Journal of Water Process Engineering 42 (2021) 102171

Contents lists available at ScienceDirect

Journal of Water Process Engineering

journal homepage: www.elsevier.com/locate/jwpe

Performance and microbial community analysis of anaerobic sludge digestion enhanced by in-situ microaeration

Zhen Zhou [a,c], Qiang Ming [a], Ying An [a,*], Danian Ruan [a], Guang Chen [b], Haijuan Wei [b], Mengyu Wang [a], Zhichao Wu [c,d]

[a] Shanghai Engineering Research Center of Energy – Saving in Heat Exchange Systems, College of Environmental and Chemical Engineering, Shanghai University of Electric Power, Shanghai 200090, China
[b] Shanghai Chengtou Wastewater Treatment Co., Ltd, Shanghai 201203, China
[c] Shanghai Institute of Pollution Control and Ecological Security, Shanghai 200092, China
[d] State Key Laboratory of Pollution Control and Resource Reuse, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China

ARTICLE INFO

Keywords:
Microaeration
Anaerobic digestion
Methane
Enzymatic activity
Microbial community

ABSTRACT

The effectiveness of in-situ microaeration was investigated with varied air dosages [0–25 mL/(L·d)] to enhance mesophilic anaerobic digestion (AD) of sludge in semi-continuous systems in terms of specific biogas production and volatile solids (VS) removal rate, focusing on performance evaluation, microbial community analysis, and underlying mechanisms. The optimal dosage of 12.5 mL/(L·d) increased specific biogas production by 15.8 ± 7.3% and enhanced VS removal by 18.3 ± 1.6%. In-situ microaeration stimulated activity of hydrolytic enzymes, enlarged sludge particle size, and improved sludge dewaterability. *MiSeq* sequencing analysis showed that dominant bacterial and archaeal communities were remarkably different between in-situ microaeration and obligate AD process. The presence of oxygen enriched relative abundances of Deltaproteobacteria, Anaerolineae, Clostridia, Synergistia and Caldilineae, enabling the acid-producing process to metabolize more types of substrates. Due to the selective enrichment, in-situ microaeration significantly enriched aceticlastic methanogens rather than hydrogenotrophic methanogens in the AD reactor. Analysis of coupling microaeration pretreatment and in-situ microaeration for anaerobic digestion indicated that excessive microaeration decreased VS removal rate and methane yield owing to substrates consumption by facultative bacteria. In-situ microaeration-based AD could be a promising process for sludge treatment and bioenergy recovery.

1. Introduction

Anaerobic digestion (AD), a mature and promising technology [1,2] with enormous developmental potentiality for waste activated sludge (WAS) treatment [3], has drawn extensive attentions in recent years for its low-cost inputs (low investment, low energy consumption and low operation) [4] and high-effect outputs (largely WAS mass and volume reduction, effectively carbon footprint mitigation, inactivation of pathogenic microorganisms, and bioenergy recovery) [5]. However, the anaerobic conversion efficiency of WAS treatment was of poor quality in China compared with that in developed countries [3,6] because of its low organic matter content [7], and high concentrations of metal ions (e.g. Ca^{2+}, Fe^{3+}, Al^{3+} and Mg^{2+}) and grit (50%–65%) [8]. The long solid retention time (10–30 days) [3] of AD process and insufficient investment for WAS management [6] further baffled the application and popularization of AD process. So the proportion of municipal wastewater treatment plants (WWTPs) adopting AD technology was only about 2% in China, greatly lower than the average ratio (above 50%) in developed countries [2].

To overcome these bottlenecks and make progress of AD, effective measures should be taken in terms of technology and management. As for the AD process itself, three main approaches, such as optimization of operational conditions [9], supplement of additives [3] and pretreatment of WAS, have been proposed to improve both efficiency and bioconversion degree of WAS. In fact, no matter which method was adopted, the strengthening mechanism should be to maintain a balance in kinetics and energy during the four stages (hydrolysis, acidification, acetogenesis, methanogenesis) of anaerobic reactions so as to improve the overall stability of AD system as well as enhance methane production [1,9]. Besides, the pretreatment methods, including physical, chemical,

* Corresponding author.
E-mail address: anying007@163.com (Y. An).

https://doi.org/10.1016/j.jwpe.2021.102171
Received 9 March 2021; Received in revised form 18 May 2021; Accepted 1 June 2021
Available online 11 June 2021

biological technologies or their combinations [3,5], were also employed to promote hydrolysis rate and improve system stability [10]. The additives (e.g., nutrient, zero valent iron, nanomaterials and conductive materials) [3] were used to benefit biogas production from sludge [11]. However, certain pretreatment processes or supplement of additives usually required additional energy and/or chemicals, which not only increased operational costs and complexity, but also caused a series of environmental problems [12]. Developing a more environmentally friendly and economically feasible strategy should be taken into consideration.

Recent studies showed that microaeration was more environmentally friendly and cheaper than other pretreatment methods [13], and had advantages of enhancing hydrolysis [12], maintaining low volatile fatty acid (VFAs) concentration [14] and removing H_2S from biogas [1]. Besides, the activity of anaerobic bacteria could not be harmfully affected by microaerobic condition owing to the rapid and almost entire consumption of injected O_2 [15]. Fu, et al. [16] found limited oxygen supply significantly increased cellulase activity and cumulative methane production (CMP) by 10.9–49.0% and 10.2%, respectively. Although previous literatures have studied effects of microaerobic condition on AD of corn straw [17], food waste [18], cellulose and *Miscanthus* [13], etc., there was limited information about effects of in-situ microaeration on the shift of microbial community structure and enzymatic activity of AD for WAS. So, incisive understandings of the mechanisms and microbial metabolism of in-situ microaerated AD systems are still needed.

