Temperature-Phased Biological Hydrolysis and Thermal Hydrolysis Pretreatment for Anaerobic Digestion Performance Enhancement

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Abstract: Thermal hydrolysis (TH) and biological hydrolysis (BH) are two main and growing anaerobic digestion pretreatment technologies. In this study, municipal wastewater sludge samples were collected from the Guelph Wastewater Treatment Plant (WWTP) in Ontario, Canada. The effects of temperature on BH treatment, including BH at 42 °C (BH42), 42 °C followed by 55 °C (BH42+55), 55 °C followed by 42 °C (BH55+42), and 55 °C (BH55) were evaluated for anaerobic digestion performance enhancement and compared with TH treatment at 165 °C. The TH, BH42, BH42+55, BH55+42, and BH55 treatments caused the reduction of volatile suspended solids (VSS) by 22.6%, 17.5%, 24.6%, 23.1%, and 25.9%, respectively. The soluble chemical oxygen demand (sCOD) content of the sludge increased by 377.5%, 323.8%, 301.3%, 286.9%, and 221.7% by the TH, BH55, BH42+55, BH55+42, and BH42 treatments, respectively. Volatile fatty acids (VFA) constituted around 40% of the sCOD in the BH-treated sludge and 6% in the TH-treated sludge. The cumulative methane yields (NmLCH4/g COD fed) of sludge treated by BH55+42 and TH were respectively 23% and 20% higher than that of the untreated sludge. For BH pretreatment, sludge treated by BH55+42 produced more methane than those treated by BH42+55, BH55, and BH42. The methane yields of the combined sludge treated by the TH and BH55+42 treatments were in the ranges of 248.9 NmLCH4/g COD to 266.1 NmLCH4/g COD fed, and 255.3 NmLCH4/g COD to 282.2 NmLCH4/g COD fed, respectively.

Keywords: activated sludge; anaerobic digestion; methane generation; sludge pretreatment; temperature-phased biological hydrolysis; thermal hydrolysis

1. Introduction

The anaerobic digestion (AD) of wastewater sludge has become increasingly attractive due to its capacity to recover energy from wastewater [1,2]. Sludge produced from wastewater treatment processes includes the primary sludge from the primary sedimentation tank and waste activated sludge (WAS) from the secondary biological treatment process. The volatile suspended solids (VSS) of WAS is typically between 60–80% of the total solids with an energy content of 19–23 kJ/gVSS [2]. Anaerobic digestion converts sludge organic content into energy-rich methane gas through rather specific reactions, including hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Hydrolysis is a critical extracellular enzymatic reaction step to break down particular solids and macromolecules into small soluble compounds, which makes them utilisable by organism cells for methane production through intracellular metabolic reactions. Since WAS mainly consists of aggregated bacterial cells and extracellular polymeric substances (EPS), the hydrolysis of WAS has been widely accepted to be a rate-limiting step for methane production from WAS. One of the practical approaches to enhance the
hydrolysis of WAS is to integrate anaerobic digestion with sludge pretreatment technologies that can efficiently degrade the non-readily biodegradable solid content of sludge for methane production [3,4]. Various sludge pretreatment technologies, including the ultrasonic homogenizer [5,6], thermal hydrolysis [7–9], biological/enzymatic hydrolysis (BH) [10–12], high-pressure homogenization [5,13], and ozonation [8,14,15] have been evaluated for AD performance enhancement. Among these technologies, thermal hydrolysis (TH) and biological hydrolysis (BH) have been proven to be relatively effective in destroying volatile solid (VS), increasing the biogas production of anaerobic digestion, producing class A type biosolids for sludge land application, and improving the dewaterability of digested sludge [16–19].

Studies indicate that the optimum TH temperature for sludge treatment is between 150–180 °C. Wilson et al. [17] showed that the TH treatment of combined primary sludge and WAS at 150 °C and 170 °C increased the biogas by 24% and 59%, respectively. Valo et al. [20] reported that a combined chemical–TH pretreatment of WAS at 130 °C and 170 °C for one hour could enhance the biogas production by 21% and 45%, respectively. Bougrier et al. [21] showed that the methane yield of WAS increased from 145 L/kg VS to 238 L/kg VS and 256 L/kg VS. After the sludge was treated by TH at 150 °C and 170 °C for 30 min, respectively. While many studies showed a significant positive impact of TH pretreatment at temperatures around 170 °C on the methane production, it is also well-known that TH treatment at high temperatures could cause the caramelization of sugars, the production of inhibitive refractory compounds, and high energy consumption [22,23].

BH treatment is characterized by anaerobic digestion at temperatures below 70 °C for a retention time of around three to five days. The biological hydrolysis of sludge involves the disintegration of flocculated sludge, the extracellular hydrolysis of macromolecules (proteins, polysaccharides, and lipids) into oligomers and monomers, and the fermentation of small soluble molecules (peptides, amino acids, sugars, glycerol, etc.) by acidogenic and acetogenesic bacteria. The products of acetate, formate, H₂, and CO₂ of BH treatment can be utilized directly by methanogens to produce methane and carbon dioxide. BH temperature affects sludge disintegration and the microbial community structure, so it is considered to be one of the most crucial operating parameters of BH [23–25].

Many studies have been carried out to investigate the effect of temperature on biological hydrolysis. Most of those studies were based on a two-stage configuration, which consisted of an either mesophilic (37–42 °C) or thermophilic (45–70 °C) BH stage followed by an AD process. The BH–AD process that enhances the hydrolysis of WAS has demonstrated improvement in biogas production, VS reduction, and pathogen deactivation [26–28]. Ge et al. [29] studied the treatment of primary sludge by BH–AD in a pilot scale and demonstrated that the BH pretreatment at 60 °C and 70 °C achieved a VS reduction of 41% and 48%, respectively. Wu et al. [30,31] reported that a VS reduction of 44.8% and a methane yield of 240 L CH₄ per kg of VS were achieved by a pilot-scale BH–AD process that was operated at a BH temperature of 70 °C.

The above-mentioned studies showed that both TH and BH treatments can be effective pretreatment processes of AD for performance enhancement. Comparing to TH, BH has advantages of lower energy consumption, and is less likely to produce refractory organics. However, the optimal temperatures and retention times for BH treatment are still open to explore.

