A comparative study on mesophilic and thermophilic anaerobic digestion of different total solid content sludges produced in a long sludge-retention-time system

YI WANG (✉ wangyi1003@sina.com)

Xi'an University of Architecture and Technology <https://orcid.org/0000-0002-2511-2266>

Miao Wang

Xi'an University of Architecture and Technology

Jun Peng

Xi'an University of Architecture and Technology

Linping Wang

Xi'an University of Architecture and Technology

Jie Yang

Xi'an University of Architecture and Technology

Xiaomei Kou

Power China-northwest engineering corporation limited

Baohua Chai

power China-northwest engineering corporation limited

Lijuan Gao

power China-northwest engineering corporation limited

Xiaofeng Han

power China-northwest engineering corporation limited

Research Article

Keywords: Waste sludge, Long SRT, Thermophilic anaerobic digestion, Mesophilic anaerobic digestion, Lower TSC

Posted Date: March 3rd, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1391343/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Most waste sludge belongs to long sludge-retention-time (SRT) sludge in China, but its outlet is becoming a severe environmental problem nowadays. In this study, the characteristics of mesophilic and thermophilic anaerobic digestion of long SRT sludge was investigated under different total solid content (TSC) of 4%, 6%, and 8% in batch reactors. The results showed that the methane production potential (P_0) and methane production rate ($R_{\max}$) of thermophilic anaerobic digestion (TAD) were increased by about 60% and 200% compared with that of the mesophilic anaerobic digestion (MAD) at 4% TSC. In TAD groups, the P_0 of 240.95 L/kg-VS and $R_{\max}$ of 22.17 L/kg-VS/d were achieved in the reactor of 4% TSC while the inhibition concentration of propionate and free ammonia reached 1022 mg/L and 603 mg/L in the reactor of 8% TSC. Although the diversity of microbial community was poor in TAD, *Coprothermobacter* was enriched in TAD, which could degrade protein efficiently. In addition, the dewaterability of digested sludge in TAD was poorer than that in MAD, and both got worse with the increased TSC. Therefore, it is appropriate for the long SRT sludge to anaerobic digestion under thermophilic condition and lower TSC. So, thermophilic anaerobic digestion with lower TSC might be an alternative approach for the treatment and resource utilization of long SRT sludge.

Statement Of Novelty

Although several studies have been focused on sludge anaerobic digestion, there are few tests on the comparison of mesophilic and thermophilic anaerobic digestion for long sludge-retention-time (SRT) sludge under different total solid content (TSC). Therefore, we compared the differences in gas production performance, hydrolysis performance, microbial community and dewaterability of the digested sludge during mesophilic and thermophilic anaerobic digestion under different TSC, and determined the better conditions of long SRT sludge for anaerobic digestion finally.

Introduction

With the decrease of water environmental capacity and the stricter sewage discharge standards, biological nutrient removal process (BNR), such as oxidation ditch or A2O craft, is used widely in wastewater treatment plants (WWTPs) of China for removal of nitrogen and phosphorus. One of the characteristics of BNR process is to produce massive waste sludge which has a sludge retention time (SRT) of more than 10 days. This waste sludge has high content of nitrogen and phosphorus due to the long generation cycle of nitrifying bacteria [1], which is significantly different from the traditional activated sludge. Furthermore, the amount of waste sludge production is increasing rapidly with the increasing quantity of municipal wastewater. Therefore, the development of an appropriate disposal approach for the waste sludge from BNR process is very crucial.

Anaerobic digestion (AD) is widely used for stabilizing and resourcing waste sludge of WWTPs due to its effective energy recovery and sludge volume reduction. The total solid content (TSC) of waste sludge is an indispensable parameter for the design and operation of AD, which is usually controlled at about 4%

as the pumping and mixing of sludge becomes challenging at higher TSC [2]. It is easy to understand that low TSC of waste sludge often means large digester volume due to low organic loading rate (OLR). So, high TSC of waste sludge has become a focus of research in recent years due to the improvement of the treatment capacity of digesters [3]. However, it is reported that the mixing and stirring at a TSC of more than 9% can become difficult because a significant viscosity value mutation occurs with increasing TSC from 8–9% [4]. Duan et al. and Liao et al. also found that the degradation of TS and VS remained unchanged with the increasing TSC in the above range [5, 6]. Wang et al. [7] reported the inhibitory concentration for free ammonia and total ammonia of thermophilic AD under different TS contents. The current studies on AD of waste sludge with different TSC are still very limited, especially for the waste sludge of BNR process. So, the effect of different TSC on AD process needs further study and explore.

Temperature is another important factor affecting the anaerobic digestion process, which usually includes psychrophilic ($< 25\text{ }^{\circ}\text{C}$), mesophilic ($25\text{ }^{\circ}\text{C} \sim 40\text{ }^{\circ}\text{C}$), and thermophilic ($> 45\text{ }^{\circ}\text{C}$) conditions [8]. Psychrophilic anaerobic digestion is rarely applied due to its low efficiency, while mesophilic anaerobic digestion (MAD) has been applied widely because of its good stability. As for thermophilic anaerobic digestion (TAD), on the one hand, its application has been limited due to its high energy demand and poor stability [9, 10]. On the other hand, TAD is favored due to its advantages of fast biogas production rate, high sludge degradation rate, and high methane production potential, which are based on faster hydrolysis under higher temperature [11, 12]. In addition, TAD is more effective in inactivating pathogens than MAD [13], which is very helpful for the final disposal of digested sludge.

