Biogas Production and Microbial Communities in the Anaerobic Digestion of Sewage Sludge Under Hydrothermal Pretreatment with Air and a Catalyst

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Abstract

Hydrothermal pretreatment (HTP) of sewage sludge (SS) has been shown to improve the subsequent biogas production by anaerobic digestion (AD), but the effect of catalysts on HTP performance was less explored. This study intended to investigate the SS pretreatment by wet air oxidation (WAO) with the addition of K_2CO_3 as a catalyst on the performance of methane production by AD. WAO was found to improve the solubilization of SS, the soluble chemical oxygen demand, dissolved organic carbon, and total dissolved nitrogen. The methane yield from WAO increased from 202 mL/gVS$_{in}$ with no catalyst added to 277 mL/gVS$_{in}$ with 10 wt% of K_2CO_3 added at 180 °C with 30 min of residence time. Under this pretreatment condition, the highest methane production rate could achieve 15.8 mL/gVS$_{in}$ day, and the percentage of methane reached 73%. The structure of the microbial community involved in the AD was affected by the residence time, working gas, and catalyst of the HTP process. The results showed that Bacteroidetes, Bacteroidia, and *SC103* were the dominant phylum, class, and genus of bacteria, respectively, of almost all of the samples. In addition, the most abundant archaeal order was Methanosarcinales, while *Methanosaeta* was the dominant archaeal genus of most of the samples. However, *Methanosarcina* largely increased the relative abundance, corresponding to the amount of K_2CO_3 catalyst used. The findings in this study demonstrated the potential use of K_2CO_3 during WAO of SS and implied the link between shift of methanogen community and the enhanced methane yield in AD.

Keywords Methane production · Thermal hydrolysis · Wet air oxidation · K_2CO_3 catalyst · Microbial community

Abbreviations

AD	Anaerobic digestion
AS	Anaerobic sludge
CH$_4$	Methane
COD	Chemical oxygen demand
CO$_2$	Carbon dioxide
DI	De-ionized
DGGE	Denaturing gradient gel electrophoresis
DOC	Dissolved organic carbon
FISH	Fluorescent in situ hybridization
HTP	Hydrothermal pretreatment
N$_2$	Nitrogen
OTU	Operational taxonomic units
PCR	Polymerase chain reaction
RT	Reaction time
RT-PCR	Reverse transcription polymerase chain reaction
SCOD	Soluble chemical oxygen demand
SS	Sewage sludge
TDN	Total dissolved nitrogen
TH	Thermal hydrolysis
TS	Total solids
VFAs	Volatile fatty acids
VS	Volatile solids
VSR	Volatile solids reduction
WAO	Wet air oxidation
WO	Wet oxidation

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Introduction

Sewage sludge (SS), composing of primary sludge, secondary sludge, and other sludge depending on the wastewater source and the treatment technology employed, is one of the main by-products of the activated sludge process in wastewater treatment plants [1]. SS contains a large quantity of organic compounds and has emerged as a suitable material for energy recovery [2, 3]. However, SS also includes heavy metals, microorganisms, and hazardous organic compounds [4], which can jeopardize the environment and public health if SS is not strictly treated [2, 3]. To date, agricultural activity, landfills, and incineration have been the main technologies for disposing of the sludge used in wastewater treatment plants [2, 5]. However, each of these methods carries its own advantages and limitations and may result in different environmental and economic effects [6]. Despite the longer retention time [7], anaerobic digestion (AD) is advantageous as compared with methods for stabilizing sludge due to its lower cost, lower environmental footprint, and moderated performance [8]. The biogas product of AD can be applied to cooking, heating, electricity production, and other applications. With the rapid increase in the volume of SS in recent years [9, 10], AD has proven to be an efficient technology for treating SS. This technique can transform organic wastes into green power, produce fertilizer, and reduce the environmental hazards of disposal sludges through the activity of a consortia of microorganisms, comprising both bacterial and archaeal groups [11]. The bacterial group first converts the organic compounds into hydrogen and organic acids, which are then further transmuted into methane and carbon dioxide by the archaeal group [12]. Thus, the activity of the microbial community involved in AD plays an important role in influencing the effect of biogas production.

The AD process involves four main steps, namely hydrolysis, acidogenesis, acetogenesis, and methanogenesis [1, 2, 5], and functions in the absence of oxygen. However, hydrolysis, in which complex organic matter is converted into soluble organic molecules, is the rate-limiting step for AD [2, 13, 14]. In order to enhance the performance of AD, pretreatment methods (i.e., hydrothermal [15, 16], thermal [17], chemical [18], biological [19], physical [3], and several combinations such as thermochemical [18] and physicochemical [20]) are useful ways of solving the abovementioned issue. By the application of these pretreatment methods, organic matter becomes easier for anaerobic bacteria to use, improving the digestion, enhancing the biogas production, and reducing the waste sludge released into the environment [21, 22]. Among the available pretreatment methods, hydrothermal pretreatment (HTP) is favorable by virtue of its effectiveness and simplicity of application and sanitation effects [7]. It is performed in an aqueous solvent at evaluated temperature and pressure to break down the organic and inorganic components of sludge or under inert (thermal hydrolysis (TH)) or oxidizing (wet oxidation (WO) or wet air oxidation (WAO))

atmospheres [23]. In general, the TH process, using nitrogen as the working gas, is normally applied as a pretreatment step in sludge processing, prior to AD; meanwhile, the WAO and WO processes work using air and oxygen, respectively, and can be used as the final step in the complete destruction of sludge [5, 23]. On the other hand, low-pressure WAO or WO is also used as a pretreatment step to process SS in the water phase at raised temperatures and pressures, which improves the physical and chemical properties of sludge and enhances AD [23]. In recent years, many studies have shown that water is highly reactive under the conditions of HTP; thus, this pretreatment method easily converts large molecules into smaller molecules [23–25] and, as a result, biogas production from sludge is improved over conventional mesophilic AD.

In an effort to enhance the effect of the anaerobic process, several conditions of HTP have been investigated. For example, Kim et al. [24] studied the effect of HTP temperature from 150 to 270 °C (1.5–3.5 MPa) on the characteristics of activated sludge and the effect of biogas production. They found that the optimum temperature for HTP was 180 °C. Donoso-Bravo et al. [21] evaluated the solubilization of macromolecular compounds and the anaerobic biodegradability of sludge from 0 to 30 min of hydrolysis time (170 °C and 8 bar). They found that the hydrolysis time in this range had a small positive effect on sludge solubilization, and a slight increase in the total biogas production was observed with increasing HTP time. Most of these studies have shown that the range of optimal temperature on HTP to enhance the effect of the AD process for SS is 160–180 °C [23, 26–28] with the time ranging from 20 to 60 min [29]. It is because above 180 °C, non-biodegradable compounds begin to form [21] and reduce sludge biodegradability in the AD process. Abe et al. [30] studied the influence of low-pressure WO on the thermophilic AD of secondary sludge. The highest rate of biogas production in this study was achieved at 150 °C with 40% of the theoretical oxygen required to oxidize the organic carbon content of low-pressure WO.

Alkali pretreatment of SS is a chemical technique, whereby strong alkali is used to break cell walls and membranes, thus improving the solubility of organic matter in high pH conditions [31, 32]. Kim et al. [13] showed that alkali pretreatment increased the solubilization of sludge with the effective order: $NaOH > KOH > Mg(OH)_2$ and $Ca(OH)_2$. Alkali pretreatment is sometimes combined with thermal treatment, which enhances the efficiency of the AD of higher biogas generation and solid reduction [10, 33–35]. On the other hand, in the hydrothermal liquefaction study, the weak alkali K_2CO_3 is often used as the catalyst to enhance the yield of oil products [36, 37], but it is not widely used in hydrothermal pretreatment for AD. Furthermore, the effect of pretreatment processes of SS on microbial communities in AD has not been well studied until recently. High-throughput sequencing technique has helped researchers to shed light on the microbial community structure of AD for the alkaline pretreatment of SS [38] and

the HTP of SS [39]. Generally, previous works have primarily focused on the effect of HTP on the AD of SS on the basis of changes in temperature and time, and most study lacked comprehensive analysis of the bacterial community involved in the AD. Therefore, there is limited information regarding the microbial community structures behind the enhanced biogas production caused by different factors of HTP. In addition, most HTP used nitrogen as the working gas and did not include catalysts K_2CO_3 in HTP.

