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Post-treatment of dewatered digested sewage sludge by thermophilic high-solid digestion for pasteurization with positive energy output

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ABSTRACT

This study investigated the possibility to use thermophilic anaerobic high solid digestion of dewatered digested sewage sludge (DDS) at a wastewater treatment plant (WWTP) as a measure to increase total methane yield, achieve pasteurization and reduce risk for methane emissions during storage of the digestate. A pilot-scale plug-flow reactor was used to mimic thermophilic post-treatment of DDS from a WWTP in Linköping, Sweden. Process operation was evaluated with respect to biogas process performance, using both chemical and microbiological parameters. Initially, the process showed disturbance, with low methane yields and high volatile fatty acid (VFA) accumulation. However, after initiation of digestate recirculation performance improved and the specific methane production reached 46 mL CH_4/g VS. Plug flow conditions were assessed with lithium chloride and the hydraulic retention time (HRT) was determined to be 19–29 days, sufficient to reach successful pasteurization. Degradation rate of raw protein was high and resulted in ammonia-nitrogen levels of up to 2.0 g/L and a 30% lower protein content in the digestate as compared to DDS. Microbial analysis suggested a shift in the methane producing pathway, with dominance of syntrophic acetate oxidation and the candidate methanogen family WSA2 by the end of the experiment. Energy balance calculations based on annual DDS production of 10 000 ton/year showed that introduction of high-solid digestion as a post-treatment and pasteurization method would result in a positive energy output of 340 MWh/year. Post-digestion of DDS also decreased residual methane potential (RMP) by>96% compared with fresh DDS.

1. Introduction

Anaerobic digestion (AD) of sewage sludge is a commonly applied approach to stabilize sewage sludge and simultaneously produce biogas. In 2017, 753 GWh of biogas were produced at Swedish wastewater treatment plants (WWTP), representing 36% of all biogas produced in Sweden, with the remainder deriving from food waste, slaughterhouse waste, and other industrial wastes (SwedishEnergyAgency, 2018). This represented an increase of 6% compared with the previous year (SwedishEnergyAgency, 2018). Biogas produced at WWTP plants is often used for generation of electricity and heat and can supply a substantial proportion of the energy used at the treatment plant (Jenicek et al., 2012). In addition, the biogas can be used as vehicle fuel after upgrading (normally > 97% methane content) (SiS, 1999). This is common practice in Sweden (SwedishEnergyAgency, 2018), with 65% of all biogas produced at WWTPs in 2017 being upgraded to vehicle fuel (Energimyndigheten, 2017). Increased production and upgrading of biogas at WWTPs is one important step in reaching Sweden's target of a fossil-free vehicle fleet by 2030.

In addition to biogas, AD results in a nutrient-rich digestate that can be used as a fertilizer in agriculture (Arthurson, 2009; Nkoa, 2013). In 2017, >2.7 million metric tons of digestate were produced in Sweden, of which 83% were used as fertilizer. Of the digestate

Abbreviations: AD, Anaerobic digestion; DDS, Dewatered digested sludge; CSTR, Continuously stirred tank reactor; FAN, Free ammonia-nitrogen; GWP, Global Warming Potential; HRT, Hydraulic retention time; NMR, Nuclear magnetic resonance; OLR, Organic loading rate; OM, Organic matter; RMP, Residual methane potential; SRT, Sludge retention time; TS, Total solids; VS, Volatile solids; VFA, Volatile fatty acids; WWTP, Wastewater treatment plant.

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produced at WWTPs in that year, 31% (187 kton) was used as agricultural fertilizer (Energimyndigheten, 2017). The use of digestate from biogas plants in Sweden is regulated by two different certification systems, one for digestates from WWTPs (Revaq) and one for plants operating with other organic materials, often in co-digestion (Sweden, 2013). Both systems require e.g., analysis of indicator pathogens, metal concentrations, visible impurities, and nutrient levels. In addition, biogas plants operating with animal-based waste are obliged to use a pasteurization step for use of the digestate as a fertilizer. These regulations are set by European Union (EU) regulations (EG) no. 1069/2009 and (EU) no. 142/2011) to avoid spread of pathogens, such as enterococci or salmonella. These regulations currently does not apply to sludge from WWTPs. However, the Swedish Environmental Protection Agency has proposed new directives that will make it mandatory to pasteurize all sludge from WWTPs (Naturvårdsverket, 2013). Consequently, stabilization by storage for 6 months, as used today, will not be allowed for sewage sludge in the future. If the new regulations come into force, major changes may be needed to meet the pasteurization requirement at many WWTP plants in Sweden. There are several common methods for pasteurization of sludge (and other biomass types) at biogas plants. The most common method in Sweden is heating at $\geq$ 70 °C for 1 h (prior or post digestion) (Bagge et al., 2005). In addition, applying a thermophilic digestion temperature of 52 and 55 °C can be used, with a exposure time of at least 24 and 8 h, respectively (Naturvårdsverket, 2013). This method e.g. *in situ* pasteurization, can give a similar reduction in pathogenic indicator organisms to external pasteurization (Norin, 2007). Considering the high water content of sludge, thermophilic digestion, instead of pre-treatment, are likely to be the most cost-effective method to pasteurize sludge at WWTPs. Operation at thermophilic conditions can also be of interest since it has the potential to improve degradation rates as compared with mesophilic conditions, thus allowing shorter retention times (Gebreeyessus and Jenicek, 2016). Moreover thermophilic, as compared to mesophilic temperature, give a decreased viscosity and reduced cost of stirring (Gebreeyessus and Jenicek, 2016). However, thermophilic temperature also poses some challenges, e.g., it increases the risk of process instability due to comparatively high free ammonia levels (FAN) (Gebreeyessus and Jenicek, 2016; Wu et al., 2020).