The waste sludge of long SRT was extremely difficult to hydrolyze due to the accumulation of inert products in the sludge, which in turn leads to a longer digestion time under MAD [14]. Some researchers have studied the utilization of thermal hydrolysis pre-treatment (THP) to solve the difficult hydrolysis problem of long SRT sludge [15–17]. However, the studies on hydrolysis and methanogenesis of TAD for long SRT sludge from BNR process are few.

The purpose of this study was to compare the difference of MAD and TAD for long SRT waste sludge in terms of gas production potential, hydrolysis characteristics, and dewaterability of digested sludge under different TS contents in MAD and TAD. The results would provide a meaningful reference for the practical application of AD in treating long SRT waste sludge from the BNR process.

Materials And Methods

Substrates and inoculum

Long SRT waste sludge (dewatered sludge) was collected from the No. 3 wastewater treatment plant in Xi'an, where the orbital oxidation ditch was adopted as the sewage treatment craft and the SRT of more 18 days was controlled during the operation. The seed sludge was obtained from a laboratory scale anaerobic reactor ($35 \pm 2\text{ }^{\circ}\text{C}$) and then subjected to a period of domestication. The physicochemical properties of the dewatered sludge and inoculum sludge are listed in Table 1.

Table 1
Physical and chemical properties of dewatered sludge and
inoculum sludge.

Parameter	Inoculum sludge	Dewatered sludge(DWS)
pH	7.14	6.89
TS(g/kg)	39.43	167.32
VS(g/kg)	18.39	92.95
VS/TS (%)	46.64	55.55
TCOD(g/Kg)	46.15	160.69
TN (% of TS)	/	4.66
TP (% of TS)	/	1.90
TOC (% of TS)	/	12.71

Experimental setup

Serum bottles (100 mL) with working volume of 60 mL were used to collect and analyze gas production, and serum bottles (250 mL) with working volume of 200 mL were used to collect the liquid samples for hydrolysis analysis in this experiment. Total solid content (TSC) of 4%, 6%, and 8% were set for both MAD and TAD. Serum bottles (100 and 250 mL) were numbered as M-4%, M-6%, and M-8% for MAD and T-4%, T-6%, and T-8% for TAD, respectively. The ratio of dewatered sludge to inoculum was set to 7:3 (w/w VS), and the mixed sludge was diluted to the TSC of 4%, 6%, and 8% with deionized water. The serum bottles were purged by nitrogen gas for 2 min to remove the oxygen and then placed into water bath at 36 °C for MAD and 52 °C for TAD at a rate of 140 r/min. Blank experiment with inoculum only was used for deducting the gas production from the seed sludge. Each experiment was conducted in triplicates.

Analytical methods

Physical and chemical analysis

Biogas production was measured daily with a calibrated glass syringe and its composition (CH_4 , CO_2 , N_2 , and H_2) was analyzed by GC (Agilent6890N, TCD, TDX-01). The liquid samples were collected once every 7 days, and 20 mL of the liquid samples were extracted from the serum bottle each time. The pH was determined by a portable pH meter (PHS-3C). TS and VS were determined by using the weight method. Total ammonia nitrogen (TAN) was determined with a standard method [18] and chemical oxygen demand (COD) was determined by colorimetric analysis using dichromate reflux and phenol-sulfuric acid methods. Analyses of total organic carbon (TOC) were conducted using a Vario EL element analyzer (Germany). Total nitrogen (TN) and total phosphorus (TP) of sludge were determined by reference to the soil test method [19].

Volatile organic acids of acetate, propionate, butyrate, and valerate (VFAs) were assayed by GC (Agilent6890N, FID) after all the samples were centrifuged at 10000 r/min for 10 min and passed through a cellulose acetate filter (pore size = 0.22 µm). In addition, the liquid samples were taken, and the dewaterability of digested sludge was determined by the Brinell funnel for the specific resistance to filtration (SRF) of sludge at the end of the experiment.

Microbial community extraction and analysis

To identify the hydrolysis and acid-producing bacteria in MAD and TAD, samples were collected from M-4%, M-8%, T-4%, and T-8% groups at the end of experiments and placed in dry ice for transportation at low temperature. The total DNA was extracted from sludge using the OMEGA kit (Life, USA), whereas its completeness was tested by agarose gel electrophoresis. Genomic DNA was accurately quantified using the Qubit 2.0 DNA Assay Kit (Life, USA) to determine the amount of DNA that should be added to the PCR reaction. PCR was amplified with the primers of 341F (5'-CCCTACACGACGCTCTTCCGATCTG-3') and 805 R(5'-GACTGGAGTTCCTTGGCACCCGAGAATTCCA-3'). Illumina MiSeq high-throughput sequencing (HTS) was performed by Jingneng Biotechnology Co., Ltd. (Shanghai, China).