The current study intends to conduct evaluation of less discussed factors of HTP, including working gas and the catalyst, on the performance of the following AD process. In order to evaluate the effect of catalysts added during the HTP process on the performance of anaerobic biodegradability, K_2CO_3 was tested for the thermal treatment experiments in this study. Since high energy requirement is a limitation of HTP [40] and reduction of residence time will decrease energy consumption, our study plans to explore whether using catalyst in reaction will have potential to reduce the residence time of HTP. Moreover, the bacterial and archaeal communities will be further investigated to elucidate the impact of different factors of HTP on the microbial communities of the subsequent AD. Overall, this study aims to enhance our knowledge of the potential effects of HTP on the AD process and the resulting shift of microbial communities.

Materials and Methods

Materials

The SS and anaerobic sludge (AS) used were respectively collected from the concentrated sludge and anaerobic digester of Dihua Wastewater Treatment Plant in Taipei, Taiwan. Table 1 shows the properties of these sludges. After the

Table 1 Properties of the sludge

Parameters	Sewage sludge	Anaerobic sludge
TS (mg/L)	22,040 ± 100	21,493 ± 110
VS (mg/L)	17,250 ± 70	15,940 ± 105
DOC (mg/L)	113 ± 39	252 ± 55
TDN (mg/L)	61 ± 1	515 ± 0
pH	6.72 ± 0.02	7.00 ± 0.01
COD (mg/L)	26,600 ± 850	22,650 ± 212
SCOD (mg/L)	350 ± 71	475 ± 71
C%	42.38 ± 1.31	37.96 ± 0.22
N%	6.93 ± 0.16	5.92 ± 0.02
S%	0.95 ± 0.07	1.08 ± 0.01
H%	6.14 ± 0.11	5.72 ± 0.05
C/N	6.12 ± 0.07	6.41 ± 0.05

samples were obtained from this plant, they were stored at 4 °C if not immediately used in experimental analyses.

Methods

Hydrothermal Pretreatment Process

The HTP process was performed in a reactor (HP/HT pressure reactor, 4563 Bench Top Reactor, Parr Instrument, Moline, 1 L), which was equipped with a temperature controller and pressure indicator. After adding 200 mL of SS, nitrogen or air was fed into reactor at 345 kPa for 2 min in order to replace prior gas before the HTP reaction occurred. In each test, the sample was mixed by an agitator at 100 rpm. The temperature for the HTP reactions was 180 °C at 648.1 kPa (94 psi) while the reaction time for the constant temperature reaction period was 30, 60, or 90 min. On the other hand, K_2CO_3 was added at 0, 5, or 10 wt% of total solids (TS) in the samples before HTP, which then checked the effect of the catalyst on the performance of the process. During the HTP process, the temperature of the samples increased from 25 to 180 °C at which it remained during the residence time of 30, 60, or 90 min before being cooled down to 50 °C by air and then to ambient temperature by cold water to end the experiment. The time for the increasing of the temperature to 180 °C was 15 min, but 110 min was recorded for the period for reducing the temperature from that to ambient temperature. The HTP process was conducted following the schematic diagram for the experimental apparatus required for HTP presented by Chang et al. [37]. The reactions of the HTP are shown in Table 2.

AD Assays

The batch AD of the SS was conducted at a mesophilic temperature (35 °C) in a water bath. To maintain the constant temperature, the water level in the bath was adjusted to the upper level of the sludge height in the bottles. In this process, 800 mL of the raw (as control) or treated SS or de-ionized (DI) water (as background) and 200 mL of the AS were added to

Table 2 Reactions of HTP processes

Sample	Reaction temperature (°C)	Resident time (min)	Working gas	Catalyst
1	180	30	N_2	-
2	180	60	N_2	-
3	180	90	N_2	-
4	180	30	Air	-
5	180	30	Air	$m_{K_2CO_3} = 5\%TS$
6	180	30	Air	$m_{K_2CO_3} = 10\%TS$

the batch reactors (1-L glass bottles). This inoculum/substrate ratio was approximately 0.2, which was also within the ratios of previous study [41]. One-hundred milliliter bottles containing 80 mL of the treated SS and 20 mL of the AS were also set up for the confirmation of the trend of biogas production (Fig. S1 in Supplementary Information, SI). SS was the substrate of this process, while AS was the inoculum of the AD. The nitrogen gas was supplied to the bottles for 5 min to remove air from the head space and ensure the presence of anaerobic conditions. In order to minimize the effects of settling during AD, the bottles were manually mixed twice a day. The biogas was collected in a 1-L gas bag, which was then measured in terms of the volume and concentration of methane and carbon dioxide, once or two times per week. The volume of biogas gas was measured manually using the 50-mL Hamilton syringe. After 35 days of AD, the cumulative methane and carbon dioxide generation, as well as volatile solids reduction (VSR), were quantified.

Analytical Methods

Chemical oxygen demand (COD) and soluble chemical oxygen demand (SCOD) were measured with a COD kit (DR3900, HACH, USA) while the dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a TOC analyzer (1080 TOC analyzer, O·I·Analytical, USA). The samples were then filtered through a 0.45-μm pore size membrane filter and used for the analysis of DOC, SCOD, and TDN. The TS, volatile solids (VS), and pH were then measured in accordance with the Standard Methods [42]. All of the analyses were determined three times in order to assess the precision and data errors. The results were then presented as mean values. The contents of methane (CH_4) and carbon dioxide (CO_2) in the biogas were analyzed by a gas chromatography/thermal conductivity detector (GC/TCD, 8900, China Chromatography Co., Taipei, Taiwan) with a packing column (60/80 Carbonxen-1000, 4 ft. × 1/8 in SS, Sigma-Aldrich, Saint Louis, MO, USA). The pH of the control and pretreated samples was measured by pH meter (HQ40d, HACH, USA) and then adjusted to 7.0 prior to AD.

In order to exhibit the transfer of COD from the particulate form to the soluble one of sludge, the solubilization COD was determined by the following formula [43]:

$$\text{Solubilization COD} = \frac{SCOD_T - SCOD_0}{COD_0 - SCOD_0} \times 100\% \quad (1)$$

where $SCOD_T$ is the soluble COD of sludge after HTP (mg/L), and $SCOD_0$ and COD_0 are the soluble COD and COD of the sludge before HTP (mg/L).

The VSR was determined by following equation [24]:

$$\text{VSR} = \frac{VS_0/TS_0 - VS_T/TS_T}{VS_0/TS_0 - (VS_0/TS_0 \times VS_T/TS_T)} \times 100\% \quad (2)$$

where VS_0/TS_0 is the VS/TS before AD and VS_T/TS_T is the VS/TS after AD.

The COD mass balance was determined according to Riau et al. [44] with the following formula:

$$PCOD_I + SCOD_I = PCOD_F + SCOD_F + B \quad (3)$$

where $PCOD_I$ and $SCOD_I$ are particulate and soluble COD (%) before anaerobic digestion, $PCOD_F$ and $SCOD_F$ are particulate and soluble COD (%) after anaerobic digestion, and B is biogas converted from particulate and soluble COD in anaerobic digestion (%).

DNA Extraction and 16S rRNA Gene Amplification and Sequencing

The microbial community structures of the AD samples (35 days) were analyzed using 16S rRNA gene amplicon high-throughput sequencing. The DNA was extracted from SS by the DNeasy PowerSoil Kit (QIAGEN, Germany). The hypervariable V3 and V4 regions of microbial 16S rRNA genes were amplified using the universal primers: 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACT ACHVGGGTATCTAATCC-3′) in the conditions of an initial denaturation at 95 °C for 3 min, followed by 30 cycles at 95 °C for 30 s, annealing at 57 °C for 30 s, with elongation at 72 °C for 30 s and a final extension at 72 °C for 5 min.

The polymerase chain reaction (PCR) products were mixed equally and then checked by means of agarose (2.0%) gel electrophoresis. The 16S rRNA gene amplicons were sent to a sequencing company (Genomics, Taiwan), and the library sequencing was carried out on an Illumina Miseq platform (Illumina Inc., San Diego, CA, USA), generating 300 bp paired-end reads. UIIME is the main tool of the workflow [45]. The raw reads were quality trimmed by Trimmomatic with PEAR. OTU (Operational Taxonomic Unit) was analyzed by mothur v.1.39.5 with 97% [46]. Chimeric sequences were identified by UCHIME v4.2 [47].

Results and Discussion

Effect of Residence Time in HTP Process on the Performance of AD

Characteristics of Pretreated Sewage Sludge by the HTP

The reaction time (or the residence time "RT") for the constant temperature reaction is an important factor in the HTP of SS,

which results in some influence on the energy balance and operating costs of the system. Therefore, this study first investigated the effect of residence time in the range of 30 to 90 min for HTP on the performance of AD at the previous suggested optimal temperature of 180 °C, under 648.1 kPa and nitrogen as the working gas.