Anaerobic digestion of organic material requires different microorganisms with different metabolic functions, working in a synchronized manner (Schnurer, 2016). For an efficient process, high diversity is suggested to be important, allowing multiple metabolic pathways to be active at the same time (Schnürer, 2019). Many different parameters have been shown to affect development of the microbial community and microbial diversity, including substrate composition, operating conditions, and reactor design (Schnürer, 2019). The most common design in Sweden and Europe at present is the continuously stirred tank reactor (CSTR), which is used for treatment of sludge from WWTPs and different organic waste streams from the food and feed industry, and from agriculture (Energimyndigheten, 2017; Scarlat et al., 2018). These processes operate with a relatively low content of total solids (TS; 2–15%) and the substrate is typically fed into the reactor by pumping. The degree of degradation in such processes varies significantly, depending mainly on the character of the ingoing material. During AD of sludge, typically only 50–60% of organic matter (OM), or even less, is converted to biogas, compared with 70–80% in digestion of food waste (Appels et al., 2008; Nordell and Karlsson, 2011). This low level of degradation is related to the presence of recalcitrant OM in secondary sludge (biological sedimentation sludge), combined with an often low hydraulic retention time (HRT) at WWTPs (Zhen et al., 2017). This low efficiency of sludge digestion represents a loss of methane potential and may also pose a risk of methane losses during storage of digestate (Nordell and Karlsson, 2011). However, as shown in many studies, the degradation efficiency and biogas production from sludge can be increased by different thermal, mechanical, chemical, or biological pre-treatments (as reviewed in Neumann et al. (2016) and Zhen et al. (2017). In addition, some studies suggests post-anaerobic digestion (PAD) as another way to improve sludge digestion and process efficiency (Svensson et al., 2018; Yang et al., 2019). Moreover, treatment of sludge by anaerobic digestion after dewatering using high solid digestion (dry digestion) has also been suggested as method for improved degradation (Duan et al., 2012; Li et al., 2018; Wu et al., 2020). This technique allows stabilization of organic waste with a high total solids concentration (>15%) and provides some advantages over wet digestion systems, such as less reactor volume, and thus less need for heating, and higher organic load compared with CSTR processes (Kothari et al., 2014; Lei et al., 2015). Moreover, during high solid digestion the digestate has a comparatively lower water content and thus also higher nutrient content per unit wet weight, making it more attractive as a fertilizing agent.

The goal of this study was to mimic high solid thermophilic digestion for post-treatment of dewatered digested sewage sludge (DDS) at a Swedish WWTP. The technique has been tested before for dewatered sewage sludge (DS) but not for DDS, and shown good results in terms of degree of degradation, biogas production and energy balance, at both mesophilic and thermophilic conditions (Duan et al., 2012; Li et al., 2018; Wu et al., 2020). The specific aims of this study was to evaluate to possibility to use high solid thermophilic digestion of DDS for simultaneous biogas production, sanitization and stabilization of DS. More specifically a plug-flow reactor was constructed and process operation was evaluated in regard to biogas production, rest gas potential and pasteurization effect, using indicator organisms. In addition, process performance was evaluated by analysis of the bacterial and archaeal communities using Illumina sequencing. The process was also evaluated by qualitative analysis of changes in solid-phase organic matter characteristics by solid-state ^{13}C cross-polarization magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy. Furthermore, to estimate the energy cost for the post treatment of the DDS the net energy used for heating was related to the energy produced in biomethane during the high sold treatment.

2. Material and methods

2.1. Reactor design and setup of pilot-scale experiment

A cylinder-shaped reactor (stainless steel) with an active volume of approximately 38 L, with dimensions 120 cm length and diameter 20 cm, was used. The reactor was operated in plug-flow mode, meaning that substrate (DDS) was inserted at one end and digestate was removed at the other end. The process temperature was set to 52 °C and the reactor material was heated using a water-heating jacket. Temperature was measured manually with PT-100 thermometers (Traceable, VWR, Sweden) at two different test points in the reactor at least once every day. Stirring was performed continuously with a through-rod with propeller blades, which moved the sludge radially at < 1 rpm. The reactor was inoculated with fresh DDS from the full-scale Nykvarnsverket WWTP and filled to approximately 50% of its volume. Substrate, i.e., DDS, was then collected on a daily basis from the full-scale plant directly after dewatering (Fig. 1) and fed to the reactor, which reached full active volume after approximately 30 days. Total solids (TS) and volatile solids (VS) concentration in the substrate was on average 27 ± 5% and 64 ± 5% (VS of TS), respectively. The average organic loading rate (OLR) was 6.1 ± 2.4 kg VS/m^3 d, with the variations caused by differences in TS/VS ratio of the substrate

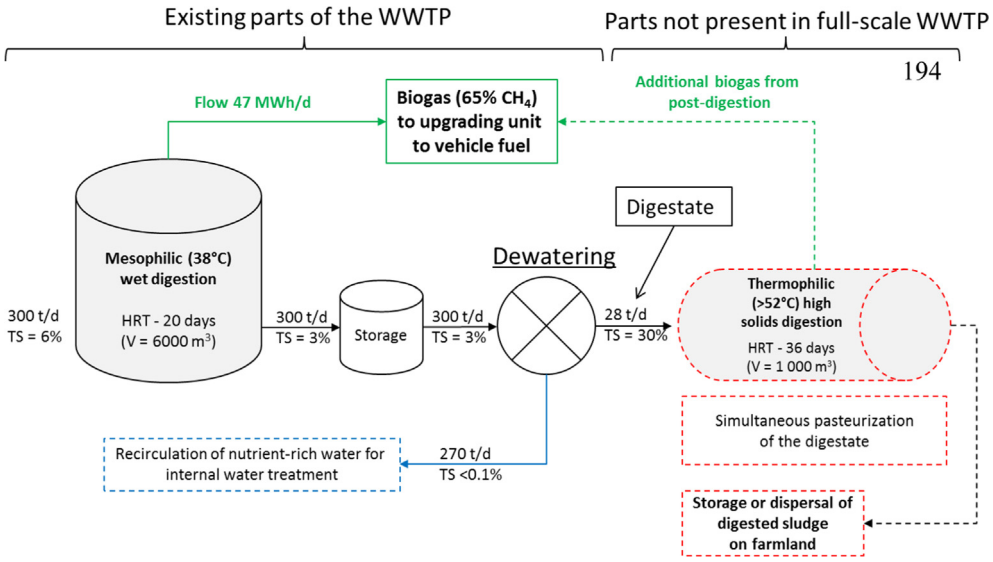

Fig. 1. Schematic overview of the digestion part of Nykvarnsverket wastewater treatment plant (WWTP). Dotted boxes are not parts of the WWTP, but were simulated at pilot scale in this study.

collected from the full-scale plant during the experiment. Up to day 134, no digestate was re-circulated to the inlet feed. From day 135 to 176, 10% of the digestate (TS: 23%±1.7%, VS: 59%±1.5%) from the plug flow (w/w) was re-circulated to the inlet, together with fresh DDS, to achieve continuous re-inoculation. No separation or treatment of the digestate was performed prior to re-circulation. The calculated retention time based on intake and outtake volumes was on average 30 days. To validate this value and to secure actual plug flow with no material passing through the reactor in shorter times than this, the DDS was on one occasion supplemented with 2900 mg lithium chloride ($LiCl_2$) per kg TS (VWR, Sweden) giving a maximum concentration in the reactor 22.2 mg /L of lithium. Lithium concentration in the outflowing digestate was then analyzed, to determine when the concentration started to change, according to Swedish Standard SS 028150–2/ICP-AES. Statistical analyses are based on 95% confidence interval, unless otherwise stated. Lithium chloride was used as a tracer as it is not degraded in the process and the concentration was set sufficiently low to minimize risks for inhibition. According to a previous study continuous exposure to Lithium at 250 mg/L have only minor effects on methanogenic activity (Anderson et al., 1991).

