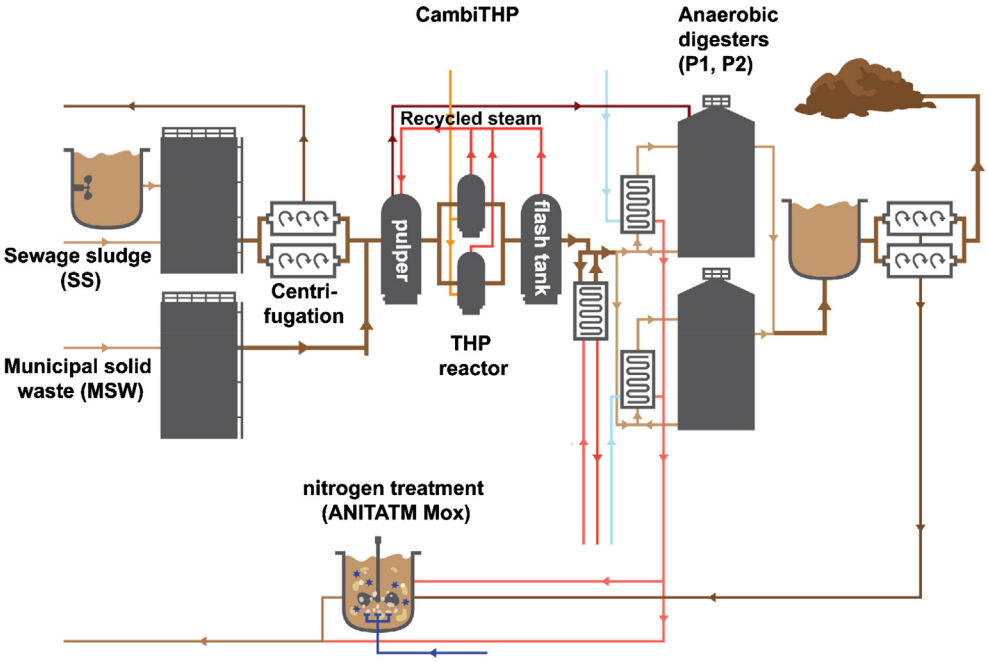

Fig. 1. Illustration of substrate flow and thermal hydrolysis process (THP) pre-treatment at the industrial-scale plant after installation of the CambiTHP™ technology.

and lignocellulose, respectively, with the reference and THP period inoculum. Three replicate cultures without substrate were used as controls for each set of assay conditions.

2.3. Laboratory-scale anaerobic digester operation

Identical laboratory-scale continuously stirred tank digesters (Belach Bioteknik, Stockholm, Sweden) with active volume 5 L were operated for 100–175 days (Fig. 2). The digesters were fed semi-continuously in a batch-wise manner (once a day, six days a week). In order to make the experiment practically feasible, large batches of feedstock for the laboratory-scale digesters were taken from the industrial-scale plant at two time points. The composition of the influent substrate was thus relatively similar throughout the experimental trial. The laboratory-scale digesters, designated D_{REF}, D_{OLR}, D_{ct}, D_{THP}, were inoculated with sludge taken from P1 and P2 during the reference period. During a start-up period (days 0–77), all digesters were operated at conditions similar to those in the industrial-scale plant, i.e. SS:MSW 57:43, 37 °C, OLR 2–3 g VS/L/day and HRT 18 days (Fig. 2A). Thereafter, digester D_{OLR} continued operating with SS:MSW, whereas D_{ct} and D_{THP} started to receive centrifuged (ct) SS:MSW and centrifuged/THP-treated SS:MSW, respectively. As these experiment were done before the installation of the THP at the industrial scale plant the treatment of the substrate was done in a laboratory-scale reactor (Cambi AS), in which batches of 3 kg were treated each time. This reactor operated under the same conditions as the industrial scale equipment (165 °C and 6 bar for 20 min) with the difference that the steam generated during the treatment was released and not recirculated to the substrate. A reference digester, D_{REF}, continued to be operated under conditions similar to those in the industrial-scale plant during the reference period. D_{OLR}, D_{ct} and D_{THP} operated with an OLR of 3 g VS/L/day, resulting in HRT ranging from 18 to 27 days depending on the influent TS level. Between days 98–126, OLR was then gradually increased to reach 5 g VS/L/day at day 126, resulting in HRT of 11–17 days (Table 2). Between days 126–175, the digesters were operated continuously at these conditions and the temperature was kept at 37 °C (Fig. 2).

A second laboratory-scale digester experiment was started at a later stage to evaluate the potential to increase OLR in the industrial-scale digesters in the THP period and effects by operating at 42 °C instead of 37 °C. During this experiment the THP-treated substrate were taken directly from the industrial-scale process. At this time the substrate composition was also changed towards a higher ratio of SS versus MSW (76:24 THP-treated ct_SS:MSW) compared to the ratio used in the first laboratory-scale digester experiment. This second experimental set-up involved digesters D_{THP2} and D_{42}. D_{THP2} was a continuation of D_{THP} but now fed 76:24 THP-treated ct_SS:MSW. D_{THP2} continued to operate at 37 °C in order to distinguish the impact by changing

