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Enhancing methane production of anaerobic sludge digestion by microaeration: Enzyme activity stimulation, semi-continuous reactor validation and microbial community analysis

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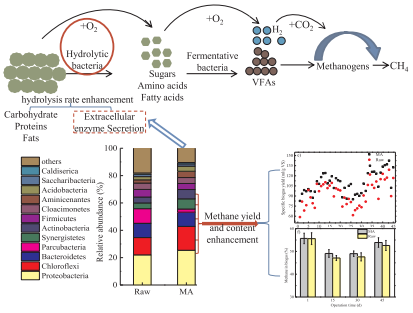
GRAPHICAL ABSTRACT



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ABSTRACT

Effects of microaeration pretreatment on sludge hydrolysis, biogas production and microbial community structure in anaerobic digestion (AD) were investigated by bench-scale tests and semi-continuous experiments. Bench tests showed that microaeration led to the release of dissolved organic matters, generation of volatile fatty acids and stimulation of enzyme activity. Correlation analysis showed that methane production was significantly correlated with the activity of α-glucosidase at 0.01 level, and with protease activity, released polysaccharides and VFAs at 0.05 level. Semi-continuous experiments showed that microaeration accelerated the utilization of organic matters, increased biogas production by 16.4%, enhanced methane content in biogas, and improved sludge dewaterability. Microbial community structure analysis showed that microaeration promoted enrichment of hydrolytic and fermentative bacteria in AD reactor rather than methanogenic bacteria, and aceticlastic methanogenesis was the main methanogenic pathway for methane production.

1. Introduction

Activated sludge process has been extensively applied for wastewater treatment in the world owing to its high efficiency and low energy consumption (Tchobanoglous et al., 2003). Waste activated sludge massively generated from the process has become a pressing

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problem in recent years (Niu et al., 2016). AD is a highly desirable technology for waste activated sludge stabilization and reduction, with concomitant production of digester gas to meet most of the energy requirement for plant operation, and thus to curtail greenhouse gases emissions (Nguyen and Khanal, 2018). Despite the aforementioned merits, hydrolysis with slow rate is generally rate limiting step in sludge AD process because cell walls of sludge are resistant to be decomposed (Shrestha et al., 2017), resulting in some common issues such as long retention time and low efficiency.

Various sludge pretreatment methods have been applied to accelerate hydrolysis, including thermal, chemical, biological and mechanical methods and their combinations (Liu et al., 2019). Thermal hydrolysis is a successful approach to making sludge more amenable to AD by altering rheological, properties, improving biodegradation and enhancing dewaterability of sludge, and have been successfully applied in full-scale 75 °C plants (Barber, 2016). Appels et al. (2010) found that thermal treatment efficiently solubilized organic and inorganic compounds and increased biogas production at high temperature of 90 °C. Li et al. (2017) reported that alkaline hydrothermal pretreatment for sludge AD can realized self-sufficiency in energy at the cost of a proper amount of CaO. Although efficient to enhance AD efficiency in lab-scale to full-scale investigations, these physicochemical approaches have several drawbacks, such as requirement of expensive chemicals and energy, high investment of equipment, and complicated operation. Therefore, more economic, simple and environment-friendly pretreatment method should be developed to improve AD performance of sludge.

Traditionally, oxygen or air is not allowed to mix with the digester biogas due to its negative effects on growth and activity of obligate anaerobes, especially methanogens, and the mixture of methane and oxygen has an explosive risk (Nguyen and Khanal, 2018). Recently, microaeration treatment has been applied to accelerate hydrolysis in both AD (Montalvo et al., 2016) and sludge *in situ* reduction process (Niu et al., 2016). Microaeration is applied as pretreatment step or dosing a small amount of air or oxygen into an AD system (Nguyen and Khanal, 2018), and has been successfully applied to improve the methane yield of AD for corn straw (Xu et al., 2018), Miscanthus floridulus (Peng et al., 2017), brown water and food waste (Lim et al., 2014), etc. Of course, the presence of oxygen in AD system or pretreatment stage also resulted in consumption and degradation of organic matters owing to competition between facultative and strict anaerobes (Nguyen and Khanal, 2018). Those intensive efforts mentioned above illustrated successful application of microaeration to AD enhancement, but most of the previous studies were batch test for organic waste. More detailed investigations are required for waste sludge to better understand effects of microaeration. Previous studies usually elucidated enhancement mechanisms from the perspective of intracellular substances release, accumulation of volatile fatty acids (VFAs) and disintegration of microstructure, but to our knowledge, effects of enhancement strategies on enzyme activity were scarcely found in literatures. Enhancement pretreatment also had a significant influence on bacterial and archaeal community structure, and systematic investigations on variations of microbial community structure and enzyme activity are also required to deepen the understanding of enhancement mechanisms.

In this study, effects of microaeration on sludge AD performance were investigated by bench-scale tests and semi-continuous experiments. Bench-scale tests were initially employed to analyze impacts of aeration rate (AR) and aeration time on release of dissolved organic matters (DOM), enzyme activity and methane yield, and correlation analysis between these indices after microaeration were carried out to clarify key matters affected AD greatly. Semi-continuous systems were operated under optimized conditions to validate enhancement efficiency, and the *Illumina-MiSeq* platform was applied to investigate shifts of functional bacteria responsible for hydrolysis, fermentation and methanogenesis.