2.2. Pasteurization effect

To evaluate the pasteurization effect during digestion of the DDS, different indicator pathogens were added to the pilot reactor. These were *Salmonella*, *Escherichia coli* (>10^5 cfu/g w/w), *Enterococcus* (>10^5 cfu/g) and eggs from the roundworm *Ascaris suum,* which were added to the DDS to achieve high amounts of pathogens in the inlet. The pathogens were then analyzed after different time intervals in the digestate leaving the plug-flow reactor, to evaluate degree of inactivation during digestion. The *Ascaris suum* eggs were added in nylon bags (10 000 eggs per bag) surrounded by steel mesh so that they could be transported through the reactor without breaking. After 1 HRT (30 days), the bags were collected at the end of the reactor and the eggs were cultivated in 0.1 M sulfuric acid (30 days at 28 °C) to evaluate whether they were active or not. *Escherichia coli* was analyzed according to (NMKL, 2005), with detection limit 10 cfu/g, *Enterococci* was analyzed according to (NMKL, 2011), with detection limit 100 cfu/g, and *Salmonella* was analyzed according to (NMKL, 1999) (detection of growth from 25/50 g). F-specific coliphages and somatic coliphages were analyzed by the Department of Energy and Technology, Swedish University of Agricultural Sciences. The methods used were standard ISO10705-1:1995 and 10705–2:2000 with *E. coli* (ATCC 13706) and *S.* Typhimurium WG 49 (ATCC 700730), respectively, as host for the phages and with detection limit 10 pfu/g.

2.3. Microbial analysis

Samples were taken from the reactor outlet after 19, 33, 54, 108, 133, and 166 days of operation and frozen at −20 °C until analysis. Before extraction, the samples were thawed at room temperature and triplicate aliquots of 0.3 g were extracted as described previously (Sun et al., 2015). To analyze the microbial community structure, 16S rRNA amplicon libraries for Illumina sequencing were constructed and bioinformatic analysis of sequencing data was carried out as described previously (Müller et al., 2016). An additional library was constructed for archaeal 16S rRNA gene, using primers arch516F (5′-TGYCAGCCGCCGCGGTAAHACCVGC-3′) and arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (Takai and Horikoshi, 2000). All 16S rRNA amplicon libraries were sequenced at SciLifeLab Uppsala, Sweden, using the MiSeq Illumina sequencing platform. The microbial community had 639 049 and 122 468 sequences after quality trimming and chimera check, with 21 381–46 096 and 1454–12 955 sequences per sample for the bacterial and archaeal community, respectively. The triplicates were pooled *in silico* and randomly subsampled according to the sample with the lowest number of sequences reads (72 809 and 4999 for the bacterial and archaeal community, respectively). The sequences have been deposited in the NCBI Sequence Read Archive (SRA) under accession number SRP156546.

2.4. Gas detection and analytical methods

Volumetric gas production from the plug flow reactor and methane content of the gas produced were measured online with a Ritter milligas counter (MGC-10/20, Ritter, Germany) and gas sensor (BlueSens GmbH, Germany), respectively. The measured data were entered in the program BacVis (BlueSens, Germany). Extraction and management of the data collected were performed according to Moestedt et al. (2015). All volumetric gas data pre-

sented here were converted to standard conditions at pressure 1.01325 bar and temperature 273.2 K. Analyses of TS and VS in substrate and digestate were performed according to Swedish standard SS 28113. The pH was measured at 25 °C (WTW level 2, inoLab, Germany) according to Swedish standard SS-EN ISO 10523:2012. Volatile fatty acids (VFA) were analyzed by gas chromatography according to a previously published method by Jonsson and Borén (2002). Ammonium (NH_4-N) was analyzed according to FOSS Tecator application sub-note 3502 with Kjeltec 8200 equipment (FOSS in Scandinavia, Sweden). The method gives the concentration of total dissolved free ammonium, including dissolved ammonia (NH_4^+–N (aq) + NH_3–N (aq)). Ammonia-nitrogen concentration was then calculated by the formula in Hansen et al. (1998), using concentration of ammonium-nitrogen, pH, and reactor temperature (52 °C). Raw protein content was estimated as the difference between Kjeldahl-nitrogen content and ammonium-nitrogen content multiplied by 6.25 (FAO, 2002). Analysis of carbohydrates (five sugars), hemicellulose and cellulose were made by Eurofins Environment Testing AB (Lidköping, Sweden) according to the method PSLK6. The five sugars quantified by SCAN-CM were xylose, mannose, glucose, galactose and arabinose, respectively. Samples for analysis of raw protein and organic compounds were withdrawn during the start-up phase (day 0–54) and during the stable phase (day 108–176), and three samples from each period were mixed to one sample before analysis. Solid-phase OM structural groups in DDS and in post-digestate samples were analyzed using solid-state ^{13}C cross-polarization magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) spectroscopy, after removing interfering paramagnetic matrix by HCl as described previously (Shakeri Yekta et al., 2018). Duplicate HCl-treated samples were freeze-dried and 70 mg of the dried samples were packed inside 4 mm ZrO2 MAS rotors. The NMR spectra were obtained using a Bruker 500 MHz Avance III spectrometer with operational ^{13}C frequency of 125.75 MHz, a spin-rate of 10 kHz and a sweep-width of 350 ppm.

2.5. Residual methane potential in untreated and treated DDS

To evaluate and compare the risk of methane emissions from fresh (untreated) and post-digested DDS during storage prior to spreading on farmland, their residual methane potential (RMP) was evaluated. For this, 250 g of the material were incubated in triplicate 450-mL anaerobic bottles in AMTPSII © (BioProcess Control AB, Sweden). The test was performed twice with fresh DDS and on digested DDS taken on day 36 and 73 of reactor operation. The incubation temperature was 30 °C, selected as it represented the highest temperature measured during storage of the DDS at the investigated WWTP. The test was run for a total of 40 days, during which the quantity of gas produced in each experimental bottle was measured continuously using a volumetric method (Badshah et al., 2012). In short, the biogas produced was passed through a bottle with NaOH, which captured carbon dioxide, while the methane produced was measured with AMTPSII flow meters. Gas data were converted to standard conditions at pressure 1.01325 bar and temperature 273.2 K.

2.6. Energy balance calculation

For the energy balance calculation, the thermal energy for heating the annual production of sewage sludge (10 000 ton/y) was compared to the energy in the biogas being produced, according to the experiment. Additionally the annual energy requirement for stirring and pumping of the simulated plant (using the estimated effect of 25 kW, received from dry-digestion biogas plant manufacturer) was included according to the equation below:

$$\text{net energy [Kwh]} = \text{produced biogas [Kwh]} - \text{heating [Kwh]} - \text{stirring [Kwh]}$$

$$\text{produced biogas [kWh]} = \frac{\text{biogas } [Nm^3]^*}{\text{ton}} \times \text{methane}[\%]^* \times 9.97\left[\frac{\text{kWh}}{Nm^3}\right] \times \text{sludge } [m^3]$$

$$\text{heating } [kWh] = \Delta T[^\circ\text{C}] \times 1.163\left[\frac{\text{kWh}}{m^3 \times ^\circ\text{C}}\right] \times \text{sludge } [m^3]$$

*Data acquired from the experimental simulation in the present study

The thermal heat requirement for water (1.163 kWh/m^3) was used for the sludge and energy content of methane (9.97 kWh/Nm^3) was applied. No recirculation of heated sludge nor application of heat –exchangers on the outgoing sludge was considered.