Recently, a temperature-phased biological hydrolysis (TPBH) process, which was characterized by a mesophilic treatment at 42 °C for a solid retention time (SRT) of 1.5 days followed by a thermophilic treatment at 55 °C for 1.5 days (BH42+55), was introduced as a full-scale BH sludge pretreatment technology [27]. Ding et al. [28] showed that the biological hydrolysis (BH) of secondary sludge at 42 °C for six days increased the scOD from 175.2 ± 38.2 mg/L to 3314.5 ± 683.4 mg/L, and the concentration of acetic acid from 41.5 ± 2.1 mg/L to 786.0 ± 133.2 mg/L. Chen and Chang [25] showed that sludge treatment by BH at 35 °C, 42 °C, and 55 °C resulted in different microbial community structures and functional bacterial groups for protein and carbohydrate degradation.

While the condition of BH42+55 has been applied to the full-scale TPBH processes, limited studies have been conducted to assess the effects of the different phase temperatures on the BH performance
and biogas production. Meanwhile, TH sludge pretreatment has proven its superiority over many other pretreatment methods, but well-designed comparative studies of TH and BH treatments have yet to be published. Thus, the objectives of this study are: (1) to investigate the effects of BH temperature on hydrolysis performance, biogas production, and the destruction of volatile suspended solids (VSS); (2) to compare BH and TH (at 165 °C) treatment in terms of sludge solubilization, biogas production, and solids reduction; and (3) to study the effects of the BH treatments on microbial community structures.

2. Materials and Methods

2.1. Sludge Sample Collection and Preparation

Municipal wastewater sludge samples were collected from the Guelph Wastewater Treatment Plant (WWTP) in Ontario, Canada. The wastewater treatment process at the Guelph WWTP consists of preliminary screening and grit removal, primary sedimentation, extended aeration activated sludge treatment, secondary clarification, rotating biological contactors, and sand filtration tertiary treatment. According to the annual average wastewater characteristics from 2011 to 2015, the average daily wastewater flow treated by the Guelph WWTP was 50.02 ± 15.6 million liter per day with a Carbonaceous Biochemical Oxygen Demand (cBOD₅) at 193.4 ± 15.6 mg/L, total suspended solids (TSS) at 257.2 ± 27.1 mg/L, total phosphorus at 5.14 ± 0.38 mg/L, total Kjeldahl nitrogen (TKN) at 38.5 ± 2.9 mg/L, and total ammonia nitrogen (NH₃-N) at 22.3 ± 1.6 mg/L.

Samples of the primary sludge, secondary sludge, and digested sludge were taken respectively from the wasting line of the primary clarifier, recycle line of the secondary clarifier, and the feed line of the anaerobic digester. The sludge samples were transported to the lab about half an hour after sampling. The primary, secondary, and anaerobic digested sludge samples were filtered through a standard sieve with the average opening size of 4.76 mm. The combined sludge was made in the lab by mixing the sieved primary and secondary sludge in a 1:1 volume ratio; then, it was used for the hydrolysis and biological methane potential (BMP) experiments. The sludge samples were characterized by measuring the total solids (TS), VS, TSS, VSS, total chemical oxygen demand (TCOD), sCOD, volatile fatty acids (VFA), pH, and alkalinity according to the standard methods [32].

2.2. Bench-Scale Sludge Hydrolysis Pretreatment

Parallel BH and TH experiments were carried out to assess the effect of temperature conditions on BH performance, as well as compare the performances of BH and TH in the treatment of the same batch of sludge samples. The BH temperature conditions tested included: 42 °C for three days (BH42); 42 °C for 1.5 days followed by 55 °C for 1.5 days (BH42+55); 55 °C for three days (BH55); and 55 °C for 1.5 days followed by 42 °C for 1.5 days (BH55+42).

The BH experimental steps included filling the combined sludge in two-liter plastic bottles, flushing the mixed sludge for one minute using nitrogen (N₂) gas, capping the testing bottles with airtight lids, placing the capped sludge-filled bottles in the incubators at preset temperatures, and shaking at 100 rpm. The TH treatment was carried out at 165 °C for 30 min using an oil bath and a self-made cylindrical stainless steel vessel with a working volume of 80 mL. After 30 min of heating, the TH reactor was immediately taken out of the oil bath and soaked in water to cool down to room temperature. The effects of the BH and TH treatments were characterized by analyzing the change in the concentrations of TS, VS, TSS, VSS, TCOD, sCOD, VFA, pH, and the alkalinity of the treated sludge.

The VFA compositions were measured using gas chromatography (GC-HP6890, Agilent Technologies, Santa Clara, CA, USA) equipped with a Supelco-24108 column and a flame ionization detector (FID). Nitrogen was used as a carrier gas. The injector and detector temperature were maintained at 200 °C and 250 °C, respectively. The oven temperature was increased at a rate of 10 °C from 100 °C to 200 °C and then held at 200 °C for 10 min. VFA mix standard was purchased from Sigma-Aldrich, Canada, which included acetic, propionic, isobutyric, butyric, isovaleric, valeric
4-methylvaleric (isocaproic), hexanoic (caproic), and heptanoic acid; these were used for the peak identification and standard curve determination. The VFA standard curves were then prepared by analyzing a series of VFA mix solutions obtained by diluting five mmol/L of the standard VFA mix.

2.3. Biological Methane Potential (BMP) Test

The effects of TH and BH pretreatments on biogas production and VSS reduction were assessed using batch BMP tests. The BMP methods that were used in this study were adapted from the methods used by Angelidaki et al. [33], Hansen et al. [34], and Owen et al. [35] with some modifications. The BMP experiments used 160-mL standard serum bottles, and all the BMP bottles contained 50 mL of the AD sludge as inoculum and 15 mL of sludge substrate. Untreated sludge (control bottles), BH-treated sludge (BH testing bottles), TH-treated sludge (TH testing bottles), or distilled water (blank bottles) were used as BMP substrate to assess the effect of pretreatment on biogas production. Triplicates were used for all the testing, control, and blank bottles in the BMP tests. The food (g TCOD in substrate) to microorganism (g VSS in inoculum) ratio was initially evaluated and then controlled between 0.8–0.84. The inoculums used in the BMP tests were the sludge samples taken from the anaerobic digester of the Guelph WWTP, which were operated at a SRT of 15 days.