Figure 1 shows the changes in the characteristics of the SS following HTP (values are available in Table S1 in SI). According to Fig. 1a, the value of TS and VS was nearly unchanging, and the value of VS/TS remained constant around 80% with increasing time in the pretreatment stage. However, Fig. 1b exhibits the increase in the TDN, which increased from 61 mg/L in the raw sludge to 374, 384, and 475 mg/L in the treated SS in 30, 60, and 90 min. Figure 1b also shows that the value of the DOC is 113 mg/L under untreated conditions, followed by a substantial increase to 2136, 2266, and 2670 mg/L after pretreatment at 180 °C during the residence times of 30, 60, and 90 min, respectively. Like DOC, the value of SCOD increased from 350 mg/L in the control to 7050 (RT30), 7115 (RT60), and 8450 mg/L (RT90) in Fig. 1c. Figure 1d reveals the decrease in pH when the reaction time increases during the HTP process. The value of the pH in the raw SS sample was 6.72; however, this value dropped considerably to 6.17, 6.01, and 5.95 after the HTP reaction at 180 °C in 30, 60, and 90 min of residence times, respectively. The pH decrease is considered due to the formation of the volatile fatty acids (VFA) as shown in the previous study [48, 49].

The main purposes of HTP are the solubilization and cell disruption of substrates [24]. Accordingly, as is shown in Fig. 1, there was a rapid increase in the value of TDN, DOC, and SCOD after the HTP process, and the COD solubilization was revealed to increase with the increase in the reaction time: 25.52% (RT30), 25.9% (RT60), and 30.86% (RT90). The results illustrate the positive effect of HTP on the characteristics of treated sludge. Qiao et al. [50] also showed that organic carbon in the solid phase was converted into the liquid phase after increasing the temperature and pressure of HTP. Previous reports showed that HTP changed the physical characteristics of the sludge [24] and broke up its long carbon chain to improve the solubilization of organic matter [51]. The longer time the HTP was carried out, the more the solubilization of sludge was improved.

AD of Substrates

The VSR is an important value in AD and can be used to assess the performance of the AD process. Figure 2a illustrates the VSR of pretreated and non-pretreated samples following AD. The increase in the VSR value when the residence time increased, shown in Fig. 2a, revealed the positive effect of the residence time on the performance of the AD process. The percentage of VSR in the raw SS was 28.8%; however, this increased rapidly to the following: 44.2%, 47.7%, and finally peaked at 50.3% after 30, 60, and 90 min HTP, respectively. In addition, according to COD mass balance in Fig. 2c, only

Fig. 1 Effects of HTP time on the characteristics of SS at 180 °C and N_2 as working gas. a TS and VS. b DOC and TDN. c COD, SCOD, and solubilization COD. d pH. Control, RT30, RT60, and RT90 present the characteristics of the untreated SS and treated SS at 180 °C, 648.1 kPa, and with nitrogen as the working gas during the residence time: 30, 60, and 90 min, respectively
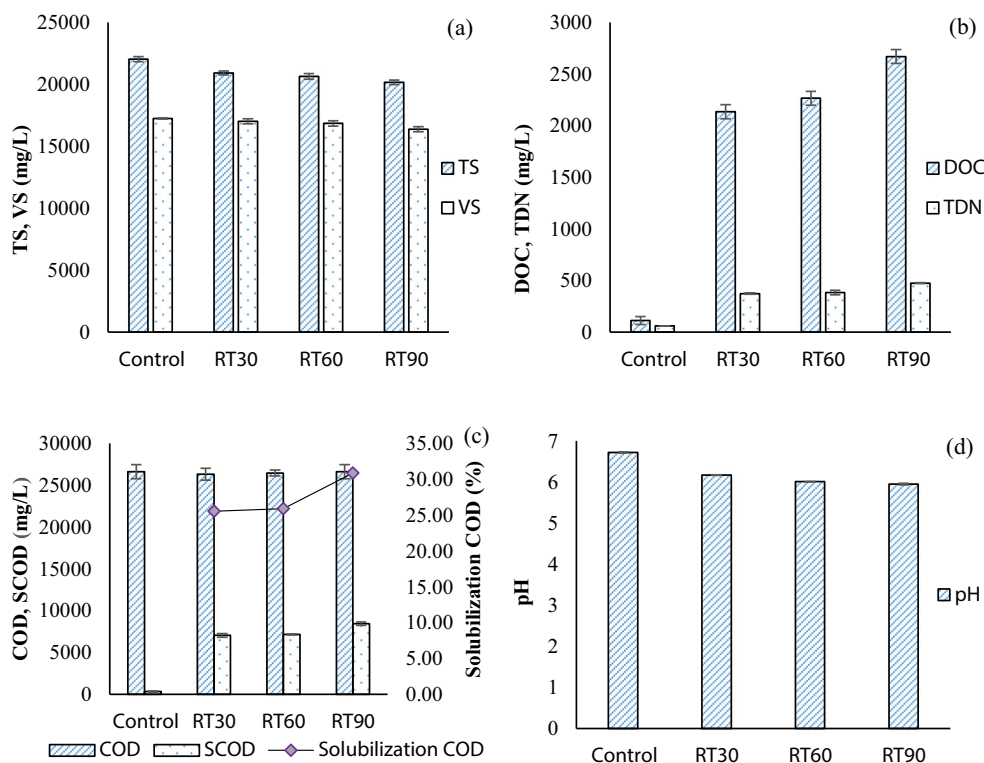
Fig. 2 VS reduction after AD (**a**); volume and percentage of methane and carbon dioxide (**b**); COD mass balances in AD (**c**). Control, RT30, RT60, and RT90 represent the untreated SS and treated SS at 180 °C, 648.1 kPa, and with nitrogen as the working gas during the residence time: 30, 60, and 90 min, respectively
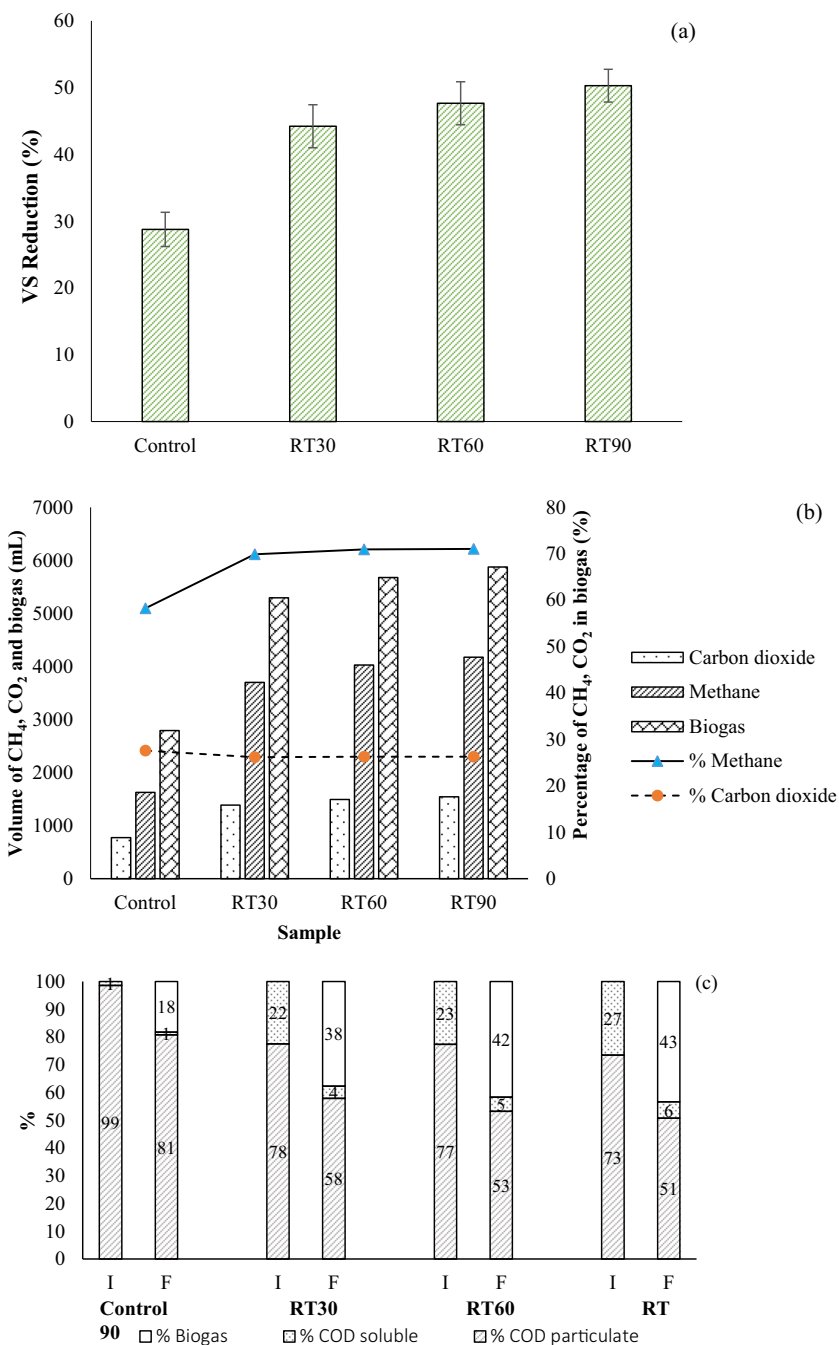
18% of COD was converted into biogas in the raw SS; however, this percentage increased sharply in pretreated samples to 38%, 42%, and 43% after 30, 60, and 90 min HTP, respectively.