3. Results and discussion

3.1. Process performance

After approximately one sludge retention time (SRT ~ 30 days), the first digestate from the plug-flow reactor was discharged from the outlet, indicating that the reactor was filled up. Plug-flow conditions were the investigated by a lithium test. A perfect plug flow would result in a normally distributed recovery of the added lithium around the designed SRT (in this case 36 days). This was not completely reached in the study. Instead, the detection of lithium concentration was similar to normal distributed around day 30, with tails reaching about 10 days and with no lithium at all detected until day 19, when the plug reached the outlet (Fig. S1). This result suggests almost optimal plug flow, with actual exposure time just a bit less that the designed 36 and an SRT in the reactor reaching in average 28–29 days. These results confirmed that no short-circuit occurred in the reactor between ingoing substrate and outgoing digestate. During the start-up phase (day 0–54), the process suffered from severe process disturbance, as methane production was very low (10–15 mL/g VS) and the VFA concentration was high (45 g/L) (Fig. 2). The VFAs present in the highest concentrations were acetic acid, propionic acid, and butyric acid (and their iso- forms). The VFA pattern was similar in samples taken from the middle of the reactor and from the outlet (data not shown). However, as the experiment proceeded the VFA concentration decreased somewhat, which correlated with increased methane production and a change in pH from 7.8 (during the start-up phase) to 8.4 in the stable phase (day 108–176) (Fig. 2). The highest methane production was reached during the stable phase, 46 mL CH_4/g VS, corresponding to approximately 6% of the total methane yield in the main reactors at the full-scale Nykvarnsverket plant (annual biomethane production of 1.8 million m^3 in 2017). In comparison, dry digestion of dewatered, but as yet undigested, sludge results in methane yield of between 18 and 157 mL CH_4/g VS (Duan et al., 2012; Li et al., 2018).

The calculated protein content in the substrate and digestate was 82 and 55 g/kg TS, respectively, suggesting efficient protein degradation (Fig. 3). This was in line with the simultaneous increase in the concentration of ammonium-nitrogen in the stable phase from approximately 18 to 33 g/kg TS, which was 183% higher than for the fresh DDS. These results suggest that thermophilic dry digestion of the DDS improved the degradation of proteins and amino acids compared with the preceding full-scale mesophilic CSTR process. The ^{13}C NMR spectroscopy analysis of the solid-phase organic matter further showed that ^{13}C resonances within ^{13}C chemical shift interval of 167 – 220 ppm; (Hertkorn

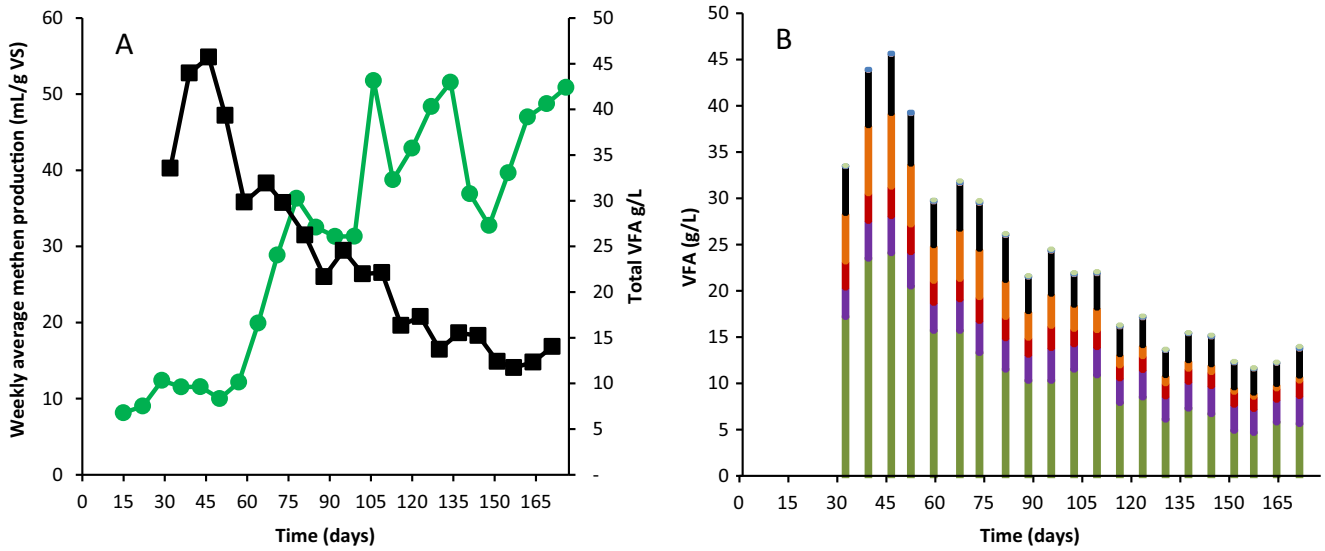

Fig. 2. A) Weekly average methane production (●) and total volatile fatty acids (VFA) concentration (■) during thermophilic dry digestion of dewatered digested sewage sludge. B) VFA profile in the digestate (outlet) from the plug-flow reactor. Acetic acid (■), propionic acid (■), *iso*-butyric acid (■), butyric acid (■), *iso*-valeric acid (■),valeric acid (■), *iso*-hexanoic acid (■) and hexanoic acid (■).