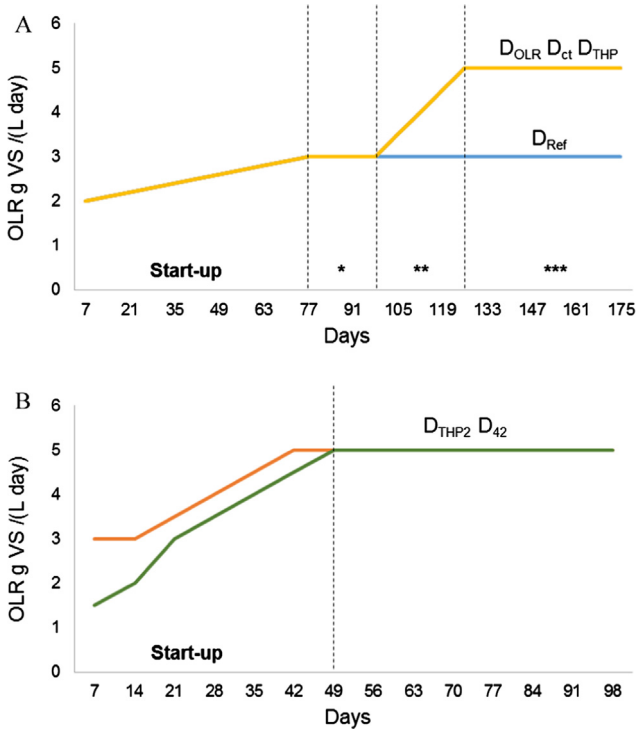

Fig. 2. Operating conditions of laboratory-scale digesters. (A) Start-up period – laboratory-scale digesters D_{REF}, D_{OLR}, D_{ct} and D_{THP} operated at similar conditions, i.e. fed 57% sewage sludge (SS) and 43% municipal solid waste (MSW). *Feed to D_{ct} and D_{THP} changed to centrifuged (ct_SS:MSW) and thermal hydrolysis process (THP)-treated ct_SS:MSW, respectively, **Organic loading rate (OLR) increased, ***Constant parameters. (B) Digesters D_{THP2} (changed fed composition) and D_{42} (changed fed composition and operated at 42 °C).

the feedstock composition without altering operating temperature. D_{42} was inoculated with sludge from P1 taken in the THP period, received the same substrate as D_{THP2} (76:24 ct_SS:MSW) but was operated at 42 °C in order to mimic the conditions in the industrial-scale digesters after THP installation. The OLR was gradually increased from 2–3 to 5 g VS/L/day and HRT decreased from 28 to 17 days during the initial 50 days and thereafter D_{THP2} and D_{42} were operated at constant parameters until day 100 (Fig. 2B).

Gas production and composition were analysed daily and digester effluent was sampled weekly for chemical analyses. Values obtained for over 2 HRT at constant operating parameters were used for evaluation of biogas digester performance.

2.4. Analytical procedures and calculations

Substrate characteristics (TS, VS, total and organic nitrogen content, ammonium-nitrogen, carbon, total phosphorus, potassium, magnesium, calcium, sodium, sulphur) were analysed by Agri Lab AB. At the industrial-scale plant, the alkalinity was determined using an existing protocol (VAV, 1984), total volatile fatty acid (VFA) concentration using Hach-Lange LCK 365. Ammonium-nitrogen was analysed according to the International Organization for Standardization's guidelines (ISO, 2013) and TS and VS following the method from the Swedish Standards Institute (SS, 1981). Total gas production was analysed daily and pH, biogas composition, TS, VS and ammonium-nitrogen in the laboratory-scale digesters and batch trials were determined as described previously (Westerholm et al., 2012). For VS calculation, VFA losses were compensated for using the rough method recommended by Vahlberg et al. (2013). Free ammonia (NH_3) levels were calculated as reported elsewhere (Hansen et al., 1998). Methane content in the gas was monitored using gas chromatography (GC) and VFA (acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, capronate and isocapronate) concentration using high performance liquid chromatography (HPLC) (Westerholm et al., 2012). The CO_2, O_2 and H_2S concentrations in the gas were analysed using a Biogas 5000 Analyser (Geotechnical Instrument). All volumetric gas values presented are converted to standard conditions at pressure 1.01 bar and temperature 273 K.

Degree of degradation was calculated using Eq. (1) that accounts for the gas weight that is released during AD, which lowers the effluent digestate volume in relation to the influent substrate volume:

$$\text{Degree of degradation} = \left(\frac{(TS_S \times VS_S) - (A \times (TS_D \times VS_D))}{(TS_S \times VS_S)}\right) \times 100 \quad (1)$$

where subscripted S and D are values for substrate and digestate, respectively. A was calculated according to Eq. (2):

$$A = \frac{m_s}{m_s - m_g} \quad (2)$$

where m_g is weight of produced gas calculated by assuming biogas has a density of 1.1–1.2 g/mL (depending on CH_4 (ρ_{CH4} = 0.7 kg/Nm^3) and CO_2 content (ρ_{CO2} = 2.0 kg/Nm^3) in the produced gas). m_s is weight of ingoing substrate during a set time period.

2.5. Sample collection and DNA sequencing

Digester sludge samples (50 mL) for molecular analyses were collected from the industrial-scale digesters P1 and P2 at three sampling points in the reference period (~30 days between each sampling) and 5–6 sampling points in the THP period (5–196 days between each sampling) (Table S1). Samples from the laboratory-scale digesters were taken on three different occasions after 2 HRT of operation with constant operating conditions (with 7 days between each sampling). All samples were stored at −20 °C until further use. DNA extraction, construction of 16S amplicon libraries and Illumina MiSeq sequencing were carried out on triplicate samples from each sampling point and digester as described previously (Müller et al., 2016).

2.6. Data processing and statistical analyses

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 and Holmes, 2013) was used to organise the data into a single data object and for production of graphics in R Studio v. 1.1.423 (http://www.r-project.org, TeamR RStudio, 2016) as described previously (Westerholm et al., 2018). Richness and evenness estimates were compared using analysis of variance (ANOVA, *aov* function). Non-metric distance scaling (NMDS) plots of microbial community profiles were generated using Bray-Curtis distances (R package vegan, Oksanen et al., 2017). Permutational ANOVA (PERMANOVA) was performed to evaluate the effect of operating parameters on microbial community structure using

Table 2
Laboratory-scale digester operating parameters, feed characteristics and process performance (between operating days 126–175). Column "Inoculum" designates if sludge for start-up of the digesters was taken before or after installation of the thermal hydrolysis process (THP) at the production-scale plant. Operating parameters differing in relation to the reference digester D_{REF} are in bold. Error bars represent standard error of the mean based on at least three sampling occasions.