The volumes of methane and carbon dioxide in the AD of control and pretreated sludge are shown in Fig. 2b. In the control sample, the volumes of CH_4 and CO_2 were 1626 and 771 mL, which accounted, respectively, for 58% and 28% of the volume of the biogas. Besides CH_4 and CO_2, the rest of the biogas volume might include nitrogen, water vapor, hydrogen sulfide, ammonia, and hydrocarbons [15, 52]. However, the volumes of CH_4 and CO_2 were largely improved after the HTP process. When the residence time was 30 min, the volume of CH_4 and CO_2 was increased to 3699 mL (70% of biogas) and 1384 mL (26% of biogas), respectively. Moreover, Fig. 2b showed that the percentage of CH_4 and CO_2 in the biogas of the treated samples after 30, 60, and 90 min of residence time was almost the same value. The highest volumes of CH_4 and CO_2 were 4172 mL and 1544 mL in the sample treated for 90 min, followed by 4027 mL (CH_4) and 1491 mL (CO_2) of the sample pretreated for 60 min, respectively. Compared with the sample pretreated

for 30 min, the percentage of CH_4 increased by 8.9% and 12.8%, respectively, for samples pretreated for 60 and 90 min. According to Fig. S2a, the CH_4 yield of the pretreated sludge was 220, 242, and 256 mL/gVS$_{in}$, corresponding to 30, 60, and 90 min of residence time, while the yield of the raw SS was 96 mL/gVS$_{in}$. Moreover, the value of CO_2 was 82 mL/ gVS$_{in}$ (30 min), 89 mL/gVS$_{in}$ (60 min), 95 mL/gVS$_{in}$ (90 min), and 45 mL/gVS$_{in}$ (raw sludge). The highest CH_4 production rates were all observed in the second week of AD, with the values of 5.5 mL/gVS$_{in}$ day, 14.4 mL/gVS$_{in}$ day, 15.3 mL/gVS$_{in}$ day, and 15.8 mL/gVS$_{in}$ day for raw SS, 30, 60, and 90 min of residence time, respectively (Fig. S3a–d).

According to the above results, the 90 min of residence time had higher CH_4 yield than that of 30 min, but they had similar percentage of CH_4 around 70% of the biogas. Since the following study intends to compare the effect of working gas and the catalyst of HTP, in order to save input energy and time in the laboratory, 30 min of residence time will be chosen for the experiments afterwards to test the two factors of HTP. This time was also used for the HTP in order to enhance the yield of biogas with other substrates. For example, Huang et al. [7] carried out HTP after 30 min of residence time to improve the biogas yield of manure waste. Carrère et al. [33], meanwhile, reviewed HTP technologies to enhance AD for activated sludge. Their results showed an optimal temperature being between 160 and 180 °C following a treated time of 30– 60 min of TH.

## Effect of Working Gas in the HTP Process on the Performance of AD

Characteristics of Pretreated Sewage Sludge by the HTP

By employing nitrogen and air as the working gases for HTP, this study evaluated the effect of TH and WAO on the efficiency of the AD process. The characteristics of the raw SS and pretreated SS are depicted in Fig. 3. The HTP was carried out at 180 °C, 648.1 kPa, and with 30 min of residence time. As shown in Fig. 3, while the value of TS, VS and COD remained almost constant in all of the samples, the value of DOC, TDN, and SCOD changed substantially after the hydrothermal reactions were carried out. Compared with the control, there was a considerable increase of, respectively, 18.9, 6.1, and 20.1 times in the value of the DOC, TDN, and SCOD of pretreated SS using nitrogen as the working gas. In addition, with the pretreated SS using air as the working gas, the concentration of DOC, TDN, and SCOD rose rapidly to 20.2, 6.4, and 20.3 times, respectively, by comparison with the raw SS. It is worth noting that the values of the DOC, TDN, and SCOD of pretreated SS using air increased and were comparable with the values of pretreated SS using nitrogen.

AD of Substrates

As is shown in Fig. 4a, there was a substantial increase in the percentage of VSR, from 28.8% (control) to 44.2% (TH), and

Fig. 3 Effects of HTP working gas on the characteristics of SS at 180 °C during 30 min. **a** TS and VS. **b** DOC and TDN. **c** COD, SCOD, and solubilization COD. **d** pH. TH and WAO represented HTP using nitrogen and air as the working gas
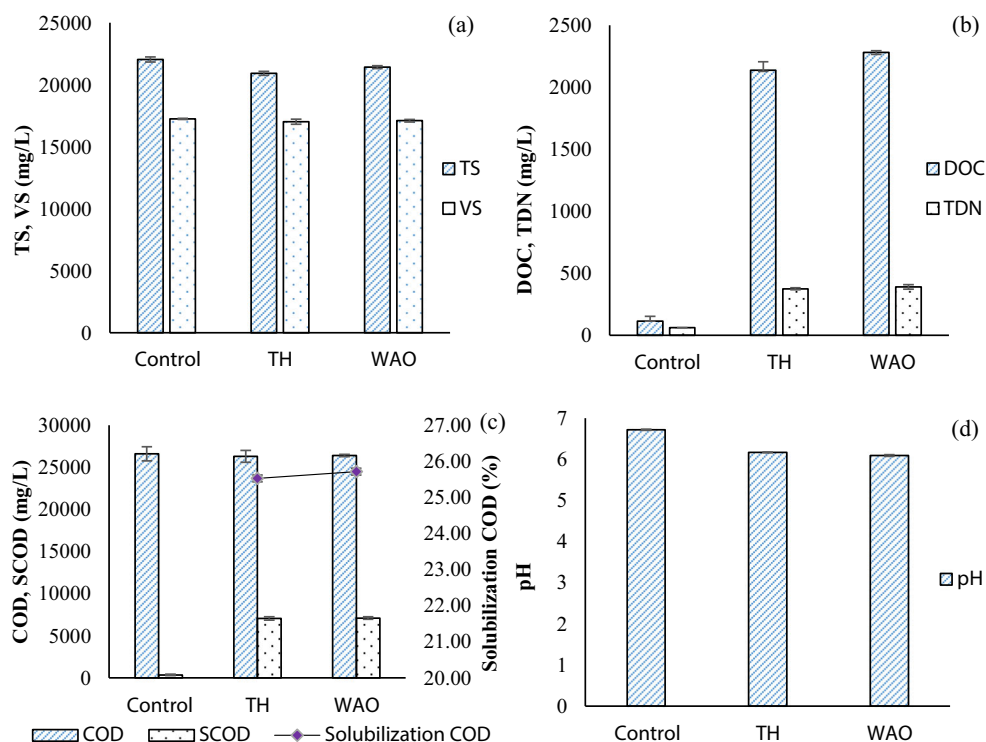
Fig. 4 VS reduction after AD (**a**); volume and percentage of methane and carbon dioxide (**b**); COD mass balances in AD (**c**). Control, TH and WAO represent the untreated SS and HTP at 180 °C, 648.1 kPa and 30 min using nitrogen and air as the working gas, respectively
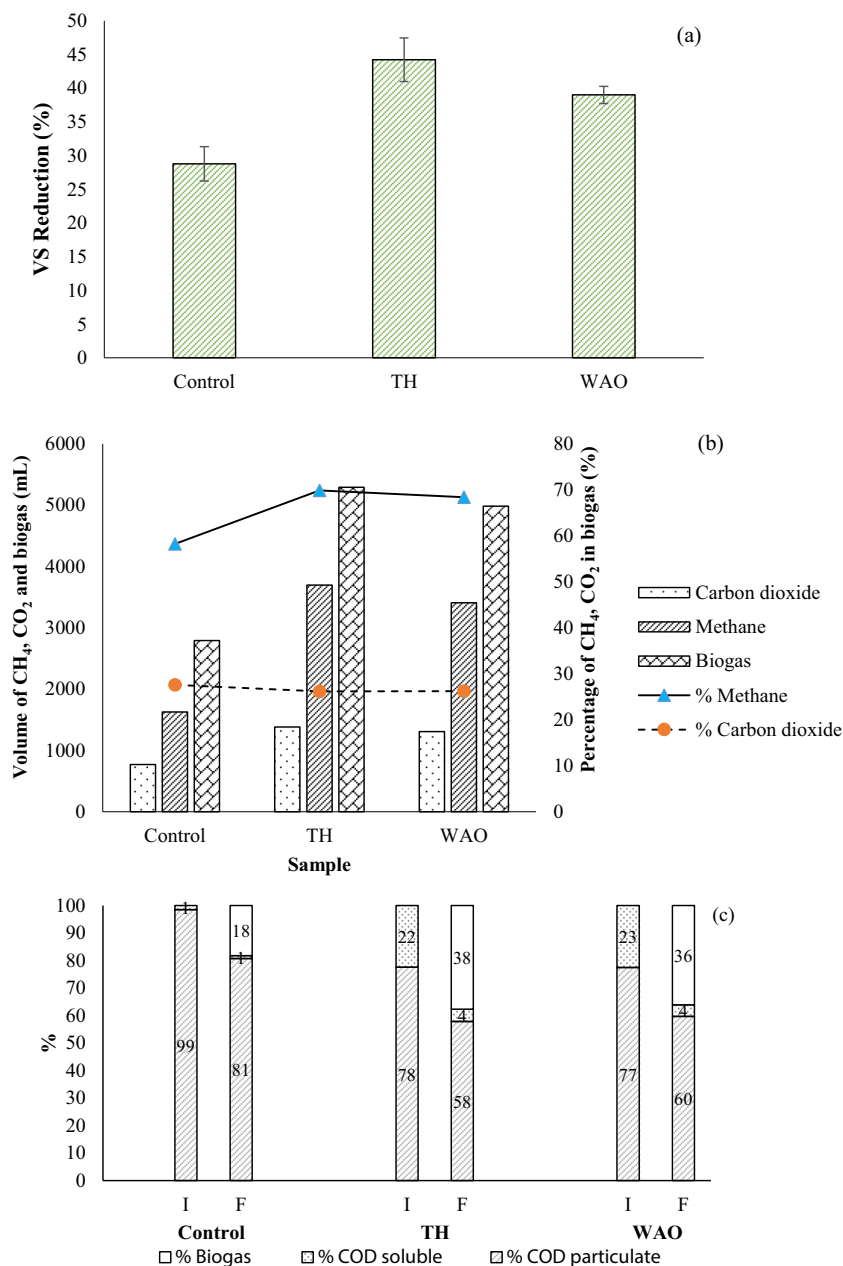
39.0% (WAO); thus, the methane yield increased to 220 and 202 mL/gVS_{in} after HTP using nitrogen and air as the working gases, respectively, as depicted in Fig. S2b. Meanwhile, the yield of carbon dioxide increased slightly, from 45 mg/L (control) to 82 mL/gVS_{in} (nitrogen) and 87 mL/gVS_{in} (air) after HTP. The highest CH_4 production rates were all observed in the second week of AD, with the values of 5.5 mL/gVS_{in} day, 14.4 mL/gVS_{in} day, and 13.6 mL/gVS_{in} day for raw SS, TH, and WAO, respectively (Fig. S3a, b, e).

