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Hydrothermal pretreatment of sewage sludge for enhanced anaerobic digestion: Resource transformation and energy balance

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ABSTRACT

The objective of this study was to evaluate the effect of hydrothermal pretreatment (HTP) at 90, 125 and 155 °C on the extent of anaerobic digestion (AD) of sewage sludge, energy balance, and the distribution and availability of N and P. AD was evaluated with four bench-scale mesophilic, semi-continuous digesters with 20-d (Phase 1) and 10-d (Phase 2) solids retention time (SRT), fed with raw sludge (R1), 90 °C (R2), 125 °C (R3) and 155 °C (R4) HTP sludge. The two sludge mixtures used in this study had high ultimate biodegradability (58.3 for Phase 1 and 56.9% for Phase 2). With the exception of Phase 2 R4, HTP increased organic matter destruction and methane production. HTP of Phase 2 sludge mixture at 155 °C resulted in the formation of high propionate levels, which led to a lower methane production. HTP significantly changed the digesters' bacterial communities, but had a minor effect on the archaeal communities. Abundance of well-known propionate degraders in all digesters was very low; however, *Proteiniphilum*, which is involved in the degradation of intermediates in the propionate degradation pathway, was found in relatively high abundance in Phase 2 R4. The highest net energy gain (ΔE) was obtained with the control (i.e., raw sludge AD without HTP) for both phases. HTP heat recovery greater than 85% is required to attain the same net energy as the control or higher. HTP at 155 °C followed by AD led to increased solids reduction, overall crude protein removal and release of ammonium N. HTP-AD decreased P availability. Thus, P and N recovery is recommended before and after HTP-AD, respectively.

1. Introduction

Domestic wastewater is increasingly regarded more as a resource rather than a waste, a resource for water, energy, and nutrients (N and P) [1]. In recognition of the potential significant resource recovery from wastewater, municipal wastewater treatment plants are now referred to as water resource recovery facilities (WRRFs) [2]. Large quantities of sludge, a by-product of municipal wastewater treatment, are produced. Over 12 million dry metric tons of sewage sludge are produced in WRRFs in the United States [3]. Sewage sludge must be treated before disposal or utilization to minimize negative impacts on the environment and public health, as well as to recover resources (e.g., methane as a renewable bioenergy and nutrients). Anaerobic digestion (AD) is a traditional and most commonly used sludge stabilization and treatment process. The benefits of AD include sludge mass reduction, odor removal, pathogen reduction, and energy recovery [4]. AD is a complex process consisting of four sub-processes: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is usually the rate-limiting step in the AD of particulate organic wastes, especially waste activated sludge (WAS) [4].

In order to accelerate waste solubilization and improve AD efficiency, various mechanical, thermal, chemical, and biological methods, and their combinations have been evaluated [4,5]. Among these methods, hydrothermal pretreatment (HTP) is very effective in achieving a high degree of organic matter solubilization, which may potentially result in higher sludge biodegradability [6]. The main benefits of the combined HTP-AD process include volatile solids (VS) reduction, energy and nutrient recovery, enhanced dewaterability, and destruction of pathogens [7]. The HTP temperature for sewage sludge has been from 60 to 270 °C [8]; sludge solubilization was positively correlated to HTP temperature (60–170 °C) [9]. HTP duration above a certain value, e.g., 24 h for 90 °C HTP and 90–120 min for 120–160 °C HTP [10] or 10 min for 170 °C HTP [11], did not result in additional sludge solubilization. Industrial-scale, high-temperature HTP technologies use treatment up to 160 °C for 30 min and 6 bar (Cambi™) or up to 180 °C for 60 min and 10 bar (Exelys™) [7].

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Previous studies have shown variable extent of HTP effect on sludge degradability, attributed to differences in HTP temperature and duration, as well as sludge type and characteristics. For WAS, although most studies reported an increase in the extent of methane production with low temperature HTP (<100 °C), the reported methane increase after HTP ranged widely from 1.6% to 124% [12]. High-temperature HTP (>100 °C) was originally developed to reduce sludge pathogen content and produce class A biosolids. For high-temperature HTP, compared to duration, temperature has a more pronounced effect on organic matter solubilization [12]. A low biodegradability sludge exhibited a higher increase in methane production with HTP than a more biodegradable sludge [13]. The effect of high-temperature HTP on ultimate biodegradability involves two competing processes: sludge solubilization, which should increase methane yield, and formation of soluble but refractory and/or inhibitory compounds (e.g., dioxins and melanoids) [14,15], which do not contribute to or even decrease biogas production.

In addition to the benefits of energy recovery via biomethane production, AD may also affect the release and distribution of nutrients such as N and P. Sewage sludge is an important resource for N and P recovery. Typically, sewage sludge contains 1–8% N and 0.5–5% P (dry weight basis) [16]. In a typical WRRF with enhanced biological phosphorus removal (EBPR) and precipitation, approximately 90% of P is in the sludge, making it the most abundant resource for P recovery [17]. Detailed information on N and P distribution and availability during HTP-AD is needed to mitigate the environmental impacts of nutrient runoff upon land application of biosolids as well as provide guidance for the effective recovery of N and P from sludge. HTP leads to organic N solubilization, i.e., release of soluble protein and ammonium, while AD results in partial mineralization of the organic N and release of ammonium [18]. During AD, organic P is converted to orthophosphate, which subsequently reacts with metal cations or other minerals to form phosphate minerals or mineral-adsorbed phosphates [19]. HTP-AD is an established industrial process, especially for enhancing biogas production [7]. Laboratory studies have shown that in addition to increased biomethane production, HTP-AD can lead to nutrient recovery, which has not been explored sufficiently at conditions relevant to practice [20,21]. Thus, it was important to determine the best conditions of the combined HTP-AD process for the specific sludge used in the present study in order to increase biogas production while achieving a positive energy balance, as well as to recover available N and P.

Most HTP-AD studies were conducted with either WAS or primary sludge (PS). However, in most WRRFs, WAS or thickened WAS (TWAS) and PS are combined before the sludge mixture is fed to the digesters. The characteristics of the sewage sludge mixture (i.e., PS and WAS), compared to PS and WAS, are inherently more variable due to non-consistent mixture ratios used at the plant, as well as changes of plant operational conditions. Furthermore, most HT-AD studies used batch assays rather than semi-continuously or continuously fed digesters. Previous studies suggested that the results of continuously or semi-continuously fed digesters can be quite different from those of batch assays as a result of differences in organic loading rates, as well as volatile fatty acids (VFAs) and ammonium concentrations [22]. Therefore, operation of continuously or semi-continuously fed digesters at different solids retentions times (SRTs) provides the most representative results [22], as well as representative digestate samples for microbial community analysis.

Even data from studies that used continuously or semi-continuously fed digesters varied widely in response to HTP temperature. Li and Noike [23] reported that HTP at 150 to 175 °C resulted in doubling the methane production from WAS in a continuously fed digester at 5-d SRT, compared to raw WAS. In a study by Wilson and Novak [24], when mesophilic semi-continuously fed digesters were fed with PS and WAS mixture, operated at 15–20 d SRT, the VS reduction and biogas production was slightly lower with HTP at 170 °C than at 150 °C, but higher than without HTP [24]. The results of the Higgins et al. [22] study suggested that the methane yield from mesophilic semi-continuously fed digesters, operated at 15-d SRT fed with PS and WAS mixture, increased with HTP temperature from 130 to 170 °C; however, the methane yields were not statistically different [22]. The differences among HT-AD studies may be attributed to HTP temperature and duration, sludge type and properties, as well as SRT and organic loading rate (OLR) of the digesters.

In summary, there are several knowledge gaps related to the impact of HTP on AD performance and nutrient distribution and availability. Comprehensive studies using continuously or semi-continuously fed digesters have not been performed which simultaneously evaluated digester performance in terms of VS and total chemical oxygen demand (TCOD) destruction, biogas and methane production, as well as energy balance and nutrients transformation with HTP over a wide range of temperatures and AD at typical OLRs and SRTs. The overall goal of this study was to address the following questions: 1) would HTP increase the methane production to such a level that results in a positive energy balance? 2) how do HTP and AD affect N and P distribution and availability, and what is it recommended for best N and P recovery? To accomplish the study goal, HTP at 90, 125 and 155 °C of sewage sludge mixture and subsequent AD at two SRTs (10 d and 20 d) was assessed in terms of a) AD performance and energy balance; b) microbial community structure; and c) release of N and P. The results of this study in terms of semi-continuous AD performance, microbial community composition, analysis of net energy production, along with release of N and P, provide valuable, quantitative information for the scale-up and optimization of the combined HTP-AD process for energy and nutrient recovery from sewage sludge.

2. Materials and methods

2.1. Sewage sludge

Thickened sewage sludge, a blend of PS and WAS, as well as PS and WAS, were obtained from the F. Wayne Hill Water Resources Center (FWHWR Center; Buford, GA, USA). The sludge mixture passes through a Waste Activated Sludge Stripping to Remove Internal Phosphorus (WASSTRIP®) process (retention time 6–12 h) to release P from phosphate accumulating bacteria; then, polymer is added to the sludge mixture to enhance its dewaterability. The sludge mixture is then passed through a rotary drum thickener to recover P in the filtrate and then the thickened sludge mixture is fed to mesophilic (35 °C) anaerobic digesters (ca. 20-d SRT). The sludge mixture was stored in the laboratory at 4 °C in the dark. During the entire study, sludge mixture was collected twice from the same plant and used for AD at 10-d and 20-d SRT, respectively. In addition, anaerobic digestate collected at the FWHWR Center was incubated in the laboratory at 35 °C for over 60 d until biogas production was negligible, then used as inoculum for biochemical methane potential (BMP) tests and the semi-continuous digesters.