Parameters processing and analysis

The amended Gompertz model (Eq. (1)) was applied to determine the lag phase time and methane production potential [20]. The time period to produce 90% of methane yield (T_{90}) and the time period for effective methane production (T_{ef}) can be calculated according to Eqs. (2) and (3), respectively [21]. Eq. (4) was adopted to calculate the concentration of free ammonia nitrogen (FAN) with the actual pH and temperature [22].

$$P = P_0 \cdot \exp \left\{ - \exp \left[(R_{max} \cdot e^{-\lambda} (\lambda - t) / P_0) \right] + 1 \right\} \quad (1)$$

$$T_{90} = \lambda + 3.25 \times P_0 / (R_{max} \cdot e) \quad (2)$$

$$T_{ef} = T_{90} - \lambda \quad (3)$$

$$FAN / TAN = \left\{ 1 + 10^{-pH} / \left\{ 10^{-[0.09018 + 2729.92 / T(K)]} \right\} \right\}^{-1} \quad (4)$$

where P (L/kg-VS added) is the cumulative methane yield at time t , the value of e is 2.7183., R_{max} (L/kg-VS added /day) is the maximum specific methane production rate, P_0 (L/kg-VS added) is the methane production potential, and λ (day) is the lag phase time. The parameters in this equation (P_0 , R_{max} , and λ) were estimated by the nonlinear fitting of Origin 2017. TAN (mg/L) is the total ammonia nitrogen concentration, FAN (mg/L) is the free ammonia nitrogen concentration, and $T(K)$ is the temperature (Kelvin).

Results And Discussion

Effects of total solid content (TSC) on gas production of MAD and TAD

Biogas production of MAD and TAD under different TSC

Bio-Methane Potential batch test was conducted for 35 days. The biogas production rate is shown in Fig. 1a. The gas production rate of TAD was significantly higher than that of MAD under same TSC in the first 13 days, but the biogas production rate of different TSC at the same temperature have no significant differences. The biogas production rates of M-4%, M-6%, and M-8% groups reached its maximum on day 3, which were 19.6, 23.2, and 21.7 mL/g-VS/d, respectively. However, the maximum biogas production rates of T-4%, T-6%, and T-8% of TAD were 44.5, 44.5, and 37.7 mL/g-VS/d on day 6 or 7, which were 2.27, 1.92, and 1.74 times higher than that of MAD under the same TSC, respectively. In addition, the biogas production rate fluctuated greatly at the early stage of TAD, and its duration was shorter compared with MAD. This result was consistent with the study of De Vrieze et al., who also found that the reaction rate of TAD was faster than that of MAD due to the accelerated biochemical reaction rate caused by higher temperature [23].

The cumulative biogas yield of different groups was displayed in Fig. 1b. The biogas production decreased with increasing TSC in the groups of TAD, and the biogas production in T-4%, T-6%, and T-8% were 368.4, 352.3, and 333.4 mL/g-VS, respectively, which was similar to the results of Wang et al [7]. On the contrary, the biogas production in the groups of MAD increased with increasing TSC and the final biogas yields of M-4%, M-6%, and M-8% were 235.1, 266.5, and 320.5 mL/g-VS, respectively, which was opposite to the change trend in TAD. Even so, the final biogas production of TAD was significantly higher than that of MAD under the same TSC, which meant that TAD was more powerful to produce biogas. This finding proved TAD could be capable to produce more biogas volume than MAD in the specific duration[24]. However, the TSC of sludge had different effect on biogas production of TAD and MAD.

Biogas composition of MAD and TAD under different TSC

The average contents of CH₄, CO₂, H₂, and N₂ in the total biogas volume during the whole experiment are shown in Fig. 2. The CH₄ content of biogas in M-6%, M-8%, T-4% and T-6% digester were 62%, whereas it was 59% and 55% in M-4% and T-8% digester, respectively. In addition, the contents of CO₂ and N₂ in biogas were 29% ~ 43% and 2% ~ 11%, respectively. During the whole experiment, no H₂ was detected in all groups. Some studies had shown that the higher CH₄ content in biogas was due to the more stable AD process [25, 26]. From this view, the stability of M-6%, M-8%, T-4% and T-6% digesters was better than that in M-4% and T-8% digester.

Kinetic modeling with amended Gompertz model

The kinetic parameters obtained by nonlinear curve fit using the amended Gompertz model are listed in Table 2. The λ of methane production in MAD groups was distinct from that in TAD groups, which was below 0.23 day in MAD groups, while maintained at about 0.5 day in TAD groups, indicating that the microorganisms of TAD need a longer adaptation period compared with those of MAD as reported previously [26]. In addition, the methane production rate ($R_{\max}$) of TAD was significantly greater than that of MAD under the same TSC. Being the highest one among MAD groups, the $R_{\max}$ of M-8% was 10.98 L/kg-VS/d, whereas it reached 22.17 L/kg-VS/d in T-4%, which exhibited TAD was significantly better than MAD in terms of methane production rate.

Table 2
Kinetic parameters of methane production by the amended Gompertz equation

Parameter	λ	$R_{\max}$	P_0	R^2	T_{90}	T_{ef}
Unit	d	L /kg-VS/d	L /kg-VS	/	d	d
M-4%	< 0.1	6.96	146.42	0.9856	23.52	25.16
M-6%	< 0.1	7.88	167.14	0.9840	23.53	25.36
M-8%	0.23	10.98	205.01	0.9972	22.55	22.32
T-4%	0.51	22.17	240.95	0.9953	13.51	13.00
T-6%	0.46	19.5	222.58	0.9962	14.11	13.65
T-8%	0.52	14.44	207.37	0.9909	17.68	17.16

Table 2 shows that the methane production potential (P_0) of TAD was significantly greater than that of MAD at the same TSC. However, the P_0 in TAD groups decreased with increasing TSC, while it increased with increasing TS in MAD groups. Even so, this study displayed that the highest P_0 in MAD groups was still smaller than the lowest P_0 in TAD groups. In addition, it was obvious that T_{ef} was longer in MAD than that in TAD, which was similar with T_{90} in both AD systems. Surprisingly, the shortest T_{ef} and T_{90} in MAD groups occurred in M-8%, whereas the shortest values in TAD groups were obtained in T-4%. However, both T_{ef} and T_{90} in T-4% were only about 60% of those in M-8%, which implied that the T-4% group was more efficient than others.