All of the BMP bottles were flushed with N\textsubscript{2} gas immediately after being filled with the inoculums and substrates; then, they were sealed with a rubber stopper that was tightened by an aluminum crimp. The bottles were put upside down in the BMP incubator, which was set at 35 °C and shaken at 100 rpm. The biogas produced from each of the BMP bottles was monitored volumetrically from the headspace of each bottle using a 50-mL glass syringe equipped with a luer valve. The frequency of biogas measurement was based on the biogas production rate during the BMP testing period. The blank BMP bottles were used to determine the biogas production from the added inoculum. The methane volume produced by the substrate sludge in each BMP bottle was determined by subtracting the methane volume produced by the blank bottle from the total methane volume measured from the BMP testing bottle. The accumulated methane volume generated from the substrate was normalized to standard conditions at STP [28].

The composition of the biogas was analyzed by using an Agilent 6890 gas chromatography (GC) system (HP6890, Agilent Technologies, USA) with a thermal conductivity detector (TCD) and a HP-PLOT Molesieve GC column (Agilent 19095P-MSO and 30 m × 0.530 mm, Agilent Technologies, USA) [25,28]. The carrier used argon gas at a gas flow rate of five mL/min and 4.54 psi. The injector and detector temperature were maintained at 200 °C and 150 °C, respectively. The temperature of the GC oven was held at 35 °C for 7.5 min, and then increased from 35 °C to 206 °C at a rate of 24 °C/min, and held at 206 °C for one minute, as recommended by the manufacturer. The biogas peaks were identified and quantified by comparing them with the peak areas of the standards, which consisted of hydrogen, oxygen, nitrogen, methane, and carbon dioxide at various composition fractions (Praxair, Mississauga, ON, Canada).

The BMP tests started with five testing bottles for each test condition, among which three bottles were used for the triplicate biogas measurement, and two bottles were randomly designated as the sacrificed BMP bottles. The sacrificed bottles were opened on the 10th and 20th days of the BMP tests to determine the changes in the concentrations of TS, VS, TSS, VSS, and TCOD of the sludge over the course of the BMP tests. Changes in the concentrations of the solid contents were measured at the end of the 30-day BMP tests. The reductions of the various sludge solid parameters during the BMP test periods were calculated based on Equation (1).

$$RDC_{AD} = 100 \times \left( \frac{C_F - C_M}{C_F} \right)$$

where $RDC_{AD}$ is the concentration reduction (%), $C_F$ is the concentration in the raw sludge before pretreatment, and $C_M$ is the concentration of mixed liquor in the BMP bottles on a given day.
The comparisons of the different BH temperature conditions and the performance of the TH and BH treatments were carried out in parallel experiments using the same batch of sludge sampled from the Guelph WWTP. Three batches of sludge samples were taken from the Guelph WWTP at different times to study the effects of BH and TH treatments on biogas production and VSS reduction. A consistent performance trend was observed with these three different batches of sludge.

2.4. DNA Extraction and Illumina Sequencing

Sludge samples were taken from the BH55+42, BH42+55, and control BMP bottles on the 15th day of the BMP tests for DNA extraction and sequencing analysis. The sludge samples were centrifuged at 10,000-g for 10 min, and the DNA samples were extracted from the pellet using the MO BIO Laboratories PowerSoil DNA Isolation Kits (USA) according to the manufacturer’s protocol. The extracted DNA was quantified using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Mississauga, ON, Canada) and stored at −20 °C for further use.

The primer pair sequence follows the 16S Metagenomic Sequencing Library Preparation protocol as recommended for the Illumina MiSeq System. It targets the 16S V3 and V4 regions of bacteria, and creates a single amplicon of approximately ~460 bp. The polymerase chain reaction (PCR) conditions included: denaturation at 95 °C for three minutes, followed by 25 cycles (each consisting of 95 °C for 30 seconds (s), 55 °C for 30 s, and 72 °C for 30 s) and a final extension of 72 °C for five minutes. The DNA amplification reaction agent that has a total ready volume of 25 µL contained 2.5 µL of template, 5 µL of amplicon PCR forward primer, 5 µL of amplicon PCR reverse primer, and 12.5 µL of 2x KAPA HiFi HotStart ReadyMix (supplied by VWR, Radnor, PA, USA).

The PCR Clean-Up and Index PCR, which followed the methods recommended by Illumina, used the AmPure XP beads and the Nextera XT Index kit (supplied by Illumina, San Diego, CA, USA). The library quantification, pooling, denaturing, and MiSeq sample loading used Illumina’s recommended protocols. The on-instrument classification of taxonomies of the sequence reads were based on the Greengenes database (http://greengenes.lbl.gov/) by selecting the Metagenomic workflow of the MiSeq Reporter software. The candidate sequences were classified to the taxonomy with a confidence threshold of 89%, and were clustered to operational taxonomic units (OTU) at 98% sequence identity [36]. Statistical analyses of the data were performed on BaseSpace using the 16S Illumina Metagenomic application.

3. Results and Discussion

3.1. Sludge and Inoculum Characteristics

Table 1 shows the properties of the three batches of combined sludge and BMP inoculum samples that were used in this study. The properties of the sludge sampled from the Guelph WWTP at different times varied in TCOD contents, sCOD/TCOD ratios, and VFA contents. The combined sludge was a mixture of equal volumes of the primary and secondary sludge. The TS and TCOD contents of the second batch of sludge were significantly higher than those of the first and third batches of sludge. The VS/TS ratio of the first, second, and third batches of combined sludge were 68%, 70%, and 69%, respectively, and their sCOD/TCOD ratios were 4.2%, 2.7%, and 4%, respectively. On the other hand, the characteristics of the BMP inoculum sludge were relatively consistent. Of the BMP inoculum used in the first, second, and third batches of BMP experiments, the ratios of VS/TS were 54.2%, 58.8%, and 58.3%, respectively, and the ratios of sCOD/TCOD were 3.1%, 3.0%, and 2.7%, respectively.
Table 1. Combined sludge (CS) and inoculum sludge from anaerobic digestion (AD) characteristics for the three batches. TS: total solids, VS: volatile solid, TSS: total suspended solids, VSS: volatile suspended solids, TCOD: total chemical oxygen demand, sCOD: soluble chemical oxygen demand, VFA: volatile fatty acids, ALK: alkalinity.

