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Impacts of Temperature and Solids Retention Time, and Possible Mechanisms of Biological Hydrolysis Pretreatment on Anaerobic Digestion

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Abstract: Anaerobic digestion (AD) has benefits in sludge management, energy recovery, and pathogen reduction. In order to better understand the mechanisms of biological hydrolysis (BH) pretreatment on AD, biochemical methane potential (BMP) and continuous stirred-tank reactor (CSTR) tests were utilized to compare untreated municipal combined sludge with pilot-scale BH pretreated sludge. During the BH process, there was 15%, 30%, and 33% (*w/w*) volatile solids (VS) reduction after BH at 42 °C (BH42) for 24, 48, and 72 h, respectively; under BH61 (42 °C for 36 h and 61 °C for 6 h), and there was 10% and 30% (*w/w*) overall VS reduction after 36-h and 42-h hydrolysis, respectively. BMP results showed that BH42-pretreated sludge had 22.6% enhancement of methane yield compared to untreated sludge, and BH61 pretreated sludge had 29.4% enhancement of methane yield. Both temperature and solids' retention time (*SRT*) contributed to the enhanced AD performance within 36 h, while temperature played more important roles after 36-h BH pretreatment. CSTR tests confirmed the acceleration of anaerobic digestion by BH pretreatment, and higher enhancement was observed when *SRT* of anaerobic digestion was shorter than 16 days. Through a literature review of BH-related studies, the possible mechanisms were highlighted for further optimization on the scale-up systems in order to reduce carbon footprint and operating expenditure for wastewater treatment plants.

Keywords: anaerobic digestion; biochemical methane potential (BMP); biological hydrolysis; continuous stirred-tank reactor (CSTR); resource recovery; volatile solids reduction

1. Introduction

Anaerobic digestion (AD) can recover methane gas from municipal sludge, source separated organics, or agro-waste. The on-site anaerobic digesters could save transportation or disposal costs by reducing 40–80% of biosolids or biowastes [1]. Meanwhile, biogas (mainly composed of methane and carbon dioxide) is captured and can be used for energy recovery, thus reducing greenhouse gas emissions [2]. Moreover, the processed sludge could be a good source of soil conditioner and slow-release fertilizer [3].

US Environmental Protection Agency (EPA) regulates pathogen limits and management practices for direct land application, and specific time–temperature regimes are required to obtain Class A biosolids [4]. Different disintegration methods have been developed to enhance AD performance, to accelerate the AD process, and/or to meet Class A biosolid requirements [5–7]. Among them, biological hydrolysis (BH) pretreatment has been proven to be a relatively simple and energy-efficient process, showing high potential upon further optimization of the AD process [8–10].

There are various reported studies on BH enhancement and scale-up designs [11–15], among which multi-stage hydrolysis provides a good platform to evaluate temperature and solid retention times (*SRT*) effects on sludge pretreatment and anaerobic digestion. In this study, two different BH-pretreated sludges from a 4- to 6-stage BH pilot plant were selected: (1). BH at 42 °C for 24–72 h (BH42); and (2). BH at 42 °C for 36 h and at 61 °C for 6 h (BH61). The impacts of temperature and *SRT* on BH pretreatment were investigated through comparing biogas generation and volatile solids reduction (VSR). The possible BH mechanisms and performance evaluation were discussed by summarizing previously reported studies.

2. Materials and Methods

2.1. Biological Hydrolysis-Anaerobic Digestion (BH-AD) Process Flow Design

The biological hydrolysis–anaerobic digestion (BH-AD) process flow diagram is presented in Figure 1. The BH-AD pilot plant includes 4- or 6-stage BH reactors (300 L each), and an anaerobic digester (4000 L). The sludge fed into the system was the combination of real-time primary sludge (total solids 25–30 g/L) and thickened waste-active sludge (WAS, total solids 30–35 g/L) by 1:1 volume ratio through two sludge pumps from Guelph Wastewater Treatment Plant (WWTP).