2. Material and methods

2.1. Substrate and inoculum

The mixed sludge used as substrate for all the assays was a mixture of primary sludge (60%) and waste activated sludge (40%). The inoculum sludge for batch and semi-continuous tests was obtained from the AD system with daily treatment capacity of 204 tons dried solids located in the Bailonggang Wastewater Treatment Plants (Shanghai, China). Characteristics of mixed sludge were as follows: pH of 7.1 ± 0.1, total solids (TS) of 41.1 ± 1.2 g/L, volatile solids (VS) of 25.4 ± 1.1 g/L, COD of 32800 ± 2112 mg/L, soluble COD (SCOD) of 479 ± 15 mg/L, total organic carbon (TOC) of 151.2 ± 1.8 mg/L, ammonium nitrogen (NH_4^+-N) of 67.7 ± 3.0 mg/L, VFAs of 319 ± 23 mg/L, proteins (PN) of 28.4 ± 1.3 mg/L, and polysaccharides (PS) of 15.2 ± 2.1 mg/L.

2.2. Microaeration pretreatment

A series of microaeration pretreatment tests were performed in six bench-scale reactors with effective volume of 2.5 L each at 35.0 ± 1.0 °C. In each reactor, an aeration pipe was installed at the bottom, and a vertical stirrer (60 rpm) was fixed in the middle for sludge homogenization. Mixed sludge with 4% solid content was injected into the reactor. Then aeration intensities of six reactors were maintained at 0 (raw sludge), 1, 2, 3, 4, 5 and 6 air volume per gram TS per minute (vmm) by controlling an air flowmeter into the reactor to investigate effects of AR on microaerobic hydrolysis at identical pretreatment time of 4 h. Six microaeration times of 0, 4, 8, 16, 24 and 48 h were also chosen in the other assay by controlling aeration intensity of 4 vmm.

Batch assay of AD was conducted in 250 ml digester bottles with a working volume of 200 ml. After microaerobic hydrolysis, 160 ml pretreated mixed sludge and 40 ml inoculum sludge were fed into a digester bottle. The initial pH was adjusted to 7.2 ± 0.1 with 2 M HCl and 2 M NaOH. All the bottles were flushed with N_2 for 5 min to replace the air and provide anaerobic conditions, and then incubated in a water bath at 35.0 ± 1.0 °C. In order to maintain sludge homogeneity, a moderate manual stirring for several seconds was conducted for each bottle several times per day. Biogas (methane) volume was measured by water replacement method by connecting a bottle filled with 3% w/w NaOH solution to remove CO_2 and H_2S.

2.3. Setup and operation of semi-continuous anaerobic digester

The semi-continuous AD was conducted in two continuous stirred-tank reactors (CSTR) with effective volume of 14 L each and made of double-wall high-silica glass (Fig. 1). One CSTR was fed with microaeration pretreated sludge (MA reactor) by a reactor of 1 L under optimized conditions, the other was fed with raw sludge (Raw reactor) for control. The inlet was located at the top of each CSTR, and the outlet and sampling port were at the bottom. CSTRs were kept at 35.0 ± 1.0 °C by a water jacket system using an electric heater. An agitator was mounted at the top of the digester and stirred at 80 rpm continuously for sludge mixing. pH and biogas volume were monitored by an online pH meter and a wet gas flowmeter, respectively. Mixed sludge (substrate) and digested sludge at the same volume of 0.7 L were injected into and withdrawn from the CSTR by peristaltic pumps.

2.4. Analytical methods

2.4.1. Bacterial and archaeal community

In order to understand effects of microaeration on the evolution of microbial community structure in the AD system, two sludge samples were gathered from MA and Raw reactor for MiSeq sequencing. The genomic DNA of each sample was extracted using an extraction kit

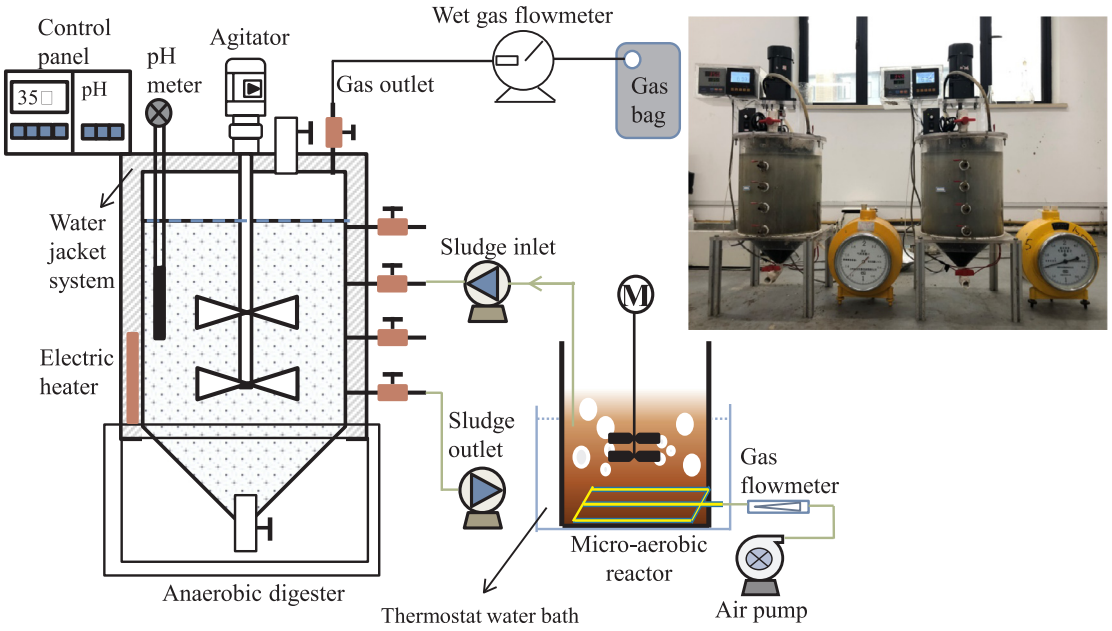

Fig. 1. A schematic of microaeration pretreatment and semi-continuous AD system.