The characteristics of PS, WAS, and Phase 1 and 2 sludge mixture are shown in Table 1. Compared to WAS, PS had higher VS-to-total solids (TS) ratio (VS/TS) and TCOD/VS ratios, and a lower total nitrogen (TN)-to-TCOD ratio (TN/TCOD). Although the sludge mixture was taken from the same WRRF, there are significant differences in characteristics among the two sludge mixtures used in the two phases of the study. Phase 1 sludge mixture had a higher soluble COD (SCOD) concentration. Although the TCOD/VS ratio of Phase 1 and 2 sludge mixtures was comparable, the VS/TS ratio of Phase 1 sludge was lower than that of Phase 2 sludge. The TN concentration was comparable, whereas the TCOD/TN ratio of Phase 1 sludge mixture was lower than that of Phase 2 sludge mixture. In addition, Phase 2 sludge had a much higher soluble orthophosphate concentration than Phase 1 sludge. The TN/TS ratio of Phase 1 and 2 sludge mixture was 6.6 and 5.2%, respectively. The total phosphorus (TP) to TS ratio (TP/TS) of Phase 1 and 2 sludge mixture was 2.3 and 2.2%. Thus, the N and P content in the two sludge mixtures used in the present study was well within the above mentioned literature reported ranges. Although the two sludge mixtures had similar P

Table 1
Characteristics of primary sludge (PS), waste activated sludge (WAS), and thickened sludge mixture.

Parameter	PS	WAS	Sludge Mixture (PS + WAS)	
			Phase 1	Phase 2
pH	5.92	6.32	7.44	5.71
SCOD (mg/L)	2232 ± 12[a]	492 ± 4	10720 ± 360	6120 ± 360
TCOD (mg/L)	38027 ± 1796	14873 ± 123	74073 ± 3455	97213 ± 1014
TS (g/L)	23.71 ± 0.56	14.16 ± 0.14	51.6 ± 0.4	62.7 ± 0.16
VS (g/L)	19.52 ± 0.50	10.39 ± 0.15	38.8 ± 0.7	52.1 ± 0.19
VS/TS (%)	82.3	73.4	75.1	83.1
TCOD/VS	1.95	1.43	1.91	1.87
VFAs (mg COD/L)	NA[b]	NA	6339 ± 13	5420 ± 16
Total nitrogen (mg N/L)	1068 ± 8	917 ± 39	3419 ± 241	3240 ± 46
Ammonium (mg N/L)	110 ± 2	52 ± 1	678 ± 15	316 ± 8
Crude protein (mg/L)	5984	5404	17,619	18,275
Total phosphorus (mg P/L)	335 ± 0	680 ± 5	1189 ± 17	1350 ± 24
Soluble orthophosphate (mg P/L)	49 ± 1	184 ± 2	14.3 ± 0.2	368 ± 3

[a] Mean ± standard deviation ($n = 3$).
[b] NA, not analysed.

content, the P distribution between solid/liquid phases was quite different. The soluble orthophosphate accounted for 1.2 and 27.3% of TP in Phase 1 and 2 sludge, respectively. As mentioned above, the sludge undergoes WASSTRIP® to release and recover P before AD. However, due to operational inconsistencies, it is possible that the P release and recovery step was not always conducted. In addition, the two raw sludge mixtures were different in terms of microbial community composition, discussed in detail in Section 3.4, below. These data suggest that even for the same plant, the thickened sludge mixture composition significantly differed over time, reflecting operational changes at the study WRRF. In preliminary BMP tests conducted in the present study, the ultimate biodegradability of PS, WAS, and Phase 1 and 2 sludge mixture was 63.2, 34.3, 58.3, and 56.9%, respectively. Based on the ultimate biodegradability results, the sludge mixtures used in the present study had estimated PS/WAS ratios of 76/24% and 71/29% on a TS basis for Phase 1 and 2 sludge mixture, respectively.

2.2. Hydrothermal pretreatment (HTP)

For each HT batch, six replicate aliquots of ca. 130 mL of raw sludge mixture were added to 200-mL polypropylene-lined stainless-steel hydrothermal reactors (COL-INT Tech., Irmo, SC, USA). The HT reactors were sealed and heated in a forced air oven (VWR; Radnor, PA, USA), which was maintained at a pre-set target temperature (90, 125, or 155 °C) for 4 h. Previously, Fang et al. [20] reported that for WAS obtained from the same WRRF, the total gas and methane production increased with HTP at 125 °C, but decreased with HTP at 225 °C, more likely due to formation of recalcitrant and/or inhibitory substances. In addition, in preliminary batch tests using sludge mixture from the same WRRF, HTP at 185 °C resulted in lower biogas production during the first 10 d of incubation and comparable biogas to the raw sludge mixture after over 70 d of incubation (data not shown). Therefore, in the present study, the highest HTP temperature tested was 155 °C. After 4 h heating, the HT reactors were removed from the oven, allowed to cool down to room temperature, and the sludge slurries were then stored in glass bottles at 4 °C in the dark until subsequent use in tasks described below. The sludge mixture without HTP was used as a control (referred to as raw sludge mixture).

The solubilization of the sludge mixture after HTP, based on COD measurements, was calculated using Eq. (1).

$$COD_s = \frac{SCOD_{HT} - SCOD_0}{TCOD_0 - SCOD_0} \times 100 \quad (1)$$

where COD_S is sludge solubilization (%); $TCOD_0$ and $SCOD_0$ are TCOD and SCOD concentrations before HTP (g/L), respectively; and $SCOD_{HT}$ is the SCOD concentration after HTP (g/L).

In preliminary BMP tests conducted in the present study, the ultimate biodegradability of Phase 1 HTP sludge at 90, 125 and 155 °C was 58.1, 60.8, and 65.5%, whereas that of Phase 2 HTP sludge at 90, 125 and 155 °C was 60.8, 63.9, and 57.7%, respectively. The lower methane production with Phase 2 HTP sludge at 155 °C may be due to formation of recalcitrant and/or inhibitory substances by HTP [5,15].

2.3. Semi-continuous digestion runs

Four semi-continuously fed digesters (R1, R2, R3, and R4) were set up using 2.8 L water-jacketed Spinner cell flasks (Bellco Glass, Inc., Vineland, NJ, USA) with a 1 L liquid, working volume. The digesters were housed in a 22 ± 2 °C room, heated by water circulation resulting in a digester mixed liquor temperature of 35 °C. The digesters' contents were continuously mixed with magnet-bearing, Teflon mixer assemblies (Bellco Glass Inc.) driven by external magnetic stirrers. The digesters were started with predigested anaerobic sludge obtained from the FWHWR Center, as mentioned above. The feed for R1, R2, R3, and R4 was raw sludge mixture and sludge mixture pretreated at 90, 125 and 155 °C, respectively. Operation of the digesters entailed two phases, each phase with a different sludge mixture and operational conditions. The nominal SRT and OLR were 20 d and 3.5 g TCOD/L-d during Phase 1, and 10 d and 7.0 g TCOD/L-d during Phase 2, thus keeping the same sludge feed concentration for both phases. Phase 2 operation was decided upon completion of the Phase 1 digestion run, which achieved a relatively high VS and degradable COD destruction at 20-d SRT. Thus, Phase 2 was initiated to evaluate the combined HTP-AD process at 10-d SRT. As a result, two different raw sludge mixtures were used in this study.

Total biogas volume, composition (CH_4, CO_2) and digester effluent pH were measured at the end of every feeding cycle (2 or 3 d). TS, VS, SCOD, TCOD, VFAs, ammonium, and orthophosphate were measured regularly. At the end of each phase of operation, the digestates were analyzed for pH, TS, VS, TCOD, SCOD, VFAs, ammonium, total nitrogen (TN), soluble TN, total phosphorus (TP), and orthophosphate. Microbial community analysis was carried out for the feed raw sludge mixture and digestates at the end of each phase of operation.

2.4. Analytical methods

pH, COD, TS and VS were determined according to Standard Methods [25]. For SCOD, ammonium, soluble TN and orthophosphate measurements, the sample liquid portion was passed through a 0.45 μm membrane filter. Ammonium was determined by the salicylate method (HACH Method 10031; HACH, Loveland, CO, USA). Free ammonia (FA) as a function of total ammoniacal N, temperature, and pH was calculated as previously described [26]. TN and soluble TN were measured by the persulfate digestion method (HACH Method 10208). Organic N was calculated as the difference between TN and ammonium N (nitrite and nitrate were not detected in any sludge samples). Crude protein was calculated as the organic N multiplied by a conversion factor of 6.25 (equivalent to 0.16 g N/g protein, based on the general protein formula of $C_{16}H_{24}O_5N_4$) [27]. TP and orthophosphate were measured by the molybdovanadate/acid persulfate digestion method (HACH Method 10127).

Total gas produced was measured by displacement of an acidified brine solution (10% NaCl w/v and 2% H_2SO_4 v/v) in graduated columns after equilibration to atmospheric pressure. Biogas composition (CH_4

and CO_2) was measured using a gas chromatography (GC) unit (Model 6890 N; Agilent Technologies, Inc., Palo Alto, CA) equipped with two columns and two thermal conductivity detectors [28]. VFAs were measured using a high-performance liquid chromatography (HPLC) unit equipped with a UV–Vis detector (Agilent 1100 Series, Santa Clara, CA) at the wavelength of 210 nm and 5 mM H_2SO_4 as the eluent [29]. The samples were prepared by centrifugation at 10,000 rpm for 15 min and filtration of the supernatants through 0.2 μm PTFE membrane filters.

Bacterial and archaeal DNA was extracted from raw sludge and digestate samples using DNeasy PowerSoil DNA Isolation Kit (QIAGEN LLC, Germantown, MD) according to the manufacturer's instructions. Detailed information on amplification, sequencing, and bioinformatic analysis is presented in Text S1.

2.5. Energy balance

Energy balance of the AD and the combined HTP-AD process was analyzed according to previously described methodology [30,31]. Two full-scale digesters were considered, each with a liquid volume of 2000 m^3 and a feed flow rate of 100 or 200 m^3/d, corresponding to 20 and 10 d SRT for Phase 1 and Phase 2, respectively. Detailed assumptions, equations, as well as the description and value of each parameter used for the energy balance calculations are summarized in Text S2 and Table S1.