In this work, different oxygen loads were firstly applied to investigate effects of in-situ microaeration on the AD performance. Then, enzymatic activity, microbial community structure and specific methanogenic activity were investigated to demonstrate the reasons for the elevated AD performance under the circumstance of in-situ microaeration. Lastly, in consideration of microaeration pretreatment enhancing methane production yield and rate of AD for WAS [19], bench-scale experiments were conducted to clarify the technical feasibility associated with energy balance and cost estimates for in-situ microaeration and its combination with microaeration pretreatment on AD for WAS.

2. Material and methods

2.1. Substrate and inoculum

Mixed sludge (MS) employed as substrate for this research consisted of 40% primary sludge and 60% WAS collected from the Bailonggang WWTP (Shanghai, China). Digestion sludge (DS) served as inoculum was taken from a pilot-scale mesophilic digester (35.0 ± 1.0 °C) with semi-continuous feeding of MS acquired from the same source. The main features of MS used were as follows: volatile solids (VS) of 23.7 ± 0.9 g/L, total solids (TS) of 40.6 ± 1.6 g/L, total chemical oxygen demand (COD) of 29,730 ± 1842 mg/L, soluble COD of 458 ± 11 mg/L, ammonium nitrogen (NH_4^+-N) of 58.7 ± 2.6 mg/L, total organic carbon (TOC) of 133.5 ± 1.4 mg/L, VFAs of 308 ± 18 mg/L, pH of 7.1 ± 0.1, polysaccharides (PS) of 19.3 ± 3.2 mg/L, and proteins (PN) of 34.5 ± 2.4 mg/L.

2.2. Setup and operation of semi-continuous anaerobic digester

The tests were carried out in two identical continuous stirred-tank reactors (CSTR) with working volume of 14 L at 35.0 ± 1.0 °C (Fig. 1). The test CSTR (R1) was amended with air at dose of 12.5 (Phase I) and 25 air volume per sludge volume per day [mL/(L·d)] (Phase II) by in-situ microaeration treatment with manual syringe, respectively. The control CSTR (R2) was operated as conventional AD without aeration. The initial MS was inoculated and acclimated for 40 days; then two reactors were continuously operated to verify the effects of in-situ microaeration on AD efficiency. A wet gas flow meter and an online pH meter were employed to monitor biogas volume and pH. The flow rate of MS (substrate) injected into each reactor was 0.7 L/d by a peristaltic pump.

Meanwhile, another CSTR fed with microaeration pretreated sludge (MA reactor) was operated to validate effect of MA on AD technology. MA was obtained under the optimum conditions (4 air volume per gram TS per minute for 4 h) on the basis of our previous research [19].

2.3. Enzymatic activity determination

Sludge samples were gathered at Days 1, 6, 12 and 18 to measure hydrolase activities (α-Glucosidase and protease) and adenosine triphosphate (ATP) concentrations by ELISA kit (Shanghai Hengyuan Biotech) on the basis of former reported methods [19].

2.4. Bacterial and archaeal community analysis

MiSeq sequencing was adopted to analyze influences of in-situ microaeration on the evolution of bacterial and archaeal community structure of sludge by collecting samples from R1 and R2 at Day 20. The

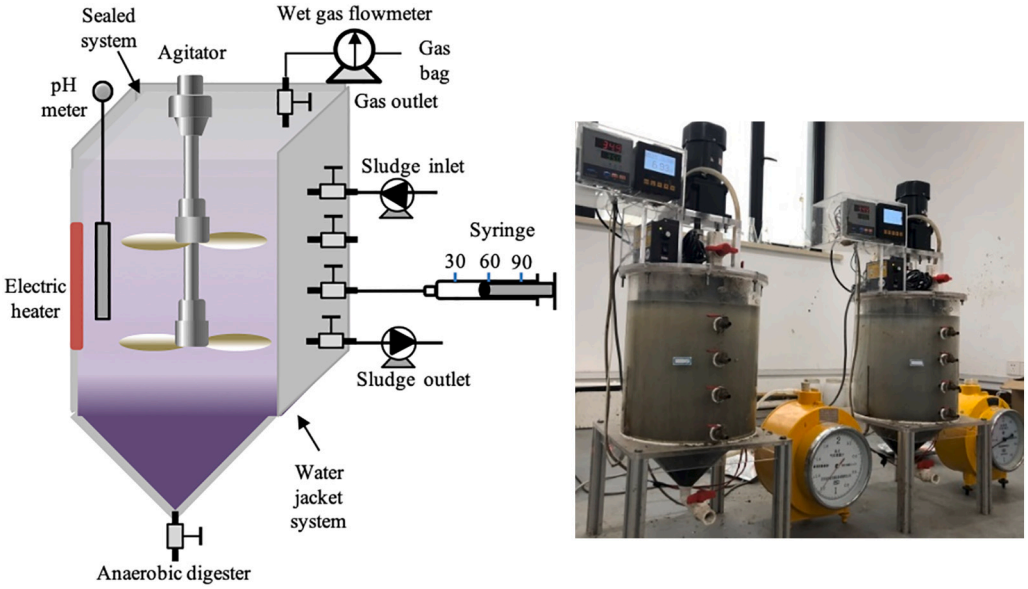

Fig. 1. Schematic of the semi-continuous AD reactors used in this research.

detailed experimental procedures of genomic DNA extraction, PCR amplification, 16S rRNA gene sequencing, biodiversity and phylogenetic analysis could be obtained in Ruan, et al. [19] and Hou, et al. [20]. Then 16S rRNA sequences were clustered into operational taxonomic units by setting a distance cut-off of 0.03 (α).