(Shanghai Majorbio Bio-Pharm Technology, China) according to reported methods (Hou et al., 2018). 16S rRNA genes fragments were amplified from the obtained DNA using a primer set, 338F (5′-ACTCC TACGGGAGGCAGCAG-3′) and 806R (5-'GGACTACHVGGGTWTCT-AAT-3′) for bacteria, and Arch524F (5-'TG YCAGCCGCCGCGGTAA-3′) and Arch958R (5-′YCCGGCGTTGAVTCCAATT-3′) for archaea. Purified amplicons were pooled in equimolar, and paired-end sequenced on an *Illumina-MiSeq* platform. Then 16S rRNA sequences were clustered into operational taxonomic units by setting a 3% distance limit.

2.4.2. Soluble and gaseous substances

Concentrations of TS, VS, COD, alkalinity and NH_4^+-N were measured according to Chinese NEPA standard methods (Chinese NEPA, 2012). The soluble components (SCOD, VFA, etc.) were filtered through 0.45 μm cellulose acetate membrane filters before analysis. pH was monitored using an HQ30d portable meter (Hach, USA). Concentrations of TOC were determined by a Multi N/C 3100 Analyzer (Analyti Jena, Germany). PN and PS were analyzed by a 2802 UV/vis Spectrometer (Unico, USA) following the modified Lowry method and anthrone method, respectively. VFAs were measured by a GC-7900P/FID gas chromatograph (Tianmei, China) with GC-flame ionization detector and a 30 m × 0.32 mm × 1.0 μm fused-silica capillary column. CH_4 content in the biogas was analyzed with GC-TCD fitted with parallel column of 1.1 m × 3/16″ Molsieve 137 and 0.7 m × 1/4″ chromosorb 108.

2.4.3. Sludge property and enzyme activity

Sludge specific resistance to filtrate (SRF) was monitored according to pressure filtration method. Capillary suction time (CST) was determined with a portable CST 304B instrument (Triton, UK) equipped with an 18 mm diameter funnel and Whatman No.17 chromatography-grade paper. The particle size was measured by SALD-2201 Laser Diffraction Particle Size Analyzer (Shimadzu, Japan). The viscosity was measured by a Brookfield Viscometer (model TD2-LVDV-1). Protease and α-Glucosidase activity of sludge liquor were measured for samples collected from microaeration reactors by using the ELISA kit (Shanghai Hengyuan Biotech) according to reported methods (Zheng et al., 2019).

2.5. Calculation methods

2.5.1. Modified Gompertz model

Cumulative methane production (CMP) curves in batch tests were calculated using non-linear regression according to modified Gompertz (MG) equation (Eq. (1)).

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

where $P(t)$ and P are CMP and maximum methane production (ml CH_4/g VS), respectively. R_m, λ and t represent maximum methane production rate (ml CH_4/(g VS·d)), lag-phase time (d) and elapsed time (d), respectively. e is the natural index. Parameters λ, R_m and P were obtained by nonlinear fitting using Origin 9.0 software.

2.5.2. Statistical analysis

One-way factor analysis of variance (ANOVA) and correlation analysis of experimental data were conducted by using SPSS 19.0 software.

3. Results and discussion

3.1. Effects of aeration rate and time on hydrolysis and AD

3.1.1. Aeration rate

Fig. 2 illustrates effects of AR on DOM release, VFAs, enzyme activity and methane yield in microaeration pretreatment. PN and PS are major constituents of macromolecular organic matters in sludge with proportion more than 60% (Neyens and Baeyens, 2003). As shown in Fig. 2a, concentrations of PN, PS and TOC increased firstly and then decreased with the rising AR, and reached the maxima of 58.2, 31.1 and 145.5 mg/L at 4 vmm, respectively. Because sludge hydrolysis is a biochemical process with slow rate, oxygen supplied at higher AR is difficult to utilize completely, and thus excessive oxygen would dissolve in liquid and consume released DOM (Nguyen and Khanal, 2018), resulting in the descending trend of PN, PS and TOC at AR above 4 vmm. In AD, VFAs are important intermediate products that can be directly utilized by methanogenic archaea for methane production. As shown in Fig. 2b, total concentrations of VFAs showed the same trend as released DOM, and reached the maxima of 351.4 mg/L at 4 vmm, in which acetate, propionate, *iso*-butyrate, n-butyrate, *iso*-valerate and n-valerate accounted for 70.8%, 19.0%, 3.0%, 0.6%, 5.0% and 1.6%, respectively. Acetate was the most abundant VFAs in all the samples, and is a key component to increase methane production during VFAs hydrolysis (Wang et al., 1999). The results verified that excessive aeration consumed released DOM and decreased VFAs supply for AD in microaeration pretreatment.