Figure 4b shows the volume of biogas after the HTP was conducted with the TH and WAO processes. After the AD stage, the volume of biogas was 2793 mL for the control sample, with 5293 and 4982 mL for the pretreated sludge

using TH and WAO, respectively. The amount of biogas of pretreated SS by TH was higher, by 89.5%, compared with untreated SS, and it was 6.24% higher than the amount of biogas in the pretreated SS that used WAO. Although WAO showed slightly better COD solubilization of SS than TH, TH could achieve higher VSR, which might result in the larger amount of biogas production from pretreated SS by TH. The COD mass balance (Fig. 4c) also supports the results, since 38% of COD was converted into biogas in the pretreated sample by TH, while it was 36% in the pretreated sample by WAO. It is notable that the percentage of methane and carbon dioxide in TH and WAO presented similar results. The percentage of methane in biogas was 70% using TH and 68%

using WAO, respectively; meanwhile, the percentage of carbon dioxide in the two cases was 26%. From what has been shown above, HTP using nitrogen and air as the working gases effectively improved the biogas yield. At the temperature of 180 °C, 648.1 kPa, and 30 min of residence time, the amount of biogas produced from SS after HTP using nitrogen was slightly higher than that which used air. Although TH is more widely used as a pretreatment technology to enhance the performance of AD, and WAO is more often used for final sludge destruction [23], our results showed the effective use of WAO pretreatment to improve AD.

## The Effect of the Catalyst Added to the HTP Process on the Performance of AD

Characteristics of Sewage Sludge Pretreated by HTP

Figure 5 shows the change in the characteristics of SS after reactions without and with the catalyst (K_2CO_3). Mouneimne et al. [53] reported that too high a concentration of K^+ may result in the inhibition of AD. Therefore, in this study, K_2CO_3 was added to the reaction at 0, 5, and 10 wt% of solids in the SS. The pH values of SS samples after spiking 5 and 10 wt% K_2CO_3 were 8.50 and 9.56, respectively. The contribution of alkaline sludge disintegration in our experiments could be relatively minor, since according to the previous study, the alkaline pretreatment of sludge will result in limited SCOD when the pH values were below 10 [54]. The ratio of VS/TS of samples in Fig. 5a remained nearly unchanged and was the same with the above two cases; by contrast, the value of pH in

Fig. 5d varied. Specifically, there was an apparent decrease in the pH, from 6.72 in the raw sample to 6.09 in the pretreated sample without the catalyst. Nevertheless, this value rose considerably, to 7.37 and 7.78, after K_2CO_3 was added at 5 and 10 wt% of the TS in the sample in the WAO reaction, respectively. As is shown in Fig. 5b, the DOC quickly increased by 20.2 times, from 113 mg/L of raw sludge to 2281 mg/L of WAO pretreated SS without the use of K_2CO_3. The concentration of the DOC also rapidly increased by approximately 28.1 times and 35.1 times, respectively, after using the catalyst corresponding to 5 and 10 wt% of TS of the SS sample. Figure 5b shows that there was a large increase in the value of TDN following WAO. This value was only 61 mg/L in the raw SS but increased to 389 mg/L after WAO pretreatment at 180 °C during the residence time of 30 min without the catalyst. In comparison with the WAO pretreated SS without the catalyst, the value of TDN increased by 38.56% and 60.92% when K_2CO_3 was added, corresponding to 5 and 10 wt% of the sample's TS.

As is depicted in Fig. 5c, the value of the COD in all of the samples remained unchanged; nevertheless, the concentration of SCOD in the WAO pretreated sludge improved sharply without or with the K_2CO_3 catalyst. The value of the SCOD jumped from 350 mg/L in the raw SS to 7100 mg/L during the WAO without the K_2CO_3. However, when the K_2CO_3 was added in the amount of 5% and 10 wt% of the TS of the sample, the value of the SCOD rose considerably by 67.6% and 102.81%, respectively. Furthermore, the increase in the SCOD was connected to the increase in the COD solubilization, and this value is shown in Fig. 5c. The value of the COD