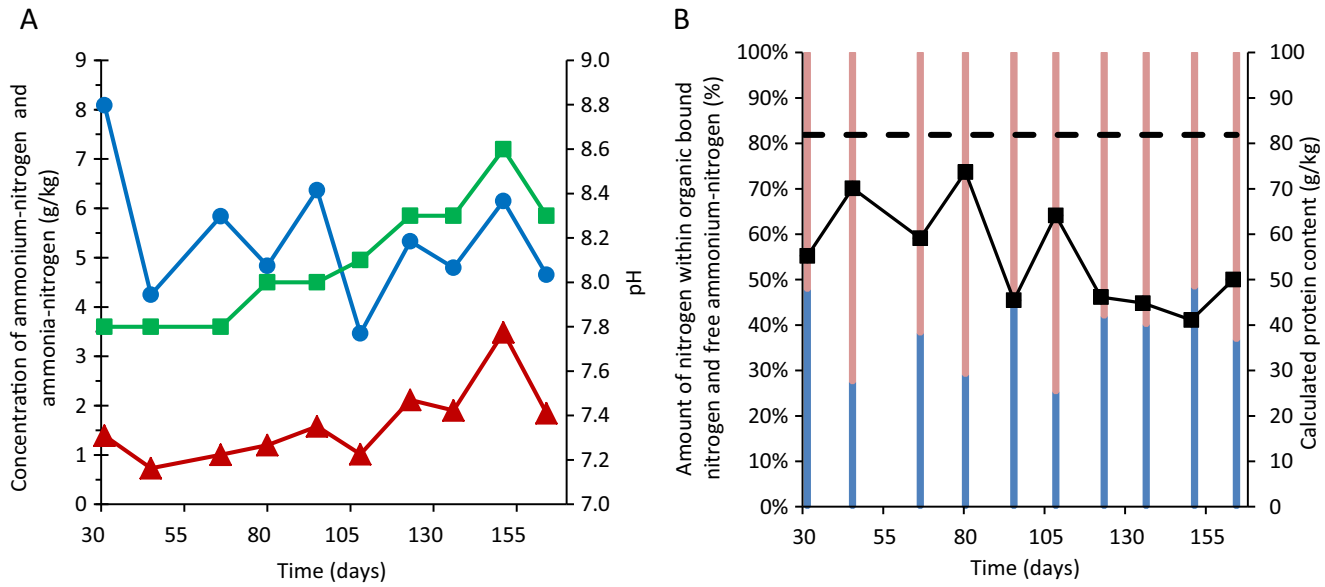

Fig. 3. Concentrations of nitrogenous compounds in outgoing digestate during thermophilic dry digestion of dewatered digested sewage sludge (DDS): A) pH (■), and ammonium-nitrogen (●), and calculated concentration of ammonia–nitrogen (▲). B) Amount of free ammonium-nitrogen (NH_4-N + NH_3-N; ■), organically bound nitrogen (■), calculated concentration of proteins in DDS (– – –), and calculated concentration of proteins in digestate (–■–).

et al., 2002) had lower intensities in the solid-phase of the DDS samples after digestion as compared to the DDS substrate (Fig. S2). The signal intensities in this range can be assigned to carbonyl C groups (C = O) in complex organic matrices (Hertkorn et al., 2002). Previous comparison of ^{13}C NMR spectra of digestates, from biogas reactors with different substrate profiles and operational conditions, indicated that resonances assigned to carbonyl C likely originate from protein content of the digestate (i.e. C = O is abundant structural constituent of amino acids and polypeptides (Shakeri Yekta et al., 2019). Accordingly, a lower intensity of protein-derived, carbonyl C signals in spectra of the digested DDS support the removal of protein-like structures during the high solid-digestion process. Therefore, it may be perceived that hydrolysis of the residual protein contents in the effluent from the preceding full-scale mesophilic CSTR process have been further enhanced upon thermophilic high solid digestion of the DDS. Similar findings, e.g. efficient protein degradation, have been reported on thermophilic high solid digestion with ethanol distillation residue and with source-separated organic household waste mixed with garden residues (Goberna et al., 2009; Huang et al., 2017).

The ammonium level, in combination with high pH and the thermophilic process temperature, resulted in high FAN levels, which increased from approximately 1 g/kg to 2 g/kg on average during the stable phase of the experiment (Fig. 3). Previous studies using dry digestion of DS have found lower FAN levels, 0.2–0.8 g/L (Duan et al., 2012; Li et al., 2018). Ammonia concentration of 1.0–1.1 g/L is often described as the upper threshold to avoid process failure (Hansen et al., 1998; Moestedt et al., 2016; Westerholm et al., 2016). Despite the high ammonia levels the process in this study showed decreasing VFA levels and increasing methane pro-

duction in the stable phase of operation. A few studies have reported even higher FAN levels (1.5 g/L) in well-functioning biogas processes (Westerholm et al., 2016), although most of those studies have been performed in semi-continuous CSTR or in batch mode, and not in dry digestion with plug flow as in the present case. Nevertheless, studies of other high solid digestion processes have shown similarly high ammonia levels as in the present study, including at thermophilic conditions (Goberna et al., 2009; Huang et al., 2017). This suggests that dry digestion might allow higher ammonia levels without risking process failure and methanogenic inhibition as compared with CSTR systems. Alternatively, the plug-flow design may have resulted in a gradient of ammonia following the length of the reactor, giving a lower level of inhibition, and thus methane production, close to the reactor inlet and a higher ammonia level and inhibition at the outlet. Despite the stable operation towards the end of experiment, VFA was present in concentrations higher than in the substrate. Thus, it was obvious that the process suffered from some restriction in functionality and were operating at inhibited steady state conditions. This VFA accumulation could have been caused by direct inhibition of ammonia or by lack of essential micronutrients, precipitated by sulfides released during the degradation of proteins.

In contrary to the observed decrease in protein contents of DDS after the digestion, the relative concentrations of carbohydrate monomers (such as mannose, glucose, xylose, galactose and arabinose) and cellulose were higher in the effluent of the plug-flow reactor compared to the fresh DDS (Table S1). Furthermore, the increased level of cellulose correlated with a decrease in hemicellulose and proteins (Table S1). Less efficient degradation of cellulose in high-protein environments has been reported previously and is suggested to be caused by high ammonia levels (Liu et al., 2017; Sun et al., 2016). These results demonstrate the importance of increased knowledge on regulation of hydrolytic enzyme activity to achieve optimum conditions for degradation of carbohydrates in the presence of high ammonia concentrations.

3.2. Pasteurization effect

The lithium test confirmed plug-flow conditions, with 19 days as the shortest time of exposure for the sludge. This, combined with the thermophilic digestion temperature set at 52 °C, far exceeded the suggested requirement for pasteurization by heat treatment, which in Sweden is exposure for > 24 h at 52 °C (Naturvårdsverket, 2013). The pasteurization effect was verified by the fact no *Enterococcus*, *E.coli*, phages, nor *Salmonella* were detected above the threshold levels in the treated sludge, although the DDS was spiked with > 10^5 cfu/g of *E. coli* and *Enterococcus*. Furthermore, eggs from the roundworm *Ascaris suum* were found to be unable to grow when incubated after the dry digestion process. The efficient killing of the different indicator organisms in the sludge in this study was likely caused by a combined effect of high temperature and high ammonia levels, as shown previously (Scaglia et al., 2014).