3. Results and discussion

Based on the aforementioned, specific objectives of the study, the obtained results and discussion are presented as follows: first, the effect of HTP on sludge characteristics is evaluated; second, the performance of semi-continuous digesters fed with raw and HTP sludge at 20 and 10 d SRTs is described; third, the results of microbial community composition performed on raw sludge and digestate samples are analyzed to provide additional information on the performance of the semi-continuous digesters; fourth, energy balance calculations based on methane production are presented to determine if HTP is energetically favorable or not; at last, data on N and P distribution and release as affected by HTP and AD are presented to identify the best approach for the effective recovery of N and P from sewage sludge.

3.1. Effect of HTP on sludge characteristics

Fig. 1 shows the effect of HTP at 90, 125 and 155 °C on SCOD for Phase 1 and 2 sludge mixture. The COD-based solubilization of the sludge mixture increased with HTP temperature, which is consistent with previous studies [32–34]. At 155 °C, solubilization reached 37.9 and 27.1% for Phase 1 and 2 sludge mixture, respectively.

Fig. 2A and B show the effect of HTP at 90, 125 and 155 °C on VFAs for Phase 1 and 2 sludge mixture, respectively. Phase 1 raw sludge had a higher VFAs concentration than Phase 2 sludge (6339 vs 5420 mg COD/L) (Table 1). Specifically, Phase 1 raw sludge had higher acetate, propionate, i-butyrate, and i-valerate concentration, but lower n-butyrate and n-valerate concentration compared to Phase 2 raw sludge. For Phase 1 sludge, acetate and propionate were the two major VFAs in all samples, except in the 155 °C pretreated sludge, where n-valerate represented ca. 40% of the total COD_{VFA} (Fig. 2A). The effect of HTP on VFAs formation was more pronounced for Phase 2 sludge mixture. For Phase 2 sludge, the VFAs concentration at 155 °C was significantly higher compared to that of raw sludge and HTP sludge at 90 and 125 °C. Propionate was the dominant VFA in sludge with HTP at 155 °C (Fig. 2B). In addition, for both Phase 1 and 2 sludge, HTP at 155 °C resulted in high n-valerate formation (Fig. 2). In a study by Xue et al. [10], the VFAs in dewatered WAS decreased after HTP at 120 °C for 3 h, and remained the same after HTP at 160 °C for 3 h, compared to raw sludge. Donoso-Bravo

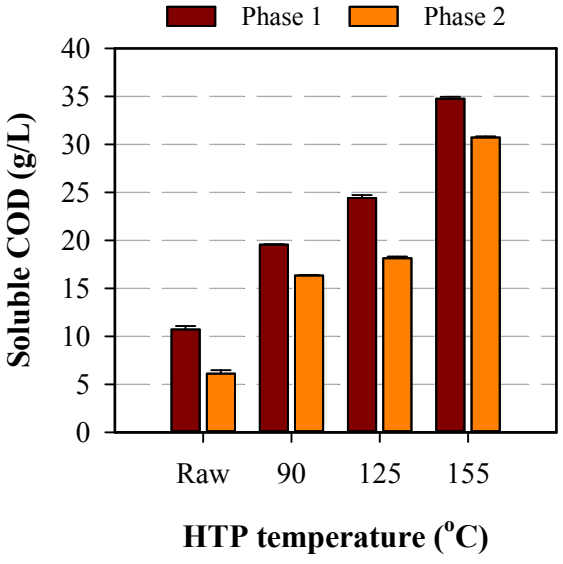

Fig. 1. Soluble COD concentration at different HTP temperatures in Phase 1 and Phase 2 sludge mixture (Error bars are mean ± standard deviation, $n = 3$).

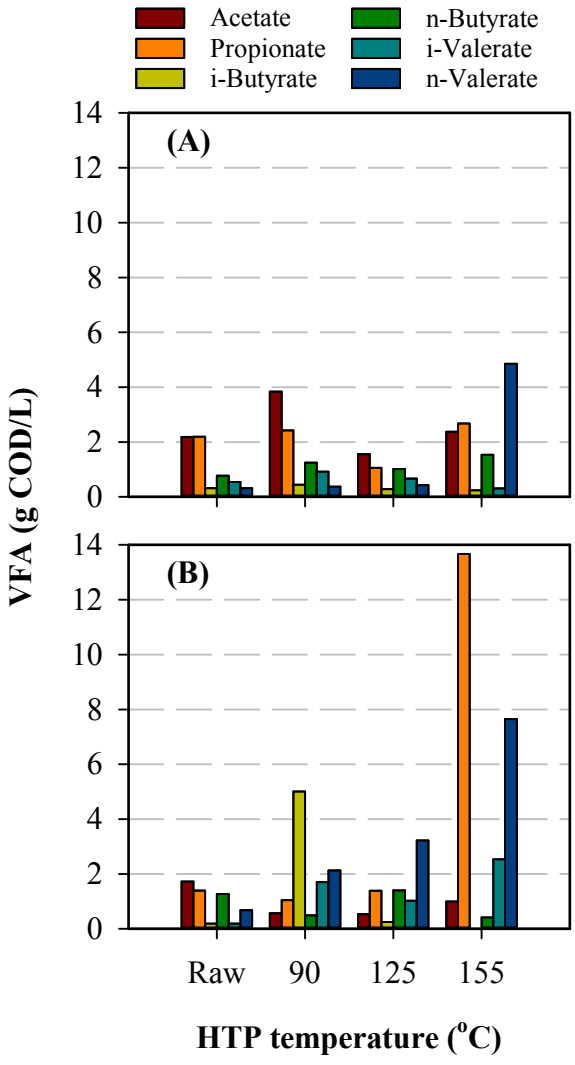

Fig. 2. VFAs concentration at different HTP temperatures in Phase 1 (A) and Phase 2 (B) sludge mixture.

et al. [11] reported that acetate increased with time, while propionate remained constant in WAS after HTP at 170 °C for up to 30 min. In both studies, among all VFAs produced, acetate was the most abundant and propionate was low (<450 mg COD/L) [10,11].

Thermal decomposition of sludge particulate matter and oxidation are two major reactions during HT; the former releases organic matter to liquid phase, while the latter transforms soluble organic matter into oxygenated organic intermediates and inorganic end-products [35]. Oxidation is carried out by free radicals in three steps, i.e., initiation, propagation, and termination. In the initiation step during HT, organic matter degrades to free radicals. In the propagation phase, organic hydroperoxides (ROOH) are formed and further transformed to intermediates with a lower carbon number. Finally, in the termination phase, persistent species with short hydrocarbon chains are formed from free radicals [36]. Specifically, acetate, monosaccharides, and amino acids are three major intermediates in HT organic matter degradation [36]. In general, VFAs are the main by-products in the HT process [37]. The type and yield of VFAs by the HT process depends on the feedstock characteristics, reaction time, amount of oxidant available, and process temperature [38]. The exact reaction pathways and kinetics, even for pure compounds, have not been fully understood due to the formation of a large number of intermediates through parallel and consecutive reactions [36]. Among the VFAs, acetate is the most stable with the lowest degree of oxidation compared to other VFAs, which becomes the main product, while propionate has the second lowest degree of oxidation [39]. Sewage sludge is very complex, mainly consisting of protein, carbohydrates, and lipids. Among these components, VFAs are produced by lipid degradation [40]. The type of the produced VFAs depends highly on the type of lipids and their long chain fatty acids. With HTP of glyceryl tristearate at 170 °C for 2 h, the predominant VFAs were n-valerate, caproate and heptanoate (89% of C2-C7 fatty acids), whereas those from glyceryl trilinolenate consisted of acetate and propionate (87% of C2-C7 fatty acids) [24]. Thus, in the present study, formation of high propionate levels with Phase 2 sludge HTP at 155 °C for 4 h may be attributed to the sludge lipid composition, as well as HTP temperature and duration.

3.2. AD performance

3.2.1. Phase 1 digestion run

During Phase 1, the SRT was 20 d and the nominal OLR was 3.5 g TCOD/L-d; however, the actual, mean OLR was 3.47 g TCOD/L-d. Phase 1 operation lasted for 69 d, corresponding to ca. 3.5 retention times (Fig. S1). Data related to the performance of the four digesters after three SRT values during Phase 1 are presented in Table 2. HTP led to increased VS and TCOD destruction, resulting to increased methane production. The VS and TCOD destruction followed the series R4 ≈ R3 > R2 > R1. Compared to R1, the VS and TCOD destruction in R4 was higher by 9.2%. An excellent agreement between TCOD destruction and COD-to-CH_4 conversion was achieved. As a result, the COD balance was ≤ ±2.4% (Table 2). The biogas methane content ranged from 69.4 to 72.6%. The specific methane yield increased with HTP temperature from 302 to 391 mL/g VS_{added} at STP.

The digesters' ammonium concentration ranged from 1257 to 1733 mg N/L (Table 3). The FA concentration ranged from 79 to 108 mg N/L. Both ammonium and FA are known inhibitors of the AD process, especially methanogenesis. However, the reported inhibitory thresholds of ammonium and FA fall in very wide ranges, from 1100 to 11,800 mg N/L, and 27 to 1450 mg N/L, respectively [41]. Thus, it is unclear if the digesters in the present study were inhibited by ammonium or FA by directly comparing their concentrations to literature thresholds. In the present study, as relatively low levels of VFAs were observed, and the

Table 3
Energy balance (GJ/d) for Phase 1 and Phase 2 AD and HTP-AD processes.

Energy component	Control	Without HTP heat recovery (HTP at °C)			With 85% HTP heat recovery (HTP at °C)		
		90	125	155	90	125	155
Phase 1 (20 d SRT)							
Input heat ($E_{i,\ heat}$)	6.0	28.9	43.6	56.1	28.9	43.6	56.1
Heat recovered ($E_{i,\ heat\ recovered}$)	NA[a]	0	0	0	19.5	32.0	42.6
Input electricity ($E_{i,\ electricity}$)	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Energy input (E_{input})	6.8	29.7	44.4	56.9	10.2	12.4	14.3
Energy output (E_{output})	37.0	39.7	42.1	43.8	39.7	42.1	43.8
Net energy (ΔE)	30.2	10.0	−2.3	−13.1	29.5	29.7	29.5
Phase 2 (10 d SRT)							
Input heat ($E_{i,\ heat}$)	11.4	57.4	86.6	111.7	57.4	86.6	111.7
Heat recovered ($E_{i,\ heat\ recovered}$)	NA	0	0	0	39.1	64.0	85.3
Input electricity ($E_{i,\ electricity}$)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Energy input (E_{input})	12.4	58.3	87.6	112.7	19.2	23.6	27.4
Energy output (E_{output})	73.3	74.5	80.7	66.4	74.5	80.7	66.4
Net energy (ΔE)	60.9	16.2	−6.8	−46.3	55.2	57.1	39.0

[a] NA, not applicable for control digester without HTP.