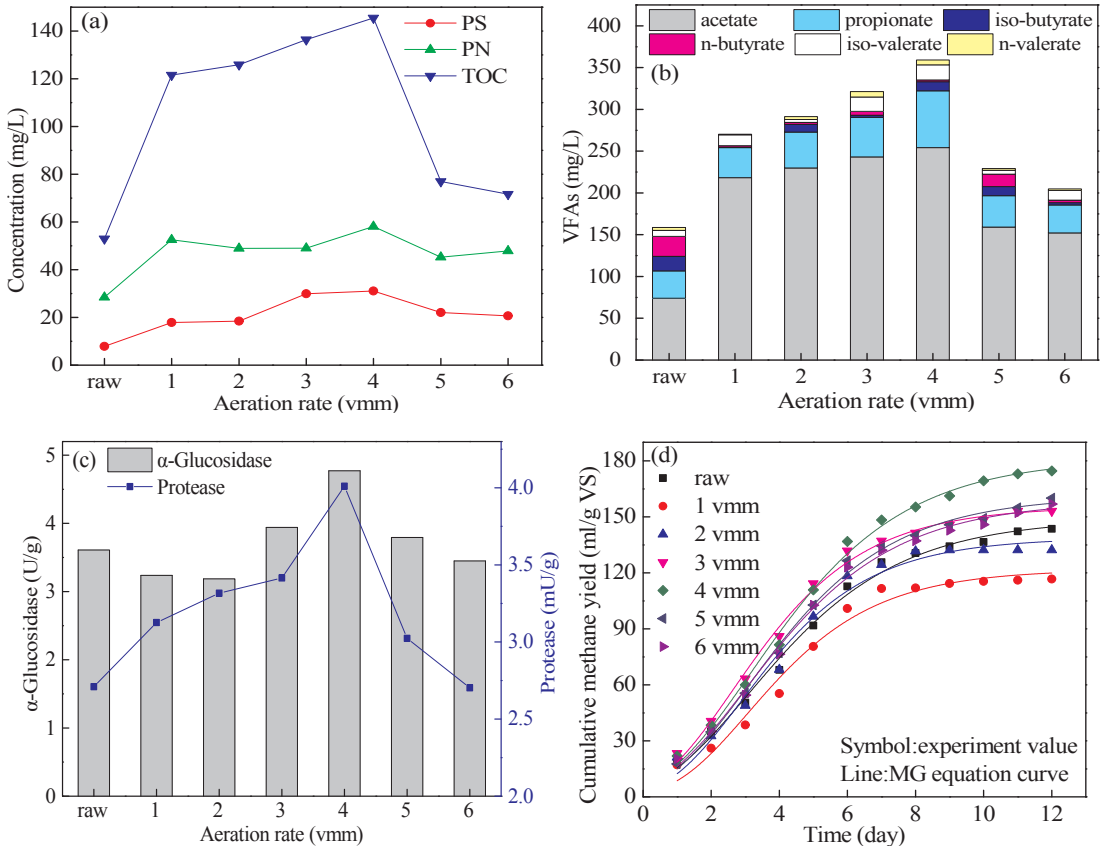

Fig. 2. Effects of AR on DOM, VFAs, enzyme activity and methane yield.

Table 1
The modified Gompertz equation fitted with methane production data.

AR vmm	*P* ml/g VS	R_m ml/(g VS·d)	λ d^{-1}	R^2	Time h	*P* ml/g VS	R_m ml/(g VS·d)	λ d^{-1}	R^2
0	149.1	21.5	0.53	0.993	0	133.8	20.7	0.57	0.995
1	121.8	21.0	0.94	0.978	4	190.4	23.7	0.85	0.993
2	138.7	24.1	0.75	0.98	8	176.9	20.2	0.76	0.992
3	155.5	26.3	0.62	0.994	16	173.5	21.3	0.54	0.994
4	181.3	26.1	0.46	0.995	24	174.2	22.1	0.56	0.994
5	161.2	24.7	0.67	0.995	48	154.9	21.4	0.60	0.994
6	158.5	23.6	0.56	0.995	–	–	–	–	–

Protease is responsible for the release of glucose and the hydrolysis of PS to monoses, while α-glucosidase breaks peptide bonds into PN molecules and decomposes PN to amino acids (Hou et al., 2018). The concentrations of protease and α-glucosidase were 4.01 and 4770 mU/g VS at AR of 4 vmm, which favored hydrolysis of PN and PS in sludge (Min et al., 2009). Further increase of AR also inhibited activities of these functional enzymes and obligated anaerobes in the subsequent AD process. The conclusion was confirmed by CMPs in Fig. 2d, which were 6.6%, 21.6%, 11.5% and 9.2% higher than that of raw sludge at AR of 3, 4, 5 and 6 vmm, respectively. Lim et al. (2014) also reported that microaeration led to the production of exoenzymes and the increased CMP in anaerobic co-digestion of brown water and food waste. The CMP data were well fitted by the modified Gompertz model (R^2 = 0.978–0.995) and the fitting results are presented in Table 1. Microaeration greatly enhanced methane production and rate, with fitted *P* and R_m both 21.6% higher than that of raw sludge at 4 vmm.