3.3. Residual methane potential

RMP was determined in fresh DDS and in the digestate from the plug-flow reactor. The results showed values below 0.05 m^3 CH_4/-ton for the digestate after dry digestion and within the range 0.94–1.25 m^3 CH_4/ton (w/w) in the untreated DDS (Table 1). The RMP in untreated DDS was similar to that in a previous study, where the values ranged between 0.09 and 0.21 m^3 CH_4/ton depending on storage time of the material, e.g., after direct sampling or after 1 HRT and storage of the DDS (Willen et al., 2016). Thus, the post digestion resulted in a > 96% reduction in RMP compared with the untreated DDS. This result was somewhat unexpected, as the digested DDS contained high levels of readily degradable organic material, such as sugars (136 g/kg; during stable phase) and VFA (22–29 g/L). Degradation of these compounds was possibly hampered by the extremely high concentration of ammonia–nitrogen, which might have inhibited microbial activity. Moreover, the RMP test was carried out at room temperature, while the microorganisms were accustomed to thermophilic conditions, which could have decreased their activity. In any case, this low RMP of the digestate is highly valuable, as methane is a 34-fold more potent greenhouse gas than carbon dioxide (IPCC, 2013). On average for the two test runs, the global warming potential (GWP) corresponded to 28 ton CO_{2eq} for DDS and only 0.6 ton CO_{2eq} for the digestate after dry digestion (IPCC, 2013).

Table 1
Residual methane potential (RMP) in fresh dewatered digested sludge (DDS) and digestate from the plug-flow reactor for two test runs.

	RMP (m^3 CH_4/ton)	RMP (kWh CH_4/ton)	Reduction compared with untreated DDS (%)
Fresh DDS run 1 (day 36)	1.25	12.4	0%
Digestate run 1 (day 73)	0.05	0.5	−96%
Fresh DDS run 2 (day 73)	0.94	9.4	0%
Digestate run 2 (day 103)	–	n.d.	−100%

3.4. Microbial community analyses

Sequencing of the microbial community by the Illumina Miseq resulted in OTUs ranging from 1762 and 3493 for the bacterial community, and from 145 to 217 for the archaeal community. For both bacteria and archaea, the lowest values were observed for the inoculum sample, after which an increase in the number of OTUs was observed (Table S2). After 54 days of operation a drop was seen for both bacteria and archaea, after which the number of observed OTUs increased again and stabilized at the end of the experiment (Table S2). The Simpson and Shannon diversity indices of both the bacterial and archaeal communities showed an a similar trend with increasing values over time, although with slightly lower values at day 43 and 108 as compared the final phase of operation (Table S2). A weighted UniFrac principal coordinate analysis (PcoA) plot and the alpha diversity indices both illustrated a shift in community composition over time (Fig. 4). The observed shift was likely a consequence of the a) higher temperature compared with the process from which the inoculum was taken and b) increasing and high ammonia level. Temperature and ammonia have been shown to be strong determining parameters for both the community structure and diversity in different biogas processes (De Vrieze et al., 2015; Müller et al., 2016; Sundberg et al., 2013). However, in contrast to present study, increasing ammonia and temperature have in many studies been shown to cause a decrease in diversity (Levén et al., 2007; Müller et al., 2016; Sun et al., 2015; Westerholm et al., 2018). One possible explanation for contradictory results could be that the concept of high solid digestion with plug flow reduce the risk for wash out of ammonia inhibited slow growing bacteria.

The bacterial community structure in the inoculum was typical for sewage sludge from WWTP and dominated by the phyla Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, and Chloroflexi (Fig. 5) (Hao et al., 2016). The observed dominance of the archaeal class Methanomicrobia, mainly comprised of the aceticlastic methanogenic genus *Methanosaeta* (>50%), was also typical for biogas processes operating with sewage sludge (Fig. 6)

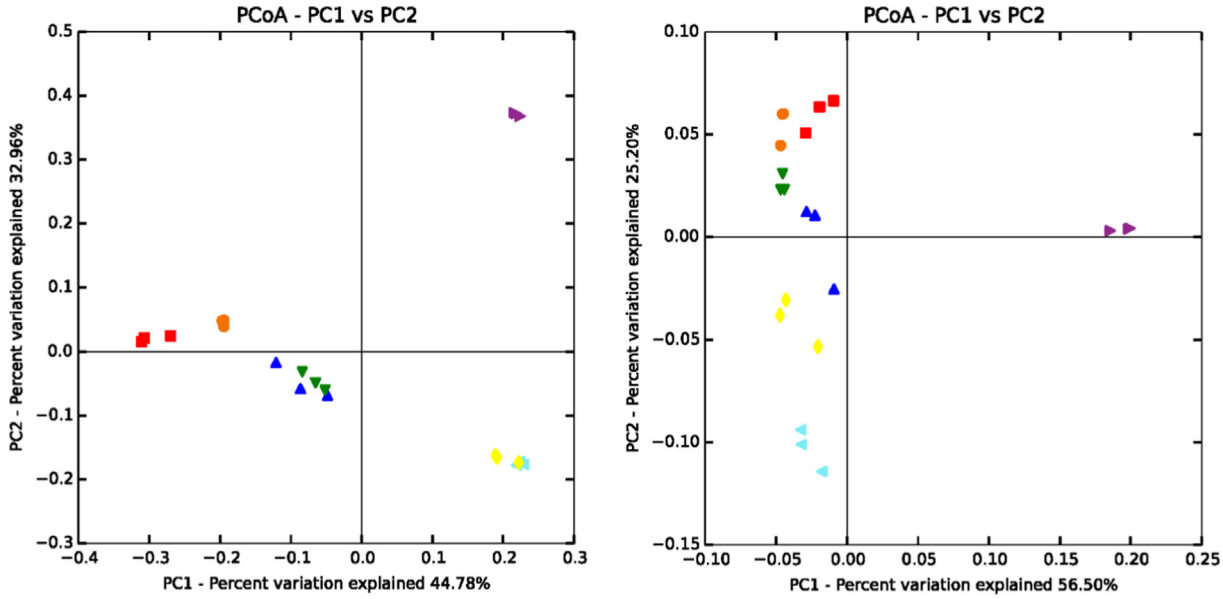

Fig. 4. Phylogenetic distance of A) bacterial and B) archaeal community between samples as determined by weighted UniFrac principal coordinate analysis (PCoA). ■ 19 days, ▲ 33 days, ● 54 days, ▲ 108 days, ◆ 131 days, ▲ 166 days; ▲ inoculum).