To sum up, it seemed TAD is generally superior to MAD in terms of methane production rate, methane production potential, and the time required for completing AD. Meanwhile, the performance of TAD would be deteriorated with the increasing TSC, but MAD showed an opposite trend with the increasing TSC.

Effects of different TSC on hydrolysis of MAD and TAD

Evolution of VFAs concentration in liquid phase

The variation of VFAs (acetate, propionate, butyrate, and valerate) under different TSC of MAD and TAD groups is illustrated in Fig. 3. Acetate and propionate were the dominant components among the

intermediate VFAs whether in TAD or MAD groups.

Figures 3a, 3b, and 3c show that no obvious accumulation of VFAs existed in the MAD groups except more than 200 mg/L accumulated in M-4% groups on 28th day. It seemed that VFAs concentration was mainly acetate in M-4% group while propionate in M-8% group, which meant that more propionate was produced in MAD at high TSC initially.

Figures 3d, 3e, and 3f show the variation of VFAs in TAD groups. VFAs in the TAD groups reached the peak value of 582.2, 937.5, and 1402.9 mg/L, respectively, on day 7 in the digesters of T-4%, T-6%, and T-8%, where the accumulation of propionate was notable, thereby showing that faster hydrolysis rate occurred in TAD initially. Subsequently, VFAs concentrations in T-4% and T-6% groups decreased obviously, but the VFAs accumulation appeared in the T-8% group continuously, although it appeared a little decrease with time. By the end of the experiment, the accumulation of propionate still remained at about 400 mg/L and acetate reached 618.5 mg/L in TAD of T-8% group. This result indicated that more propionate and acetate would be produced in TAD process, and its utilization rate decreased with the increasing TSC, which also proved that TAD may lead to higher intermediate product accumulation at higher TSC [27, 28]. This result implied that TAD of waste sludge with high TSC was more likely to be inhibited by the accumulation of intermediate products especially propionate and acetate.

Evolution of SCOD, TAN, FAN concentration, and pH in liquid phase

SCOD reflected the dissolution, hydrolysis and acidification of organic matter in AD system and the evolution of SCOD concentration in both ADs was displayed in Fig. 4a. It showed that the SCOD concentration in TAD groups was significantly higher than that in MAD groups, and both SCOD concentrations were positively correlated with the TSC. In TAD groups, SCOD concentration reached the peak value on day 7 for T-4% and T-6% as VFAs concentration aforementioned while SCOD concentration in T-8% group reached the peak at the end of the experiment. Generally speaking, SCOD in TAD group appeared a higher concentration on day 7 and then decreased slightly, but in the later stage, an obvious upward trend occurred again, especially in T-8% group. The results proved that TAD effectively improved hydrolase activity and increased the dissolution of substrates as reported previously [29, 30].

The pH value is affected by multiple factors, such as temperature, alkalinity, volatile fatty acids, ammonia, and others [31]. Figure 4b displays the pH evolution with time. The initial pH of MAD and TAD groups was approximately 7.2, which decreased on day 7 and then increased successively to the end of the experiment. Compare with the TAD groups, all the pH levels in MAD groups were low at the same TSC. In addition, the high TSC was related with high pH value in the same AD groups. This finding indicated that higher TSC of waste sludge and higher temperature of AD system led to a higher pH, which seemed inconsistent with the result of VFAs accumulation. In fact, this may be ascribed to the characteristics of long SRT sludge, which contained more protein and could release more ammonia nitrogen from the bacteria cell [20].

Figure 4c showed that TAN concentration increased with increasing TSC whether in TAD or MAD groups. Similar to SCOD concentration and pH, TAN concentration in TAD group was much higher than that of MAD group. TAN concentration of T-4%, T-6%, and T-8% peaked on day 15, then declined slightly and gradually accumulated to 1171, 1655, and 2139 mg/L, respectively. However, TAN concentrations of M-4%, M-6%, and M-8% accumulated gradually with the progress of digestion and reached 703, 1044, and 1314 mg/L, respectively. Ammonia nitrogen is a typical methane fermentation inhibitor [5, 32, 33] that ranged from 1.1 g/L to 1.18 gTAN/L [34]. The effect of ammonia on methane fermentation can be evaluated by free ammonia nitrogen (FAN), which has been suggested as the active component that causes ammonia inhibition, considering that it is freely membrane permeable [35].

The evolution of FAN is displayed in Fig. 4d, which is similar to the evolution of TAN in this study. The FAN concentration in TAD was much higher than that in MAD, and it increased with the increasing TSC whether in TAD or MAD groups. Thus, FAN concentration in T-8% group reached the maximum value of 603 mg/L at the end of the experiment. Li reported that free ammonia at 560 ~ 568 mg/L inhibited methane production by 50% in TAD [36]. Another study on TAD of manure also showed poor treatment ability, when the free ammonia was higher than 600 mg/L[37]. This indicated that the T-8% group might be affected by the inhibition of FAN, which was consistent with its poor methanogenic performance as Fig. 1 shown. So, the methane production in the TAD of BNR sludge was easily inhibited by the excessive accumulation of FAN, especially under high TSC.