2.5. Coupling microaeration pretreatment and in-situ microaeration for anaerobic digestion

Batch assays were carried out at 35.0 ± 1.0 °C in 250 mL digester bottles in order to investigate synergistic effects of microaeration pretreatment and in-situ microaeration on methane production of AD. Two different biomass sources collected from R1 and R2 at Day 20 were used as inoculums and designated as RS1 and RS2. The detailed design of batch experiments is demonstrated in Table 1. Firstly, four glass bottles were added with 40 mL inoculated sludge and 160 mL substrate sludge, respectively. Then, pH was adjusted and maintained at 7.2 ± 0.1 by using 2 M HCl and 2 M NaOH.

Lastly, all bottles were degassed with nitrogen (99.99%) for 10 min to displace air and supply absolutely anaerobic environment before starting the experiment, and then incubated at 35.0 ± 1.0 °C up to 14 days in a water bath. Besides, a moderate manual stirring for several seconds was carried out for each bottle several times per day for purpose of maintaining sludge homogeneity. The production of biogas was quantified volumetrically by water displacement test method with a bottle full of 3% w/w NaOH solution connected to absorb H_2S and CO_2.

2.6. Kinetic analysis

CMP curves during the batch assays were calculated by fitting the experimental methane production data with modified Gompertz equation (Eq. (1)).

$$P(t) = Pexp\{-exp[1 + R_m e(\lambda - t)/P]\} \quad (1)$$

where, $P(t)$ is the CMP at time t, mL CH_4/g VS; P is the maximum methane potential, mL CH_4/g VS; R_m is the maximum methane production rate, mL CH_4/(g VS·d); $e = 2.71828$; λ is the lag-phase time, d.

2.7. Other item analysis

The determination of NH_4^+-N, COD, TS and VS were done in accordance twith standard methods [21]. pH was analyzed with a portable pH meter (HQ30d, HACH, USA). TOC concentration was determined by a Multi N/C 3100 Analyzer (Analyti Jena, Germany). Extracellular polymeric substances (EPS) were extracted and stratified to Slime EPS, loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) according to reported methods [22,23]. The concentrations of PS and PN in each EPS fraction were monitored following the modified anthrone method and Lowry method [24]. VFAs were measured by a GC-7900P/FID gas chromatograph (Tianmei, China). The amount of methane was determined by GC-TCD equipped with parallel column of 1.1 m × 3/16″ Molsieve 137 and 0.7 m × 1/4″ chromosorb 108. Specific resistance to filtrate (SRF) was measured by pressure filtration method. Capillary suction time (CST) was analyzed by a portable CST 304B instrument (Triton, UK). The particle size distribution was monitored with an SALD-2201 Laser Diffraction Particle Size Analyzer (Shimadzu, Japan).

Table 1
Parameters of Modified Gomperz equation fitting with methane production data for the combination of microaeration pretreatment and in-situ treatment of MS.

Groups	Substrate	Inoculum	P (mL/g VS)	R_m [mL/(g VS·d)]	λ (d)	R^2
MS + RS2	MS[a]	RS2[c]	64.11 ± 0.12	19.17 ± 1.54	0.11 ± 0.27	0.99995
MS + RS1	MS	RS1[d]	108.32 ± 1.96	8.64 ± 0.53	−2.57 ± 0.46	0.99994
MA + RS2	MA[b]	RS2	85.57 ± 0.87	7.52 ± 0.28	−1.77 ± 0.25	0.99996
MA + RS1	MA	RS1	94.05 ± 0.71	8.45 ± 0.23	−1.43 ± 0.18	0.99998

[a] MS: the raw mixed sludge.

[b] MA: MS with microaeration pretreatment.

[c] RS2: biomass sources collected from obligate AD.

[d] RS1: biomass sources collected from AD with in-situ microaeration.

3. Results and discussion

3.1. Semi-continuous flow experiment

Fig. 2 demonstrates the changes of specific biogas yield and VS removal efficiency in the semi-continuous flow anaerobic reactors with/without in-situ microaeration. As shown in Fig. 2a, the specific biogas yield fluctuated in the two reactors mainly because of varied organic matter contents and characteristics of MS collecting from the real WWTPs. The contribution of injected air to the specific gas yield was about 0.7 ± 0.2%. Compared to R2 for control, in-situ microaeration increased the average specific biogas yield by 15.8 ± 7.3% with air dosage ranging from 0 to 12.5 mL/(L·d). The specific biogas yield increased gradually in phase II because the organic matter content of MS increased in winter. In this stage, the average specific biogas yield of R1 (138.7 ± 29.6 mL/g VS) was 13.1 ± 6.7% higher than that of R2 (123.3 ± 28.8 mL/g VS). The results suggested that high oxygen supply did not further improve the specific biogas yield, because higher aeration intensity might inhibit the activity of methanogens, increase the oxidation of intermediate soluble products and then reduce methane yield [18]. Correspondingly, the average VS removal of R1 in phase I and II were 24.9 ± 1.5% and 23.0 ± 1.5%, respectively, both higher than those of R2 (18.3 ± 1.6% and 17.9 ± 0.9%). The average specific biogas yield of MA reactor fed with microaeration pretreated sludge was 135.4 ± 22.4 mL/g VS, which was 11.7 ± 2.4% higher than that of the control reactor fed with raw sludge. In this sense, enhancement for biogas production of AD by in-situ microaeration was slightly superior to that by microaeration pretreatment.