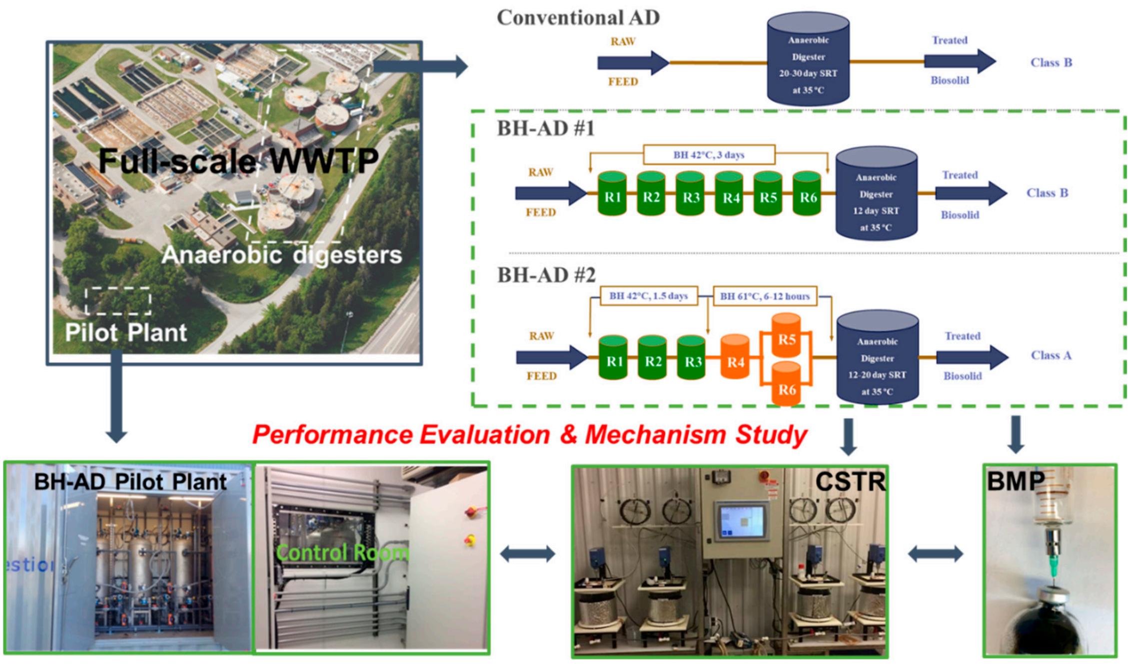

Figure 1. Biological hydrolysis–anaerobic digestion (BH-AD) process flow design and performance evaluation.

Two pilot-plant test modes under various SRTs were tested: (1). BH-AD #1 test mode: BH reactors (R1 to R6) were operated as serial plug flow mesophilic reactors at 42 °C for 72 h, followed by 12-day anaerobic digestion at 37 °C (BH42—12 day). (2). BH-AD #2 test mode: the first three BH reactors (R1 to R3) were the same as BH-AD #1 (42 °C for 36 h), and R5 and R6 were operated as in-parallel batch pasteurization reactors (61 °C for 6 h), followed by 12–20 day anaerobic digestion at 37 °C (BH61—12 day, BH61 for 16 days, and BH61 for 20 days, respectively).

2.2. Biochemical Methane Potential (BMP) Tests

Both BH42 and BH61 processes were evaluated by BMP tests, which followed that of previous studies [16,17]. Briefly, substrate sludge (BH42- or BH61-pretreated sludge) and inoculum were filled into the 160 mL serum bottles, and placed in an incubator with constant shaking at 100 rpm at 35 °C. The food to microorganism (F/M) ratio (total COD of substrate/volatile suspended solids of inoculum) was between 1.0–1.6. Biogas composition was determined by the GC (HP6890, Agilent Technologies, Santa Clara, CA, USA) with HP-PLOT Molesieve GC column (30 m × 0.530 mm, Agilent Technologies, USA) and a thermal conductivity detector (TCD). The time-temperature setting of GC oven was: 0–7 min, 35 °C; 7–13 min, increased from 35 °C to 205 °C at a rate of 28 °C/min; 13–14 min, held at 205 °C for 1 min. The temperature of the injector and detector was set at 200 °C and 150 °C, respectively. Argon was used as the carrier gas, and methane was quantified by comparing with biogas mixed standards (Praxair Inc., Saskatoon, SK, Canada). The methane yield was calculated based on Equations (1) and (2)

$$M1 = \frac{Vtc}{VSr} \tag{1}$$

$$M2 = \frac{Vtc}{tCODi} \tag{2}$$

where $M1$ is the methane yield based on VS removal (L CH_4/kg VSr), and $M2$ is the methane yield based on initial total COD of mixed liquor in each BMP bottle (L CH_4/kg COD); Vtc is the cumulative methane volume against blank (i.e., inoculum only) normalized at standard temperature and pressure (STP); VSr is the volatile solids removal (kg) during the tests; $tCOD_i$ is the initial total COD of substrate sludge in each BMP bottle.

The biodegradability of sludge was calculated based on Equation (3)

$$BD\ (\%) = \frac{Vc}{Vt} \times 100 \tag{3}$$

where BD (%) is the biodegradability; V_c is the cumulative methane volume (against blank) from 1 kg COD of selected substrate at STP; and V_t is the theoretical methane yield from 1 kg COD of 100% biodegradable substrate (i.e., 350 L methane generation/1 kg COD) at STP.

2.3. Continuous Stirred-Tank Reactor (CSTR) Tests

The performance of BH61 pretreated sludge at different BH stages was evaluated using 20 L CSTR testing units. The temperature of each unit was maintained at 37 °C, and SRT (12–20 days) were matched with the BH-AD pilot plant. The inoculum was collected from a 4000 L anaerobic digester in the pilot plant. CSTR feeding occurred on weekdays in batches with raw sludge (RS) and BH61 pretreated sludge (R2 and R6), as indicated in Table 1. CSTR units were stabilized for around 3 to 5 times the designed SRT (around 40–60 days) before evaluation, and each test mode was consistently operated for at least three times the SRT (between 38 to 90 days). Organic loading rate (OLR) was calculated based on Equation (4)

$$OLR = \frac{VSf\%}{SRT} \tag{4}$$

where $VS_f\%$ is the concentration of feeding substrate sludge (g/L) to each CSTR unit; SRT is the solids' retention time.