Bacteria community of hydrolysis

Diversity analysis of bacterial community

Table 3 shows the microbial diversity indexes of the groups of M-4%, M-8%, T-4%, and T-8%. The values of OTU, Chao, ACE, Shannon, Simpson, and Shannon even indices in the groups of MAD were higher than those of TAD. This indicated that higher temperature could reduce the diversity and abundance of microbial community during anaerobic digestion, which was also related to the poor stability of TAD according to other researchers [38]. However, M-4% group had slightly higher bacterial species index than M-8% group, whereas T-8% group had slightly higher bacterial species index than T-4% group. Therefore, TSC of waste sludge had little direct influence on the microbial community of AD compared with the temperature.

Table 3
Microbial diversity indices in the groups of M-4%, M-8%, T-4% and T-8%

Sample	Observed OTU	Chao	ACE	Shannon	Simpson	Goods Coverage	Shannoneven
M-4%	888	955	949	5.439	0.9878	0.9993	0.8011
M-8%	792	848	851	4.787	0.9582	0.9989	0.7172
T-4%	547	670	666	2.667	0.8053	0.9979	0.4230
T-8%	600	704	697	3.178	0.8554	0.9986	0.4967

Differences in the genus level of hydrolysis

The differences of microbial community on genus level are given in Fig. 5. Although the dominant genus was not obvious in the MAD groups compared with TAD groups, the diversity of microbial community in the MAD groups are significantly more than that of the TAD groups. In detail, *Bacteroidetes*, *Aminicenantes genera incertae sedis*, *liumatobacter*, and *Gammaproteobacteria* were relatively massive in MAD groups. The abundance of *Aminicenantes genera incertae sedis* was higher in the M-8% group than in the M-4% group. In addition, some bacteria that belong to *Aminicenantes* can ferment carbohydrates and proteinaceous substrates [39, 40], which may be related to the larger methane yield in M-4% group.

The dominant genera in the TAD groups were *Coprothermobacter*, *Clostridiales* and *Bellilinea*. Among them, *Coprothermobacter* is a thermophilic protein-degrading anaerobe [41] related to the degradation of abundant protein. This can explain why higher ammonia nitrogen accumulation was observed in the TAD groups. *Clostridiales* belongs to the phylum of *Firmicutes*, which could not only hydrolyze proteins, lipids, and cellulose, but also oxidize acetate. In this study, *Clostridiales* accounted for about 5% in T-4% group but 20% in T-8% group. However, *Bellilinea*'s share of T-4% and T-8% is the opposite of *Clostridiales*, which accounted for 30% in T-4% and less than 1% in T-8%. Ye et al. found a kind of *Clostridium straminisolvens* (Accession No. NR024829), which is an aerotolerant, thermophilic, anaerobic, and cellulolytic bacterium involved in hydrolysis and acidogenesis [42]. This bacterium may be related to some *Clostridiales* bacteria found in the T-8% group. *Bellilinea* can utilize a variety of carbon sources and is reportedly related to the degradation of propionate [43]. In this study, the presence of *Bellilinea* is hardly observed in T-8%. This finding reflects the continuous accumulation of propionate in the T-8% group with high TSC of TAD.

Dewaterability of the digested sludge

Specific resistance to filtration (SRF) is one of the important indexes that are used to describe dewaterability of sludge. SRF of the digested sludge in MAD and TAD groups are shown in Table 4.

Table 4
SRF of different digested sludge

parameter	unit	M-4%	M-6%	M-8%	T-4%	T-6%	T-8%
SRF	s ² /g	1.27×10 ⁸	4.69×10 ⁸	8.57×10 ⁸	1.51×10 ¹⁰	2.62×10 ¹⁰	3.38×10 ¹⁰

It can be seen that the digested sludge in M-4% group was easy to filter, whereas the digested sludge in M-8% group was moderately difficult to filter. The filtration difficulty of the digested sludge in M-6% group was between M-4% and M-8%. However, all the SRF values of digested sludges in TAD group increased up to two order of magnitudes compared with that in MAD group, which indicated the digested sludges of TAD were more difficult to filter. In addition, the difficulty degree was increased with the increasing TSC for both MAD and TAD groups, which was similar to the finding of Wang et al. [7]. Some studies also revealed that the bad dewaterability of digested sludge in the TAD was caused by high concentration of SCOD[25, 44, 45]. This study showed that TAD and high TSC led to a high concentration of SCOD, which did worsen the dewaterability of digested sludge.

Conclusion

Higher methane production potential and faster digestion rate were observed in TAD compared with MAD at the same total solid content (TSC) of long SRT sludge. In MAD groups, the methane production increased by about 40% with increasing TSC from 4–8%. However, high TSC especially 8% in TAD, may lead to an obvious accumulation of propionate and acetate. Meanwhile, TAD could enrich some specific thermophilic bacteria, such as *Coprothermobacter*, which led to produce high concentration of TAN and FAN. This high concentration of TAN and FAN may inhibit the methane production in TAD system of high TSC. In addition, the poor dewaterability of digested sludge cannot be ignored in TAD. In brief, the superiority TAD for the long SRT sludge lies in its higher methane production potential and faster digestion rate and an appropriate total solid content can guarantee the successful operation of TAD.