Fig. 5 Effects of HTP catalyst on the characteristics of SS at 180 °C, 30 min, and air as the working gas. **a** TS and VS. **b** DOC and TDN. **c** COD, SCOD, and solubilization COD. **d** pH. C0, C5, and C10 represent the values of pretreated SS that K_2CO_3 was added to the reaction at 0, 5, and 10 wt% of the TS of the sample, respectively
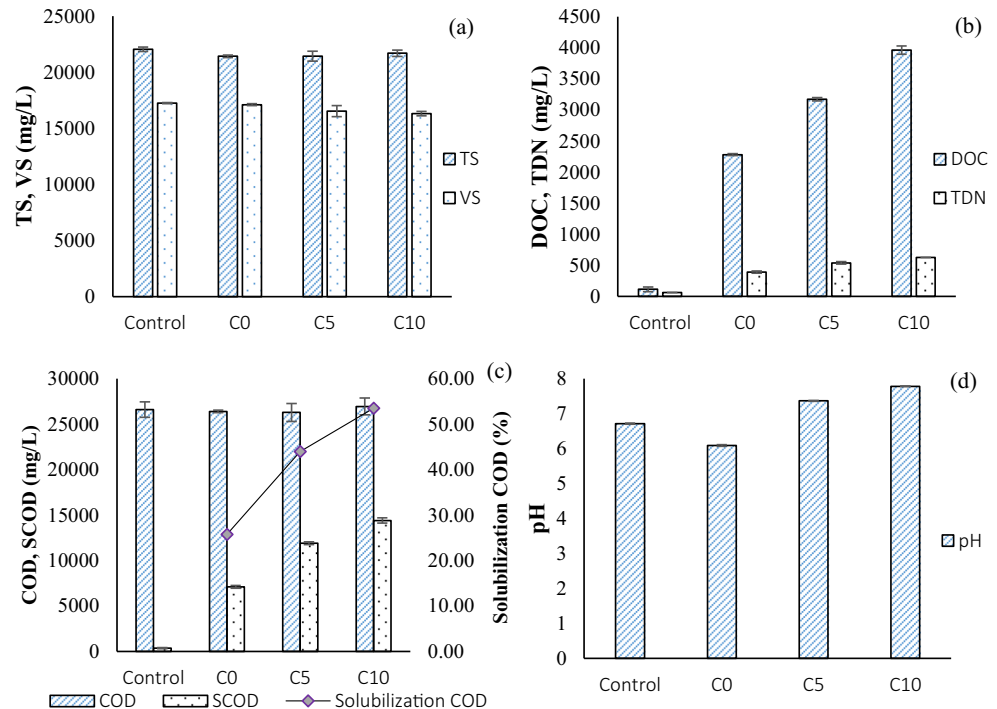
solubilization was improved to 25.71% after the WAO reaction without the catalyst and then increased to 44% and 53.52% when K_2CO_3 was added in the amount of 5% and 10 wt% of TS of the sample.

AD of Substrates

After AD, with a VSR of 28.78% (Fig. 6a), the yields of CH_4 and CO_2 in the raw SS were found to be 96 mL/gVS$_{in}$ and 45 mL/gVS$_{in}$, respectively, which is shown in Fig. S2c. Again, the yield of methane and carbon dioxide increased rapidly when HTP was applied. In the WAO pretreated SS without the catalyst (K_2CO_3), the methane yield was improved to 202 mL/gVS$_{in}$, with a VS removal of 38.99%. The results also show that using the catalyst (K_2CO_3) in WAO had a positive effect on AD. After K_2CO_3 was added in the WAO pretreatment reaction at the ratio of 5 wt% and 10 wt% of TS in the sample, the methane yield increased dramatically, to 247 mL/gVS$_{in}$ and 277 mL/gVS$_{in}$, corresponding to a VSR of 45.6% and 51.4%, respectively. Nevertheless, the yield of carbon dioxide in the WAO pretreated samples was 77, 86, and 94 mL/gVS$_{in}$, respectively, when the K_2CO_3 was added in the quantities of 0%, 5%, and 10% of the TS in the sample. The highest CH_4 production rates also increased from 13.6 mL/gVS$_{in}$ day (without K_2CO_3) to 15.7 mL/gVS$_{in}$ day (5 wt% K_2CO_3) and 15.8 mL/gVS$_{in}$ day (10 wt%

Fig. 6 VS reduction after AD (**a**); volume and percentage of methane and carbon dioxide (**b**); COD mass balances in AD (**c**). C0, C5, and C10 represent the untreated SS and WAO at 180 °C, 648.1 kPa, and 30 min with catalyst K_2CO_3 at 0, 5, and 10 wt% of the TS, respectively
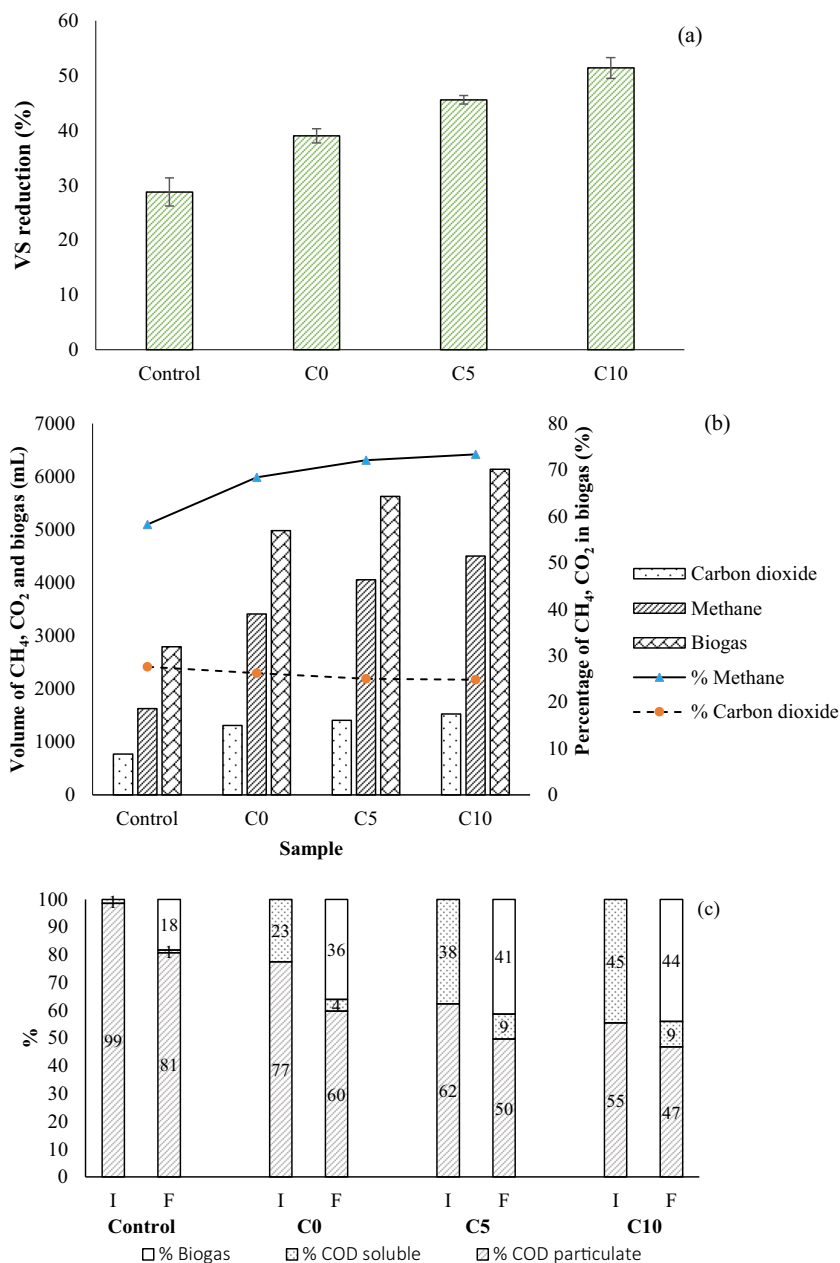
K_2CO_3) (Fig. S3e-g). These results demonstrate that using K_2CO_3 in HTP with air as the working gas was very effective to enhance COD solubilization and further improve methane yield in the subsequent AD. Since the methane yield 277 mL/gVS_{in} is the highest value observed among all treatments, especially higher than that from 90 min of residence time in HTP with nitrogen as the working gas, using K_2CO_3 in HTP with air as the working gas can reduce the residence time compared with traditional HTP.

The volume of methane, carbon dioxide, and biogas is presented in Fig. 6b. The volume of biogas in the untreated sludge was found to be 2793 mL after AD. However, the volume of biogas in the samples after the hydrothermal process was increased by 78.4% (without K_2CO_3), 101.4% (5 wt% K_2CO_3), and 119.8% (10 wt% K_2CO_3). The COD mass balance (Fig. 6c) also supports the results, since 18% of COD was converted into biogas in the untreated sludge, while it increased to 36% (without K_2CO_3), 41% (5 wt% K_2CO_3), and 44% (10 wt% K_2CO_3). Similar to this trend, the percentage of methane in the biogas was 58% with raw sludge; however, the percentage of methane increased to 68%, 72%, and 73% in the pretreated samples when K_2CO_3 was added in the amount of 0%, 5%, and 10% of the TS, respectively. It should be noted that the percentage of carbon dioxide in biogas is also similar to the above two cases, amounting to approximately 26% with and without the addition of K_2CO_3.