3.2. Variation of EPS and sludge properties

As an intricate mixture of polymers, EPS are main ingredients of WAS floc matrix and play a crucial role in sludge flocculation, sedimentation, compression and dewaterability [25]. As shown in Table 2, in-situ microaeration led to remarkable changes in the production of Slime EPS, LB-EPS and TB-EPS. Compared with R2, the total concentrations of PN and PS in Slime EPS and LB-EPS for sludge discharged from R1 both decreased, while those in TB-EPS increased. Yuan and Wang [26] revealed a notable connection between sludge reduction and the degradation of TB-EPS and cell lysis. The lower contents of PS and PN in TB-EPS for sludge discharged from R2 indicated that in-situ microaeration motivated cell breakup and then led to the diffusion of EPS from inner layer to outer layer, especially through the disintegration of PN in TB-EPS [27].

The PN/PS ratio for sludge discharged from R1 ranged from 5.2 to 5.6, whereas that for sludge discharged from R2 varied from 2.3 to 4.6. EPS with relatively high PN content would be hydrolyzed to serve as carbon and energy source for gas production [28] to ease the shortage of nutrient in substrate.

Compared to R2, SRF and CST of sludge discharged from R1 decreased by 14.7% and 14.0% (Table 2), indicating that dewaterability of MS improved by in-situ microaeration. Better sludge dewaterability of an AD process operated under microaerobic condition was also reported

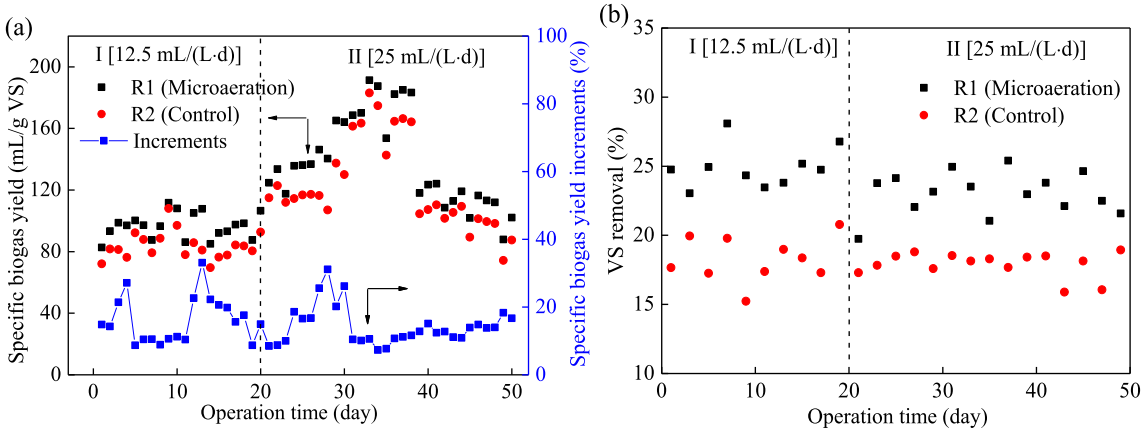

Fig. 2. Variations of specific biogas yield and VS removal during semi-continuous AD process with and without in-situ microaeration treatment.

Table 2
Characteristics of sludge discharged from two AD reactors with/without in-situ microaeration treatment at day 20.

	Slime-EPS		LB-EPS		TB-EPS		CST (s)	Median particle size (μm)	SRF (10^{12} m/kg)
	PS (mg/g VSS)	PN (mg/g VSS)	PS (mg/g VSS)	PN (mg/g VSS)	PS (mg/g VSS)	PN (mg/g VSS)			
R1	0.34 ± 0.04	1.89 ± 0.06	0.16 ± 0.04	0.81 ± 0.07	0.88 ± 0.02	4.56 ± 0.09	161.6 ± 16.2	59.3 ± 5.2	5.96 ± 0.37
R2	0.55 ± 0.02	2.00 ± 0.05	0.22 ± 0.05	1.00 ± 0.08	1.22 ± 0.05	2.86 ± 0.08	187.8 ± 19.5	52.2 ± 4.7	6.99 ± 0.49

by Jenicek et al. [29]. A higher PN/PS ratio of sludge discharged from R1 could be associated with well dewatering capacity along with hydrophobicity [30]. Moreover, LB-EPS and TB-EPS were reported to play a vital role in dominating sludge dewaterability. LB/TB-EPS ratio for sludge discharged from R1 (0.18) were lower than that from R2 (0.30), supporting the enhancement of sludge dewaterability by in-situ microaeration [23]. The decreased LB/TB-EPS ratio also manifested the strengthening of flocs structure and the improvement of sludge