3.1.2. Aeration time

Effects of microaeration time on DOM release, VFAs, enzyme activity and methane yield in pretreatment process are shown in Fig. 3. The production of PS and TOC both showed the trend of increasing firstly and then decreasing, and increased by 83.1% and 27.8% at the first 4 h, respectively. The concentration of PN in the supernatant rose gradually with microaeration time and increased by 63.4%. As shown in Fig. 2b, the production of VFA had the same trend as PS, and increased by 214.3% after 4 h microaeration. Maintaining high concentrations of acetate and butyrate in VFAs can promote methane production (Wang et al., 1999). Among all pretreated groups, the main VFAs were acetate and butyrate, accounting for more than 77% of the total VFAs, and all groups were improved compared with the raw sludge.

Activities of protease and α-glucosidase both increased after microaeration, and reached the maxima of 4.1 and 4400 mU/g VS after 4 h microaeration (Fig. 3c), which enabled 64% and 47% increase over that in the group of raw sludge, respectively. The results indicated that microaeration promoted the growth and metabolism of facultative bacteria, leading to more hydrolytic enzyme production and then higher methane production was obtained (Charles et al., 2009; Lim et al., 2014). The CMP in Fig. 3d showed that 4 h pretreatment achieved the highest yield of 169.7 ml/g VS, with 34.7% increase compared to

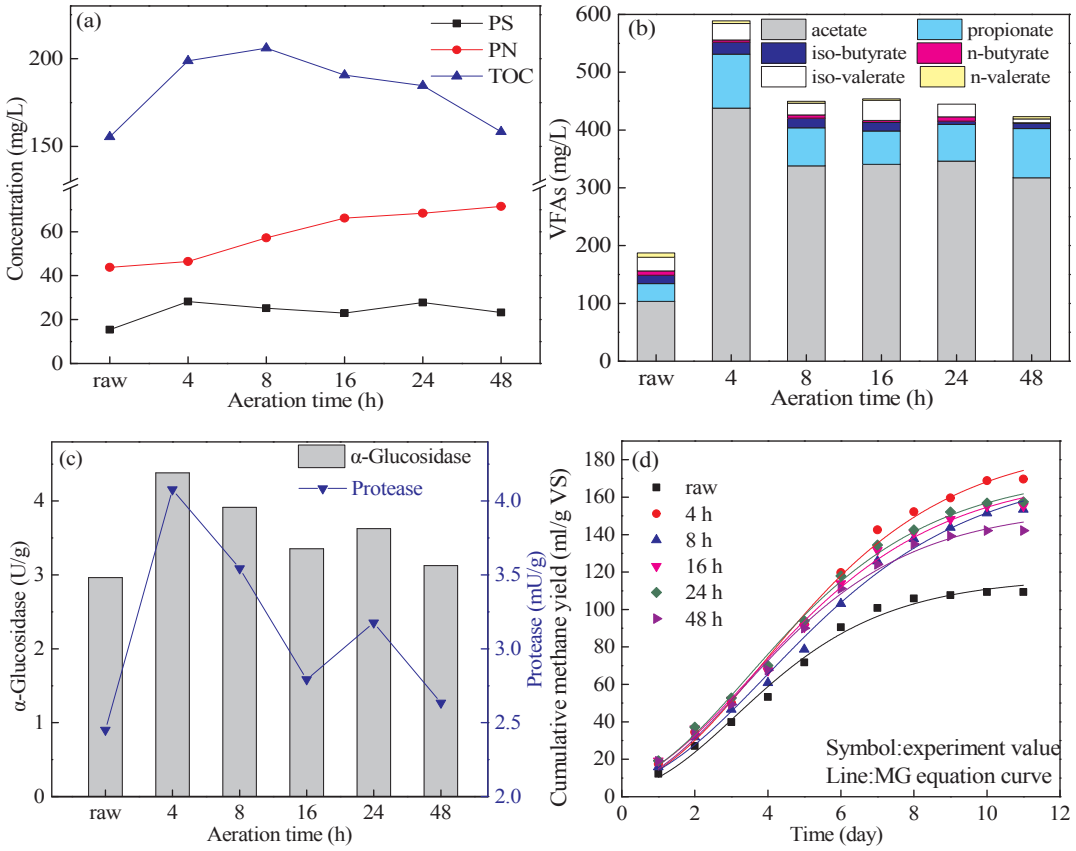

Fig. 3. Effects of aeration time on DOM, VFAs, enzyme activity and methane yield.

the control. CMPs after 8, 16 and 24 h were 21.7%, 23.1% and 25% higher than that of control, respectively, and the increase of CMP was weakened with prolonging aeration time. Fitted results of modified Gompertz model (R^2 = 0.992–0.995) in Table 1 confirmed that P and R_m values increased by 42.3% and 14.5%, respectively, and both reached the maxima after 4 h pretreatment. The results mentioned above showed that controlling pretreatment time of 4 h benefited sludge digestibility. Shorter aeration time is inadequate to stimulate activities of hydrolytic enzymes of facultative bacteria, and too long aeration will consume the released DOM, resulting in the decrease of substrates available to methanogens in AD stage (Xu et al., 2014).