| Parameters   | Batch 1     | Batch 2     | Batch 3     |
|--------------|-------------|-------------|-------------|
| TS (g/L)     | 20.9 ± 0.3  | 22.2 ± 0.1  | 27.8 ± 0.0  | 22.7 ± 0.0  | 19.7 ± 2.6  | 22.3 ± 0.5  |
| VS (g/L)     | 14.7 ± 0.3  | 12.0 ± 0.1  | 18.9 ± 0.1  | 13.3 ± 0.1  | 13.5 ± 0.2  | 13.0 ± 0.3  |
| TSS (g/L)    | 19.3 ± 0.1  | 20.8 ± 0.1  | 26.0 ± 0.4  | 21.2 ± 0.2  | 18.4 ± 0.2  | 20.6 ± 0.5  |
| VSS (g/L)    | 13.9 ± 0.2  | 12.0 ± 0.2  | 18.0 ± 0.2  | 12.9 ± 0.2  | 13.2 ± 0.2  | 12.4 ± 0.3  |
| pH           | 6.7 ± 0.0   | 7.8 ± 0.0   | 6.8 ± 0.0   | 7.7 ± 0.0   | 6.8 ± 0.0   | 7.6 ± 0     |
| TCOD (g/L)   | 24.8 ± 0.4  | 20.6 ± 0.2  | 34.2 ± 0.4  | 22.1 ± 0.1  | 23.4 ± 0.4  | 21.8 ± 0.2  |
| sCOD (mg/L)  | 672.0 ± 3   | 641.0 ± 1   | 1444.0 ± 2  | 671.0 ± 1.0 | 524.0 ± 1   | 594.0 ± 11  |
| VFA (mg/L)   | 377.0 ± 3   | 629.2 ± 2   | 852.0 ± 9   | 832.0 ± 9   | 657.5 ± 1.5 | 287.0 ± 5   | 539.1 ± 1.8 |
| ALK (mg/L as CaCO₃) | 730.5 ± 10 | 5295.0 ± 50 | 713.0 ± 5 | 5185.0 ± 100 | 763.0 ± 35 | 4593.0 ± 100 |

The effects of TH and BH treatments on sludge solubilization and biogas production were similar in experiments on each of the three batches of sludge. However, for simplicity, the results obtained for the second batch of sludge are discussed in detail in Sections 3.2–3.5 and 3.7, while a comparison of the results obtained from the three batches of sludge is summarized separately.

3.2. Effect of TH and BH Treatments on Sludge Solubilization

Figure 1 shows the percent changes in the concentrations of TS, VS, TSS, VSS, and TCOD caused by the TH and BH treatments on the combined sludge. The percent changes were determined by normalizing the concentration changes with the corresponding initial concentrations. The change in TS caused by the TH treatment was negligible. In contrast, the BH treatments caused a 4% to 6% reduction in TS, which was likely due to the BH-induced biogas release and the oven drying-induced VFA loss during the TS measurement. The GC analysis showed that the gases generated during the BH treatments consisted mainly of carbon dioxide (CO₂), whereas the methane (CH₄) content was negligible. This result is because no inoculum was added to the BH bottles, and only a short anaerobic reaction time was applied in BH treatments. The ratios of TCOD to TS maintained a very close range of 1.24 ± 0.007 for the raw as well as the TH and BH-treated sludge.

Figure 1. Reduction in biosolid concentration caused by thermal hydrolysis (TH) and biological hydrolysis (BH) treatments.
The VS reduction caused by the treatment of TH, BH42, BH42+55, BH55+42, and BH55 were 1.8%, 17.7%, 16.6%, 16.2%, and 13.1%, respectively (Figure 1). Compared to the TH treatment, the BH treatment resulted in a considerable reduction in the VS content of sludge. The TS mass balance for the BH treatment can be formulated as in Equation (2): 

$$\Delta TS_{loss} = \Delta VS + \Delta iS$$

(2)

where $\Delta TS_{loss}$ is the total TS reduction, $\Delta VS$ is the VS reduction, and $\Delta iS$ is the inorganic solids change.

When the $\Delta TS_{loss}$ observed in the BH treatments was in the range of 4% to 6%, the BH treatments resulted in a significant conversion of the VS content to the inorganic solids ($\Delta iS$). The gravimetric analysis confirmed that the $\Delta iS$ content of the sludge increased by 17% to 22% after the BH treatments (Figure 2). The main fermentation products of carbohydrates and proteins included short-chain VFAs, NH$_4$, H$_2$O, and CO$_2$. The CO$_2$ generated from the fermentation reaction would mainly exist in the form of bicarbonate under the pH condition in this case. Therefore, both of the released NH$_4$ and CO$_2$ from the sludge hydrolysis could contribute to the increase in the inorganic content of the BH-treated sludge. It is worth noting that the VS reduction caused by the TH treatment was close to the TH-induced TS reduction (Figures 1 and 2), implying that TH treatment at 165 °C might only degrade particles and macromolecules into smaller, soluble, organic molecules with only an insignificant production of CO$_2$ and NH$_3$.

Both TH and BH treatments resulted in a significant destruction of the suspended solids content (TSS) of the sludge. The TH, BH42, BH42+55, BH55+42, and BH55 treatments caused the TSS reductions of 16.9%, 12.0%, 17.4%, 16.3%, and 17.1%, respectively, and the VSS reductions of 22.6%, 17.5%, 24.6%, 23.1%, and 25.9%, respectively (Figure 1). These results showed that the BH42+55, BH55+42, and BH55 treatments achieved comparable suspended-solid solubilization to the TH treatment. The relatively lower TSS and VSS reductions obtained with BH42 might suggest that the mesophilic BH treatments could be less effective for the solubilization of sludge suspended solids. The reduction of TSS is the sum of the reductions in VSS and inorganic suspended solids. The solids measurement showed that more than 98% of the TSS reduction is caused by the VSS reduction in both TH and BH treatment. This is understandable, because the TH and BH treatments could exert only a negligible impact on the solubilization of inorganic solids.

![Figure 2](image_url). Change in inorganic matter content caused by TH and BH treatments.