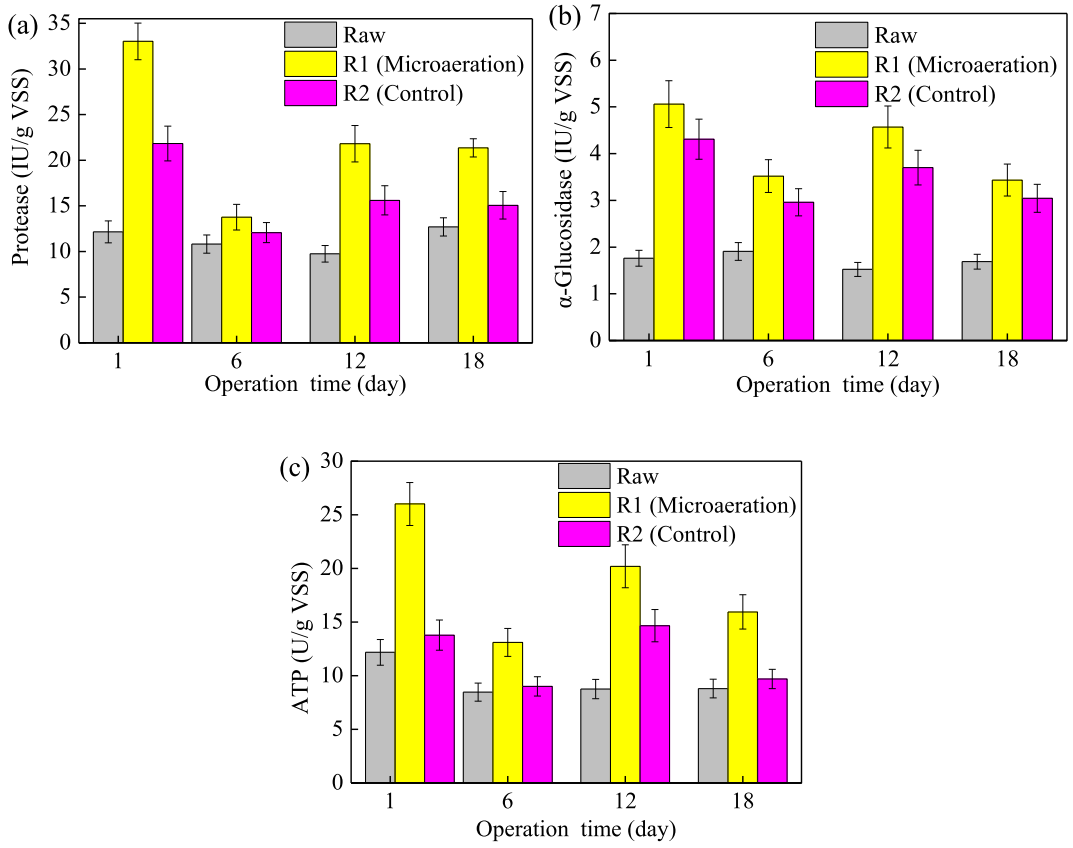

Fig. 3. Activities of extracellular hydrolase (a, b) and content of ATP (c) during semi-continuous AD process with and without in-situ microaeration treatment.

aggregation. This result was in accordance with a clear increased particle size of sludge in R1 compared with R2 (Table 2).

3.3. Influence of in-situ microaeration over enzymatic activity

Protease and α-glucosidase, main extracellular hydrolase in sludge mixture, play a central role in degrading soluble PN and PS into small molecular organic matters, such as amino acids and monosaccharide [7]. As the universal energy currency of biological realm, ATP, generated via substrate level phosphorylation [1], can be served as an indicator of biological activity and viable biomass in biological studies [22].

During the whole operation period (Fig. 3), activities of protease, glucosidase and ATP of raw MS had a small fluctuation, and were maintained at 11.4 ± 1.3, 1.7 ± 0.2 and 9.6 ± 1.8 IU/g VSS. The maximum values of protease, α-glucosidase and ATP were achieved in R1, reaching 33.0, 5.1 and 26.0 IU/g VSS on day 1, which were 2.5, 2.2 and 2.9 times of those in R2. These results indicated that in-situ microaeration enhanced secretion of hydrolase, accelerated energetic conversions of intermediates to sustain overall stability in AD processes and finally improved the AD efficiency [1]. Besides, in-situ microaerobic condition could also be a promising tactic to facilitate the generation of ATP and enhance the specific microbial activity, which had a bearing on the release of organic matters by the higher cell lysis rate in R1 [31].

3.4. Comparison on microbial community structures

3.4.1. Bacterial community

Fig. 4 depicts the bacterial composition of the two sludge samples collected from R1 and R2 at phylum and class levels. Although most abundant bacterial phyla were similar in two sludge samples, the relative abundances differed from each other. As shown in Fig. 4a, Proteobacteria (29.40%–34.89%), Chloroflexi (13.41%–21.24%), Bacteroidetes (6.57%–12.17%), Actinobacteria (6.17%–7.36%), Acidobacteria (4.93%–5.68%), Firmicutes (3.42%–3.97%), Aminicenantes (3.59%–3.72%), Saccharibacteria (3.02%–3.93%), WS6 (2.81%–4.02%), Synergistetes (2.47%–2.26%), and Parcubacteria (1.24%–2.68%) were the most dominant phyla in each sample. Among these dominant phyla, relative abundances of four phyla playing prominent roles in hydrolysis and hydrogenogenic acidogenesis during AD of sludge [32], Chloroflexi, Firmicutes, Synergistetes and Parcubacteria, in R1 were 58.4%, 16.1%, 3.4% and 116.3% higher than those in R2.