3.1.3. Correlation analysis

The Pearson's correlation coefficients (r) of cumulative methane yield with DOM, VFAs and enzyme activity after microaeration pretreatment were analyzed to clarify key matters affected AD greatly (Table 2) and are shown in Table 2. CMP was significantly correlated with the activity of α-glucosidase and protease at 0.01 and 0.05 level, respectively. The results suggested that microaeration enriched facultative bacteria and produced more extracellular enzymes to accelerate hydrolysis of macromolecules, which provides abundant digestive matrix for subsequent methanogens and indirectly improves methane production (Charles et al., 2009; Fu et al., 2018). The concentration of PS, VFAs and acetate in the liquid phase all had significant correlation with methane yield at 0.05 level, while PN and TOC had insignificant correlation with methane yield. The three indicators of DOM (PS, PN and TOC) were all significantly related to each other at 0.05 level. VFAs and acetate are significantly correlated with TOC at 0.01 level, indicating that they were intermediate products of DOM degradation, and their accumulation in liquid phase can be considered as the results of enriching hydrolytic enzyme. Compared to the accumulation of DOM, enrichment of facultative bacteria in microaeration pretreatment was the more important cause for increasing methane yield.

3.2. Semi-continuous experiments

After 20 days microbial acclimation period, the two semi-

Table 2
Correlation analysis between soluble substances, enzyme activity and cumulative methane yield after microaeration pretreatment (n = 20).

Index	CH_4	PS	PN	TOC	Glucosidase	Protease	VFAs	Acetate
CH_4	1							
PS	0.672*	1						
PN	0.213	0.632*	1					
TOC	0.222	0.558*	0.614*	1				
α-Glucosidase	0.817**	0.646*	0.001	0.201	1			
Protease	0.556*	0.666*	0.102	0.432	0.848**	1		
VFAs	0.608*	0.587*	0.557	0.824**	0.448	0.457	1	
Acetate	0.660*	0.636*	0.561	0.790**	0.494	0.489	0.990**	1

Note: ** and * means significance at 0.01 and 0.05 level (bilateral).

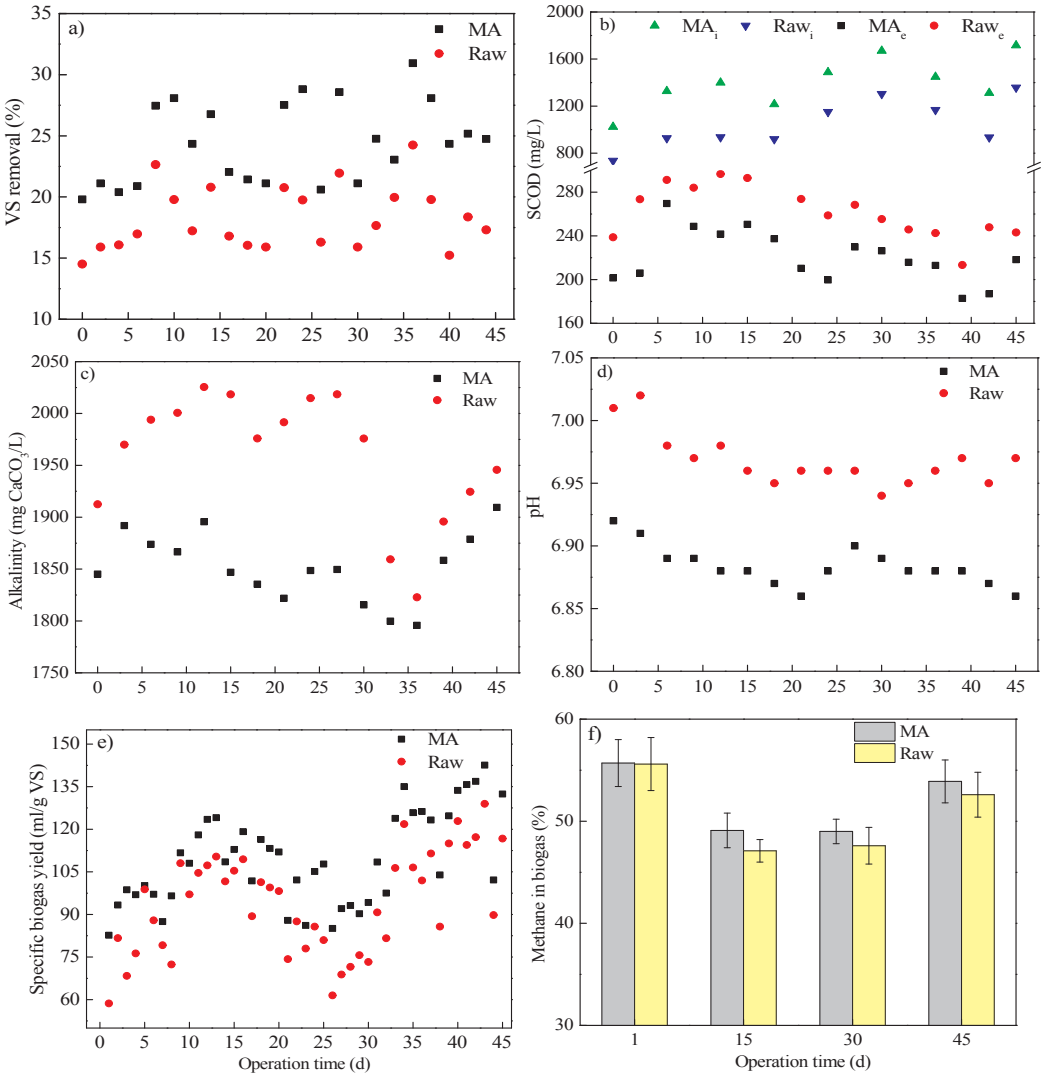

Fig. 4. Variations of VS removal, SCOD, alkalinity, pH and methane yield of two AD reactors with/without microaeration pretreatment.