Parameters	D_{REF}	D_{OLR}	D_{ct}	D_{THP}	D_{THP2}[1]	D_{42}
Inoculum[2]	ref	ref	ref	ref	cont. of D_{THP}	**THP**
Fraction SS:MSW in feedstock[3]	57:43	57:43	57:43	57:43	**76:24**	**76:24**
cf_SS[4]	–	–	+	+	+	+
$VFA_{substrate}$	4.4	4.4	5.4	2.3	nd	nd
TS_{in} (ww%)[5]	6.5	6.5	**9.4**	**10**	**10**	**10**
VS_{in} (ww%)	5.4	5.4	**8.4**	**7.9**	**8.5**	**8.5**
VS_{in} (ww%)	5.8	5.8	8.1	8.9	nd	nd
THP	–	–	–	**+**	**+**	**+**
Temp (°C)	37	37	37	37	37	**42**
OLR (g VS/L/day)	3	**5**	**5**	**5**	**5**	**5**
HRT (days)	18	**11**	**17**	**17**	**17**	**17**
Specific methane production (Nml CH_4/g VS)	337 ± 6	342 ± nd	337 ± 5	374 ± 14	424 ± 4	402 ± 3
Volumetric methane production (NL CH_4/L/day)	1.04 ± 0.04	1.70 ± nd	1.68 ± 0.03	1.87 ± 0.06	2.13 ± 0.02	2.02 ± 0.02
CH_4 (%)	62 ± 2	64 ± 1	63 ± 3	63 ± 1	67 ± 2	67 ± 2
CO_2 (%)	34 ± 2	32 ± 0.1	35 ± 1	33 ± 1	32 ± 0.2	32 ± 1
H_2S (ppm)	106 ± 27	92 ± 11	122 ± 26	156 ± 26	47 ± 39	49 ± 23
O_2 (%)	0.6 ± 0.3	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.4	0.6 ± 0.1	0.6 ± 0.1
pH	7.4 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.6 ± 0.1	7.5	7.5
Alkalinity ($CaCO_3$ g/L) $CaCO_3$/mL)	6.9 ± 0.5	5.7 ± 0.3	7.9 ± 0.6	10.7 ± 0.7	7.6 ± 0.1	8.2 ± 0.3
Total VFA (g/L)	<0.1	<0.1	<0.1	<0.1	0.3	0.7
TS_{out} (ww%)	3.8 ± 0.4	3.9 ± 0.1	5.4 ± 0.1	6.0 ± 0.1	5.4 ± 0.2	5.2 ± 0.1
VS_{out} (ww%)	2.7 ± 0.3	2.8 ± 0.01	3.9 ± 0.1	4.2 ± 0.1	3.9 ± 0.2	3.8 ± 0.1
Degree of degradation (%)	49 ± 5	48 ± 10	49 ± 1	50 ± 1	57 ± 2	59 ± 1
Tot-N (kg/ton)	2.9	2.5	4.8	4.2	4.1	3.9
NH_4-N (g/L)	1.2 ± 0.3	0.7 ± 0.1	1.6 ± 0.2	1.9 ± 0.2	1.4 ± 0.0	1.6 ± 0.1
Org-N (g/kg)	1.5	1.4	2.6	2.2	2.7	2.3
Tot-P (g/kg)	0.9	0.8	1.4	1.3	1.1	1.0
Tot-K (g/kg)	0.4	0.3	0.5	0.5	0.6	0.6
Tot-Mg (g/kg)	0.1	0.1	0.1	0.2	0.1	0.1
Tot-Ca (g/kg)	0.9	0.9	1.3	1.3	0.8	1.0
Tot-Na (g/kg)	0.2	0.2	0.3	0.3	0.4	0.4
Tot-S (g/kg)	0.3	0.3	0.4	0.4	0.4	0.4

nd = not determined.
[1] D_{THP2} is a continuation of digester D_{THP}.
[2] ref = reference period. i.e. before installation of thermal hydrolysis process (THP) at the biogas plant. Operating temp 37 °C; THP = after installation of THP. Centrifugation of wastewater sludge and changed operating temperature to 42 °C.
[3] SS = sewage sludge; MSW = source sorted municipal solid waste from households, restaurants and industries.
[4] Centrifugation of SS[1].
[5] ww=wet weight.

the Adonis function (vegan) and significant parameters were included in canonical correspondence analysis (CCA) plotting.

Raw sequences were submitted to the NCBI Sequence Read Archive (SRA) under the bioproject submission number PRJEB26813.

3. Results

3.1. Industrial-scale anaerobic digesters

Installation of THP pre-treatment, a thickening and dewatering centrifugation step and higher operating temperature in the industrial-scale digesters increased the degree of degradation from 43–47% to 48–49%, specific methane yield from about 350 to 400 NmL CH_4/gVS/day and volumetric methane production from 3500 to 3900 NmL CH_4/L/day. In line with the temperature increase caused by the THP installation a slight process instability was seen as decreased methane content and increasing VFA levels. However, the process quickly return to stable conditions and after this VFA concentrations remained at low levels (Table 1). Ammonium-nitrogen concentration, pH and alkalinity were slightly higher in both processes after the THP installation compared to before (Table 1). Calculation of free ammonia levels in the processes showed levels around 20–120 mg/L both before and after the installation.

3.2. THP effects on methane potential and degradation rate of the different substrates

The batch trials demonstrated that THP had different effects depending on substrate. For SS, the THP pre-treatment significantly ($P > 0.05$) increased specific methane potential, by 19–24%, under both sets of incubation conditions (Table 3). In addition, the number of days required to produce >80% of the methane potential was about half that required for untreated SS, irrespective of temperature. However, the THP pre-treatment had less effect on the methane potential of MSW (1–6% decrease) and the mixture of SS:MSW (1–4% decrease) (Table 3). Significantly higher methane production was obtained in batches incubated with sludge taken during the THP period compared with sludge taken during the reference period of the large-scale digesters for untreated and pre-treated SS and MSW and pre-treated SS:MSW. However, methane production did not differ in batches with different inocula during degradation of untreated SS:MSW (Table S2). Batch trials incubated with model substrates demonstrated slightly (not significant, P = 0.06) higher methane potential of albumin and cellulose when incubated at 42 °C (THP inoculum) compared with 37 °C (reference inoculum) (Table 3). Installation of THP had no effect on methane potential of triolein and straw. For all model substrates, methane production rate was considerably lower with sludge adjusted to THP treatment compared with industrial-scale digester sludge taken before THP installation (Table 3).