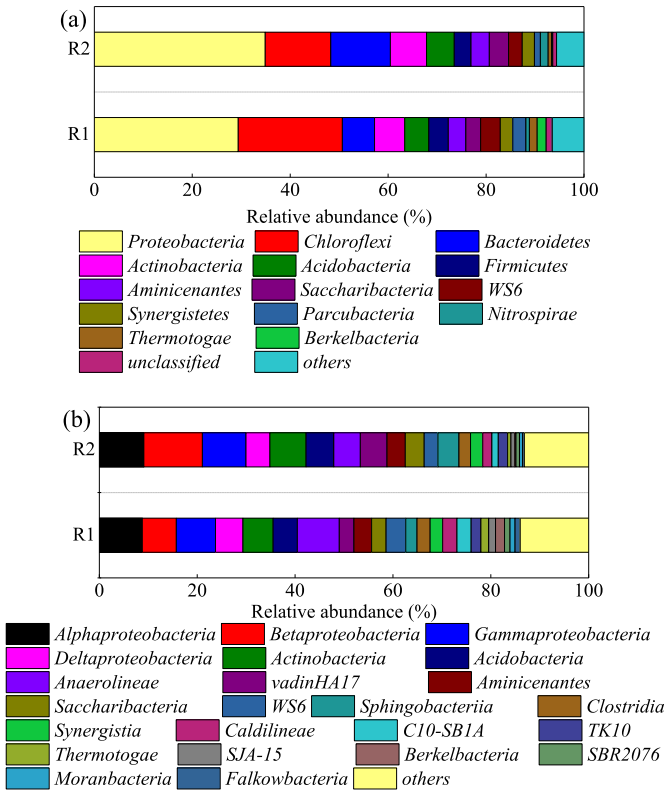

Fig. 4. Relative abundance of the predominant bacterial community at the phylum (a) and class (b) level in semi-continuous AD process with and without in-situ microaeration treatment.

Chloroflexi was reported as one of the numerically important glucose-degrading bacterial groups responsible for acidogenesis and H_2-oxidizing homoacetogenesis in AD process [33], and could utilize various carbohydrates and amino acids as substrates [34]. The relative abundances of these phyla confirmed the increase in protease and α-glucosidase production (Fig. 3) under microaerobic condition, which could enhance hydrolysis of proteins and other complex organic substrates. Firmicutes had this ability of secreting extracellular enzymes closely related to the metabolism of protein, lipids, cellulose and hemicelluloses [34]. Firmicutes was also associated with the bioconversion of PS to butyric, propionic and acetic acids [35], and had the thick cell wall to produce endospores for survival in extreme conditions [36]. Higher relative abundance of Firmicutes in R1 meant greater ability to degrade complex substrate, leading to higher hydrolysis rate under in-situ microaerobic condition [17]. Synergistetes was notable to ferment PS to acetate and H_2 [37]. Parcubacteria principally fermented simple sugars to organic acids, and some species also had the ability of degrading complex carbon sources [38]. Relatively higher abundance of the above-mentioned phyla signified that efficient hydrolysis, fermentation and acidogenesis were acquired in the anaerobic digester upon in-situ microaeration [39].

The bacteria diversity at the class level in Fig. 4b demonstrates more information on microbial community evolution between R1 and R2. Alphaproteobacteria (8.8%–9.1%), Betaproteobacteria (6.9%–12.0%), Gammaproteobacteria (8.0%–8.9%) and Deltaproteobacteria (4.9%–5.6%), affiliated to phylum Proteobacteria and functioned as dominating consumers of VFAs and glucose [19], were the predominant four classes, followed by Actinobacteria (6.2%–7.4%), Acidobacteria (4.9%–5.7%), Anaerolineae (5.4–8.6%), Clostridia (2.4%–2.7%), Aminicenantes (3.6%–3.7%), Synergistia (2.5%–2.6%) and Caldilineae (1.9%–2.9%). In comparison to R2, relative abundances of Deltaproteobacteria, Anaerolineae, Clostridia, Synergistia and Caldilineae in R1 increased by 14.5%, 57.9%, 13.6%, 3.4% and 55.4%, respectively. Most Deltaproteobacteria were well known as acetate-, butyrate-, propionate-, and glucose-utilizing microbial communities [40]. Anaerolineae and Caldilineae were in charge of hydrolysis and fermentation of organic matters [20], and are members of phylum Chloroflexi discovered to be essential for glucose utilization [40]. Clostridia, the main bacterial class of phylum Firmicutes, could utilize various carbohydrates, and transform complex macromolecules into simple products [41]. Compared to obligate anaerobic condition, hydrolysis, fermentation and acidogenesis under the presence of oxygen in the anaerobic digester gave rise to the relative abundances of Deltaproteobacteria, Anaerolineae, Clostridia, Synergistia and Caldilineae, which will enable the acid-producing process to metabolize more types of substrates.

3.4.2. Archaeal community

Fig. 5 displays the composition of archaeal community in R1 and R2 at class and genus levels. As can be seen from Fig. 5a, the dominant classes detected in R1 and R2 were aceticlastic/hydrogenotrophic Methanomicrobia and hydrogenotrophic Methanobacteria, which in total composed 97.1% (R1) and 96.4% (R2) of the entire archaeal community, respectively. Although the first two dominant classes in R1 and R2 did not shift, in-situ microaeration caused a significant decrease of Methanobacteria (27.8% for R1 and 45.1% for R2) but enriched Methanomicrobia (69.3% for R1 and 51.3% for R2) [42].