The methane yield was calculated based on Equation (5)

$$M3 = \frac{Vd}{VSrd} \tag{5}$$

where $M3$ is the CSTR methane yield based on VS removal (L CH_4/kg VSr), V_d is the total methane volume of each CSTR unit per day normalized at STP; VS_{rd} is the volatile solids reduction between feeding substrate and wasting sludge per day.

Table 1. Biological hydrolysis (BH) pretreatment conditions in BH-AD pilot plant.

BH Pretreatment	BH Pretreated Sludge	BH Stage 1 (Plug-Flow) Temperature/*SRT*	BH Stage 2 (Batch) Temperature/*SRT*
BH42 (Pilot plant was consistently operated for around 120 days)	RS′	-	-
	R2′	42 °C/24 h	-
	R4′	42 °C/48 h	-
	R6′	42 °C/72 h	-
BH61 (Pilot plant was consistently operated for around 400 days)	RS	-	-
	R2	42 °C/36 h	-
	R6	42 °C/36 h	61 °C/6 h

2.4. Sludge Analysis

Total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VSS) were determined by standard methods (Method 2540-1997, and EPA Method 160.4), and volatile fatty acids (VFA) and chemical oxygen demand (COD) were determined using Hach test vials (Hach, London, ON, Canada). All tests were conducted in triplicate, and data are expressed as the mean ± standard deviation.

3. Results

3.1. Characteristics of Substrate and Inoculum Sludge under Different Test Modes

BH42- or BH61-pretreated sludge from different reactors in BH-AD pilot plant was utilized as feed sources (Table 1) for biochemical methane potential (BMP) or continuous stirred-tank reactor (CSTR) tests. The sludge characteristics, including biosolid contents, COD and volatile fatty acids (VFA), are listed in Table 2. The sludge for BMP tests was determined based on the collected samples in duplicate from different reactors, and the sludge for CSTR tests were analyzed based on overall average of daily feeding sludge (30–90 days of each test).

Untreated raw sludge (RS) was under relatively stable condition with TS ranging from 24 to 28 g/L during the entire test. The trends of biosolid and total COD reductions during BH42 and BH61 pretreatment were presented in Figure 2. There was 15%, 30%, and 33% (*w/w*) overall VS reduction after biological hydrolysis at 42 °C (BH42) for 24, 48, and 72 h, respectively (Figure 2a). TS followed similar trends, while total COD reduction was 12%, 27%, and 40% (*w/w*) at 24, 48, and 72 h, respectively. The biosolid reduction slowed down after 48 h of BH42 process, while total COD kept decreasing over time. The trends indicated that the hydrolysis was the major stage within 48 h, while methanogenesis played a more important role after 48 h under BH42 condition. Under BH61 test mode (Figure 2b), there was 10% and 30% (*w/w*) overall VS reduction observed at 36 and 42 h *SRT*, respectively. Both TS and TCOD followed similar trends, which confirmed that the hydrolysis was dominant during 42 h BH61 processes.

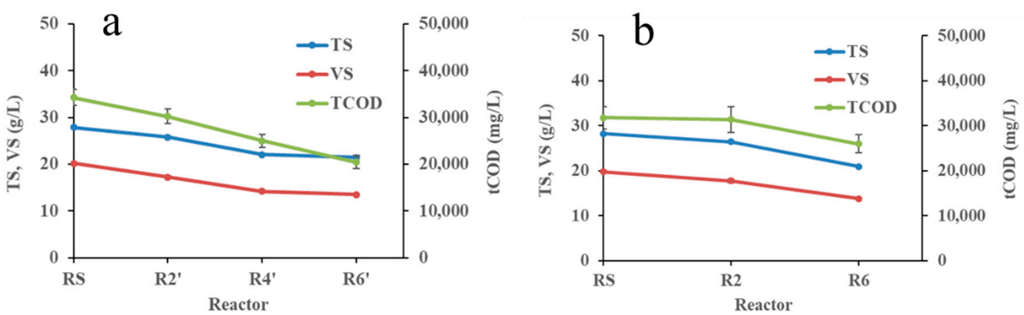

Figure 2. Sludge characteristics of tested biological hydrolysis (BH) reactors: (**a**) sludge sampled in the pilot plant during BH42 test mode for biochemical methane potential (BMP) tests, (**b**) sludge sampled in the pilot plant during BH61 test mode for BMP tests*. (*the results were calculated based on sludge samples for BMP tests (2 samples of each reactors); RS: raw sludge; R2′: BH at 42 °C for 24 h; R4′: BH at 42 °C for 48 h; R6′: BH at 42 °C for 72 h; R2: BH at 42 °C for 36 h under BH61 mode; R6: BH at 42 °C for 36 h and then 61 °C for 6 h, as listed in Table 1).