Table 2
Performance summary of the four digesters at the end of Phase 1 and Phase 2 operation.

Parameter	Phase 1				Phase 2			
	R1	R2	R3	R4	R1	R2	R3	R4
OLR (g COD/L-d)	3.46	3.48	3.48	3.46	7.68	7.05	7.05	7.22
pH	7.67 ± 0.02[a]	7.71 ± 0.05	7.70 ± 0.04	7.65 ± 0.05	7.32 ± 0.06	7.33 ± 0.03	7.34 ± 0.06	6.98 ± 0.01
SCOD (mg/L)	1036 ± 26	1762 ± 134	2455 ± 134	3728 ± 147	753 ± 66	954 ± 89	1875 ± 211	11073 ± 597
VFAs (mg COD/L)	11–66	52–86	41–92	78–89	16–45	23–43	25–60	3444–4170
Ammonium (mg N/L)	1257 ± 70	1565 ± 92	1562 ± 78	1733 ± 41	864 ± 46	917 ± 33	866 ± 38	1143 ± 68
Free Ammonia (mg N/L)	79 ± 4	108 ± 6	105 ± 5	105 ± 3	25 ± 1	27 ± 1	26 ± 1	15 ± 1
TS destruction (%)	28.1 ± 0.9	33.1 ± 1.1	35.5 ± 1.9	34.3 ± 1.1	33.3 ± 1.0	36.3 ± 0.7	34.1 ± 1.0	38.7 ± 0.5
VS destruction (%)	39.2 ± 0.8	41.8 ± 2.4	49.0 ± 1.1	48.4 ± 1.3	41.7 ± 1.1	45.0 ± 0.6	43.2 ± 1.0	48.4 ± 1.0
Degradable COD to CH_4 conversion (%)	80.1	86.7	87.7	84.5	81.7	77.6	80.0	69.2
Biogas production (mL/L-d at STP)	780 ± 28	857 ± 9	915 ± 14	965 ± 14	1773 ± 11	1735 ± 21	1829 ± 10	1630 ± 20
CH_4 production (mL/L-d at STP)	567 ± 10	613 ± 15	650 ± 19	671 ± 14	1249 ± 19	1164 ± 5	1262 ± 17	1061 ± 5
CH_4 (%)	71.9 ± 1.5	72.6 ± 1.4	72.3 ± 2.1	69.4 ± 0.9	70.4 ± 0.8	67.6 ± 1.1	69.0 ± 0.5	64.8 ± 0.8
CH_4 (mL/g VS_{added} at STP)	302 ± 5	353 ± 9	360 ± 11	391 ± 8	303 ± 5	308 ± 1	328 ± 4	284 ± 1
CH_4 (mL/g $COD_{destroyed}$ at STP)	369 ± 6	357 ± 8	346 ± 10	362 ± 7	314 ± 5	328 ± 2	386 ± 5	330 ± 2
TCOD to CH_4 conversion (%)	46.7 ± 0.8	50.4 ± 1.2	53.3 ± 1.6	55.4 ± 1.2	46.5 ± 0.7	47.2 ± 0.2	51.1 ± 0.7	39.9 ± 0.2
COD balance (%)	−2.4	−1.3	0.6	−1.9	3.4	1.1	−6.7	0.6

[a] Mean ± standard deviation ($n = 3$).

biogas CH_4 plus CO_2 content accounted for 97.2–100.3%, it is unlikely that the digesters in Phase 1 were under ammonium or FA inhibition.

The SCOD in all four digesters followed the series R4 > R3 > R2 > R1; the SCOD concentration in R4 was 3.6-fold higher than that in R1 (Table 2). The VFAs concentration was less than 92 mg COD/L in all four reactors. For R1-R3, acetate was the dominant VFA (Fig. S2). For R4, acetate was still the most abundant (35–73%), but propionate (0–30%) and n-valerate (16–41%) represented a relatively high fraction of COD_{VFA} during the entire Phase 1 operation.

3.2.2. Phase 2 digestion run

During Phase 2, the SRT was 10 d and the nominal OLR was 7 g COD/L-d; however, the actual mean OLR was 7.25 g TCOD/L-d. Phase 2 operation lasted for 35 d, corresponding to 3.5 retention times (Fig. S1). Data related to the performance of the four digesters after reaching three SRT periods during Phase 2 are presented in Table 2. HTP at 90 and 125 °C led to an increased methane production, whereas HTP at 155 °C negatively affected TCOD destruction and methane production. Compared to Phase 1 operation, the effect of HTP on AD performance during Phase 2 was less pronounced, except the observed lower methane production by R4. Compared to R1, the COD-to-CH_4 conversion in R3 exceeded by only 3.7%. The biogas CH_4 and CO_2 content ranged from 64.8 to 70.4% and from 30.7 to 34.0%, respectively. On the other hand, VS destruction followed the series R4 > R2 > R3 > R1. Thus, a significant portion of sludge particulate organic matter in R4 was hydrolyzed and acidified, but not converted to methane. The pH of the R1-R3 was between 7.2 and 7.5, whereas the pH of R4 was lower, between 6.95 and 7.0, due to higher VFA levels, in particular propionate as discussed below. The specific methane yield ranged from 284 to 328 mL/g VS_{added} at STP. Good COD balance of less than 3.4% was achieved in R1, R2 and R4, while it was higher in R3 (−6.7%) (Table 2).

The digesters' ammonium concentration ranged from 864 to 1143 mg N/L. The FA concentration ranged from 15 to 27 mg N/L, much lower than that in Phase 1 operation; FA followed the series R2 > R3 > R1 > R4. Therefore, there should be no ammonium or FA inhibition. Similar to Phase 1, the digesters SCOD increased with HTP temperature. The SCOD concentration in R4 was 14.7-fold higher than that in R1. The VFA concentration in R1-R3 was less than ca. 60 mg COD/L, but R4 had a much higher VFAs concentration, ranging from 3444 to 4170 mg COD/L. For R1-R3, acetate was the dominant VFA (Fig. S3). Propionate was the dominant VFA in R4, ranging from 77 to 83% of the total COD_{VFA} after day 20 in Phase 2. The high propionate concentration in R4 (2768–3379 mg COD/L) may be due to the high propionate levels in the feed (155 °C HTP sludge) (Fig. 2), as well as the low propionate degradation rate, which is further confirmed below based on microbial community analysis. In the present study, the R4 feed propionate concentration was ca. 9000 mg COD/L, after dilution to adjust to the target OLR. Methanogens can directly use formate, acetate, H_2/CO_2, and other methyl compounds [42]. Other VFAs formed via acidogenesis, such as propionate, n-butyrate, i-butyrate, n-valerate, and i-valerate, have to be further transformed by fermentative bacteria in a process termed syntrophic acetogenesis [42]. Propionate and butyrate are two major intermediates in this process and as precursors can account for a significant portion of the total methane produced [43]. However, fatty acid degradation is highly endergonic under standard conditions ($\Delta G^{o'}$, kJ values: propionate, +72 to +76 kJ; butyrate, +45.5 to +48.1 kJ) (Equations 2–5) [44].

$$CH_3CH_2COO^- + 3H_2O = HCO_3^- + CH_3COO^- + H^+ + 3H_2 \quad (2)$$

$$CH_3CH_2COO^- + 2HCO_3^- = 3HCOO^- + CH_3COO^- + H^+ \quad (3)$$

$$CH_3CH_2CH_2COO^- + 2H_2O = 2CH_3COO^- + H^+ + 2H_2 \quad (4)$$

$$CH_3CH_2CH_2COO^- + 2HCO_3^- = 2HCOO^- + 2CH_3COO^- + H^+ \quad (5)$$

Therefore, under well maintained methanogenic conditions, cooperation between syntrophic fatty acid-degrading bacteria and methanogenic archaea is necessary to keep the end products of VFA degradation (especially H_2 and formate) at low concentrations for the reactions to proceed, i.e., $\Delta G < 0$. There is a narrow window of H_2 concentration (10^{-4} to 10^{-6} atm) that must be maintained for the efficient propionate degradation [45]. In the present study, both formate and H_2 were not detected, and biogas CH_4 plus CO_2 content accounted for 98.7 ± 2.4%. In addition, ca. 6000 mg COD/L of feed propionate was removed in R4, which represents ca. 2/3 of the feed propionate concentration. Thus, there was not a thermodynamic limitation for propionate degradation which led to high propionate concentration in R4. Taken all together, the observed high propionate concentration in R4 was the result of the extremely high propionate concentration in the HTP 155 °C feed sludge, combined with slow propionate degradation rate, perhaps associated with a very low abundance of known propionate degraders as discussed in Section 3.3, below.

3.3. Bacterial and archaeal communities

The archaeal and bacterial DNA of ten sludge samples, that is, Phase 1 and 2 raw sludge, and digestate samples from R1-R4 for both phases, were extracted and analyzed. The number of reads for Bacteria and Archaea ranged between 65,464–118,835 and 66,865–97,208, respectively (Table S2). The operational taxonomic unit (OTU) numbers, alpha diversity including Chao, ACE, Shannon, and Simpson indices are summarized in Table S2. The coverage of Archaea and Bacteria ranged between 0.99 and 1.00. In line with the results of Liu et al. [46], compared to Bacteria, the archaeal community in each sample was less phylogenetically diverse, represented by at most 79 OTUs.