As illustrated in Fig. 5b, the obligate aceticlastic methanogenic genus

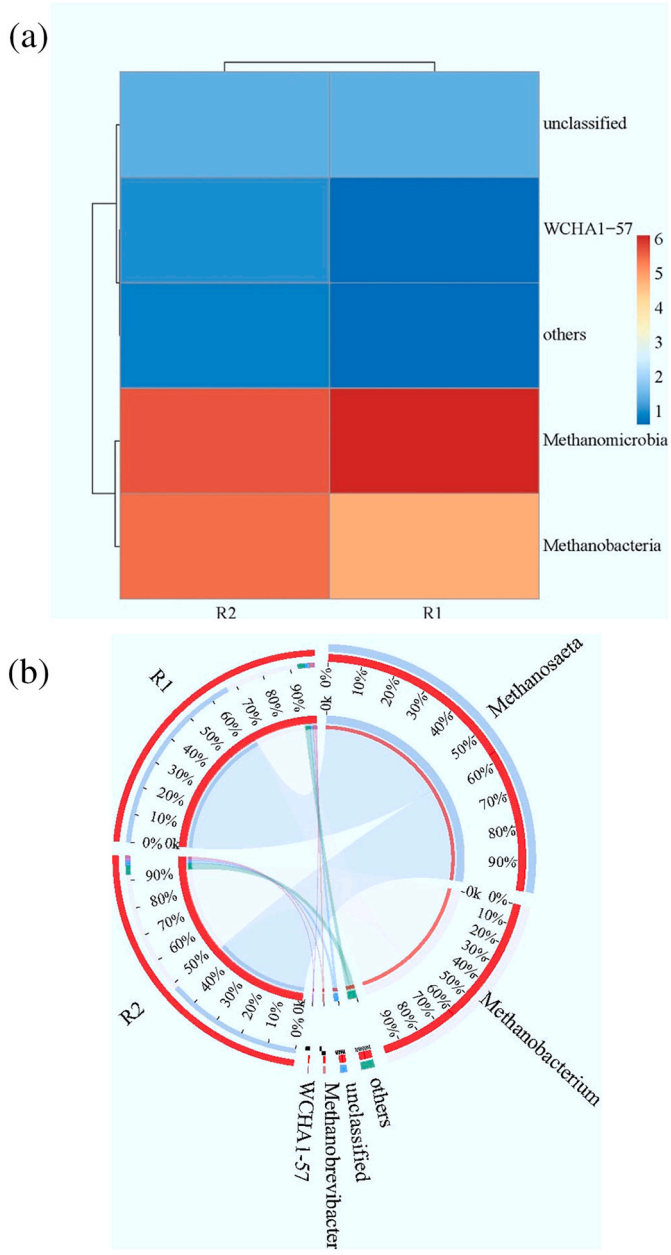

Fig. 5. Relative abundance of the predominant archaeal community at the class (a) and genus (b) level in semi-continuous AD process with and without in-situ microaeration treatment.

Methanosaeta and the obligate hydrogenotrophic methanogenic genus *Methanobacterium* [42,43] were dominant in R1 and R2, with total relative abundances of 94.0% (R1) and 93.4% (R2), respectively. The relative abundances of *Methanosaeta* and *Methanobacterium* were 67.6% and 26.4% in R1, and 49.0% and 44.4% in R2, indicating that in-situ microaeration in R1 favored the growth of *Methanosaeta* but prejudiced *Methanobacterium*. Kiener and Leisinger [44] also demonstrated that longer periods of contact with oxygen could damage the viability of *Methanobacterium*. These results also indicated that the main methanogenic pathway in R1 was aceticlastic methanogenesis. Zheng and Raskin [45] reported that *Methanosaeta* species were also observed to be the predominant archaea in a variety of anaerobic reactors at low acetate concentrations, but their numbers decreased fast as the acetate concentration increased. However, in the presence of a small amount of oxygen, acetic acid reacts with oxygen to maintain a dynamic equilibrium of acetic acid concentration, thus maintaining the higher activity and relative abundance of *Methanosaeta* [46]. As a typical hydrogenotrophic methanogen [47], *Methanobrevibacter* was reported to utilize H_2/CO_2 or formate as substrates [48], and existed in two sludge samples with low abundance ranging from 0.69% to 1.3%. Overall, the proportions of archaeal community in R1 changed significantly compared to R2 because of the in-situ microaeration, which significantly promoted enrichment of aceticlastic methanogens in AD reactor rather than hydrogenotrophic methanogens.

3.5. Energy balance and cost estimates for in-situ microaeration and its combination with microaeration pretreatment for AD

In-situ microaeration was found to be a valid method to enhance biogas production and VS removal efficiency (Fig. 2). Besides, in consideration of microaeration pretreatment strengthening methane production yield and rate [19], bench-scale experiments were implemented to investigate the techno-economic feasibility associated with the estimated energy balance and cost of in-situ microaeration and its combination with microaeration pretreatment on AD of WAS.

Fig. 6 describes variations of CMP and VS removal of raw MS and MA with inoculated sludge from R1 and R2. On day 14, the CMP obtained under various experimental conditions was in the following sequence of MS + RS1 (100.7 mL/g VS) > MA + RS1 (88.6 mL/g VS) > MA + RS2