continuous AD reactors were continuously operated for another 45 days. Variations of VS removal, SCOD, alkalinity, pH and methane yield of the two reactors are illustrated in Fig. 4. ANOVA ($p = 0.01$) showed that there was significant difference of VS removal between the two reactors, with average removal efficiencies of 18.3% ± 2.6% and 24.5% ± 3.6% before and after microaeration treatment, respectively. As shown in Fig. 4b, microaeration enhanced release of soluble substances from sludge and thus increased SCOD by 33.6% on average. Nevertheless, the average SCOD concentrations in the effluent of MA and Raw reactors were 221.8 ± 26.1 and 264.2 ± 25.1 mg/L, respectively. The results indicated that microaeration accelerated the utilization of organic matters for methane production in AD systems by improving degradability of some organic compounds (Jenicek et al., 2014) and shortening the reaction time required for hydrolysis and acidification. Alkalinity and pH are two parameters closely related to AD process and reflect buffering capacity of AD systems (Hou et al., 2018). As shown in Fig. 4c and d, the trend of alkalinity and pH corresponded well, and were both significantly lower in MA reactor than in Raw reactor ($p = 0.01$). The decreased pH and alkalinity in MA reactor were attributed to its higher efficiency of hydrolysis, fermentation and methane production for sludge after microaeration. Of course, the pH values in MA reactor fluctuated in a narrow range from 6.86 to 6.92, which had insignificant damages to methanogenic activity (Fu et al., 2018). Sufficient alkalinity (NH_3) produced from ammonification by the breakdown of PN and amino acids for sludge digestion prohibited large fluctuation of pH values in AD system (Tchobanoglous et al., 2003).

As shown in Fig. 4e, the specific biogas yields of MA reactor (109.3 ± 16.1 ml/g VS on average) were 16.4% higher than those of Raw reactor (93.9 ± 17.8 ml/g VS). The significant enhancement of biogas production in the semi-continuous MA reactor ($p = 0.01$) confirmed the results obtained from batch tests. Fig. 4f showed that methane contents in biogas from MA reactor were always higher than those from Raw reactor. The results indicated that microaeration enhanced methane concentration in biogas and had an advantage over microaeration enhanced AD process in a single reactor, in which a slightly lower ratio of CH_4/CO_2 in biogas was obtained because a small portion of oxygen is consumed during the oxidation of organic matters (Jenicek et al., 2014). Microaeration not only increased biogas production of sludge in AD system, but also improved methane content in biogas to a certain extent.

3.3. Sludge properties after microaeration pretreatment

Sludge samples fed into and discharge from Raw (Raw_i and Raw_e) and MA (MA_i and MA_e) reactors were collected to characterize organic content and dewaterability of sludge, and the results are summarized in Table 3. With equal VSS/SS ratios in the influent sludge, MA reactor

Table 3
Characteristics of sludge fed into and discharged from two AD reactors with/without microaeration pretreatment.

Sludge	VS/TS	SRF ($\times 10^{12}$ m/kg)	CST (s)	Median particle Size (μm)	Viscosity (cP)
Raw_i	0.56 ± 0.09	3.95 ± 0.45	85.4 ± 5.8	63.5 ± 2.4	11.7 ± 2.3
MA_i	0.56 ± 0.07	4.89 ± 0.36	96.5 ± 14.7	51.1 ± 3.7	25.6 ± 4.8
Raw_e	0.48 ± 0.03	7.71 ± 0.59	129.1 ± 7.8	58.9 ± 6.3	45.1 ± 7.2
MA_e	0.47 ± 0.05	5.81 ± 0.37	119.5 ± 6.1	50.1 ± 5.6	39.7 ± 6.5

obtained lower VSS/SS ratio than Raw reactor. Compared to raw sludge, microaeration pretreated sludge had higher SRF and CST, and smaller particle size, suggesting that microaeration deteriorated sludge dewaterability and disintegrated sludge aggregates to small particles, which is in accordance with previous literatures (Jenicek et al., 2014). Nevertheless, SRF and CST of sludge discharged from MA reactor were 32.7% and 8.0% lower than those from Raw reactor, respectively, indicating that dewaterability of microaerobic sludge after AD was better than that of raw sludge. The phenomena were probably related to lower SCOD concentration (Fig. 4b), and more efficient hydrolysis of extracellular polymeric substances (EPS) and particles in MA reactor (Jenicek et al., 2014). Viscosity data in Table 3 further confirmed the results of dewaterability because the viscosity of microaeration pretreated sludge was higher than that of raw sludge before AD but lower after AD treatment.

3.4. Comparison on microbial community structures

3.4.1. Bacterial community

Bacterial communities of the two samples were compared from the phylum to the genus level, and results of main groups (> 1%) are illustrated in Fig. 5. As shown in Fig. 5a, Proteobacteria (22.0–25.4%), Chloroflexi (12.7–17.5%), Bacteroidetes (10.4–10.5%), Parcubacteria (2.3–10.7%), Synergistetes (4.2–7.4%), Actinobacteria (4.3–6.8%) and Firmicutes (4.4–5.6%), which were reported to be dominant in the AD system (Nguyen and Khanal, 2018), were the most dominant phyla in each sample. Proteobacteria is an important phylum in anaerobic hydrolysis and acidification, and induced cell lysis and released intracellular substances (Cheng et al., 2018). Synergistetes can accelerate the transfer of VFAs to acetate (Zou et al., 2018), while Chloroflexi and Actinobacteria are responsible for the degradation of SMP and other complex substrates by producing hydrolytic enzymes (Cheng et al., 2018). The total relative abundance of the four phyla for hydrolysis, fermentation and acetogenesis, namely Proteobacteria, Chloroflexi, Synergistetes and Actinobacteria, in MA reactor was 15.3%, 37.9%, 77.7% and 58.3% higher than that in Raw reactor, respectively.