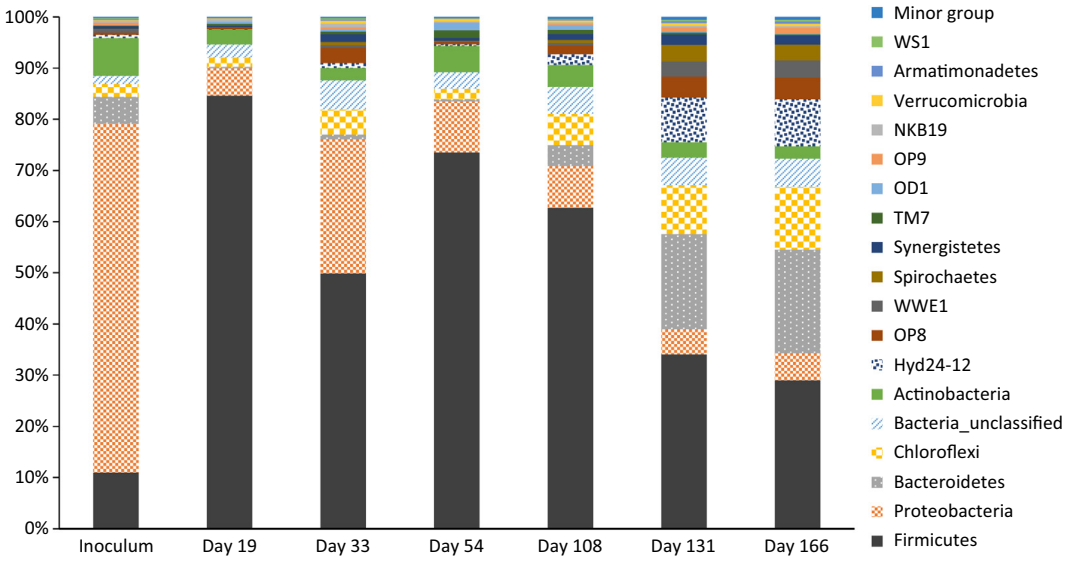

Fig. 5. Relative abundance of bacterial 16S rRNA gene at phlyum level in inoculum sludge and in the dry digestion process, arranged by time of operation (day 19, 33, 108, 131, and 166).

(Hao et al., 2016). However, over time the community changed and for the bacterial community a significant increase in the relative abundance of the phylum Firmicutes was initially observed (Fig. 5).

However, although phylum Firmicutes continuously represented a significant part of the community the abundance decreased over time and the composition also changed at lower taxonomic rank (Fig. 5, S3, S4). Within Firmicutes, an unknown genus within the order Thermoanareobacterales was initially present at high relative abundance but later this order decreased and instead members belonging to orders Caldicoprobacteraceae (genus *Caldicrobacter*), Clostridiaceae (genus *SMB3)*, and Tissierellaceae (genus *Tepidmicrobium)* increased in abundance (Fig. S4). In the later phase of operation the phyla Bacteroidetes and Choroflexi increased in relative abundance, together with the phyla Hyd24, OP8, WWE1 and Spirochaetes, initially present at very low levels (<0.7%) (Fig. 5). For archaea, the relative abundance of the genus *Methanosaeta* decreased over time and instead class Methanobacteria increased (Fig. 6). Initially this increase was caused by the family Methanobacteriaceae, genus *Methanobacterium,* but at the end of the operation this genus decrease while instead an increase in the candidate methanogen family WSA2 (~30%) was observed (Fig. 6).

The observed high relative abundance of Firmicutes agrees with findings in other studies of processes operating at high temperature and/or ammonia, including both CSTR and high solid digestion processes (Chen and Chang, 2017; Goberna et al., 2009; Lin et al., 2017; Müller et al., 2016; Sun et al., 2015; Sundberg et al., 2013; Wang et al., 2016). This phylum contain members with broad hydrolytic and/or fermentative abilities as well as syntrophic bacteria and are commonly detected in various biogas processes (Westerholm and Schnürer, 2019). The observed increase in the relative abundance of the phyla Bacteroidetes and Chloroflexi over

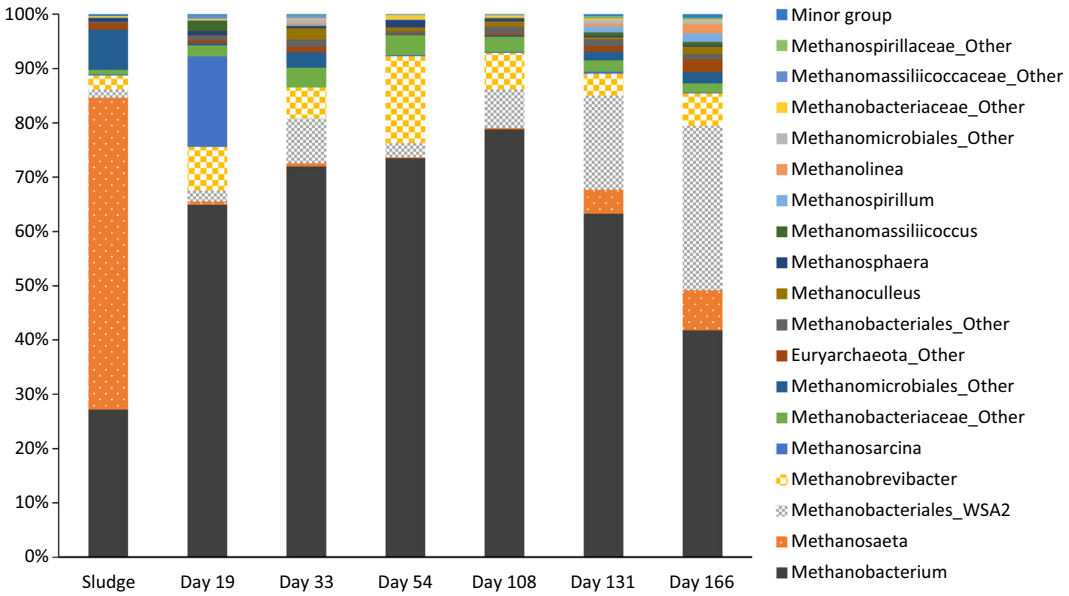

Fig. 6. Relative abundance of archaeal 16S rRNA gene at genus level in inoculum sludge and in the dry digestion process, arranged by time of operation (day 19, 33, 108, 131, and 166).

time was however somewhat more surprising as the abundance of these two phyla have been suggested to be inversely correlated with the free ammonia concentration (Campanaro et al., 2018; De Vrieze et al., 2015). Still, both are commonly detected in waste activated sludge digesters and the increase of these phyla in the present study suggests members with high ammonia tolerance. Moreover, family Anaerolinaceae, dominating in the phylum Chloroflexi, has recently also been shown to have good ability to persist during a temperature transition from mesophilic to thermophilic conditions, possibly also explaining its prevalence (Zhu et al., 2017). The increase of candidate phylum Cloacimonetes (phylum WWE1) and the genus *Treponema*, dominating within the phylum Spirochetaceae, was in line with previous studies of mesophilic CSTR and plug flow reactors operating with various substrate, such as manure and food waste at mesophilic conditions, reporting links between abundance and high ammonia levels (>5 g/L) (Bi et al., 2020; Dong et al., 2019; Müller et al., 2016; Poirier et al., 2016; Solli et al., 2014; Sun et al., 2015). The ecological function of Cloacimonetes is not established, but has been proposed to involve amino acid fermentation, syntrophic propionate oxidation, or extracellular hydrolysis (Calusinska et al., 2018). The increase of this phylum over time suggest a robust process as disappearance of members within this phylum have been suggested as an indicator for an upcoming process disturbance due to increasing TAN concentrations (Klang et al., 2019). Treponema has in similar to Cloacimonetes been proposed to be involved in hydrolysis, fermentation and acetogenesis (Li et al., 2019). In the final phase of operation, the phyla Hyd24 and OP8 also increased in relative abundance. Little is known about these phyla, as no cultivated isolates are available as yet, but Hyd24 is commonly detected in WWTP and genome analysis suggests that members of this phylum are involved in acidogenesis, producing acetate and hydrogen from fermentation of sugars (Kirkegaard et al., 2016). Candidate phylum OP8 has been observed under various environmental conditions, including extreme environments such as high temperature and saline environments, as in this study (Farag et al., 2014).