With the degree of sludge solubilization defined in this study as the ratio of $\Delta sCOD/(TCOD - sCOD_{initial})$ to reflect the change in sCOD caused solely by hydrolysis, the TH treatment showed the highest degree of sludge solubilization (17%) followed by BH55 (14%), BH55+42 (13%), BH42+55 (10%). In a study carried out to enhance anaerobic digestion by Yang et al. [37] for excess sludge hydrolysis using enzyme addition, similar sludge solubilization results were achieved.
were acetic, propionic, isobutyric, butyric and isovaleric acids. However, as shown in Table 2, Water 2018
TH-treated sludge was only 40%, which was significantly lower than the BH-treated sludge. As shown
products. In descending order, the VFA production capacity of the BH treatment methods were
detected in the BH-treated sludge, with acetic and propionic acids as the major VFA
detected in the untreated and TH-treated sludge. Acetic, propionic, isobutyric, butyric, and isovaleric
increased the total VFA concentrations in the sludge, and produced various VFAs that were not
but rather only increased the concentrations of acetic and propionic acids. However, BH treatment
sludge. It seems that the TH treatment at 165°C did not cause significant changes in the VFA species, but
rather only increased the concentrations of acetic and propionic acids. However, BH treatment

3.3. Effect of TH and BH Treatment on sCOD, VFA, and Alkalinity

Figure 3 shows the effects of the TH and BH treatments on the change in the sCOD, VFA, and alkalinity contents of the hydrolyzed sludge. The concentrations of sCOD, VFA, and alkalinity of the BH and TH-treated sludge were considerably higher than those of the untreated sludge. The highest sCOD increase of 377.5% was achieved with the TH treatment, followed by a 323.8% increase with BH55, 301.3% with BH42+55, 286.9% with BH55+42, and 221.7% with BH42 (Figure 3). The ratios of sCOD increase (ΔsCOD) to VSS reduction (ΔVSS) were in the range of 1.00 ± 0.013 for all of the BH treatments, showing consistent conversion rates of sCOD to VSS for these different treatment conditions. For the TH treatment, the ΔsCOD/ΔVSS ratio was 1.34 ± 0.024 (Figure 4), which was higher than those observed with the BH treatment, suggesting that the compositions of sCOD of the BH and TH-treated sludge were different.

Despite the considerable increase in sCOD in the TH-treated sludge, the VFA increase for the
TH-treated sludge was only 40%, which was significantly lower than the BH-treated sludge. As shown
in Figure 4, the ratios of ΔVFA (mg/L as acetic acid) to ΔsCOD (mg/L) were determined to be 39%,
40%, 37%, 40%, and 6% for the BH42, BH42+55, BH55+42, BH55, and TH treatments, respectively.

GC analysis showed that the main VFA species formed at the end of the three-day BH treatment
were acetic, propionic, isobutyric, butyric and isovaleric acids. However, as shown in Table 2,
the main VFA compositions of the untreated, TH-treated, and BH-treated sludge were different.
Both the TH-treated and untreated sludge contained mainly acetic and propionic acids, although
the concentrations of these acids were much higher in the TH-treated sludge than in the untreated
sludge. It seems that the TH treatment at 165°C did not cause significant changes in the VFA species,
but rather only increased the concentrations of acetic and propionic acids. However, BH treatment
increased the total VFA concentrations in the sludge, and produced various VFAs that were not
detected in the untreated and TH-treated sludge. Acetic, propionic, isobutyric, butyric, and isovaleric
acids were detected in the BH-treated sludge, with acetic and propionic acids as the major VFA
products. In descending order, the VFA production capacity of the BH treatment methods were
BH42, BH55+42, BH42+55, and BH55, showing a positive impact of hydrolysis temperature on VFA production. Compared to the VFA concentrations in the sludge treated by BH42+55 and BH55+42, it seems that a later stage thermophilic hydrolysis would be beneficial to VFA production. Overall, the BH treatment resulted in much higher VFA productions than the TH treatment.

The BH treatment also resulted in a significant increase in sludge alkalinity (Figure 3). Comparing the alkalinity (ALK) changes caused by the different BH methods, the sludge treated by BH55+42 showed the highest alkalinity increase, although BH42+55 and BH55 produced slightly higher VFAs. The main products of sludge hydrolysis could include VFAs, CO$_2$, soluble microbial products (SMP), and NH$_3$ released from the hydrolysis of proteins. The production of CO$_2$ and VFAs will decrease the pH, while the production of NH$_3$ will increase the pH. In this study, the initial pH of the second batch of combined sludge was 6.79, while the pH values after the TH, BH42, BH55+42, BH42+55, and BH55 treatments were 6.58, 6.78, 6.75, 6.78, and 6.45, respectively. The insignificant change in pH suggests that the pH reduction caused by CO$_2$ and VFA productions was well balanced by NH$_3$ release from protein hydrolysis. The main alkalinity contributors in the hydrolyzed sludge could include carbonate, VFA, and SMP. Since the acid dissociation constant (pKa) values of VFAs are between pH 4.7–4.8, VFAs in the hydrolyzed sludge will exist in deprotonated forms, and can thus contribute to alkalinity. SMP can also contribute to alkalinity, because SMP polysaccharides, proteins, and humic acids have negatively charged proton-binding sites [38]. However, among these alkalinity contributors, carbonate was the main species to buffer the sludge pH change in the pH range relevant to the sludge hydrolysis by TH and BH treatments.

![Figure 4. Material conversion ratios of ∆sCOD/∆VSS and ∆VFA/∆sCOD due to TH and BH treatments.](image)

### Table 2. Concentrations of VFA constituent after TH and BH treatments. BH42: BH at 42 °C, BH42+55: BH at 42 °C followed by 55 °C, BH55+42: BH at 55 °C followed by 42 °C, BH55: BH at 55 °C.

| Test Condition | Acetic (mg/L) | Propionic (mg/L) | Isobutyric (mg/L) | Butyric (mg/L) | Isovaleric (mg/L) | ALK/VFA |
|----------------|---------------|-----------------|------------------|----------------|------------------|---------|
| Control        | 334.0         | 492.0           | -                | -              | -                | 0.863   |
| TH             | 633.0         | 527.0           | -                | -              | -                | 0.532   |
| BH42           | 828.2         | 580.4           | 170.4            | 207.8          | 278.2            | 0.942   |
| BH42+55        | 988.8         | 712.0           | 216.8            | 286.9          | 375.4            | 0.820   |
| BH55+42        | 881.3         | 644.4           | 198.2            | 282.7          | 368.4            | 1.031   |
| BH55           | 1014.9        | 713.1           | 235.4            | 304.9          | 451.6            | 0.794   |
3.4. Effects of TH and BH Treatments on Methane Production

The effects of the TH and BH treatments on methane production were assessed by conducting 30-day BMP tests at 35 °C. Figure 5a,b show the COD and VS-based methane yields over the operational period of 30 days. In the first two days, the methane productions by the TH-treated and untreated sludge (control) were higher than those from the BH-treated sludge, which was likely due to inhibition from the high VFA concentrations in the BH-treated sludge to the activities of methanogens. However, the accumulated methane production from the BH-treated sludge samples started to exceed that of the untreated sludge after the third day. The TH-treated sludge reached its highest methane yield at the fifth and sixth days of the BMP tests. Eventually, sludge treated by TH and BH55+42 attained similar methane yields. Overall, for all the sludge samples that were tested, 72–83% of the total methane gas produced in the 30-day BMP tests was generated in the first five days of the BMP tests.