3.3.1. Bacterial communities

The relative abundance of bacteria at phylum and genus level are shown in Fig. 3A and B, respectively (S1 and S6 are raw sludge samples for Phases 1 and 2, respectively; S2-S5 are digestate samples from R1-R4 at the end of Phase 1 operation; S7-S10 are digestate samples from R1-R4 at the end of Phase 2 operation). The relative abundance of Bacteria at order level is shown in Fig. S4 and discussed in Text S3.

At phylum level (Fig. 3A), *Proteobacteria*, *Bacteroidetes*, and *Firmucutes* were the most abundant in the two raw sludge samples. Compared to Phase 2, the raw sludge in Phase 1 had a higher abundance of *Proteobacteria*, and a lower abundance of *Bacteroidetes* and *Firmucutes*. *Proteobacteria* play an important role in the degradation of carbohydrates and VFAs, such as propionate, butyrate, and acetate [47]. *Bacteroidetes* contains proteolytic bacteria involved in the degradation of protein and conversion to VFAs and ammonia [48]. Most proteolytic microorganisms can metabolize amino acids to produce VFAs such as acetate, propionate, succinate, and ammonia [49]. *Firmicutes* play an important role in the hydrolysis and hydrogenogenic acidogenesis during anaerobic digestion [50]. In the present study, the relative abundance of *Firmucutes* in the digestate samples increased with HTP temperature, whereas that of *Proteobacteria* decreased. Compared to the raw sludge, the relative abundance of *Bacteroidetes* increased significantly after AD. *Firmucutes* and *Bacteroidetes* dominated the digestate samples, which is consistent with the findings by Zhou et al. [51]. The phylum *Chloroflexi* contains carbohydrate degraders, found in various anaerobic digesters [47,48]; they can degrade hardly degradable organic substances. In the present work, the relative abundance of *Chloroflexi* was higher in R1 for both phases (10.6–12.7%) compared to R2-R4 (<1.8–8.2%), as well as raw sludge (0.8–3.2%). In a study by Yi et al. [48], the fraction of *Chloroflexi* was the highest in anaerobic digesters fed with food waste, and decreased as the feed TS concentration increased from 5 to 20%.

At the genus level (Fig. 3B), the major genera detected in the digestate samples were unclassified *Bacteroidetes* (*vadinHA17*) (0.1–16.6%), unclassified *Anaerolineaceae* (1.0–10.2%), *SP3-e08* (0.7–15.0%), *Sedimentibacter* (1.9–10.1%), *Proteocatella* (0.0–9.9%),

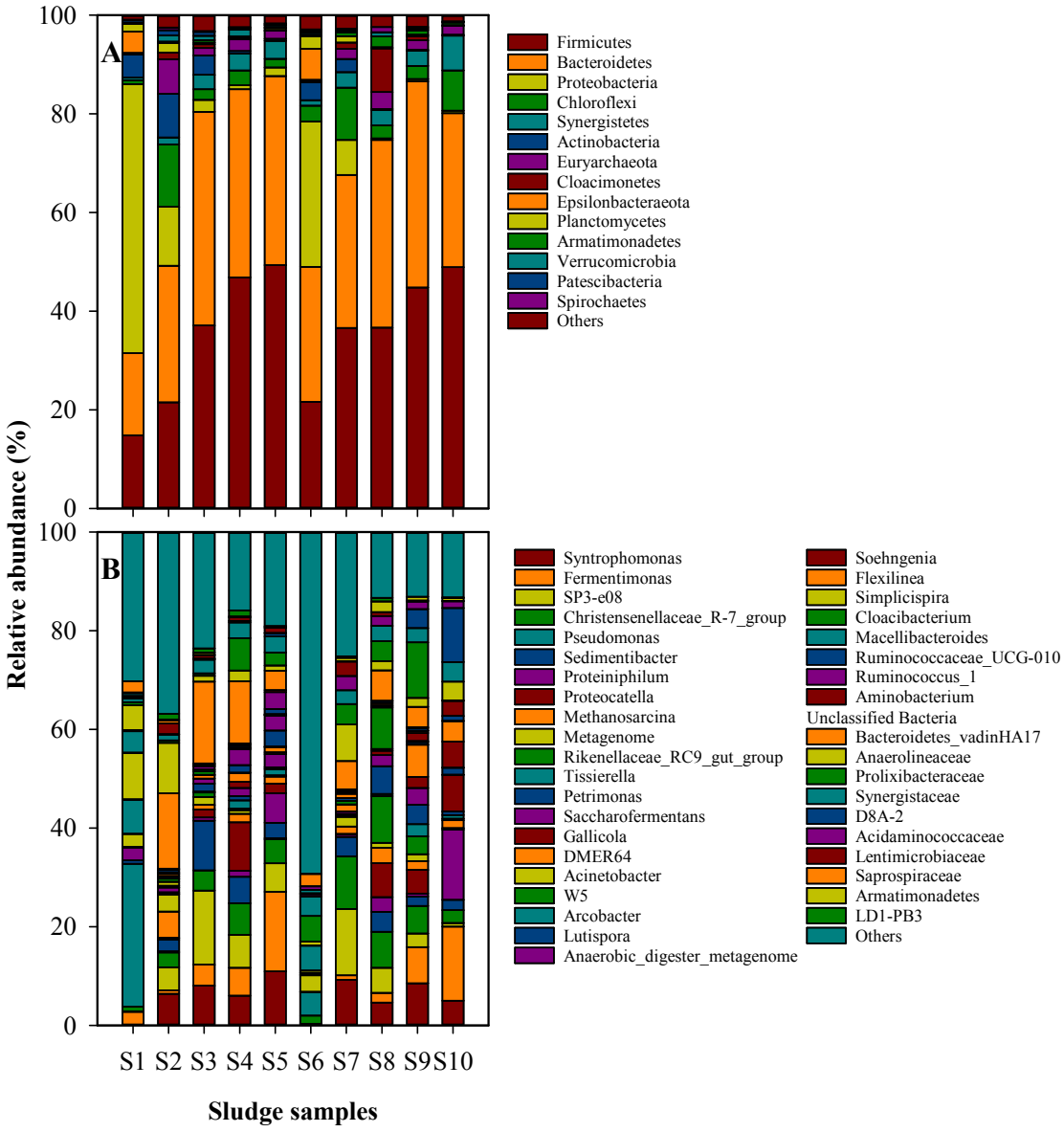

Fig. 3. Relative abundance of Bacteria at phylum (A), and genus (B) level.

Fermentimonas (0.9–16.1%), *Syntrophomonas* (4.6–10.9%), *Christensenellaceae R-7 group* (2.6–10.6%), unclassified *Prolixibacteraceae* (0.0–11.3%), *Proteiniphilum* (0.3–14.3%), and unclassified *D8A-2* (0.0–10.9%). *Pseudomonas* (29.0 and 4.7%) and *Arcobacter* (4.3 and 5.0%) were detected at relatively high levels in Phase 1 and 2 raw sludge samples. Besides, the relative abundance of *Acinetobacter* was 9.4% in Phase 1 raw sludge. However, the relative abundance of *Pseudomonas*, *Acinetobacter*, and *Arcobacter* was very low (<0.8%) in all digestate samples. The genus *Arcobacter* consists of five species, three of which are pathogenic [52]. Several *Pseudomonas* species are pathogenic [53–55]. One species of *Acinetobacter* has been implicated in a number of hospital-acquired infections, such as bacteremia, urinary tract infection, secondary meningitis, infective endocarditis, and wound and burn infections [56]. Thus, AD and HTP-AD significantly decreased the abundance of pathogens in the digestates. It is noteworthy that *Pseudomonas*, *Acinetobacter*, and *Arcobacter* all belong to the phylum *Proteobacteria*. Thus, the decrease in the relative abundance of *Pseudomonas*, *Acinetobacter*, and *Arcobacter* explains well the significant decrease of *Proteobacteria* after AD (Fig. 3A).

To date, three genera have been reported to degrade propionate: *Syntrophobacter*, *Smithella*, and *Pelotomaculum,* while *Syntrophomonas* has been reported to degrade butyrate [57]. In the present study, among the three known propionate-degrading genera, only *Pelotomaculum* was detected at a very low abundance in the digestate samples (0.0–1.3%). The highest relative abundance of *Pelotomaculum* was in Phase 1 R4 (1.3%), followed by Phase 2 R3 (0.4%), Phase 1 R1 (0.3%), Phase 2 R2 (0.2%), Phase 1 R3 and Phase 2 R1 (0.1%); it was not detected in Phase 1 and 2 raw sludge, Phase 1 R2 and Phase 2 R4 digestate samples. Specifically, although the Phase 2 feed sludge had a high propionate concentration and R4 had a persistent high propionate concentration, the known propionate degraders were not detected in Phase 2 R4. However, it is noteworthy that *Proteiniphilum* existed at a considerable abundance in Phase 2 R4 (14.3%). This species does not degrade propionate, but accelerates the propionate degradation rate as it utilizes pyruvate, an intermediate in the propionate degradation pathway [58]. Propionate degradation in the present study may have occurred in two steps: first, propionate was converted to pyruvate by unknown bacteria, which could be unclassified *Proteiniphilum* spp., or others; then, pyruvate was transformed by *Proteiniphilum* to acetate, etc. Indeed, several bacteria and fungi are able to oxidize propionate via methylcitrate to pyruvate, as

was observed with *Escherichia coli* [59]. In the present study, *Escherichia coli* was not detected. Similar results were reported by Zhang et al. [60]. In their study, ca. 2,000 mg/L propionate was degraded in BMP tests of swine manure at 37 °C without detection of the above-mentioned three genera of known propionate degraders. In addition, they found *Proteiniphilum* at a considerable abundance (2~5%); they assumed that some unknown species existed which carried out syntrophic propionate degradation by an unknown pathway [60]. Chen et al. [61] found *Proteiniphilum* at high abundance (5.4%) in a mesophilic digester fed with sewage sludge with HTP at 160 °C for 30 min. *Proteiniphilum* was thought to be responsible for enhanced degradation of amino acids [61]. In the present study, *Syntrophomonas* was detected in all four digesters in both AD phases (4.6–10.9%), in which very low levels of butyrate were observed (0–6 mg COD/L).