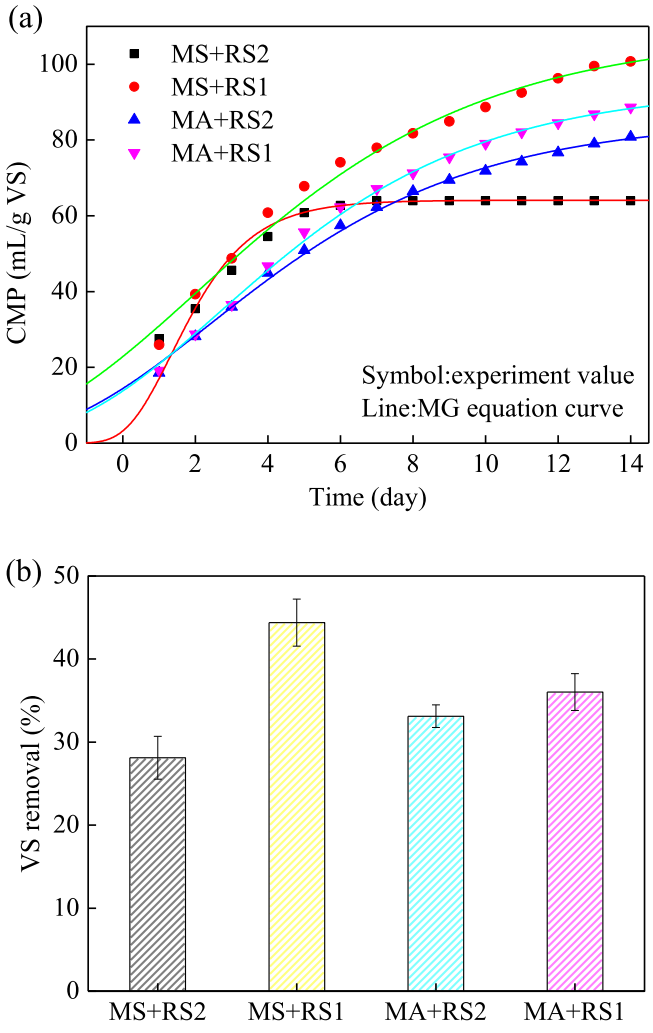

Fig. 6. Variations of the CMP (a) and VS removal (b) of different combinations of the raw mixed sludge (MS), microaeration pretreated sludge (MA), with/without in-situ microaeration treatment inoculated sludge (RS1 and RS2) for anaerobic digestion.

(80.9 mL/g VS) > MS + RS2 (64.0 mL/g VS) (Fig. 6a). The determined CMP was then regressed with the modified Gompertz equation (Eq. (1)) using non-linear regression algorithm in OriginPro 8.1 and the fitting results are displayed in Table 1. The results illustrate a well fit to experimental CMP data (R^2 = 0.9999). Compared to MS + RS2, microaerobic condition brought the change of P by +69.0%, +46.7% and +33.5%, and of R_m by −54.9%, −55.9% and −60.8% for MS + RS1, MA + RS1 and MA + RS2, respectively, showing that microaerobic condition enhanced CMP but decreased the methane production rate. Fig. 6b shows the average removal efficiency among different combination after 14 days in the sequence of MS + RS1 (44.4% ± 2.8%) > MA + RS1 (36.0% ± 2.2%) > MA + RS2 (33.1% ± 1.4%) > MS + RS2 (28.1% ± 2.6%). The trend of VS removal accorded well with the CMP.

The above results indicated that enhancement in hydrolysis and acidogenesis under in-situ microaeration might generate more substrates for methanogens, leading to higher specific methanogenic activity and final methane yield in AD process [1], while coupling microaeration pretreatment and in-situ microaeration decreased not only specific biogas yield but also VS removal efficiency in comparison to in-situ microaeration alone for sludge AD. In all above applications of microaeration, it should need to consider the substrate competition between facultative bacteria and anaerobic methanogens, in order to acquire strengthened digestion while preventing entire reduction in methane yield due to consumption of substrates by facultative bacteria [1].

It is estimated that 1 kg CH_4 can generate electricity for a capacity of 5.13 kWh [49]. Therefore, compared with MS + RS2, the enhanced methane yields for in-situ microaeration (MS + RS1), microaeration pretreatment (MA + RS2) and combination treatment (MA + RS1) generated electricity of 0.111, 0.051 and 0.074 kWh/kg VS. Meanwhile, microaeration saved costs of sludge treatment and disposal by 0.0152, 0.0047 and 0.0074 €/kg VS for MS + RS1, MA + RS2 and MA + RS1 by improving VS digestion, with typical investment of 218 €/ton dried solids [50]. So, the total investment reduced by microaeration was 0.0223, 0.0079 and 0.0122 €/kg VS considering the power consumption of 0.13 €/kWh (microaeration pretreatment) and 0.0012 €/kWh (in-situ microaeration).

4. Conclusion

This research supplied perceptions into performance, microbial community and mechanism of an innovative mesophilic AD process under in-situ microaeration treatment. In-situ microaeration obtained a 15.8 ± 7.3% gain in average specific biogas yield and a 37.1 ± 12.1% increase in VS removal efficiency at 12.5 mL/(L·d) air dosage compared to the obligate AD process. In-situ microaeration stimulated activity of hydrolytic enzymes, enlarged sludge particle size, and improved sludge dewaterability. However, excessive air dosage of 25 mL/(L·d) decreased both specific biogas yield and VS removal efficiency. Remarkable differences in dominant bacterial and archaeal species were observed between the in-situ microaeration and the obligate AD processes. The methanogenic genus *Methanosaeta* (67.6%) predominated the archaeal community in the in-situ microaeration AD process, and facilitated the accelerated degradation of diverse substrates via aceticlastic pathway. Techno-economic analysis associated with energy balance and cost estimates for in-situ microaeration and its combination with microaeration pretreatment for AD also indicated that excessive microaeration could cause reduction in methane yield and VS removal efficiency owing to substrates consumption by facultative bacteria.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by National Natural Science Foundation of China (51878403), Shuguang Plan of Shanghai (19SG49), and Science and Technology Commission of Shanghai Municipality of China (19DZ1204904).

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