Microbial Community Analysis

AD is a biological decomposition technology involving groups of microorganisms in the absence of oxygen. The taxonomic classification of the microbial community in AD samples is shown in Fig. 7. In order to understand the bacterial and archaeal communities in AD for raw and pretreated SS, the bacterial phylum, class, and genus and the archaeal order and genus were identified after sequencing for all of the samples. The phylum, class, order, and genus are shown in Fig. 7 in terms of the percentage of the amount of archaea or bacteria present. The treatment method considerably affected the microbial community structure, and therefore, in order to evaluate the effect of the HTP conditions on the microbial communities, Fig. 7 is separated into Figs. S4, S5, and S6, which correspond to samples of different HTP residence times, different working gases, and different amounts of the catalyst in the following sections. In those figures, M1 depicts the AD of raw SS; M2, M3, and M4 represent the AD of pretreated SS under 180 °C, at 648.1 kPa, with no catalyst, N_2 as the working gas, and 30, 60, and 90 min of residence time, respectively; M5, M6, and M7 show the microorganisms in AD of pretreated SS under 180 °C, 648.1 kPa, and 30 min of residence time, air as working gas and K_2CO_3 added 0, 5, and 10 w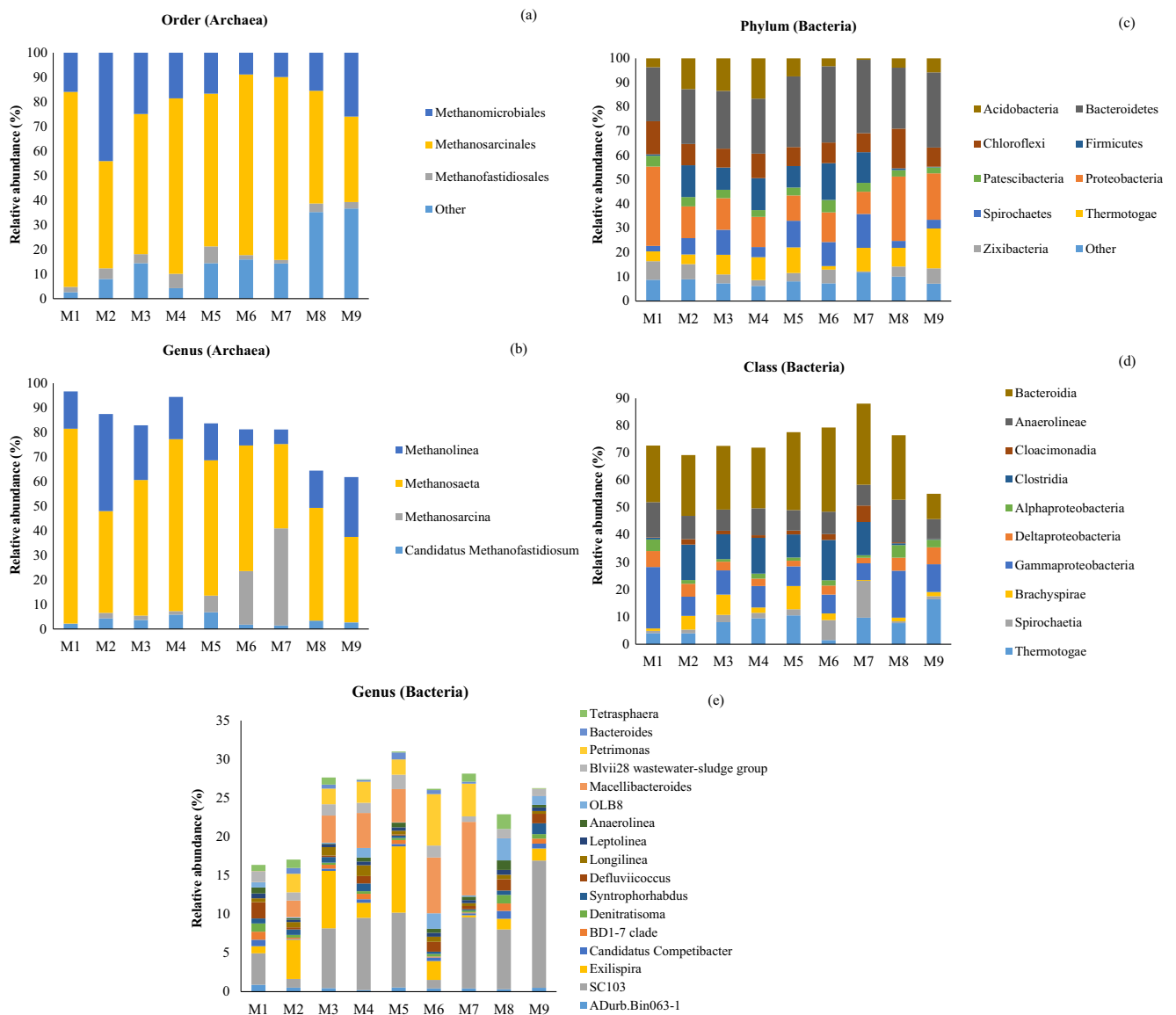t% of TS, respectively; M8 shows the microbial community of AS; and M9 shows the bacterial and archaeal communities of background AD (20% AS and 80% DI water).

Effect of the HTP Residence Time on the Microbial Community in AD

The microbial communities of archaea and bacteria in AD with the SS of the control, 30 min, 60 min and 90 min of the HTL residence times are shown in Fig. 7 and Fig. S4. The results of the taxonomic assignment demonstrate that Methanosarcinales was the most abundant order of archaea, accounting for 79.3, 43.6, 57.0, and 71.3% of the archaeal communities under these four respective conditions. The relative abundance values of Methanomicrobiales are 15.9, 44.1, 24.9, and 18.6% under these conditions, respectively (Fig. S4a). In Fig. S4b, the two dominant genera under these four conditions were *Methanolinea* (order Methanomicrobiales) and *Methanosaeta* (order Methanosarcinales), accounting for 15.2% and 79.2%, 39.5% and 41.4%, 22.2% and 55.2%, and 17.2% and 69.9% of the relative abundance values. In the short pretreatment time (30 min), the relative abundance of *Methanolinea* was increased considerably from 15.2 to 39.5%. Compared with *Methanolinea*, *Methanosaeta* decreased from 79.2 to 41.4%. Furthermore, when the residence time of the HTP increased, the percentage of *Methanolinea* decreased to 22.2% and 17.2% after 60 and 90 min. Unlike *Methanolinea*, the percentage of *Methanosaeta* increased to 55.2 and 69.9%, corresponding to a residence time increase of 2 and 3 times, respectively.

The relative abundance of bacterial communities is shown in Fig. S4c-e, in which the phylum, class, and genus levels for all of the samples were chosen for the analysis. Proteobacteria made up 32.7% of the bacterial community, which was the most abundant phylum of the control sample. Gammaproteobacteria was the most abundant class in this phylum, which made up 22.5% of the total community in the untreated sample (Fig. S4c, d). The most abundant bacterial phylum was Bacteroidetes in the three pretreated samples, which constitutes 22.7, 23.8, and 22.7% of the relative abundance values for the 30, 60, and 90 min pretreating times, respectively. Moreover, Bacteroidia was the dominant class in this phylum in the 30, 60, and 90 min residence times, which accounted for 22.3%, 23.3%, and 22.2% of the relative abundance values. The most abundant bacterial population at the genus level was *SC103* (within class and phylum: Thermotogae), which constituted 4.1, 7.7, and 9.3% of the relative abundance values for the control, 60, and 90 min pretreating times. *Exilispira* (within phylum Spirochaetes and class Brachyspirae) was predominant for 30 min of residence time, which was 5.0% of the total community.

Methanogenesis is the final step in the AD process, by which methane is produced from three main methanogenic pathways, namely acetate by acetoclastic, methyl compounds

Fig. 7 Taxonomic classification of microbial community. **a** Order (archaea). **b** Genus (archaea). **c** Phylum (bacteria). **d** Class (bacteria). **e** Genus (bacteria). M1 depicts the AD of raw SS. M2, M3, and M4 represent the AD of pretreated SS under 180 °C, at 94 PSI, with no catalyst, N_2 as the working gas, and 30, 60, and 90 min of residence time, respectively. M5, M6, and M7 show the microorganisms in AD of pretreated SS under 180 °C, 94 PSI, 30 min of residence time, air as working gas, and K_2CO_3 added 0, 5, and 10 wt% of TS of sample, respectively. M8 shows the microbial community of AS; M9 shows the bacterial and archaeal communities of background (20% AS and 80% DI water)

by methylotrophic and hydrogen/carbon dioxide, and formate by hydrogenotrophic methanogens [55]. The genus *Methanolinea* was shown to be the major hydrogenotrophic pathway [56] while *Methanosaeta* constituted the major acetoclastic pathway [55]. These experimental results show that the acetoclastic pathway was the main pathway of biogas production in the AD of control or pretreated samples when the residence time was extended to 60 and 90 min. Previous kinetic values found for *Methanosaeta* suggest that *Methanosaeta* will dominate at low acetate concentrations [57]. On the other hand, although *Methanosarcina* can use different pathways for methane production, high acetate

concentrations will favor the dominance of *Methanosarcina.* The predominance of *Methanosaeta* suggests the low acetate concentrations in our AD. In addition, the hydrogenotrophic pathway became equally important as the acetoclastic pathway in the sample with 30 min of pretreating time, implying more hydrogen or formate could be in the specific AD system. Moreover, a novel genus of methanogens *Candidatus* Methanofastidiosum was found increasing after HTP even though it is present as a minor methanogen. Since *Candidatus* Methanofastidiosum performs methanogenesis only through methylated thiol reduction [58], the result implied more methylated thiol was formed in AD of the SS after

HTP with 30–90 min of residence time. Therefore, changing the residence time in HTP could change the archaeal and bacterial community of the samples.