Table 3
Biochemical methane potential (BMP) and number of days required for formation of 50%, 80% and >90% of total methane potential during batch degradation of untreated and thermal hydrolysis process (THP)-treated substrate. Biochemical methane potential was also determined for model substrates without THP-treatment (cellulose, albumin, triolein and straw). Batch trial analyses were run on two occasions: at 37 °C and with inoculum taken from production-scale digesters during the reference period and at 42 °C and with inoculum taken 6 months after THP installation. Each set represents triplicate batch cultures. Statistical analyses were conducted using General Linear Models and significance was set to $P > 0.05$.

Inoculum	Incubation temp	Substrate (fraction)[1] or model substrate	**Without THP**				**With THP**				Change in methane potential by THP (*P*-value)[2]
			Methane potential (Nml CH_4/g VS)	Days to reach partial production (%)[1]			Methane potential (Nml CH_4/g VS)	Days to reach partial production (%)[1]			
				50%	80%	>90%		50%	80%	>90%	
Reference	37 °C	SS	356 ± 6	5	28	53	443 ± 3	4	14	28	24% (0.002)
		MSW	509 ± 9	4	14	28	475 ± 3	4	14	28	−6% (0.03)
		SS:MSW (67:33)	386 ± 24	4	14	36	381 ± 3	3	14	36	−1% (ns)
		Cellulose	305 ± 25	8	14	28	–	–	–	–	–
		Albumin	333 ± 3	3	14	28	–	–	–	–	–
		Triolein	900 ± 65	14	28	36	–	–	–	–	–
		Straw	291 ± 8	28	53	78	–	–	–	–	–
THP	42 °C	SS	424 ± 4	12	46	60	506 ± 11	8	30	46	19% (P > 0.001)
		MSW	625 ± 11	8	20	46	603 ± 2	8	20	46	−4% (ns)
		SS:MSW (67:33)	564 ± 7	8	20	46	542 ± 6	8	20	46	−4% (ns)
		Cellulose	396 ± 48	12	30	46	–	–	–	–	–
		Albumin	420 ± 57	12	30	46	–	–	–	–	–
		Triolein	864 ± 110	20	30	60	–	–	–	–	–
		Straw	293 ± 11	30	60	75	–	–	–	–	–

[1] SS = sewage sludge; MSW = source sorted municipal solid waste from household, restaurants and industries and a mixture of both (SS:MSW).
[2] ns = not significant. $P \leq 0.05$.

3.3. Laboratory-scale continuous biogas digesters

Throughout the experimental period, all laboratory-scale digesters had 61–62% CH_4 and 89–156 ppm H_2S in the gas and VFA levels below detection (<0.1 g/L). Volumetric and specific methane production in D_{REF} was 1.04 ± 0.04 NL CH_4/L/day and 337 ± 6 NmL CH_4/g VS, respectively (Table 2). With the OLR increase from 3 to 5 g VS/L/day in D_{OLR}, D_{ct} and D_{THP}, volumetric methane yield increased by 62, 61 and 79%, respectively, relative to D_{REF} (Table 2). The specific methane yield in D_{OLR} and D_{ct} was maintained at 337–342 NmL CH_4/g VS after the OLR increase, whereas it was significantly ($P < 0.05$) higher in D_{THP} than in D_{REF} (Table 2).

In the second experimental set-up involving D_{THP2} and D_{42}, significantly higher volumetric and specific methane yield were obtained (Table 2). The average acetate concentration was 0.3 g/L in D_{THP2} and 0.7 g/L in D_{42} (remaining VFAs < 0.5 g/L). All digesters had stable pH around 7.5 (Table 2). The alkalinity was significantly higher for D_{THP} compared to the other digesters (Table 2). Analyses of the digestate revealed higher levels of total nitrogen, ammonium-nitrogen, phosphorus and minerals in D_{ct} and D_{THP} than in D_{REF} and D_{OLR}. The degree of degradation was higher in D_{THP2} and D_{42} (57–59%) than in D_{REF}, D_{OLR}, D_{ct} and D_{THP} (48–50%, Table 2).

3.4. Microbial community diversity and structure

The NMDS analysis demonstrated clear divergence of the microbial communities between the reference and THP periods in both parallel industrial-scale digesters (Fig. 3). Interestingly, although the laboratory-scale digester D_{REF} operated with similar parameters as the industrial-scale digesters in the reference period, these samples clustered separately. Furthermore, with every operational change made (i.e. in D_{OLR}, D_{ct}, D_{THP}), the distance from the reference digester (D_{REF}) increased. Similar separation between laboratory- and industrial-scale digesters was not observed in the second experimental set-up, where D_{42} positioned more closely to the industrial-scale digesters in the THP period (Fig. 3). The alpha diversity analysis revealed significantly ($P < 0.05$) lower observed richness and Shannon diversity index in industrial-scale digesters in the THP period compared with the reference period (Table S3). However, the Simpson diversity remained similar in both periods (Fig. S1).

The taxonomic profiling of industrial-scale digesters showed a relatively even distribution of the dominant phyla Firmicutes (17–24%), Bacteroidetes (26–35%) and Chloroflexi (7–20%) in the reference period (Fig. S2). Minor fractions of the phyla *Candidatus* Cloacimonetes, Fibrobacteres, Proteobacteria, Synergistetes, Acidobacteria, Actinobacteria, Armatimonadetes, Spirochaetes and Verrucomicrobia were also detected (1–8% in at least one of the industrial-scale reference samples) (Fig. S2). At class level, Clostridia (15–22%), Anaerolineae (6–18%), Bacteroidia (15–29%) and Bacteroidetes_vadinHA17 (14–10%) dominated (Fig. 4). After the installation of THP, the proportion of Clostridia significantly increased to 47–69%, Bacteroidetes_vadinHA17 remained at similar levels as before THP installation, whereas Bacteroidia and Anaerolineae significantly decreased, to 4–16% and 1–6%, respectively (Fig. S3A, Table S3). The classes Proteobacteria and Fibrobacteres decreased below detection following THP installation and instead members within the phyla Verrucomicrobia and Thermotogae appeared (~3%) (Figs. 4 and S2). Caldicoprobacteraceae, Family_XI, Ruminococcaceae and Syntrophomonadaceae (Firmicutes phylum) were the dominant families in the THP period (Fig. S4). The genera *Fastidiosipila* and *Sedimentibacter* (Clostridia class) were classifiable genera that significantly increased in relative abundance after THP installation, whereas the family Draconibacteriaceae (Bacteroidetes phylum) significantly decreased (Figs. S3B, S5 and S6, Table S3).