The BMP tests showed that BH temperature could significantly affect methane production enhancement. As shown in Figure 5a,b, sludge treated by BH55+42 produced highest methane out all the BH conditions. Methane production with the BH55+42 treatment was also slightly higher than that with the TH treatment after 10 days of BMP tests. At the 15th day of the BMP test, the standardized (standard temperature and pressure, STP) methane yields of sludge treated by BH55+42 and TH were 23% and 20% higher than the control, respectively, while the other BH treatment conditions led to enhancement between 12–15%.

![Figure 5. CH4 yields of untreated and TH and BH-treated sludge substrate; (a) CH4 NmL/g COD fed and (b) CH4 NmL/g VS fed.](image-url)
The 30-day methane yields at STP for the untreated, TH, BH42, BH42+55, BH55+42, and BH55 sludge samples were 223.80 NmLCH₄/g COD, 259.45 NmLCH₄/g COD, 239.54 NmLCH₄/g COD, 240.6 NmLCH₄/g COD, 266.4 NmLCH₄/g COD, and 259.71 NmLCH₄/g COD fed, respectively and 404.9 NmLCH₄/g VS, 469.4 NmLCH₄/g VS, 433.4 NmLCH₄/g VS, 435.4 NmLCH₄/g VS, 482.0 NmLCH₄/g VS, and 469.9 NmLCH₄/g VS fed, respectively.

This study also assessed sludge biodegradability, which is defined by the ratio of the actual CH₄ produced to the theoretical CH₄ per gram COD. Based on the methane gas volume obtained during the first 15 days of the BMP tests, the biodegradability of the sludge treated by BH55+42 and TH was determined to be 73% and 71%, respectively, compared to 59% for the untreated sludge. Similarly, sludge biodegradability measurements obtained at the end of the 30-day BMP tests for the sludge treated for the BH55+42 and TH were 76% and 74%, respectively, but only 64% for the untreated sludge. Accordingly, sludge digestion coupled with BH55+42 or TH pretreatments can achieve a higher biodegradability in 15 days than sludge digestion without pretreatment in 30 days.

3.5. VSS Reduction by Anaerobic Digestion

Figure 6 presents the reduction of VSS concentrations in sludge at the 10th, 20th, and 30th days of the BMP tests. At the end of the BMP tests, total VSS reductions in the sludge-treated BH42, BH42+55, BH55+42, BH55, and TH, as well as that of the untreated sludge, were 45.8%, 46.3%, 47.7%, 43.9%, 43.1%, and 42.7%, respectively, (Figure 6). Thus, the BH and TH pretreatment did not demonstrate an evident enhancement of the overall VSS reduction in this study. The change in the VSS content of BMP mixed liquor reflected the growth of anaerobic microbes in the anaerobic digestion system and the destruction rate of VSS contents of the feedstock. VSS destruction during the BMP stage might be offset by the growth of anaerobic microbes. Thus, the reduction of VSS in the anaerobic digestion stage can be affected by the feed sludge solid contents, sludge composition, solid loading rates, SRT, and microbial community structures of the anaerobic digestion systems. Thus, further studies are needed to determine the optimal process conditions of anaerobic digestion for VSS reduction.

3.6. Comparison of the Results with the Three Batches of Sludge for Selected TH/BH-AD Conditions

The first batch test on BH and TH treatments showed that BH55+42 can result in a higher methane production than BH42+55, and comparable methane production enhancement can be achieved by TH and BH treatment. In order to verify these results, two other batches of tests were conducted using sludge taken from the same sources at different times. Among the total three batches of tests, TH was included in the first two batches while the third batch did not include TH but a BH treatment at 75 °C (BH75). As shown in Table 1, the raw sludge used in the second batch test had higher TS, TCOD, sCOD, VFA, and TSS concentrations than the first and third batches of sludge. Similar hydrolysis performance trends during the BH and TH treatment tests were observed in terms of VSS reduction and the production of sCOD and VFAs. The sludge solubilization for TH, BH42+55, BH55+42, and BH42...
for the batch 1 and 2 treatments were 17%, 13%, 13%, and 10% for batch 1, and 19%, 15.8%, 15.4% and 12.7% for batch 2, respectively. For the third batch, the sludge solubilization was 11% for BH42, 13.6% for BH42+55, 14.6% for BH55+42, 15.3% for BH55, and 20.6% for BH75. These results demonstrate the consistent hydrolysis performance trend caused by the BH and TH treatment for sludge solubilization.

The BMP tests that were conducted using all three batches of sludge consistently showed that the sludge treated by BH55+42 had the highest methane production enhancement (Table 3). Methane yields from the first, second, and third batches of the sludge treated by BH55+42 were 282 ± 9.5 NmL/g, 255 ± 3.4 NmL/g, and 267 ± 3 (with an overall average of 268) NmL/g COD fed at the 15th day of BMP incubation, which was 21%, 23%, and 19% higher, respectively, than those with the controls. Accordingly, methane yields from the two batches of TH-treated sludge (the third batch did not have a TH condition) were 266 ± 2.4 NmL/g and 249 ± 3.7 NmL/g COD fed, corresponding to 14% and 20% enhancement, respectively, compared to the controls. Similar tendencies were also observed at the 30th day of the BMP test. Overall, although methane yields with the first batch sludge were slightly higher than the other two batches, all three batches of sludge showed that the BH55+42 treatment had the highest methane production enhancement compared to other BH treatment conditions.