3.3.2. Archaeal communities

The relative abundance of Archaea at order and genus levels is depicted in Fig. 4A and B, respectively. In raw feed sludge samples (S1 and S6 for Phase 1 and 2, respectively), the four major orders of Archaea were *Methanosarcinales*, *Methanomassiliicoccales*, *Methanobacteriales*, and *Methanomicrobiales*. In R1-R4 digestate samples of both AD phases (samples S2-S5, and S7-S10), the predominant order was *Methanosarcinales*.

Several genera of methanogens existed in the two raw sludge samples, while the predominant genera in the digestate samples were *Methanosarcina* (66–88%) and *Methanosaeta* (7–30%). *Methanosaeta* is a

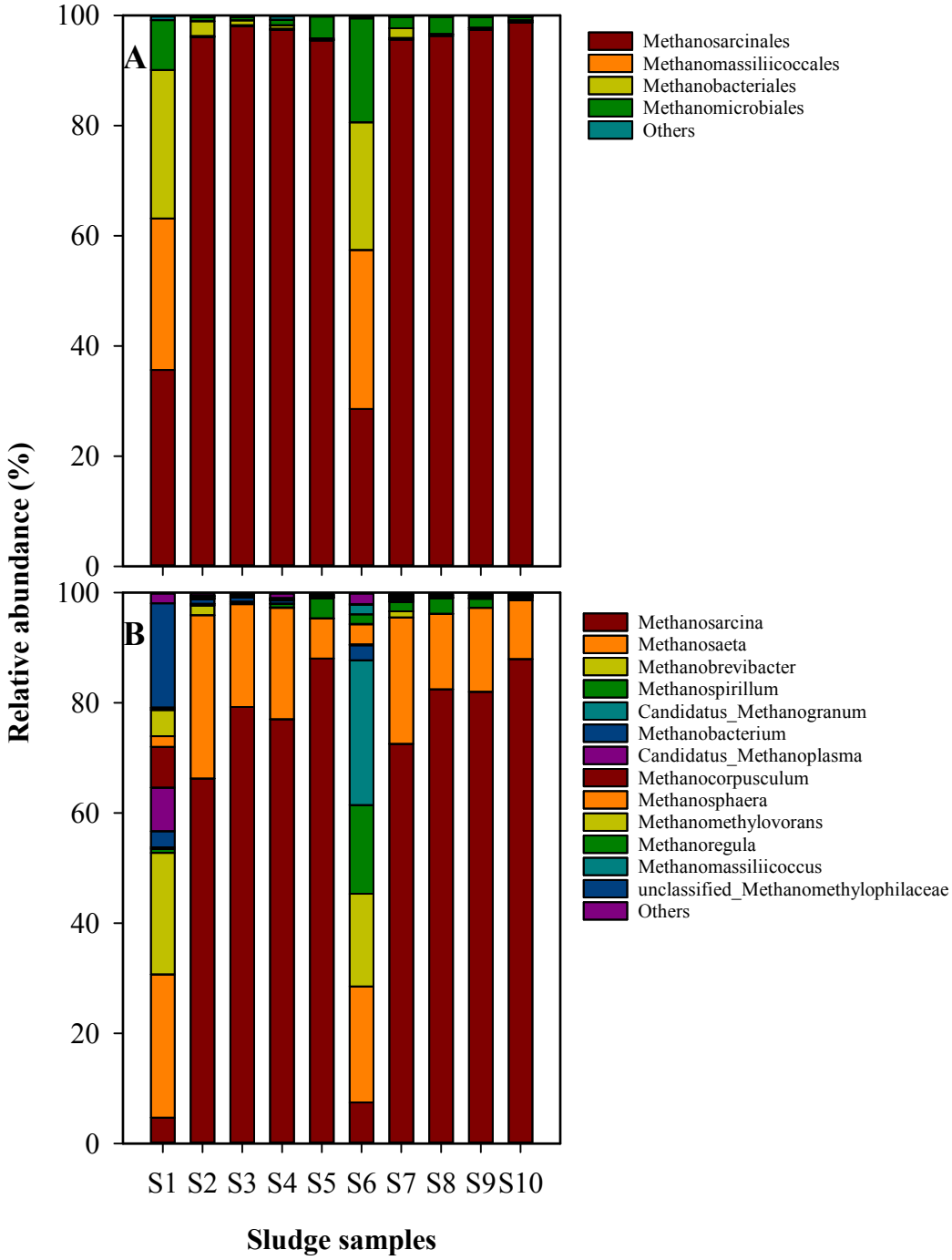

Fig. 4. Relative abundance of Archaea at order (A) and genus (B) level.

strictly acetoclastic methanogen, while *Methanosarcina* can use acetate, H_2, CO_2, and methylated one-carbon compounds producing CH_4 by means of three metabolic pathways [62,63]. The relative abundance of *Methanosarcina* in the digestate samples increased with HTP temperature while that of *Methanosaeta* decreased, which may be attributed to the type of substrate availability. *Methanosarcina* and *Methanosaeta* compete for acetate and free ammonia [64]. Compared to *Methanosarcina*, *Methanosaeta* has a higher affinity to acetate but a slower growth rate [65]. The Monod half saturation coefficient of acetate (K_s) and maximum specific growth rate (μ_{max}) of *Methanosarcina* and *Methanosaeta* are 300 and 30 mg COD/L, and 0.71 and 0.12 d^{-1}, respectively [66]. Based on kinetics, *Methanosarcina* has an advantage over *Methanosaeta* at acetate concentrations ca. above 25 mg COD/L [66]. In the present work, acetate levels in the digesters during the 3-d or 2-d feeding cycles based on feed and effluent VFAs ranged from 31 to 565 mg COD/L for Phase 1, and from 17 to 277 mg COD/L for Phase 2. Therefore, the relatively high acetate levels contributed to higher relative abundance of *Methanosarcina* than *Methanosaeta*. Chen et al. [67] reported that HTP at 160 °C led to a shift from strict acetoclastic methanogenesis to acetoclastic/hydrogenotrophic methanogenesis, which favored *Methanosarcina* than *Methanosaeta* as the former uses a wider range of substrates, while the latter is a strict acetoclastic methanogen. *Methanobrevibacter*, which uses H_2, CO_2, and formate, existed at a relatively high abundance in the two raw sludge samples (17–22%), but its abundance was very low in the digestate samples (0.0–1.7%). *Methanospirillum*, which uses H_2, CO_2, and formate, was detected in low abundance in the digestate samples (0.27–3.64%).

3.3.3. Principal component analysis (PCA)

PCA was conducted for both bacterial and archaeal communities at order level to compare the similarity and difference in terms of microbial communities between the raw and digestate samples with results shown in Figs. S5 and S6, respectively. The results of PCA confirm that the bacterial communities in the two raw sludge mixtures, as well as Phase 1 and 2 R1 digestate samples were significantly different from each other, and also differed from the communities in all digestate samples of digesters fed with hydrothermally treated sludge. The bacterial communities of Phase 1 and 2 R2-R4 digestate samples were similar (Fig. S5).

The results of PCA confirm that the archaeal communities in the two raw sludge mixtures were significantly different, and also differed from the communities in all digestate samples of digesters fed with hydrothermally treated sludge (Fig. S6). The archaeal communities of the digestate samples were very close, consistent with relative abundance results of Archaea, which showed that *Methanosarcina* and *Methanosaeta* were the two predominant genera of methanogens in all the digestate samples. In summary, PCA results suggest that HTP significantly changed the bacterial communities in the digestates, but had a minor effect on the archaeal communities. A similar observation was reported by Chen et al. [67].

3.4. Energy balance

Energy balance of the AD and HTP-AD processes was calculated based on the experimental results (Table 2) with and without HTP heat recovery, following previously described methodologies [30,31], summarized in Text S2. For the latter case, 85% heat recovery was used in the energy balance calculations as initially suggested by Lu et al. [31], and subsequently used in other studies [30,68]. Anastasakis et al. [69] reported that the heat recovery by a heat exchanger used for sewage sludge ranged from 72.8 to 78.5% with a mean value of 75.3%. Energy balance results are shown in Table 3. For both AD phases, without HTP heat recovery, the net energy production (ΔE, GJ/d) decreased from positive to negative as the HTP temperature increased. For Phase 1, with 85% HTP heat recovery, the ΔE of the HTP-AD processes ranged from 29.5 to 29.7 GJ/d, slightly lower than that of AD without HTP (30.2 GJ/d). For Phase 2, even with 85% HTP heat recovery, all HTP-AD processes had a ΔE significantly lower than that of AD without HTP (60.9 GJ/d). The ΔE for R4 with HTP at 155 °C was much lower than that for the other reactors due to the high HTP energy input and the lower energy output (i.e., methane production). Based on data from the present study, in order to achieve the same net energy as the control, the HTP heat recovery efficiency at HTP of 90, 125, and 155 °C needs to reach 88, 86, and 86% for Phase 1, and 97, 90, and 107% for Phase 2, respectively. The required HTP heat recovery efficiency at HTP temperature of 155 °C for Phase 2 is higher than 100%, indicating that even with full HTP heat recovery the net energy production of HTP-AD at 155 °C would be significantly lower than that of the control. In addition, for all other HTP-AD processes, the required HTP heat recovery efficiency (86–97%) is higher than a reported range of 72.8 to 78.5% by Anastasakis et al. [69] for sewage sludge and thus will not be possible to achieve. It should be noted that in terms of net energy production, the ineffectiveness of HTP observed in the present study is very much related to the fact that both sludge mixtures had a relatively high ultimate biodegradability (58.3 for Phase 1 and 56.9% for Phase 2). However, HTP did contribute to higher solids reduction and N release/availability as discussed in Section 3.5, below.