In D_{REF} and D_{OLR}, the Bacteroidia (34–53%) and Clostridia (18–26%) classes dominated, followed by classes within the phylum Cloacimonetes (4–12%) (Fig. 4). Statistical analysis revealed significantly higher levels of Clostridia in D_{ct}, D_{THP}, D_{THP2} and D_{42} than in D_{REF} (Fig. S3C, Table S3). A considerably higher level of Clostridia (48–63%) and lower level of Bacteroidia (16–30%) distinguished D_{THP2} and D_{42} from the other laboratory-scale digesters (Figs. 4 and S3C). Closer examination revealed dominance of the family Draconibacteriaceae (Bacteroidetes phylum) in all 37 °C D-digesters (Fig. S6). The family distribution within Bacteroidetes in D_{42} instead resembled that in digesters P1 and P2 in the THP per-

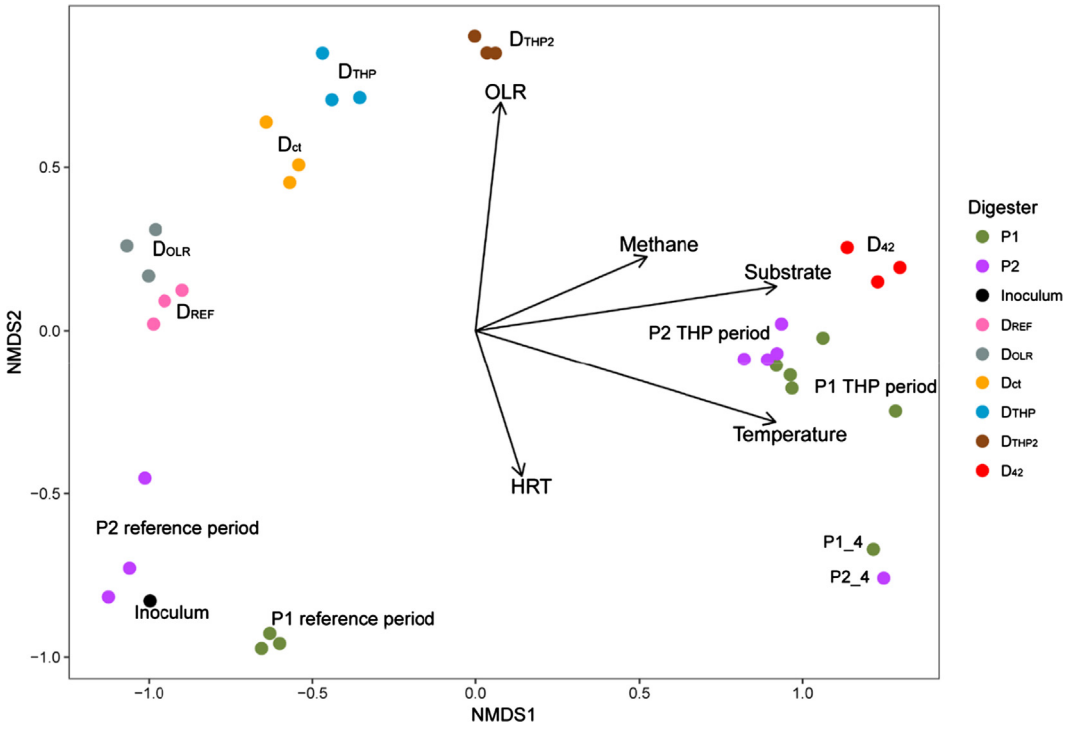

Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot calculated from Illumina sequencing data. Digester parameters significantly associated with changes in microbial community structure are plotted as vectors, where the length and direction indicate the contributions of the variable to the principal components.