Declarations

Acknowledgments

This work was supported by the Science and technology innovation demonstration project of social development of Xi'an Science and Technology Bureau (Project No. 20SFSF0011), Key Research and Development Program of Shaanxi Province (Grant number: 2021ZDLSF05-04) and National Natural Science Foundation of China (Grant number: 52170172).

Conflict of interest The authors declare that they have no conflict of interest.

Availability of data and material Data for this study are available.

References

1. Li, X., Chen, H., Hu, L., Yu, L., Chen, Y., Gu, G.: Pilot-scale waste activated sludge alkaline fermentation, fermentation liquid separation, and application of fermentation liquid to improve biological nutrient removal. *Environ. Sci. Technol.* **45**, 1834–1839 (2011). <https://doi.org/10.1021/es1031882>
2. Di Capua, F., Spasiano, D., Giordano, A., Adani, F., Fratino, U., Pirozzi, F., Esposito, G.: High-solid anaerobic digestion of sewage sludge: challenges and opportunities. *Appl. Energ.* **278**, 115608 (2020). <https://doi.org/10.1016/j.apenergy.2020.115608>
3. Álvarez, C., Colón, J., Lópes, A.C., Fernández-Polanco, M., Benbelkacem, H., Buffière, P.: Hydrodynamics of high solids anaerobic reactor: Characterization of solid segregation and liquid mixing pattern in a pilot plant VALORGA facility under different reactor geometry. *Waste Manage.* **76**, 306–314 (2018). <http://dx.doi.org/10.1016/j.wasman.2018.02.053>
4. Yao, Y., Huang, Y., Hong, F.: The influence of sludge concentration on its thermophilic anaerobic digestion performance based on low temperature thermal hydrolysis pretreatment. *Procedia Environ. Sci.* **31**, 144–152 (2016). <https://doi.org/10.1016/j.proenv.2016.02.020>
5. Duan, N., Dong, B., Wu, B., Dai, X.: High-solid anaerobic digestion of sewage sludge under mesophilic conditions: feasibility study. *Bioresour Technol.* **104**, 150–156 (2012). <https://doi.org/10.1016/j.biortech.2011.10.090>
6. Liao, X., Li, H., Cheng, Y., Chen, N., Li, C., Yang, Y.: Process performance of high-solids batch anaerobic digestion of sewage sludge. *Environ. Technol.* **35**, 2652–2659 (2014). <https://doi.org/10.1080/09593330.2014.916756>
7. Wang, T., Chen, J., Shen, H., An, D.: Effects of total solids content on waste activated sludge thermophilic anaerobic digestion and its sludge dewaterability. *Bioresour Technol.* **217**, 265–270 (2016). <https://doi.org/10.1016/j.biortech.2016.01.130>
8. Shin, J., Jang, H.M., Shin, S.G., Kim, Y.M.: Thermophilic anaerobic digestion: effect of start-up strategies on performance and microbial community. *Sci. Total Environ.* **687**, 87–95 (2019). <https://doi.org/10.1016/j.scitotenv.2019.05.428>
9. Anjum, R., Grohmann, E., Krakat, N.: Anaerobic digestion of nitrogen rich poultry manure: impact of thermophilic biogas process on metal release and microbial resistances. *Chemosphere.* **168**, 1637–1647 (2017). <https://doi.org/10.1016/j.chemosphere.2016.11.132>
10. Mao, C., Feng, Y., Wang, X., Ren, G.: Review on research achievements of biogas from anaerobic digestion. *Renew. Sust Energ. Rev.* **45**, 540–555 (2015). <https://doi.org/10.1016/j.rser.2015.02.032>
11. Arras, W., Hussain, A., Hausler, R., Guiot, S.R.: Mesophilic, thermophilic and hyperthermophilic acidogenic fermentation of food waste in batch: Effect of inoculum source. *Waste Manage.* **87**, 279–287 (2019). <https://doi.org/10.1016/j.wasman.2019.02.011>
12. Wu, L., Li, X., Yang, F., Zhou, Q., Ren, R., Lyu, Y.: One-step acquirement of superior microbial communities from mesophilic digested sludge to upgrade anaerobic digestion. *Chemosphere.* **263**, 128047 (2021). <https://doi.org/10.1016/j.chemosphere.2020.128047>
13. Ryue, J., Lin, L., Kakar, F.L., Elbeshbishy, E., Al-Mamun, A., Dhar, B.R.: A critical review of conventional and emerging methods for improving process stability in thermophilic anaerobic digestion. *Energy*