Table 2. Characteristics of substrate and inoculum sludge at various test modes.

Parameter/Test Mode		pH	TS g/L	TSS g/L	VS g/L	VSS g/L	TCOD mg/L	SCOD mg/L	VFA mg/L	OLR g VS/L/d	F/M TCOD/VSS
BMP *: BH42 test mode	Inoculum	7.69 ± 0.05	18.35 ± 0.12	16.69 ± 0.07	10.78 ± 0.19	10.03 ± 0.08	15,610 ± 1513	687 ± 50	241 ± 12	-	-
	RS	6.55 ± 0.01	27.85 ± 0.27	26.53 ± 0.25	20.22 ± 0.26	19.99 ± 0.93	34,275 ± 1722	2448 ± 211	1489 ± 349	-	1.65
	R2′	7.13 ± 0.07	25.75 ± 0.24	24.39 ± 0.21	17.25 ± 0.30	16.5 ± 0.22	30,225 ± 1534	3688 ± 341	1974 ± 283	-	1.63
	R4′	7.25 ± 0.08	22.04 ± 0.10	21.2 ± 0.13	14.22 ± 0.11	13.88 ± 0.13	24,975 ± 1428	3320 ± 237	1394 ± 154	-	1.35
	R6′	7.49 ± 0.07	21.42 ± 0.10	20.5 ± 0.17	13.5 ± 0.12	13.02 ± 0.09	20,450 ± 1410	2148 ± 203	696 ± 76	-	1.10
BMP *: BH61 test mode	Inoculum	7.95 ± 0.02	20.74 ± 0.26	20.11 ± 0.28	12.43 ± 0.24	12.02 ± 0.30	19,775 ± 1809	871 ± 30	686 ± 120	-	-
	RS	7.01 ± 0.15	28.19 ± 0.11	27.55 ± 0.15	19.76 ± 0.21	18.07 ± 0.34	31,780 ± 2519	2348 ± 138	1232 ± 208	-	1.19
	R2	7.31 ± 0.11	26.43 ± 0.25	25.75 ± 0.40	17.71 ± 0.34	17.39 ± 0.31	31,400 ± 2908	4116 ± 221	1986 ± 107	-	1.11
	R6	7.82 ± 0.14	20.95 ± 0.16	19.73 ± 0.29	13.78 ± 0.14	12.91 ± 0.23	25,975 ± 1975	6420 ± 285	3068 ± 308	-	1.07
CSTR **: BH61–12 day	Inoculum	-	-	-	-	-	-	-	-	-	-
	RS	7.22 ± 0.12	27.8 ± 0.36	-	19.48 ± 0.21	-	-	-	-	1.62	-
	R6	7.72 ± 0.15	19.5 ± 1.59	-	12.31 ± 0.9	-	-	-	-	1.02	-
CSTR **: BH61–16 day	Inoculum	-	-	-	-	-	-	-	-	-	-
	RS	6.96 ± 0.15	26.13 ± 1.8	-	17.5 ± 1.24	-	-	-	-	1.09	-
	R6	7.72 ± 0.18	19.78 ± 0.38	-	12.38 ± 0.29	-	-	-	-	0.77	-
CSTR **: BH61–20 day	Inoculum	-	-	-	-	-	-	-	-	-	-
	RS	7.01 ± 0.15	23.92 ± 2.26	-	17.28 ± 1.78	-	-	-	-	0.86	-
	R6	7.82 ± 0.24	17.8 ± 0.82	-	11.08 ± 0.53	-	-	-	-	0.55	-

(* The results were based on sludge sampled in the pilot plant for BMP tests in duplicate; ** The results were based on overall average of sludge sampled in the pilot plant during 30–90 days CSTR tests. OLR: organic loading rate; F/M: food to microorganism ratio; BMP: biochemical methane potential; CSTR: continuous stirred-tank reactor; RS: raw sludge; R2′: BH at 42 °C for 24 h; R4′: BH at 42 °C for 48 h; R6′: BH at 42 °C for 72 h; R2: BH at 42 °C for 36 h; R6: BH at 42 °C for 36 h and then 61 °C for 6 h, as listed in Table 1).

3.2. Evaluation of BH42 or BH61 Pretreatment by BMP Tests

The BH42 or BH61 pretreated sludge were evaluted by BMP tests (Figures 3 and 4), and methane yield based on VS removal (L CH_4/kg VSr) are presented in Figures 3a and 4a. BH42 pretreatment at 24 h (R2′), 48 h (R4′), and 72 h (R6′) had 8.1%, 15.8%, and 22.6% enhancement, respectively, compared to untreated sludge (Figure 3a). BH61 pretreatment at 36 h (R2) and 42 h (R6) had 11.4%, and 29.4% enhancement, respectively (Figure 4a). It is worth noting that the methane generation (after subtracting blank) from VS reduction (g) was assumed to be fully contributed by the substrate; however, the interference or synergistic effect between substrate and inoculum was not captured.