The net energy production is highly affected by the methane production rate. The minimum methane production rate to achieve zero energy balance, i.e., $E_{input} = E_{output}$, with 85% or without HTP heat recovery are presented in Table 4. If the actual methane production is higher than the minimum methane production, positive net energy production could be obtained. Therefore, positive net energy could always be achieved with 85% HTP heat recovery in the present study. As the actual methane production without HTP heat recovery was lower than the minimum methane production required to achieve zero energy balance, the net energy balance for R3 and R4 was negative for both AD phases (Table 3). In the case of microalgal biomass HTP-AD, in order to achieve zero energy balance, the methane production rate was estimated to be at least 0.16 L CH_4/L-d with HTP at 75 °C and at least 0.18 L CH_4/L-d with HTP at 95 °C, 20-d SRT for both cases [70].

As the methane production rate is affected by the feed VS and COD content, and its ultimate biodegradability, the net energy production is highly related to feed degradable VS and COD concentration [30]. The minimum feed VS concentration to obtain a positive energy balance was calculated as 4.4% (w/v) for microalgal biomass treatment at 130 °C for 15 min, followed by AD at 20 d SRT [30]. Bjerg-Nielsen et al. [71] stated that for pre-digested sludge, the minimum feed VS concentration to obtain a positive energy balance was 5.5 and 7.3% (w/v) for HTP at 120 °C and 170 °C, respectively. Yuan et al. [68] estimated that the minimum feed VS concentration of concentrated PS that could result in a positive ΔE was $\geq$1.7 and 2.7% (w/v) for HTP at 130 °C with pre-treatment and post-treatment, respectively. It is noteworthy that the above-mentioned studies considered 85% HTP heat recovery. The minimum feed VS concentration to achieve a positive energy balance for the experimental setup used in the present study, with 85% or without HTP heat recovery is presented in Table 4. The actual feed VS concentration was higher than the minimum VS concentration for all digesters in Phase 1 and 2 with 85% HTP heat recovery. Therefore, positive energy balance could always be achieved with 85% HTP heat recovery in the present study.

Theoretically, increasing the feed VS or TCOD concentration by thickening and for a fixed SRT, or shortening the SRT with the same feed concentration, or increasing the feed concentration by the addition of high-strength waste, as practiced in the case of co-digestion, are three ways to increase the methane production rate and thus obtain a more positive energy balance. Realization of the positive effect of concentrated feed to anaerobic digesters has been the impetus behind efforts to achieve more concentrated digesters' feedstock [72]. Indeed, during the last decade, high-solids mesophilic AD with dewatered WAS has been put forward, with SRT as low as 9–12 d and OLR as high as 8.5 g VS/L-d, resulting in methane production rate as high as 1.63 L CH_4/L-d [73]. Dewatered WAS co-digested with food waste at an OLR of 18.5 g VS/L-

Table 4
Minimum methane production rates and feed VS concentrations to achieve positive energy balance in Phase 1 and Phase 2 digesters.[a]

Phase	Digester	Minimum methane production rate (L CH_4/L-d)		Actual methane production rate (L CH_4/L-d)[b]	Minimum feed VS concentration (%, w/v)		Actual feed VS concentration (%, w/v)
		Without HTP heat recovery	With 85% heat recovery		Without HTP heat recovery	With 85% heat recovery	
1	R1	0.11	NA[c]	0.57	0.73	NA	3.78
	R2	0.46	0.16	0.62	2.60	0.90	3.50
	R3	0.69	0.20	0.65	3.84	1.11	3.62
	R4	0.88	0.23	0.68	4.48	1.17	3.46
2	R1	0.19	NA	1.14	0.63	NA	3.76
	R2	0.90	0.30	1.16	2.91	0.97	3.75
	R3	1.36	0.37	1.25	4.15	1.13	3.81
	R4	1.75	0.43	1.03	6.17	1.52	3.63

[a] Actual methane production rates and feed VS concentrations obtained in the present study;
[b] Methane production rates (at STP) adjusted to nominal OLR of 3.5 g COD/L-d for Phase 1 and 7.0 g COD/L-d for Phase 2;
[c] NA, not applicable (digesters fed with raw sludge mixture).

d at 8 d SRT resulted in a methane production rate of 5.62 L CH_4/L-d [74]. In another study by Yi et al. [48], the high-solids AD system fed with food waste at an OLR of 9.4 g VS/L-d at 20 d SRT resulted in a methane production rate of 4.52 L CH_4/L-d.

As mentioned above, the effect of HTP on energy balance of the HTP-AD process depends on the sludge feed concentration and its degradability. The key question is if the increased methane production due to HTP can compensate for the heat energy input for HTP. For the experimental setup used in this study, HTP had a negative effect on energy balance; very high HTP heat recovery (86–97%) would be required for the HTP-AD processes to obtain a net energy balance comparable to that of the control (i.e., AD without HTP). In order to make the HTP-AD process more net energy positive, it is recommended that the sewage sludge be concentrated to a higher degree than is presently practiced at the study WRRF.

3.5. Effect of HTP and AD on N species release and distribution

The effect of HTP at 90, 125 and 155 °C on the release and distribution of N species in Phase 1 and 2 feed and digestate is depicted in Fig. 5. Compared to Phase 1 sludge, Phase 2 sludge had a lower ammonium and soluble organic N concentration. Particulate N was the dominant N fraction in both Phase 1 and 2 raw sludge mixture. Overall, HTP increased the ammonium and soluble organic N concentrations, consistent with literature reports in which ammonium concentration increased with HTP temperature [10,24]. The ammonium N/soluble TN ratio decreased as HTP temperature increased, suggesting that protein was mostly solubilized instead of being degraded, consistent with previous studies [10,11].

During AD, organic N is converted to ammonium N. In the present study, the net ammonium N production after subtracting the feed ammonia concentration was 615, 757, 690, and 688 mg N/L for Phase 1 R1-R4, and 636, 635, 605, and 685 mg N/L for Phase 2 R1-R4, respectively. The effect of HTP at 90, 125 and 155 °C on crude protein

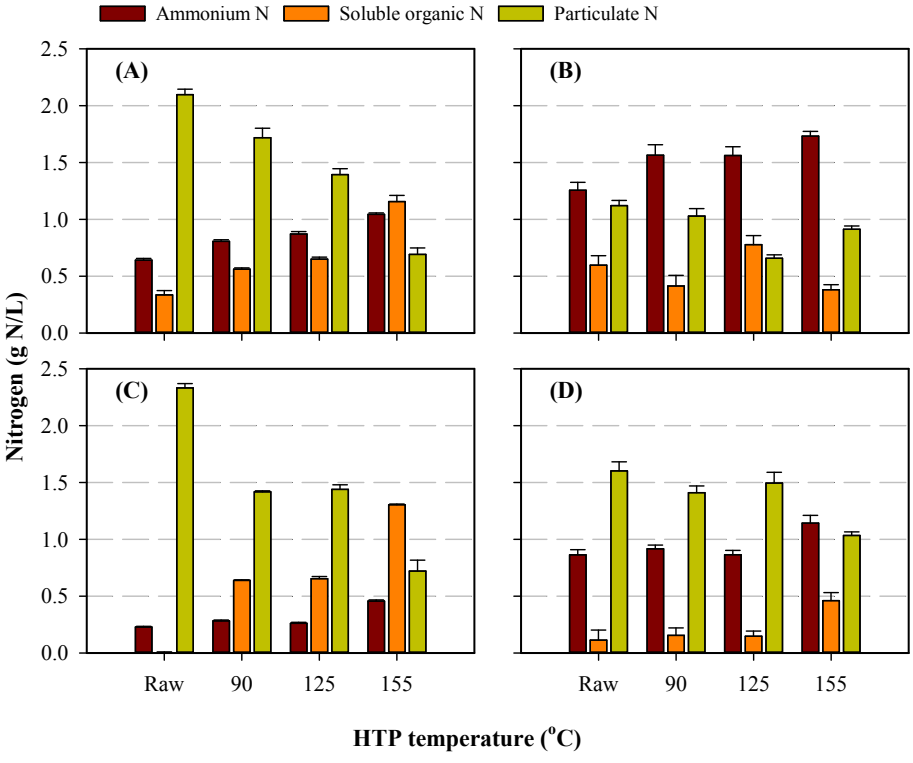

Fig. 5. Nitrogen species in Phase 1 feed (A) and digestate (B), and Phase 2 feed (C) and digestate (D) (Error bars are mean ± standard deviation, n ≥ 3).

concentration and distribution in Phase 1 and 2 feed and digestate is depicted in Fig. 6. HTP breaks microbial cell walls and leads to protein solubilization and destruction [10,11]. Crude protein solubilization had a similar trend to that of COD solubilization in both Phase 1 and 2 sludge. Crude protein destruction due to HTP was more pronounced for Phase 1 sludge, compared to Phase 2 sludge. For Phase 1 sludge, crude protein destruction due to HTP increased with HTP temperature and reached 24% at 155 °C. For Phase 2 sludge, the highest crude protein destruction due to HTP was only 6% at 155 °C. The difference between Phase 1 and Phase 2 sludge in terms of crude protein destruction due to HTP is likely related to the compositional difference of the two sludge mixtures.

During AD, protein is degraded in three steps: first, it is converted to peptides and amino acids by extracellular enzymes (proteases) [75]; then, the amino acids are degraded either through the Stickland reaction by obligate anaerobic bacteria (e.g., *Clostridium* species), which involves paired amino acids, or by hydrogen-utilizing bacteria, degrading single amino acids [76]. The degradation products of amino acids are short-chain, linear and branched organic acids, which are further transformed by fermentative bacteria to acetate, formate, H_2, and CO_2, finally resulting in methane production. AD alone resulted in 29.4–36.6% and 21.6–26.4% crude protein removal in Phase 1 and Phase 2, respectively. For Phase 1 sludge, crude protein removal by AD was comparable (29–30%) for raw sludge mixture and HTP sludge at 125 and 155 °C, which was lower than that for HTP sludge at 90 °C (36.6%). Considering both HTP and AD, the overall crude protein removal by HTP-AD ranged from 29.4 to 46.7% in Phase 1. HTP-AD resulted in higher overall crude protein removal compared to only AD. The highest overall crude protein removal (46.7%) was achieved with HTP at 155 °C. For Phase 2 sludge, the highest crude protein removal by AD (26.4%) was achieved for raw sludge mixture, compared to HTP sludge at 90, 125, and 155 °C (21.6–25.8%). The overall crude protein removal by HTP-AD ranged from 21.6 to 30.3% in Phase 2. HTP-AD at 90 and 125 °C resulted in comparable or lower overall crude protein removal relative to only AD. The highest overall crude protein removal (30.3%) was achieved with HTP at 155 °C. Thus, except for Phase 1 HTP sludge at 90 °C, HTP did not increase crude protein removal in AD. For both phases, HTP at 155 °C followed by AD resulted in the highest overall crude protein removal. For both AD phases, good TN balance was achieved (<8.4%). The ratio of net ammonium produced to protein removed ranged from 0.14 to 0.23, comparable to the typically used value of 0.16 in estimating crude protein from organic N measurements [77].