Table 3. Comparison of first, second, and third batches of the BMP tests.

| Batch Test | Condition | CH₄ Volume (NmL) | CH₄ Yield (NmL/g COD Fed) | CH₄ Yield (NmL/g VS Fed) | Sludge Solubilization (%) | CH₄ Enhancement (%) |
|------------|-----------|------------------|---------------------------|--------------------------|---------------------------|---------------------|
| Batch 1    | Control   | 87.1 ± 1         | 234.3 ± 5                 | 395.1 ± 7                | -                         | -                   |
|            | BH42+55   | 90.2 ± 2         | 242.1 ± 7                 | 410.4 ± 7                | 15.0                      | 3.5                 |
|            | BH55+42   | 105.1 ± 2        | 282.2 ± 10                | 477.4 ± 10               | 16.0                      | 21.0                |
|            | TH        | 99.3 ± 1         | 266.1 ± 2                 | 449.9 ± 4                | 19.0                      | 14.0                |
| Batch 2    | Control 2 | 107.4 ± 1        | 208.1 ± 2                 | 377.1 ± 5                | -                         | -                   |
|            | BH42+55   | 122.3 ± 5        | 238.2 ± 9                 | 431.3 ± 8                | 13.0                      | 15.0                |
|            | BH55+42   | 131.2 ± 2        | 255.3 ± 3                 | 461.1 ± 6                | 13.0                      | 23.0                |
|            | TH        | 128.4 ± 2        | 248.9 ± 4                 | 451.4 ± 7                | 17.0                      | 20.0                |
| Batch 3    | Control 3 | 105.2 ± 1        | 223.8 ± 3                 | 387.1 ± 5                | -                         | -                   |
|            | BH42+55   | 116.3 ± 3        | 248.4 ± 5                 | 429.4 ± 8                | 13.6                      | 11.0                |
|            | BH55+42   | 125.4 ± 2        | 267.4 ± 3                 | 462.4 ± 6                | 14.6                      | 19.0                |
|            | BH75      | 126.5 ± 3        | 269.8 ± 6                 | 467.1 ± 10               | 20.0                      | 20.0                |

3.7. Microbial Community Structures of the Anaerobic Mixed Liquor

Selected samples were taken from the BMP bottles for sequencing by the Illumina MiSeq system to characterize the microbial community structures under different BH-AD conditions. Figure 7a shows the dominant bacteria phyla identified in the control (untreated sludge), and sludge treated with BH42+55, and BH55+42. The bacterial phyla of Bacteroidetes, Proteobacteria, Firmicutes, Thermotogae, Chloroflex, Cyanobacteria, and Verrucomicrobia were identified as the dominant phyla in all of the tested sludge samples, but the distribution of these phyla varied with the BH methods. The phyla of Bacteroidetes, Proteobacteria, and Firmicutes showed the highest OTU percentages in the untreated sludge, followed by the sludge treated with BH55+42, and then BH42+55.

At the genus level, Sphingobacterium, Flavobacterium, Pedobacter, Sedimentibacter, Clostridium, and Bellilinea presented in all the tested samples with the OTU percentages higher than 3% (Figure 7b). The Sphingobacterium genus contained bacteria that are non-formative and non-proteolytic, but some Sphingobacterium can hydrolyze carbohydrates [39]. The genus of Flavobacterium contains aerobic bacteria, but some species, (e.g., F. hydatis and F. succinicans) can grow anaerobically under certain conditions. Most of the Flavobacterium species can degrade polysaccharides and proteins [40].
Figure 7. Taxonomic classification of the dominant microbial communities; (a) classification at phylum level, and (b) classification at genus level.

Compared to other tested sludge, sludge treated with BH42+55 showed higher proportions of Pedobacter, Sedimentibacter, and Clostridium. Pedobacter bacteria are aerobic chemoorganotrophic with an oxidative type of metabolism, and the mechanism that it presented in the BMP sludge is not clear. The genus Sedimentibacter consists of amino acid and pyruvate-utilizing anaerobic bacterium [41], while Clostridium contains many species that are acetogens, which can produce acetate from H₂–CO₂ and glucose-fermenting pathways [42]. Clostridium was only detected as a major genus (OUT > 3%) in the sludge treated with BH42+55 and the control.

The Methanosaeta and Methanolinea were the two most dominant methanogenic genera in all of the tested sludge samples. The members of Methanosaeta are acetoclastic methanogens that split acetate methane and CO₂ [43], while Methanolinea are hydrogenotrophic methanogens that utilize H₂ and formate for growth and methane production [44]. Acetate-utilizing methanogens normally dominate in mesophilic digestion systems. However, the presence of hydrogenotrophic methanogens in mesophilic anaerobic digestion systems is also critical for achieving high methane production, because they can help maintain a low system H₂ partial pressure by serving as H₂ scavengers. Methanogens can only use acetate, CO₂, H₂, and some one-carbon organics. The conversions of some fermentation productions...
(e.g., propionic and butyric acids) to methane are only thermodynamically favorable at low \( \text{H}_2 \) partial pressure. Thus, the presence of \( \text{H}_2 \)-consuming organisms in anaerobic digestion systems is important to achieve an effective conversion from organics to methane gas.

### 3.8. Discussion

This study focused on the effects of BH and TH pretreatment on the sludge solubilization and methane production of the combined primary and secondary sludge. The pretreatment results showed that the TH treatment at 165 °C for 30 min degraded around 17% of particulate COD to sCOD, and the BH treatment at temperatures between 42–55 °C caused around 10% to 14% of particulate COD dissolution. VFAs were identified as an important end product of both the BH and TH treatment of the combined sludge. For the TH treatment, VFAs are mainly produced from the degradation of unsaturated lipids [7], while for the BH treatment, VFAs are produced in the acidogenesis and acetogenesis of the sludge. The GC analysis showed that there was a significant amount of propionic acid presented in both TH and BH-treated sludge. The accumulation of propionic acid in anaerobic digestion could cause the inhibition of the activity of fermenting bacteria and methanogens, causing process instability. In this study, the ratios of propionic to acetic acids were determined to be 0.83 for the TH treatment, and between 0.7–0.73 for the BH treatments (Table 2). These values are higher than those reported in activated sludge treated by BH at conditions similar to this study. Ding et al. [28] and Chen and Chang [25] showed that the ratios of acetic acid to propionic acid were 0.29 and 0.41, respectively, in activated sludge treated by BH at 42 °C. The high propionic acid concentration in the combined sludge could be caused by the high propionic acid concentration in primary sludge. In this study, the combined sludge initially contained 492.0 mg/L of propionic acid, compared to only 18.7 mg/L in activated sludge reported in [28]. The relative high propionic concentrations in the BH-treated sludge also indicates the limited degradation of propionic acid during the three-day BH period. The biological oxidation of propionic acid to acetic acid has a standard free energy change of +76 kJ/mol, and is only possible at a hydrogen partial pressure lower than \( 10^{-4} \) atm [45]. Since \( \text{H}_2 \) is one of the main end products of fermentation, the degradation of propionic acid can only be achieved by an effective syntrophic association between propionic-oxidizing bacteria and hydrogen-consuming methanogens.