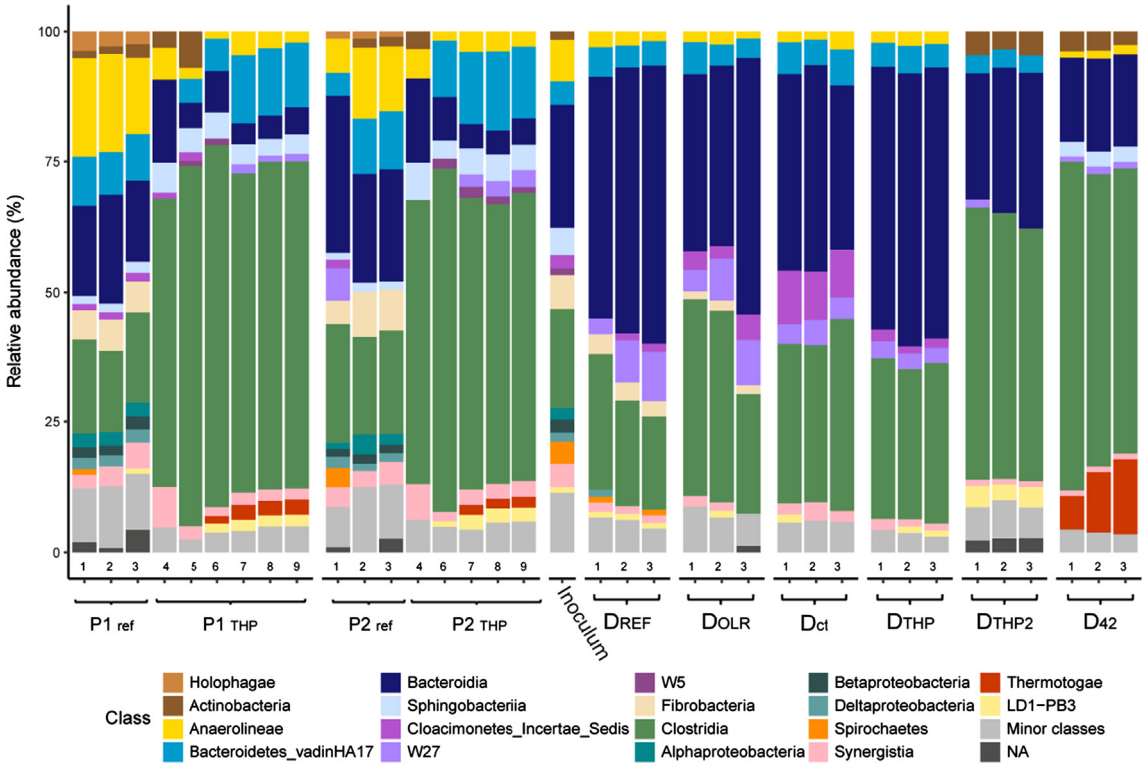

Fig. 4. Relative abundance of microbial classes (based on total bacterial and archaeal sequences) in production-scale (P1 and P2) and laboratory-scale (D_{REF}, D_{OLR}, D_{ct}, D_{THP}, D_{THP2}, D_{42}) digesters. Inoculum was taken from P1 and P2 during the reference period to initiate the processes in D_{REF}, D_{OLR}, D_{ct} and D_{THP}. Brown, yellow, blue, purple, beige, green, turquoise and light yellow bars represent classes within the phyla Actinobacteria, Chloroflexi, Bacteriodetes, *Candidatus* Cloacimonetes, Fibrobacteres, Firmicutes, Proteobacteria Verrucomicrobia, respectively. Phylum affiliation of uncultivated phylogenetic clades (W27, W5 and LD1-PB3) is indicated by colouring. Classes representing <1% (including archaeal sequences) are grouped into the grey bar section designated "Minor classes". Reads that were not phylogenetically assigned (NA) are included in minor classes except when representing >1%. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

iod, but with higher representation of Porphyromonadaceae in D_{42} (Fig. S6).

At class level, the D_{42} community resembled the parental digester community (THP period of industrial-scale digesters), with 55–63% Clostridia, but contained higher levels of the classes Bacteroidia and Thermotogae (6–14%, Fig. 4). However, in contrast to industrial-scale digesters, the genus *Gallicola* was the most abundant classified genus within the Clostridia class in D_{42} (14–16% of total sequences) (Fig. S5).

The Firmicutes:Bacteriodetes abundance ratio was significantly higher in the industrial-scale digesters after THP installation. This ratio was also higher in D_{THP2} and D_{42} than in the other laboratory-scale digesters (Table S3). Statistical analyses of the communities in both the laboratory- and industrial-scale digesters revealed a positive correlation between Firmicutes:Bacteriodetes ratio and methane yield (Table S4).

The methanogenic community structure was relatively similar over time in the industrial-scale digesters, with dominance of the hydrogenotrophic genus *Methanobacterium* (0.08–0.8% of total sequences) and the aceticlastic *Methanosaeta* (up to 0.3%) (Fig. S7). In the laboratory-scale digesters, the methanogenic profile diverged from that in the industrial-scale digesters by a higher ratio of *Methanosaeta* to *Methanobacterium* in D_{REF}, D_{OLR} and D_{42}, whereas in D_{THP} and D_{THP2} *Methanobacterium* was the dominant methanogen. In D_{THP2}, *Methanosarcina* was also detected, at 0.1–0.2% (Fig. S7).

4. Discussion

This study investigated the importance of THP in achieving high treatment capacity of SS and MSW in a biogas plant, by analysing industrial-scale and laboratory-scale process performance and by determining microbial community structure. Installation of THP at industrial-scale resulted in a slightly improved degradation, seen as an increased methane yield and ammonium level in the digestate as well as an overall higher VS reduction. The increase in biogas production corresponded to 500 kWh/t TS substrate, i.e. ~15% higher gas production compared to the period before the installation. However, it is unclear if this improvement was caused by the THP treatment as such or by the increase in operational temperature, caused by a high temperature of the substrate and non-sufficient cooling system at the plant. The thermal hydrolysis process increased the fuel consumption at the plant and the installation required pellets for burning corresponding to 400 kWh/t TS substrate (Schnürer et al., 2017). Consequently, the energy gained by higher methane yield after THP-treatment was more or less consumed by the THP operation. However, in Sweden the cost for pellets is currently lower than the profit from biogas, which is sold as transportation fuel after upgrading. Thus, the economic balance became positive for the plant. Furthermore, aspects such as improved dewaterability of feedstock and generation of sanitised digestate with better fertiliser value was other benefits of THP installation highly appreciated by the plant operators.

The comparable levels of specific and volumetric methane yield in the industrial-scale and the corresponding laboratory-scale reference digesters (D_{REF}, D_{42}) indicate high applicability of the results obtained. The laboratory-scale digester operating with similar conditions as the industrial-scale digester in the reference period and exposed to enhanced OLR (D_{OLR}) demonstrated no signs of overloading within the study period. This implies that capacity at the industrial-scale plant could have been increased without affecting process stability even before THP installation. Moreover, the degree of degradation and biogas yield of the laboratory- and industrial-scale digesters in the reference period were within typical ranges for anaerobic degradation of wastewater sludge and food waste (Björn et al., 2017; Cavinato et al., 2013; Silvestre et al., 2015; Sosnowski et al., 2008), indicating that the observed potential for increased capacity can apply at many plants. Nevertheless, due to low TS in the influent, the HRT of D_{OLR} was only 11 days. Even though digester D_{OLR} operated with stable performance throughout the experimental period (~3 HRT), it is strongly recommended that the long-term effects of operating at such low HRT should be evaluated by chemical and biological monitoring, as there is a risk of wash-out of essential nutrients and key microorganisms in the long-term (Wandera et al., 2018). The present study showed that substrate dewatering and THP treatment could be effective ways to increase the HRT (to 17 days) and reduce the potential risk of wash-out. Other important effects of THP were that it simplified pumping of material due to the decrease in viscosity. Moreover, THP seemed to improve the degradation of proteins, based on the increased level of plant-available nitrogen in the digestate, which enhanced its quality as a fertiliser. It is important to bear in mind that increased level of ammonium-nitrogen implies a risk for process inhibition by free ammonia (NH_3), which is accentuated with the increase in temperature and pH (Westerholm et al., 2016). However, in the present industrial-scale processes free ammonia concentrations never exceeded the levels previously reported to cause ammonia-related process problems (Westerholm et al., 2016). Nevertheless, the increase in ammonium-nitrogen level in the process resulted in an increased pressure and reduced efficiency of the preceding nitrogen treatment (ANITATM Mox, Fig. 1) of reject water after sludge centrifugation. To solve this problem, the reject water had to be diluted 1:1 with fresh water at the industrial-scale plant.