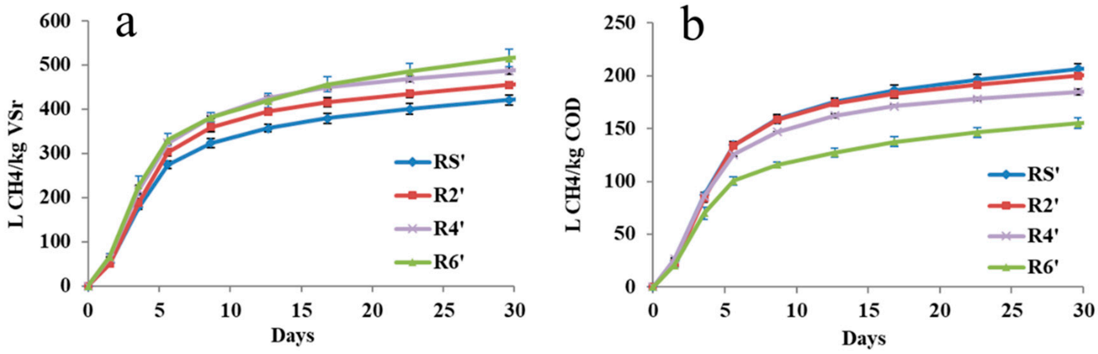

Figure 3. Methane yields of BH42-pretreated sludge by biochemical methane potential (BMP) tests: (**a**) methane yields (*M*1) based on VS removal in the substrate (L CH_4/kg *VSr*); (**b**) methane yields (*M*2) based on initial total COD of the substrate (L CH_4/kg TCOD). (the methane yields have been normalized at standard temperature and pressure (STP); RS′: raw sludge; R2′: BH at 42 °C for 24 h; R4′: BH at 42 °C for 48 h; R6′: BH at 42 °C for 72 h as listed in Table 1).

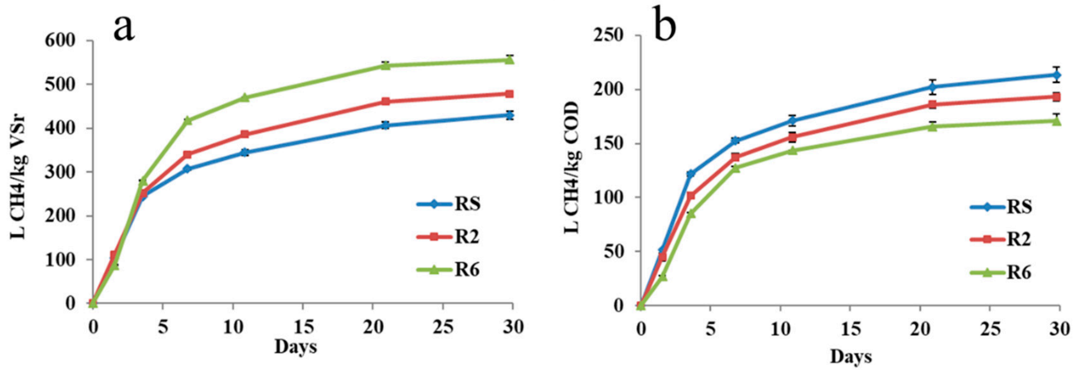

Figure 4. Methane yields of BH61 pretreated sludge by biochemical methane potential (BMP) tests: (**a**) methane yields (*M*1) based on VS removal in the substrate (L CH_4/kg *VSr*); (**b**) methane yields (*M*2) based on initial total COD (kg COD) of the substrate (L CH_4/kg TCOD). (the methane yields have been normalized at STP; RS: raw sludge; R2: BH at 42 °C for 36 h; R6: BH at 42 °C for 36 h and then 61 °C for 6 h as listed in Table 1).

The curves regarding biodegradability of mixed liquor (i.e., inoculum and substrate) of various BH pretreatments are presented in Figures 3b and 4b. The biodegradability of untreated raw sludge was 57.1% and 60.9%, respectively, after 30-day BMP tests. The difference (3.7%) in biodegradability was mainly caused by the changes in sludge characteristics during two BH test modes in the pilot plant. The biodegradability of BH42-pretreated sludge at 24 h (R2′), 48 h (R4′), and 72 h (R6′) was 57.1%, 52.9%, 44.3%, respectively; the biodegradability of BH61-pretreated sludge at 36 h (R2) and 42 h (R6) was 55.2% and 48.8%, respectively, after 30-day BMP tests.

As aforementioned, the volatile solids (i.e., organic matter) in raw sludge gradually decreased during BH processes (Figure 2), and the components left were less biodegradable. BH61 at 42 h (R6) had higher enhancement than BH42 at 72 h (R6′), which indicated that both temperature and *SRT* contributed to the enhanced performance within 36 h, while temperature (up to 61 °C in this study) might play more important roles after 36-h BH. Moreover, BH61 pretreatment showed an advantage in pathogen reduction in order to meet Class A biosolid requirements, and energy efficiency compared with other energy-intensive techniques.