Based on the results of this study, it is recommended that N recovery from sewage sludge is practiced after HTP and AD. For the experimental setup used in this study, HTP at 155 °C followed by AD resulted in the highest N release and mineralization in both AD phases.

3.6. Effect of HTP and AD on P species release

Unless stabilized sewage sludge is directly used by land application, release/solubilization of P is required for its recovery. The release strategy and its effectiveness depend highly on the P species in the sludge [78]. Some studies reported that non-apatite inorganic P (NAIP) (e.g., Fe, Al and Mn-bound P) was the dominant P species in WAS [79,80], while polyphosphate was the major P species in sludge produced in EBPR processes [81]. In a study by Wang et al. [21], strengite (23.0%), $AlPO_4$ (32.5%), alumina-adsorbed phosphate (14.5%), hydroxylapatite (9.2%), and phytic acid (16.0%) were the main P species in the raw sludge mixture obtained from the same WRRF as in the present study.

TP and soluble orthophosphate were measured for both feed and R1-R4 digestates during Phase 1 and 2 (Table 5). The soluble orthophosphate in Phase 1 feed sludge was much lower than that in Phase 2. After HTP at 90 °C, the soluble orthophosphate decreased from 13.6 to 8.8 mg P/L in Phase 1 sludge; it also decreased after HTP at 125 °C from 291 to 139 mg P/L in Phase 2 sludge. In a study by Liu et al. [78], soluble orthophosphate decreased after HTP at 170 °C for 2–3 h when significant sludge solubilization was observed; the decrease in orthophosphate concentration was attributed to adsorption on the disintegrated sludge as the number of adsorption sites increased, and precipitation/coagulation induced by the release of cations or biopolymers from the ruptured sludge flocs. Tao and Huang [82] observed a decrease in

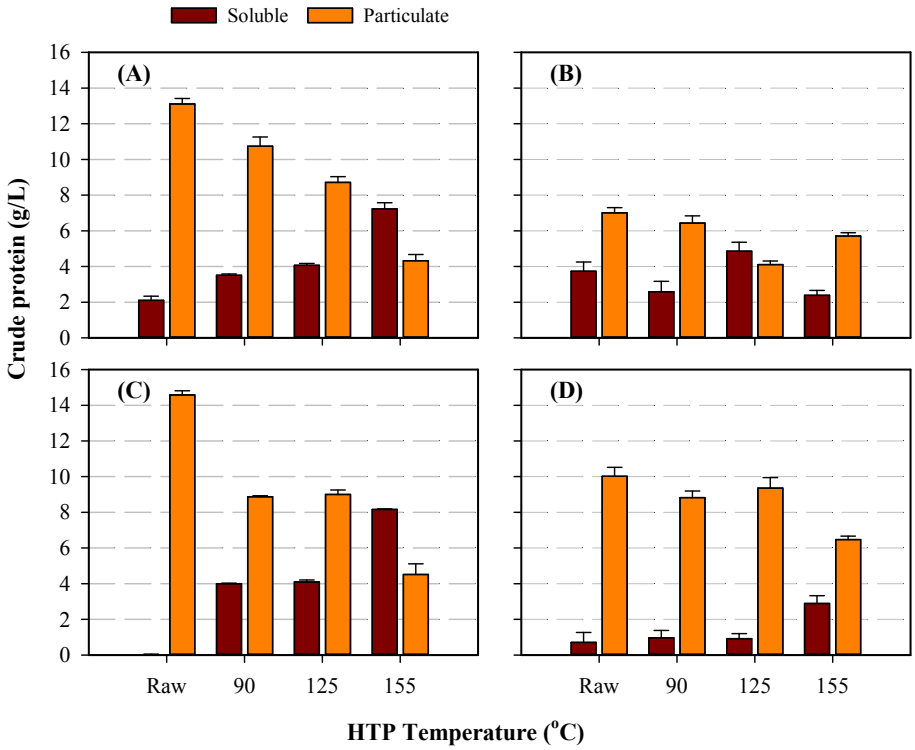

Fig. 6. Crude protein in Phase 1 feed (A) and digestate (B), and Phase 2 feed (C) and digestate (D) (Error bars are mean ± standard deviation, n ≥ 3).

Table 5
Influent and effluent TP and phosphate for Phase 1 and Phase 2 digesters.

Phase	Digester	Influent TP (mg P/L)	Effluent TP (mg P/L)	Influent orthophosphate (mg P/L)	Effluent orthophosphate (mg P/L)	P balance (%)
1	R1	1126 ± 16[a]	1111 ± 8[a]	13.6 ± 0.2	1.7 ± 0.7	1.3
	R2	1202 ± 24	1181 ± 16	8.8 ± 0.1	2.0 ± 1.5	1.7
	R3	1072 ± 34	1150 ± 50	11.3 ± 0.1	1.9 ± 0.8	−7.2
	R4	1124 ± 16	1089 ± 88	15.8 ± 0.2	2.5 ± 0.5	3.1
2	R1	1066 ± 19	1102 ± 82	291 ± 3	32 ± 3	−3.4
	R2	930 ± 22	885 ± 10	196 ± 4	26 ± 5	4.8
	R3	961 ± 38	961 ± 20	139 ± 2	29 ± 4	0
	R4	958 ± 30	1038 ± 28	206 ± 5	118 ± 8	−8.4

[a] Mean ± standard deviation ($n = 3$).

soluble orthophosphate with thermal hydrolysis, attributed to formation of calcium phosphate precipitates.

In Phase 1, after AD at 20 d SRT, the soluble orthophosphate decreased by 77.3–87.5%, while in Phase 2, after AD at 10 d SRT, it decreased by 79.1–89.0% in R1-R3 and 42.7% in R4. In Phase 2, R4 had a much higher soluble orthophosphate concentration compared to R1, R2 and R3. P solubilization under AD conditions is strongly affected by pH [78]. Latif et al. [83] observed a 3.6-fold increase in P solubility during the anaerobic digestion of WAS at pH below 5.7. In a study by Liu et al. [78], an increase in soluble orthophosphate was observed during the hydrolysis and acidification stages of AD at pH below 7; however, the soluble orthophosphate concentration decreased during the methanogenesis stage at pH above 7.3 [78]. In addition, P solubilization may be affected by the concentration of VFAs. P release at 90–100 mg P/L was obtained by adding ca. 400 mg/L of acetate during AD of EBPR sludge [84]. Thus, in the present study, the higher soluble orthophosphate concentration in Phase 2 R4 compared to R1-R3 may be attributed to a lower pH (6.98 vs 7.32–7.34) and higher VFAs levels in R4 (3,444–4,170 vs 16–60 mg COD/L). For both AD phases, good TP balance was achieved (<8.4%).

The observed decrease in soluble orthophosphate is consistent with the molecular level change of P speciation observed in recent studies using P K-edge X-ray absorption spectroscopy (XAS) and ^{31}P nuclear magnetic resonance (NMR) spectroscopy [20,21]. Specifically, Fang et al. [20] and Wang et al. [21] observed transformation of complex P species into orthophosphate and subsequent adsorption of orthophosphate on mineral phases or precipitation of metal phosphates. These processes result in P associated with Al/Ca/Fe minerals and overall reduce P mobility and availability. Although the above two studies were conducted using different sludge (WAS) and batch AD assays, the overall trend in P speciation as a result of HTP and AD is likely similar in the present study.

In summary, although HTP and AD did not significantly improve P solubilization and release, they did facilitate the transformation of P to orthophosphate. P recovery after AD in the form of P minerals is problematic due to difficulties in the separation of the fine-particle P minerals [78]. Thus, P recovery from sewage sludge is recommended before HTP and AD. Other options for P recovery may include: 1) in situ crystallization in AD systems, for example as struvite; and 2) re-dissolution of sludge-associated phosphate precipitates. However, as the latter choice requires additional reactors and chemicals, it is usually complex and non-economical [78].

4. Conclusions

Sludge mixture collected from the same WRRF at different times varied in composition and resulted in significant differences in terms of HTP solubilization, VFAs formation, and AD performance. The two sludge mixtures used in this study had high ultimate biodegradability (58.3 for Phase 1 and 56.9% for Phase 2). With one exception (Phase 2 R4), HTP increased both organic matter destruction and methane production. High levels of propionate (13,668 mg COD/L), formed with HTP at 155 °C for Phase 2 sludge attributed to sludge lipid content, negatively affected methane production in Phase 2 R4. HTP significantly changed the bacterial communities in the digestates, but had a minor effect on the archaeal communities. The predominant genera in the digestate samples were *Methanosarcina* (66–88%) and *Methanosaeta* (7–30%). The highest net energy gain (ΔE) was obtained with the control (i.e., raw sludge AD without HTP) for both phases, a result attributed to the relatively high ultimate biodegradability of the two sludge mixtures. For the experimental setup used in this study, HTP heat recovery greater than 85% is necessary to attain the same net energy as the control (i.e., AD without HTP) or higher. In order to make HTP more energy beneficial, it is recommended that the sewage sludge be concentrated to a higher degree than is presently practiced at the study WRRF. For both phases, HTP at 155 °C followed by AD resulted in the highest overall crude protein removal and release of ammonium N. P availability decreased after AD and HTP-AD. Thus, N recovery after HTP and AD is recommended, while P recovery should be practiced before HTP and AD.

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 data

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