Concordantly with the present results showing higher methane yield by THP-treatment of MW and SS at industrial-scale, positive effects of THP pre-treatment have been demonstrated previously in AD of *Salix* or SS (Bauer et al., 2014; Dereix et al., 2006; Estevez et al., 2012). However, in the batch experiments in the present study THP pre-treatment only enhanced the methane potential from SS and had little or no effect for MSW or the mixture of SS and MSW. In general, MSW contains a high proportion of easily degraded and soluble compounds, which can lower the effect of pre-treatment (Carlsson et al., 2012). An additional aspect that may have contributed to the diverging results for laboratory- and industrial-scale AD in the present study was loss of VFA by vaporization during THP pre-treatment in the laboratory. In the industrial-scale digesters, the VFA vaporised during THP was introduced into the process and was thus available for microbial degradation. In the first laboratory-scale digester experiment, technical constraints prevented a similar set-up and volatile organic compounds (likely primarily formed from MSW) were lost in THP pre-treatment. The substrate used for D_{THP2} and D_{42} was THP-treated on-site at the industrial-scale plant and thus probably contained more easily degraded organic compounds. Consequently, a combination of higher ratio of SS in the feed (increasing the positive effects of THP) and a higher fraction of easily degradable organic compounds was likely the reason for the higher methane yield in D_{THP2} and D_{42} compared with the other laboratory-scale digesters.

Anaerobic digestion at temperatures just above the commonly used mesophilic range (37–40 °C) has been demonstrated to cause temporary instability, resulting in VFA accumulation and reduced methane yield (Bousková et al., 2005; Moestedt et al., 2017; Pap et al., 2015; Westerholm et al., 2018). However, in the industrial-scale processes in the present study, the temperature increase from 37 °C to 39–42 °C in association with THP installation appeared not to impair the long-term stability, as also reported in AD of animal manure and straw (Hupfauf et al., 2018; Risberg et al., 2013). Laboratory-scale digesters D_{THP2} and D_{42}, which were included to evaluate the separate influence of increased temperature and

changed feedstock (THP and SS:MSW ratio), showed slightly lower methane yield at the higher temperature, which is in line with findings on biogas production from MSW at high ammonia concentrations (Westerholm et al., 2015) and from sewage sludge (Moestedt et al., 2017). In other studies, operating at 44–45 °C instead of 38 °C has been observed to have positive effects on methane yield (8–20%) in AD of a large range of substrates (Hupfauf et al., 2018; Lindorfer et al., 2008; Moestedt et al., 2014).

4.1. Effects of THP installation and of temperature and scale changes on microbial communities linked to methane production

NMDS analysis using the 16S rRNA gene sequence data revealed distinctly different communities in the industrial-scale digesters before and after installation of the THP system (Fig. 3). This strong division between samples of the two periods indicated a deterministic impact of the change in operating parameters. Although the Clostridia class (phylum Firmicutes) was significantly more abundant in the laboratory-scale digesters fed dewatered SS or THP pre-treated substrate, only D_{THP2} and D_{42} had similar levels of Clostridia to the industrial-scale THP samples (Fig. 4). This suggests that the increased ratio of THP-treated SS in the feedstock contributed to the higher level of Clostridia, which contradicts previous findings of increased proportion of Clostridia in association with a step-wise increase in MSW (versus waste activated sludge) and OLR (Jang et al., 2016). Here, it might be more revealing to consider the Firmicutes:Bacteriodetes ratio. Decreasing abundance of Bacteroidetes, and thus increasing Firmicutes:Bacteriodetes ratio, has been found to be linked to decreased richness of lignocellulolytic enzymes (Güllert et al., 2016). In the present study, it can be speculated that a reduced need for hydrolytic performance could have resulted in the higher Firmicutes:Bacteriodetes ratio in P1, P2, D_{THP2} and D_{42}, receiving THP-treated substrate. THP pre-treatment performed in the laboratory, on substrate with a higher fraction of MSW, appeared not to have a similar effect. Furthermore, the positive correlation between Firmicutes:Bacteriodetes ratio and methane yield in both laboratory- and industrial-scale digesters is in line with previous observations (Ahlberg-Eliasson et al., 2018; Chen et al., 2016), and could be linked to a higher level of more easily degradable compounds in these digesters.