3.3. Evaluation of BH61 Pretreatment by CSTR Tests

In order to better understand the acceleration of the BH61 process on AD, CSTR tests at various *SRT* (12–20 days), anaerobic digestion was conducted. As presented in Figure 5a, BH61-pretreated sludge (R6) showed an overall average of 34.1% enhancement on methane generation (L CH4/kg *VSr*) compared with untreated raw sludge (RS) under 12-day *SRT* CSTR tests. The average methane yield (i.e., 521 L CH4/kg *VSr*) and overall VS reduction (i.e., 57.0%) of BH61-pretreated sludge under 12-day SRT was comparable with RS under 20-day *SRT* tests (Figure 5a,c).

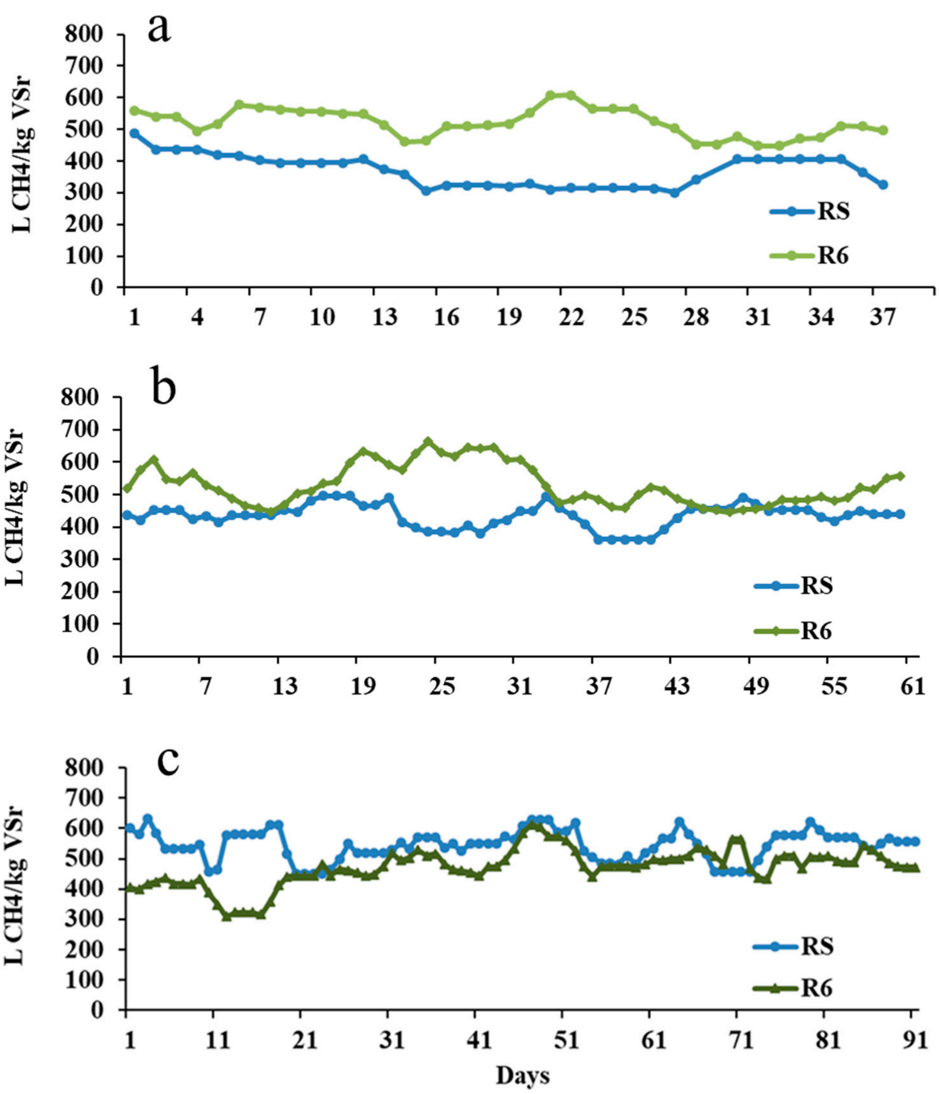

Figure 5. Continuous stirred-tank reactor (CSTR) tests on BH61 pretreated sludge: (**a**) methane yields (*M*3) based on VS removal in the substrate (L CH_4/kg *VSr*) under 12-day *SRT* anaerobic digestion; (**b**) methane yields (*M*3) under 16-day *SRT* anaerobic digestion; (**c**) methane yields (*M*3) under 20-day *SRT* anaerobic digestion. (the methane yields have been normalized at STP; RS: raw sludge; R6: BH at 42 °C for 36 h and 61 °C for 6 h as listed in Table 1).

Under 16-day *SRT* anaerobic digestion, BH61-pretreated sludge showed an overall average of 21.7% enhancement on methane yield (Figure 5b). However, there was no significant difference ($p < 0.05$) in methane generation under 20-day *SRT* CSTR tests (Figure 5c). The average organic loading rate (OLR) is listed in Table 2. The difference of OLR was mainly caused by the changes of *SRT*, and reduced total COD (kg/day) of BH-pretreated sludge (under the same feeding volume) in CSTR tests. In general, BH61 pretreatment had a more remarkable enhancement of biogas generation and VS reduction when the *SRT* of anaerobic digestion was less than 16 days, and the results confirmed that BH pretreatment could accelerate anaerobic digestion by up to 42.9%.