Effect of HTP Working Gas on the Microbial Community in AD

The microbial communities of the archaea and bacteria are shown in Fig. 7 and Fig. S5. Methanomicrobiales and Methanosarcinales were the dominant archaeal orders in the control and pretreatment with TH and WAO, making up 95.2, 87.6, and 78.8% of the relative abundance, respectively (Fig. S5a). The relative abundance of the genus *Methanosaeta* (order: Methanosarcinales) largely dropped, from 79.2% in the control sample to 41.4% in that which was pretreated with TH and 55.0% in that pretreated with WAO. The genus *Methanosarcina* (order: Methanosarcinales) slightly increased from 0.12% in the control to 2.2 and 6.8% with TH and WAO pretreatment. The relative abundance of *Methanolinea* (order: Methanomicrobiales) increased to 39.5% of the TH pretreatment but slightly decreased to 15.03% of the WAO pretreatment in comparison with 15.2% of the control (Fig. S5b).

The most abundant phylum in the bacterial community in the untreated sample was Proteobacteria, which had a relative abundance of 32.7% (Fig. S5c). The dominant class of this sample was Gammaproteobacteria (phylum: Proteobacteria), which amounted to 22.5% (Fig. S5d). As can be seen in Fig. S5e, the genus *SC103*, belonging to the class and phylum Thermotogae, was the most abundant genus of bacteria in the control sample (4.1%). Furthermore, the relative abundance of Bacteroidetes was 22.7 and 29.1% with TH and WAO pretreatment and was the most abundant phylum of the bacterial community in these two AD samples (Fig. S5c). Moreover, Bacteroidia (phylum: Bacteroidetes) was the predominant class, which accounted for 22.29 and 28.50% of the bacterial community with TH and WAO pretreatment (Fig. S5d). In Fig. S5e, the relative abundance value of *SC103* was 9.7% for the sample with WAO pretreatment. The relative abundance of *Exilispira* (phylum: Spirochaetes and class: Brachyspirae) was 5.0%, which was the most abundant genus of the sample with TH pretreatment.

From the results outlined above, it can be seen that the gas conditions of HTP play important roles for the bacterial and archaeal communities. Methane was mainly formed from both the acetoclastic and hydrogenotrophic pathways with HTP using nitrogen as the working gas and the acetoclastic pathway with HTP using air as the working gas. In addition, *Methanosarcina* and *Candidatus* Methanofastidiosum were both found increasing in AD of SS following HTP using air as the working gases, suggesting more acetate and methylated thiol could be present in this AD system.

Effect of the HTP Catalyst on the Microbial Community in AD

The microbial communities of the batch AD samples after WAO with and without K_2CO_3 were analyzed (Fig. 7; Fig. S6). Methanosarcinales was predominant in the archaeal order, which amounted to 79.3% in the control sample, 62.1% for WAO pretreatment without added K_2CO_3, 73.5% for WAO pretreatment with 5 wt% added K_2CO_3, and 74.4% for WAO pretreatment with 10 wt% added K_2CO_3 (Fig. S6a). Pretreatment and the adding of K_2CO_3 increased the relative abundance value of the genus *Methanosarcina* (order: Methanosarcinales), which increased from 0.12% in the control sample to 6.8% after WAO pretreatment without K_2CO_3, 21.8% of WAO pretreatment with 5 wt% K_2CO_3, and 39.5% of WAO pretreatment with 10 wt% K_2CO_3, respectively (Fig. S6b). Meanwhile, the relative abundance value of the genus *Methanosaeta* (order: Methanosarcinales) decreased when the samples were pretreated and more K_2CO_3 was added, dropping from 79.2% in the control sample to 55.0% in pretreatment without K_2CO_3, 51.1% in pretreatment with 5 wt% K_2CO_3, and 34.3% in pretreatment with 10 wt% K_2CO_3. As with *Methanosaeta*, the value of *Methanolinea* (order: Methanosarcina) also went down from 15.2% of the control to 15.0% in pretreatment without K_2CO_3, 6.6% in pretreatment with 5 wt% K_2CO_3 and 6.0% in pretreatment with 10 wt% K_2CO_3 (Fig. S6b).

Proteobacteria was the predominant bacterial phylum in the control sample, which had a relative abundance value of 32.7%. Moreover, Gammaproteobacteria was the abundant class in this phylum with 22.5% of the relative abundance value. The genus *SC103* of the class and phylum Thermotogae was predominant in the control and WAO pretreatment without K_2CO_3, making up 4.1 and 9.7% of the relative abundance, respectively. The most abundant phylum of bacteria in all of the pretreated samples was Bacteroidetes, which constituted 29.1, 31.4 and 30.2% of the bacteria community in WAO pretreatment with 0, 5, and 10 wt% K_2CO_3, respectively (Fig. S6c). Nevertheless, Bacteroidia (phylum Bacteroidetes) was the predominant class and amounted 28.5% in WAO pretreatment without K_2CO_3, 30.8% in WAO pretreatment with 5 wt% K_2CO_3, and 29.7% in WAO pretreatment with 10 wt% K_2CO_3 (Fig. S6d). However, *Macellibacteroides* (phylum: Bacteroidetes and class: Bacteroidia) was the most abundant genus of bacteria under higher ratios of K_2CO_3 addition, showing 7.2 and 9.5% in the 5 and 10 wt% K_2CO_3 conditions (Fig. S6e).

The results show that K_2CO_3 addition influenced the bacterial and archaeal community structures in AD. Methane was formed mainly from the acetoclastic pathway if the amount of K_2CO_3 was equal to and less than 10 wt% of the TS in the WAO. However, *Methanosarcina* population gradually increased as we increased the K_2CO_3 addition in WAO and exceeded *Methanosaeta* population when 10 wt% K_2CO_3

was in WAO. As reported in the previous literature, high acetate concentrations will favor the dominance of *Methanosarcina* [57], this result implied more acetate was produced in the AD. Volatile fatty acid (VFA) generation has been reported during thermal pretreatment of sludge with oxygen [30]. A previous study has reported that VFA yield could be improved in the anaerobic fermentation of heat-alkaline pretreated SS, and acetate was detected as the major VFA [59]. Therefore, higher concentrations of acetate could be formed after WAO or during AD of pretreated sludge. In addition, spiking K_2CO_3 in WAO would increase salt concentration, and the elevated osmotic pressure due to high salt concentrations will affect archaeal community and likely selectively enrich *Methanosarcina*, since the species of *Methanosarcina* were reported tolerant to high levels of salt [60]. Further study is still needed to elucidate the mechanisms of the increasing *Methanosarcina*, since currently, there is limited information regarding the possible reactions for HTP when air is the working gas and K_2CO_3 is added.

Conclusions

In this study, HTP was demonstrated to enhance the efficiency of the AD of SS. HTP changed the characteristics of the sludge, increasing the values of SCOD, TDN, DOC, and COD solubilization. The WAO process at low pressure served as an effective pretreatment to improve the yield of methane in AD, but the yield of methane from WAO alone was less than TH. However, this process largely improved the yield of methane to 277 mL/gVS$_{in}$ and percentage of methane to 73% at 180 °C and 30 min of residence time when K_2CO_3 was added during WAO in the amount of 10 wt% of the TS in the sample. Furthermore, this study shows that the residence time, working gas, and catalyst of HTP had an effect on the structure of the microbial community in AD. Bacteroidetes, Bacteroidia, and *SC103* were, respectively, the dominant phylum, class, and genus of bacteria in most of the samples. The most abundant archaeal order was Methanosarcinales, while *Methanosaeta* was the most abundant archaea genus in almost all of the samples. In addition, the relative abundance of the order Methanosarcinales and genus *Methanosarcina* increased when the amount of K_2CO_3 rose during the WAO process. Our results warrant the need to further understand the mechanisms behind the enhanced methane yield and shift of methanogen community in AD when K_2CO_3 was added during HTP of SS using air as the working gas.

Compliance with Ethical Standards

Conflict of Interest   The authors declare that they have no competing interests.

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