Thermophilic temperatures have been shown to cause a shift from methane formation via acetoclastic methanogenesis to syntrophic acetate oxidation (SAO) coupled with hydrogenotrophic methanogesis (Hansen et al., 1998; Hao et al., 2017; Moestedt et al., 2016; Schnürer et al., 1994; Schnürer and Nordberg, 2008; Sun et al., 2014; Westerholm et al., 2016, 2018; Nordell et al., 2013). A similar shift occurs in mesophilic conditions in response to increasing ammonia levels. It is currently unclear whether the dominance of SAO-mediated methanogenesis at thermophilic temperature is a consequence of increasing ammonia levels due to a shift in the equilibrium with ammonium, or a consequence of the temperature itself (Westerholm et al., 2016). An ammonia-induced shift to SAO is suggested to occur at around 150–200 mg/L NH_3-N (Westerholm et al., 2016), far below the levels obtained in the present study. The initial sharp increase in the relative abundance of the orders Thermoanaerobacterales and Methanobacterium and the decrease of the aceticlastic methanogen *Methanosaeta* suggest a shift to SAO-driven methanogensis in this study. *Methanosaeta* is well known for its low ammonia tolerance (Westerholm et al., 2016) and loss of this species is believed to promote development of SAO (Westerholm et al., 2016). Moreover, *Methanobacterium* is typically observed as the hydrogen-consuming partner in thermophilic SAO-dominated processes (Westerholm et al., 2016). In addition, Thermoanaerobacteriales contains the known SAO bacteria genera *Syntrophaceticus* and *Thermoactetogenium*, both previously observed in thermophilic high ammonia processes (Westerholm et al., 2016). In the later phase of operation the order Clostridiales, dominated by *Tepidmicrobium*, increased in abundance and this genus has also been highlighted in several recent papers as an ammonium-tolerant genus containing potential SAO bacteria (Müller et al., 2016; Poirier et al., 2016). In line with present study this genus was also seen to be high in a thermophilic high solid digestion process operating with DS and at FAN around 702 mg/L (Wu et al., 2020). However, members of this genus are also capable of oxidizing both proteinaceous substrates and various carbohydrates, and could thus be a part of the hydrolytic/fermentative community (Niu et al., 2009). SAO-mediated methanogenesis has also been suggested before in several other studies of thermophilic high solid digestion reporting presence of known SAO bacteria or bacterial genus know to harbor SAOB; *Clostridium ultunense* (Goberna et al., 2009; Huang et al., 2017), and the genera *Syntrophaceticus* and *Tepidanaerobacter* (Wang et al., 2018) and *Thermoacetogenium* (Lin et al., 2017). Interestingly, Ruiz-Sanchez et al. (2018) suggested that the phyla

Chloroflexi and Bacteroidales encompass new, currently undescribed, SAOB/formate-producing species. Thus, in the present study a shift in SAO bacteria might have occurred over time in line with the increasing ammonia level.

Interesting and novel results in this study were the significant increase in the candidate methanogen family WSA2 in response to the increasing ammonia levels and thermophilic conditions. The activity of this methanogen has recently been suggested to be limited to methanogenesis via methylated thiol reduction (Nobu et al., 2016). In the present study, its abundance was probably enriched as a result of the high degree of protein hydrolysis, resulting in release of methyl thiol. High relative abundance of this family has not been reported previously and contradicts findings in other studies of high solid digestion at thermophilic temperature (50–55 °C) and similar high ammonia levels (1.8–2.2 g/L), which report dominance of the hydrogenotrophic methanogen *Methanoculleus* (order Microbiales) (Goberna et al., 2009; Huang et al., 2017; Wang et al., 2018). In addition to *Methanoculleus*, the genera *Methanosarcina* and *Methanothermobacter* have also been reported to be abundant during thermophilic high solid digestion (Li et al., 2014; Meng et al., 2018; Wang et al., 2018), but were both present at low levels in this study. In addition to WSA2, *Methanobacterium* and *Methanosaeta* were present at levels of around 40 and 6%, respectively. *Methanobacterium* is often found in themophilic processes, including at high ammonia levels. However, *Methanosaeta* is well known for its low ammonia tolerance (Westerholm et al., 2016), and is thus typically not found in the conditions prevailing in this study. The presence of this methanogen might be related to the recirculation of process liquid reducing the risk of washout of less active and slow-growing microbes (Calusinska et al., 2018).

3.5. Energy balance

In the full-scale Nykvarnsverket WWTP, the digestate from the mesophilic process has a temperature of 38 °C and after dewatering approximately 30 °C. To reach 52 °C in the dry digestion unit, the energy input needed was at least $\Delta T > 22$ °C. The amount of energy needed to heat 1 ton of DDS was estimated to be 25 kWh (without any heat recovery from the outgoing digestate), which can be set against the biogas production of approximately 80 kWh/ton of DDS. There is also a high energy demand for stirring and pumping in a hypothetical full-scale dry digestion reactor, which was estimated to 210 MWh/year, corresponding to 21 kWh/ton of DDS. The net energy production would thus be 34 kWh per ton of DDS treated. Based on DDS production of 10 000 ton/year (actual output from Nykvarnsverket WWTP, 2016), the net energy balance for the pasteurization method with dry digestion would be 340 MWh/year. In Linköping city and at the WWTP, district heating from an incineration plant is used, and thus most of the energy consumed in a dry digestion unit would already be secondary energy.

4. Conclusions

This pilot-scale experiment clearly demonstrated that thermophilic high solid digestion of DDS give a good sanitization effect and simultaneously can increase biogas production at a WWTP, making this pasteurization method a net producer of energy. The specific methane production was 46 mL CH_4/g VS, corresponding to a 6% increase compared with that in Nykvarnsverket WWTP. Moreover, the resulting digestate had a low residual biomethane potential, likely caused by a very high degradation efficiency of protein fraction in the DDS. The efficient protein degradation resulted in high ammonium concentrations, which is positive for the use of the digestate as a fertilizer. The resulting ammonia levels were far above levels typically reported to be inhibitory. However, in spite this the microbiological analyses illustrated a functionally diverse community and stable methanogenesis, likely driven by members within the candidate methanogen family WSA2. Overall, these results suggest that thermophilic plug-flow dry digestion of DDS can meet the upcoming requirement on pasteurization of sludge from WWTP in Sweden, and simultaneously produce energy and reduce the risk of methane emissions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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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.2020.09.028.

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