- Sustain. Dev. **54**, 72–84 (2020). <https://doi.org/10.1016/j.esd.2019.11.001>
14. Li, Y., Chen, Y., Wu, J.: Enhancement of methane production in anaerobic digestion process: A review. *Appl. Energ.* **240**, 120–137 (2019). <https://doi.org/10.1016/j.apenergy.2019.01.243>
  15. Zhang, L., Gong, X., Wang, L., Guo, K., Cao, S., Zhou, Y.: Metagenomic insights into the effect of thermal hydrolysis pre-treatment on microbial community of an anaerobic digestion system. *Sci. Total Environ.* **791**, 148096 (2021). <https://doi.org/10.1016/j.scitotenv.2021.14809>
  16. Xu, D., Han, X., Chen, H., Yuan, R., Wang, F., Zhou, B.: New insights into impact of thermal hydrolysis pretreatment temperature and time on sewage sludge: Structure and composition of sewage sludge from sewage treatment plant. *Environ. Res.* **191**, 110122 (2020). <https://doi.org/10.1016/j.envres.2020.110122>
  17. Koupaie, E.H., Lin, L., Lakeh, A.B., Azizi, A., Dhar, B.R., Hafez, H., Elbeshbishy, E.: Performance evaluation and microbial community analysis of mesophilic and thermophilic sludge fermentation processes coupled with thermal hydrolysis. *Renew. Sust Energ. Rev.* **141**, 110832 (2021). <https://doi.org/10.1016/j.rser.2021.110832>
  18. APHA: Standard methods for the examination of water and wastewater / American Public Health Association, American Water Works Association, Water Environment Federation, 22nd edition. ed. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington (D.C.), U.S: (2012)
  19. Fan, P., Wang, Y., Wang, W., Chai, B., Lu, X., Zhao, J.: Release characteristics of nitrogen and phosphorus from sediments formed under different supplemental water sources in Xi'an moat, China. *Environ. Sci. Pollut R.* **26**, 10746–10755 (2019). <https://doi.org/10.1007/s11356-019-04537-z>
  20. Nguyen, D.D., Jeon, B., Jeung, J.H., Rene, E.R., Banu, J.R., Ravindran, B., Vu, C.M., Ngo, H.H., Guo, W., Chang, S.W.: Thermophilic anaerobic digestion of model organic wastes: evaluation of biomethane production and multiple kinetic models analysis. *Bioresour Technol.* **280**, 269–276 (2019). <https://doi.org/10.1016/j.biortech.2019.02.033>
  21. Andriamanohiarisoamanana, F.J., Saikawa, A., Tarukawa, K., Qi, G., Pan, Z., Yamashiro, T., Iwasaki, M., Ihara, I., Nishida, T., Umetsu, K.: Anaerobic co-digestion of dairy manure, meat and bone meal, and crude glycerol under mesophilic conditions: Synergistic effect and kinetic studies. *Energy Sustain. Dev.* **40**, 11–18 (2017). <https://doi.org/10.1016/j.esd.2017.05.008>
  22. Yang, Z., Wang, W., He, Y., Zhang, R., Liu, G.: Effect of ammonia on methane production, methanogenesis pathway, microbial community and reactor performance under mesophilic and thermophilic conditions. *Renew. Energ.* **125**, 915–925 (2018). <https://doi.org/10.1016/j.renene.2018.03.032>
  23. De Vrieze, J., Smet, D., Klok, J., Colsen, J., Angenent, L.T., Vlaeminck, S.E.: Thermophilic sludge digestion improves energy balance and nutrient recovery potential in full-scale municipal wastewater treatment plants. *Bioresour Technol.* **218**, 1237–1245 (2016). <https://doi.org/10.1016/j.biortech.2016.06.119>

24. Tian, Z., Zhang, Y., Li, Y., Chi, Y., Yang, M.: Rapid establishment of thermophilic anaerobic microbial community during the one-step startup of thermophilic anaerobic digestion from a mesophilic digester. *Water Res.* **69**, 9–19 (2015). <https://doi.org/10.1016/j.watres.2014.11.001>
25. Wang, P., Wang, H., Qiu, Y., Ren, L., Jiang, B.: Microbial characteristics in anaerobic digestion process of food waste for methane production—A review. *Bioresour Technol.* **248**, 29–36 (2018). <https://doi.org/10.1016/j.biortech.2017.06.152>
26. De la Rubia, M.A., Riau, V., Raposo, F., Borja, R.: Thermophilic anaerobic digestion of sewage sludge: focus on the influence of the start-up. A review. *Crit. Rev. Biotechnol.* **33**, 448–460 (2013). <https://doi.org/10.3109/07388551.2012.726962>
27. Song, Y., Kwon, S., Woo, J.: Mesophilic and thermophilic temperature co-phase anaerobic digestion compared with single-stage mesophilic-and thermophilic digestion of sewage sludge. *Water Res.* **38**, 1653–1662 (2004). <https://doi.org/10.1016/j.watres.2003.12.019>
28. Riau, V., De la Rubia, M., Pérez, M.: Temperature-phased anaerobic digestion (TPAD) to obtain class A biosolids: a semi-continuous study. *Bioresour Technol.* **101**, 2706–2712 (2010). <https://doi.org/10.1016/j.biortech.2009.07.072>
29. Xiong, H., Chen, J., Wang, H., Shi, H.: Influences of volatile solid concentration, temperature and solid retention time for the hydrolysis of waste activated sludge to recover volatile fatty acids. *Bioresour Technol.* **119**, 285–292 (2012). <https://doi.org/10.1016/j.biortech.2012.05.126>
30. Hao, J., Wang, H.: Volatile fatty acids productions by mesophilic and thermophilic sludge fermentation: biological responses to fermentation temperature. *Bioresour Technol.* **175**, 367–373 (2015). <https://doi.org/10.1016/j.biortech.2014.10.106>
31. Chen, J.L., Ortiz, R., Steele, T.W., Stuckey, D.C.: Toxicants inhibiting anaerobic digestion: a review. *Biotechnol. Adv.* **32**, 1523–1534 (2014). <https://doi.org/10.1016/j.biotechadv.2014.10.005>
32. Cho, S., Im, W., Kim, D., Kim, M., Shin, H., Oh, S.: Dry anaerobic digestion of food waste under mesophilic conditions: performance and methanogenic community analysis. *Bioresour Technol.* **131**, 210–217 (2013). <https://doi.org/10.1016/j.biortech.2012.12.100>
33. Lv, Z., Wu, X., Zhou, B., Wang, Y., Sun, Y., Wang, Y., Chen, Z., Zhang, J.: Effect of one step temperature increment from mesophilic to thermophilic anaerobic digestion on the linked pattern between bacterial and methanogenic communities. *Bioresour Technol.* **292**, 121968 (2019). <https://doi.org/10.1016/j.biortech.2019.121968>
34. Cardona, L., Mazeas, L., Chapleur, O.: Zeolite favours propionate syntrophic degradation during anaerobic digestion of food waste under low ammonia stress. *Chemosphere.* **262**, 127932 (2021). <https://doi.org/10.1016/j.chemosphere.2020.127932>
35. Tao, B., Donnelly, J., Oliveira, I., Anthony, R., Wilson, V., Esteves, S.R.: Enhancement of microbial density and methane production in advanced anaerobic digestion of secondary sewage sludge by continuous removal of ammonia. *Bioresour Technol.* **232**, 380–388 (2017). <https://doi.org/10.1016/j.biortech.2017.02.066>