4. Discussion

Anaerobic digestion (AD) involves four major stages (e.g., hydrolysis, acidogenesis, acetogenesis, and methanogenesis), and other possible pathways (anaerobic oxidation, homoacetogenesis, etc.) [18,19]. The degradation rate was reported to be mainly limited by the first step, in which complex macromolecules (polysaccharides, proteins, lipids, or humic acids, etc.) are hydrolyzed into soluble compounds with lower molecular weights [20]. Most sludge pretreatment or disintegration methods aimed to separate out the hydrolysis stage to enhance AD performance for energy-neutral operation and/or accelerate the overall process for increasing treatment capacity.

The reported BH mechanisms or findings are summarized in Table 3. Through reviewing the proposed mechanisms, as well as analyzing BMP and CSTR test results, some highlights of BH pretreatment are addressed as follows:

Table 3. Review of previous studies on biological hydrolysis (BH)-correlated processes and proposed mechanisms/findings.

Year	Substrate Sludge	BH Pretreatment	Proposed Mechanism/Findings	Reference
1981	Primary sludge	35 °C, 3 days	Separation of acid and methane phases, and the carbohydrates (mostly cellulose) were more degraded in the acid phase, while lipids were not degraded. The overall process was primarily determined by the rate of hydrolysis, not the bacterial growth kinetics.	[21]
1989	Combined sludge	37 °C, 2 days	VS reduction was not affected by the feed sludge concentration, and reduction of *SRT* (from 2 days to 1 day) in the acid phase resulted in similar amount of VFA, slight decrease in VS reduction, and a significant decrease in CH4 content.	[22]
1991	Waste activated sludge (WAS)	36.8 °C, 3.1 days	*SRT* between 3 and 4 days were optimum for acid-phase digestion of WAS; pre-hydrolysis, acidification, sulfate and nitrate reductions were the predominant reactions in the acid digester.	[23]
1996	Combined sludge	35 °C, 2–2.7 days	There was 3.9–25.6% enhancement of VS reduction; and sludge origins largely affected VS reduction (such as industrial waste tested in the feed sludge contributed to lower overall VS reduction).	[24]
1997	WAS	60–120 °C, 5–60 min	No significant difference of BH process between 60–120 °C for 5–60 min.	[25]
2003	Primary sludge & WAS	70 °C, 1–7 days	The ratio of primary to secondary sludge was important for the selection of BH pretreatment *SRT* and temperature.	[26]
2004	Sewage sludge and food waste	55 °C, 5 days	Longer *SRT*, fast hydrolysis, higher CH4 conversion rate, and balanced nutrient condition of co-substrate contributed the enhanced performance.	[27]
2005	Primary sludge	70 °C, 2 days	VSS reduction was mainly took place in BH pretreatment.	[28]
2006	Combined sludge	47–60 °C, 2 days	BH pretreatment of WAS up to 54 °C for 2 days did not show any benefits compared to the pretreatment at 60 °C, while the 6 °C increase resulted in a 43% and 31% increase in COD and VS removal, respectively.	[29]
2006	Combined sludge	57–58 °C, 6–8 days	The thermophilic reactor accounted for nearly 80% of the overall VS reduction with the mesophilic stage contributing the remaining 20%.	[30]