Several of the previously characterised genera in the Clostridia class that increased following THP installation are known to be fermentative, acid-producing bacteria, including *Fastidiosipila* and *Sedimentibacter*. *Sedimentibacter* was recovered at similar levels from samples from the THP period of industrial-scale digesters and from D_{42}, and is known to convert pyruvate and amino acids to acetate and butyrate (Breitenstein et al., 2002; Imachi et al., 2016). As discussed above, the increased level of ammonium-nitrogen in the digestate indicated that THP improved the degradation of proteins, which can be a reason for the increased level of *Sedimentibacter*. *Fastidiosipila* has previously been detected in high relative abundance in mesophilic (35 °C) AD of MSW (Cardinali-Rezende et al., 2016), in a mesophilic (37 °C) membrane reactor for landfill leachate treatment (Xie et al., 2014) and in anaerobic layers of municipal solid waste landfill (Wang et al., 2017). Species of this genus have so far not been isolated from AD digesters and there are therefore uncertainties about their role in these processes. However, considering the dominance of this genus in the THP period of the industrial-scale digesters, it probably plays an important role in degradation of THP-treated SS:MSW at 42 °C. In the laboratory-scale digesters D_{THP2} and D_{42}, the genus distribution within the Clostridia class differed from that in the industrial-scale digesters by higher representation of uncultivated members of the Christensenellaceae family and the genus *Gallicola*, respectively. The genus *Gallicola* has been detected in mesophilic AD of MSW and of chicken faeces (Westerholm et al., 2018; Ziganshina et al., 2017). So far, the *Gallicola* genus only contains one characterised species, isolated from chicken faeces and shown to use purine to produce acetate and butyrate (Ezaki et al., 2001; Schiefer-Ullrich and Andreesen, 1985). This suggests a role as an acid-forming bacteria in biogas processes. The high level of Christensenellaceae in D_{THP2} is likely an effect of the inoculum taken from D_{THP2}, which contained high relative abundance of this family. Known species have been isolated from human faeces (Morotomi et al., 2012) but members of the family Christensenellaceae prevalent in AD also remain to be isolated and characterised, which hampers speculation about their ecological functions in the process. Nevertheless, the comparable performance of the industrial-scale and laboratory-scale digesters indicates that some of the different genera in the Clostridia class occupy similar niches in the different systems.

The microbial analyses of the industrial-scale digesters showed significantly lower diversity in the THP period than in the reference period (Fig. S1). Several previous AD studies have shown lower microbial diversity in thermophilic than in mesophilic temperature conditions (Guo et al., 2014; Levén et al., 2007). Restricted diversity indices have been noted after only a few degrees of temperature increase (Westerholm et al., 2018), which indicates that the higher temperature in the THP period could have caused the lower diversity in the industrial-scale digesters. However, at laboratory scale there was no difference in richness between D_{42} and several of the laboratory-scale 37 °C-digesters and the microbial diversity in those was likely controlled by other operating variables. Instead, the transition from industrial- to laboratory-scale seemed to reduce microbial richness (Fig. S1). Higher microbial richness has been suggested to contribute to resilience of the microbiota towards changes in operating conditions (Guo et al., 2014; Weiland, 2010). However, in the present study, an OLR increase was still possible in laboratory-scale digesters with low diversity, indicating that a relatively robust microbiota was still present in the processes.

The methanogenic abundance profile, with dominance of the genera *Methanobacterium* and *Methanosaeta*, agrees with previous taxonomic findings for mesophilic biogas digesters with similar feedstock composition (De Vrieze et al., 2015; Sundberg et al., 2013; Zamanzadeh et al., 2016). The aceticlastic *Methanosaeta* is a common methanogen in SS degradation, characterised by low acetate levels (De Vrieze et al., 2016). *Methanosarcina*, that was detected in D_{THP2}, includes species using a wider substrate spectrum for growth (including acetate, H_2/CO_2, methanol and methylamine), but also species that convert acetate at a faster rate, but with a lower threshold level, compared with *Methanosaeta* (De Vrieze et al., 2012). Installation of THP had a low impact on methanogenic community structure in both industrial- and laboratory-scale systems. The operating conditions of D_{REF} were intended to mimic those in the industrial-scale digesters during the reference period. However, for practical reasons the down-scaling to a laboratory-scale system involved a slight change in feeding regime (from continuous to semi-continuous). Impacts on bacterial community structure of altered feeding regime have been noted in mesophilic (38 °C) AD processes (Mulat et al., 2015) and this could be the cause of the microbial differences between samples from D_{REF} and from the reference period of the industrial-scale digesters in this study. Considering the close clustering of D_{42} and THP samples from industrial-scale digesters, the changes in feeding regime apparently did not have a similar influence on community structure when operating at 42 °C. Moreover, increasing OLR in D_{42} had no marked microbial impact. This indicates that the 5 °C temperature increase was a strong deterministic factor shaping the microbial communities in this study. This has also been observed in AD of food waste and in AD of thin stillage at high ammonia levels (Moestedt et al., 2014; Westerholm et al., 2018;

Westerholm et al., 2015). This confirms the applicability of the results obtained by operating D_{42} at higher OLR, which suggest that industrial-scale processes operating at 42 °C and fed THP pre-treated substrate can increase their OLR to meet the estimated future population increase within the municipality served by the industrial-scale plant studied here.

5. Conclusions

Based on estimated future population increases, the industrial-scale plant studied here must increase its current feeding rate of 3 g VS/L/day to 5 g VS/L/day. Laboratory-scale investigations indicated that the large-scale plant could reach this target by operating under all the parameters investigated in this study (i.e. with and without THP, centrifugation of substrate and operation at 37 °C or 42 °C). Still, substrate dewatering and THP pre-treatment proved to be effective ways to reach high OLR without reducing the HRT and thus decrease the potential risk of wash-out of essential nutrients and key microorganisms in long-term operation. THP pre-treatment had the largest positive effect on substrate with a higher fraction of sewage sludge. Microbial analyses demonstrated that THP pre-treatment, SS centrifugation, the relative fraction of wastewater and household waste used and the operating temperature were highly influential factors differentiating the microbial communities in the digesters studied. Statistical analyses also revealed higher Firmicutes:Bacteriodetes ratio in the industrial-scale and laboratory-scale digesters fed THP-treated substrate and showed that this ratio was positively correlated with methane yield. Several previously characterised genera in the Clostridia class (Firmicutes phylum) that are known to be fermentative and acid-producing bacteria, including *Fastidiosipila* and *Sedimentibacter* (industrial-scale) and uncultivated members of the Christensenellaceae family and the genus *Gallicola* (laboratory-scale), were present in higher relative abundance in digesters fed THP-treated substrate with a higher level of sewage sludge and higher methane yield. These findings can enable more accurate prediction of the consequences of THP installation for biogas process conditions and optimisation of the process.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.wasman.2019.06.004.

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