36. Li, X., Chen, S., Dong, B., Dai, X.: New insight into the effect of thermal hydrolysis on high solid sludge anaerobic digestion: Conversion pathway of volatile sulphur compounds. *Chemosphere*. **244**, 125466 (2020). <https://doi.org/10.1016/j.chemosphere.2019.125466>
37. Chang, S., Filer, J.: Thermal Hydrolysis to Enhance Anaerobic Digestion Performance of Wastewater Sludge. *Curr. Pollution Rep.* **6**, 1–16 (2020). <https://doi.org/10.1007/s40726-020-00163-3>
38. Chen, Z., Li, W., Qin, W., Sun, C., Wang, J., Wen, X.: Long-term performance and microbial community characteristics of pilot-scale anaerobic reactors for thermal hydrolyzed sludge digestion under mesophilic and thermophilic conditions. *Sci. Total Environ.* **720**, 137566 (2020). <https://doi.org/10.1016/j.scitotenv.2020.137566>
39. Wu, Z., Lin, Z., Sun, Z., Gou, M., Xia, Z., Tang, Y.: A comparative study of mesophilic and thermophilic anaerobic digestion of municipal sludge with high-solids content: Reactor performance and microbial community. *Bioresour Technol.* **302**, 122851 (2020). <https://doi.org/10.1016/j.biortech.2020.122851>
40. Plugge, C.M., Balk, M., Zoetendal, E.G., Stams, A.J.: *Gelria glutamica* gen. nov., sp. nov., a thermophilic, obligately syntrophic, glutamate-degrading anaerobe. *Int. J. Syst. Evol. Micr.* **52**, 401–407 (2002). <https://doi.org/10.1099/00207713-52-2-401>
41. Dombrowski, N., Seitz, K.W., Teske, A.P., Baker, B.J.: Genomic insights into potential interdependencies in microbial hydrocarbon and nutrient cycling in hydrothermal sediments. *Microbiome*. **5**, 1–13 (2017). [10.1186/s40168-017-0322-2](https://doi.org/10.1186/s40168-017-0322-2)
42. Ye, N.F., Lü, F., Shao, L.M., Godon, J.J., He, P.J.: Bacterial community dynamics and product distribution during pH-adjusted fermentation of vegetable wastes. *J. Appl. Microbiol.* **103**, 1055–1065 (2007). <https://doi.org/10.1111/j.1365-2672.2007.03321.x>
43. Jang, H.M., Park, S.K., Ha, J.H., Park, J.M.: Microbial community structure in a thermophilic aerobic digester used as a sludge pretreatment process for the mesophilic anaerobic digestion and the enhancement of methane production. *Bioresour Technol.* **145**, 80–89 (2013). <https://doi.org/10.1016/j.biortech.2013.01.094>
44. Zhang, J., Li, N., Dai, X., Tao, W., Jenkinson, I.R., Li, Z.: Enhanced dewaterability of sludge during anaerobic digestion with thermal hydrolysis pretreatment: new insights through structure evolution. *Water Res.* **131**, 177–185 (2018). <https://doi.org/10.1016/j.watres.2017.12.042>
45. Wang, Q., Xu, Q., Du, Z., Zhang, W., Wang, D., Peng, Y.: Mechanistic insights into the effects of biopolymer conversion on macroscopic physical properties of waste activated sludge during hydrothermal treatment: Importance of the Maillard reaction. *Sci. Total Environ.* **769**, 144798 (2021). <https://doi.org/10.1016/j.scitotenv.2020.144798>

Figures

Figure 1

Evolution of biogas production rate (a) and cumulative biogas yield of unit VS (b) at different TSC in MAD and TAD

Figure 2

Average content of CH_4 , CO_2 , H_2 and N_2 in the biogas at different TSC in MAD and TAD

Figure 3

Change in VFAs in the liquid phase of MAD and TAD groups: a. M-4%; b. M-6%; c. M-8%; d. T-4%; e. T-6% and f. T-8%

Figure 4

Change of SCOD, TAN, FAN concentration and pH in the liquid phase of MAD and TAD groups: a. SCOD; b pH; c. TAN; d. FAN

Figure 5

Microbial community analysis on genus level

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [GraphicalAbstract.png](#)