Table 3. *Cont.*

Year	Substrate Sludge	BH Pretreatment	Proposed Mechanism/Findings	Reference
2008	Combined sludge and WAS	35~70 °C	The hydrolysis and acidogenesis stages are separated out of methanogenic stage during BH comparing with conventional mesophilic anaerobic digestion, and thus BH pretreatment could provide optimal conditions for hydrolysis and acidification.	[12]
2010, 2011	Primary sludge	50–65 °C, 2 days	The improved performance was due to an increased apparent hydrolysis rate rather than overall degradability. Possible mechanisms involved stimulated growth of the microorganisms or production of extracellular hydrolytic enzymes.	[8,9]
2011	Thickened WAS	Four-stage anaerobic digestion (37–55 °C)	Higher VS removal did not result in more biogas generation in earlier stage; soluble organics generated were consumed in the subsequent reactors, resulting in more gas production. More biogas generation was observed from thermophilic systems.	[31]
2011	Combined sludge	55 °C, 2 days	Microwave pretreatment had synergistic effects, and showed better performance compared with two-phase AD.	[32]
2013	WAS	Amylase and protease addition, 2–28 h	Amylase showed best enhancement compared with protease or mixed enzyme in terms of sludge solubilization and acidification.	[33]
2014	WAS	Bacterial inoculum (as enzyme), 40 °C, 42 h	Sodium dodecyl sulfate (SDS) acted as an enzyme modulator molecule, which increased the availability of substrates to bacteria.	[20]
2017	WAS	55 °C, 70 °C, 6 days	BH process (up to 70 °C) did not substantiallyaccelerate degradation or solubilization in the BH stage, however, degradation was improved in anaerobic digester.	[34]
2017	WAS	42 °C, 55 °C, 3–6 days	The methane yield of BH pretreated WAS at 15-day BMP test was comparable with untreated WAS after 30-day BMP. Extracellular polymeric substances (EPS) from untreated WAS contained three different molecular weight fractions, and high-MW fraction decreased from 134 kDa to 25 kDa during 6-day BH at 42 °C.	[16]
2018	Cattle slurry and maize silage	37–72 °C, 2 days	Solubilization mostly took place during the first 24 h, but there was no correlation between COD solubilization and methane production rate. BH might affect the accessibility of particulate matter (not only its solubilization) in the high-solids temperature phased anaerobic digestion (TPAD) system.	[35]
2018	Food waste	35 °C, 4 days	Single-phase configuration showed an advantage in food waste without pH control at high organic loading rate. Microbial community shifted with operational conditions.	[36]
2018	Combined sludge	42 °C, and 55 °C, 3 days	High-low temperature combination during lab-scale BH (55 °C to 42 °C) showed higher methane enhancement.	[37]
2019	Combined sludge	Enzyme addition	The optimal pre-treatments were due to protein degradation using proteases. Enzyme addition increased the biogas generation up to 3.65 and 5.77 times, respectively, compared with control.	[13]
2019	WAS	37 °C, 2 days	Mixing rate might have effects on biological hydrolysis or anaerobic digestion. *Firmicutes* and *Actinobacteria* increased with elevated mixing intensity, and *Fusobacteria* and *Chloroflexi* could contribute to hydrolysis and acidification.	[38]
2019	Combined sludge	70 °C, 5 days	Methanogens (*Sporosarcina* and *Methnosarcina*) were positively correlated to VS removal and methane yield, and negatively correlated to volatile fatty acids' accumulation.	[39]
2019	Municipal Solid Waste	Bacterial (*Aspergillus niger*) fermentation	Synergistic effect of varied hydrolytic enzymes (cellulases, hemicellulases, etc.) on carbohydrate compounds.	[15]
2020	Agro-waste digestate	65 °C, 2–5 days	Post-treatment (65 °C) in digestate increased the biodegradability of complex organic compounds for anaerobic digestion.	[14]
2020	WAS (high salinity)	60–120 °C, 12 h	Pretreatment of WAS at 80 °C was confirmed to be more economically viable for tested anaerobic digestion. Higher temperature (120 °C) and longer *SRT* were benefit for protein and carbohydrate solubilization, while lower temperature could help ammonia and phosphorus release.	[10]

(1) *SRT* and temperature played critical roles in the BH process; onsite pilot or full-scale tests are of practical importance to achieve the optimum condition in VS reduction, biogas generation, and/or pathogen reduction after AD;
(2) Substrate characteristics, especially the ratio between primary sludge and waste active sludge (or other sources of biosolid/biowaste), were essential for the selection of *SRT* and temperature for BH pretreatment;
(3) BH process involved stimulated growth of the microorganisms and production of extracellular hydrolytic enzymes; the increased apparent hydrolysis rate was reported to be more significant than overall degradability after AD, and higher VS removal might not result in more biogas generation in earlier stages;
(4) In this study, *SRT* and temperature simultaneously contributed to enhanced AD performance; either longer *SRT* or higher temperature pretreatment had higher overall biogas generation and VS reduction than untreated sludge;
(5) Compared with BH at 42 °C for 3 days (BH42), the combination of low–high temperature (from 42 to 61 °C, up to 2 days) had higher overall biogas generation and VS reduction after 30-day BMP tests;
(6) The enhancement is less significant when increasing BH *SRT* longer than 48 h, while temperature (up to 61 °C) played more important roles starting from 36-h *SRT* of BH pretreatment.

5. Conclusions

In order to utilize the most efficient biodegradation kinetics, a combination of 2-day BH61 pretreatment with 12–16 days AD process (overall 14–18-day *SRT*) is recommended based on current study. Moreover, BH61 pretreatment could meet the US EPA Class-A Biosolids' requirements. The correlated research, such as high–low temperature combination for the BH processes, thickened substrate sludge, and scale-up tests are currently under investigation, which will be presented in the following studies.

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Abbreviations

AD	Anaerobic Digestion
BH	Biological Hydrolysis
BMP	Biochemical Methane Potential
CH_4	Methane
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred-tank Reactor
GC	Gas Chromatography
OLR	Organic Loading Rate
RS	Raw Sludge
SRT	Solid Retention Time

STP	Standard Temperature and Pressure
TCOD	Total Chemical Oxygen Demand
TS	Total Solids
TSS	Total Suspended Solids
VFA	Volatile Fatty Acid Concentration
VS	Volatile Solids
VSR	Volatile Solids Reduction
VSS	Suspended Volatile Solids
WAS	Waste Activated Sludge
WWTP	Wastewater Treatment Plant

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