In the BH treatment, the short retention time could have limited the population of slow growing \( \text{H}_2 \)-consuming methanogens, which could cause a low degradation of propionic acid in the BH-treated sludge. However, there was no accumulation of propionic acid observed in the subsequent BMP test, indicating that there was a balanced population of syntrophic propionate-oxidizing bacteria and hydrogen-consuming methanogens. The Illumina sequencing also confirmed the co-existence of the syntrophic bacteria group (Figure 7b) and hydrogenotrophic methanogens in the BMP mixed liquid.

With a direct comparison between TH and BH, this study showed that the BH treatment at temperatures between 42–55 °C for three days and the TH treatment at 165 °C for 30 min can achieve comparable biogas production enhancements that range from 10% to 23%, which are comparable to the results reported by other research studies. Ferrera et al. [46] reported that BH treatment at 70 °C for nine hours could enhance biogas production by 30% in a 20-day thermophilic AD treatment of the thickened combined sludge. Bolzonella et al. [47] reported that the BH pretreatment at 65 °C for two days in the thermophilic AD (55 °C) of activated sludge enhanced methane production by 8.9%, while Ge et al. [29] showed a 25% enhancement of methane production by BH in the mesophilic digestion (35 °C) of primary sludge. For the TH pretreatment, Valo et al. [20] reported that the TH treatment at temperatures between 130–170 °C for 30 min could enhance methane production by 21% to 45% in the 20-day mesophilic AD (35 °C) of activated sludge, while Wilson et al. [17] reported a biogas enhancement up to 24% to 59% by the TH pretreatment at 150 °C and 170 °C, respectively, in the anaerobic digestion of mixed sludge at temperatures between 35–42 °C. While these studies showed that the TH treatment can achieve more than 50% enhancement in methane production, Ding et al. [28] reported around 10% enhancement on methane production by both TH treatment at 165 °C for 30 min and BH treatment between 42–55 °C. Thus, the enhancement of biogas production by the BH and TH
treatments could vary significantly with the pretreatment conditions, sludge properties, mixture ratio of primary and secondary sludge, and AD process conditions.

This study also showed that BH55+42 could achieve better methane production enhancement than BH42+55. The apparent difference in biogas production enhancement achieved by BH55+42 and BH42+55 might suggest that the carryover of the microbial population from the BH stage to the AD stage might affect the performance of the whole BH-AD process. Chen and Chang [25] showed that the protein-fermenting and carbohydrate-fermenting bacteria obtained in BH35, BH42, and BH55 were significantly different, and that the bacterial community structure that was developed at BH 42 °C had a higher similarity to that formed at BH 35 °C than the one developed at BH 55 °C. The BH55+42-AD treatment includes BH treatment at 55 °C for 1.5 days, followed by BH at 42 °C for 1.5 days, and then, the 30-day BMP test at 35 °C AD. With such a treatment sequence, an initial higher BH temperature could accelerate the dissolution of extracellular polymeric substances (EPS), and the disintegration of sludge floc aggregates. Meanwhile, BH at 42 °C could establish a microbial community that can be easily adapted to the subsequent 35 °C AD condition. Additionally, Nakasaki et al. [48] found that the methane production rate of AD can be correlated with archaea cell density in the digester, whereas no clear relationship was found between bacterial population and methane production. The BH55+42 and BH42+55 conditions could result in different archaea to bacterial ratios in the subsequent AD processes, which could further affect the methane production. Although the Illumina sequencing method in this study identified the dominant bacterial and methanogen genera, the population densities of different organism groups are yet to be determined in the future study.

In this study, mass balance analyses were also conducted to determine the ratios of conversion of COD to methane. The average COD conversions to CH\(_4\) were determined to be 0.20 NLCH\(_4\)/g COD, 0.30 NLCH\(_4\)/g COD, 0.31 NLCH\(_4\)/g COD, and 0.32 NLCH\(_4\)/g COD removal for the control, BH42+55, BH55+42, and TH pretreatments, respectively. These values were lower than the ideal COD to methane conversion rate of 0.35 LCH\(_4\)/g COD, implying that around 8.6% to 15.0% COD might be consumed through other bacterial metabolism pathways rather than by methanogenesis. For example, it is well known that acetate can be completely oxidized to CO\(_2\) by sulfate reducers through the acetyl–CoA pathway or a modified acetyl–CoA pathway [49]. In addition, some organisms, such as anaerobic methanotrophic archaea (ANME), were recently identified to be able to oxidize methane in coupling with a sulfate reducer through a two-step reaction: the formation of methyl sulfide from CH\(_4\) and CO\(_2\) by archaea and the subsequent consumption of methyl sulfide by sulfate reducers [50]. Thus, the microbiological mechanisms of the methane production enhancement by TH and BH treatments still needs to be explored in order to understand the relationship between methane production and microbial consortia in BH-AD systems.

4. Conclusions

This study assessed BH treatments with four different temperature designs (42–55 °C) for a total three days of SRT and TH at 165 °C for 30 min by using the bench-scale hydrolysis and BMP test methods. The results showed that the BH temperatures and the sequential order of BH processes of different temperatures can significantly affect the hydrolysis performance and biogas production. The TH treatment caused VSS reduction by 22.6%, and the BH treatments caused VSS reduction by 17.5% to 25.9%; the VSS reduction increased with BH temperature. The soluble chemical oxygen demand (sCOD) content of the sludge increased by 377.5%, 323.8%, 301.3%, 286.9%, and 221.7% by the TH, BH55, BH42+55, BH55+42, and BH42 treatments, respectively. The ratio of ΔVFA/ΔsCOD was determined to be approximately 40% for BH-treated sludge, while it is only 6% for TH, indicating that the BH pretreatment can produce much more VFA than the TH treatment, which is considered an advantage for the methanogenesis stage. BMP test results also showed that sludge treated by BH55+42 achieved higher methane production enhancement (up to 23% higher than the untreated sludge) than other BH conditions, and was comparable with that achieved by the TH treatment at 165 °C (20% methane enhancement). The methane yields of the combined sludge treated by TH and BH55+42
were in the ranges of 248.9 NmLCH$_4$/g COD to 266.1 NmLCH$_4$/g COD fed and 255.3 NmLCH$_4$/g COD to 282.2 NmLCH$_4$/g COD fed. Hence, this study is of significance for the optimization of BH pretreatments for AD performance enhancement.

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