At the class level (Fig. 5b), the relative abundance of Alphaproteobacteria, Gammaproteobacteria and Betaproteobacteria, belonging to phylum Proteobacteria and acting as main consumers of glucose and VFAs (Ariesyady et al., 2007), in MA reactor increased by 23.8%, 17.7% and 34.3% in comparison to Raw reactor, respectively. Specifically, the presence of Gammaproteobacteria had an important effect on PN degradation and EPS secretion (Ittisupornrat et al., 2014). Norank_Aminicenantes, which is related to the production of VFAs and decomposes sugar into galactose (Robbins et al., 2016), is also an dominant class in the two reactors, and the abundance in MA reactor (4.6%) was about twice of that in Raw reactor (2.3%). Compared to Raw reactor, the relative abundance of Anaerolineae and Caldilineae, two classes belong to phylum Chloroflexi and responsible for the hydrolysis and fermentation of organic matters (Hou et al., 2018), in MA reactor increased by 21.6% and 34.4%, respectively.

Hierarchically clustered heatmap analysis at the genus level is showed in Fig. 5c. *Thermovirga* is an anaerobic bacterium metabolizing amino acids by reducing elemental sulfur, and was enriched in MA reactor with relative abundance (6.1%) greatly higher than Raw reactor (2.8%). The total relative abundance of *norank_Anaerolineaceae* and *norank_Caldilineaceae*, which belong to phylum Chloroflexi and could utilize sucrose, glucose and N-acetyl-glucosamine (Ariesyady et al., 2007), were increased by 49.9% and 34.4% in AD reactor after microaeration, respectively. *Bacteroidetes_vadinHA17* was responsible for hydrolysis and fermentation of various pollutants in AD system (Chen et al., 2017), and was enriched in MA reactor with relative abundance 56.5% higher than that in Raw reactor. The genus *Candidatus_Microthrix,* which was related to lipid metabolism by using long-chain fatty acids as a carbon and energy source (Levantesi et al., 2010), was increased by 92% in MA reactor compared to Raw reactor.

3.4.2. Archaeal community

The relative abundances of archaeal genera in the two samples are visualized using CIRCOS in Fig. 5d. *Methanosaeta* (66.4–68.4%), *norank_WCHA1-57* (12.1–12.6%), *Methanobacterium* (8.0–8.8%) and *Methanobrevibacter* (2.6–4.3%) were the most four dominant genera in each sample. The dominant position of *Methanosaeta*, a specialist that could utilize acetate for methanogenesis exclusively (Yi et al., 2016), in the two AD reactors indicated that aceticlastic methanogenesis was the main methanogenic pathway. The relative abundance of *Methanosaeta* in MA reactor was 3.0% higher than that in Raw reactor. The total relative abundances of *Methanobacterium*, *Methanospirillum*, *Methanobrevibacter* and *Methanolinea*, typical hydrogenotrophic methanogens tending to live in an environment with high hydrogen concentration (Kurade et al., 2019), were 15.0% and 13.6% in Raw and MA reactors, respectively. Among the four hydrogenotrophic methanogens, *Methanobacterium* had the highest and close relative abundance in the two reactors, *Methanospirillum* and *Methanobrevibacter* were enriched in RAW reactor, while *Methanolinea* showed 86.5% higher relative abundance in MA reactor. WCHA1-57 was reported to use formate or H_2/CO_2 as substrates (Rakia et al., 2005) and had slightly higher relative abundance in MA reactor. Compared to bacterial community, MA had insignificant effect on changes of archaeal community. These results indicated that microaeration pretreatment promoted enrichment of hydrolytic and fermentative bacteria in AD reactor rather than methanogenic bacteria, which enhanced hydrolysis and acidification of organic matters and biogas production.

3.5. Economic analysis

At aeration time of 4 h and AR of 4 vmm, power consumption for microaeration was 0.014 kWh/kg, and enhanced methane production of anaerobic AD was 9.3 L/kg VS, which generated electricity of 0.028 kWh/kg VS. Furthermore, microaeration induced VSS removal by 0.061 kgVS/kg VS, equivalent to sludge reduction by 3.5%. Therefore, microaeration saved costs of sludge treatment and disposal by 0.0057 €/kg VS, with the route of thickening, anaerobic digestion, dewatering and landfill (typical cost of 218 €/ton dried solids) (Jiang et al., 2018). Therefore, the total cost saved by microaeration was 0.0075 €/kg VS at power cost of 0.13 €/kWh by comprehensively considering energy consumption of aeration, methane production enhancement and sludge reduction. Compared to chemical, thermal and mechanical pretreatment methods, microaeration shows advantages of simple, easy to handle, low investment and energy consumption, and no additional chemicals required, and is a promising approach for full-scale applications as a pretreatment unit for AD of sludge and organic

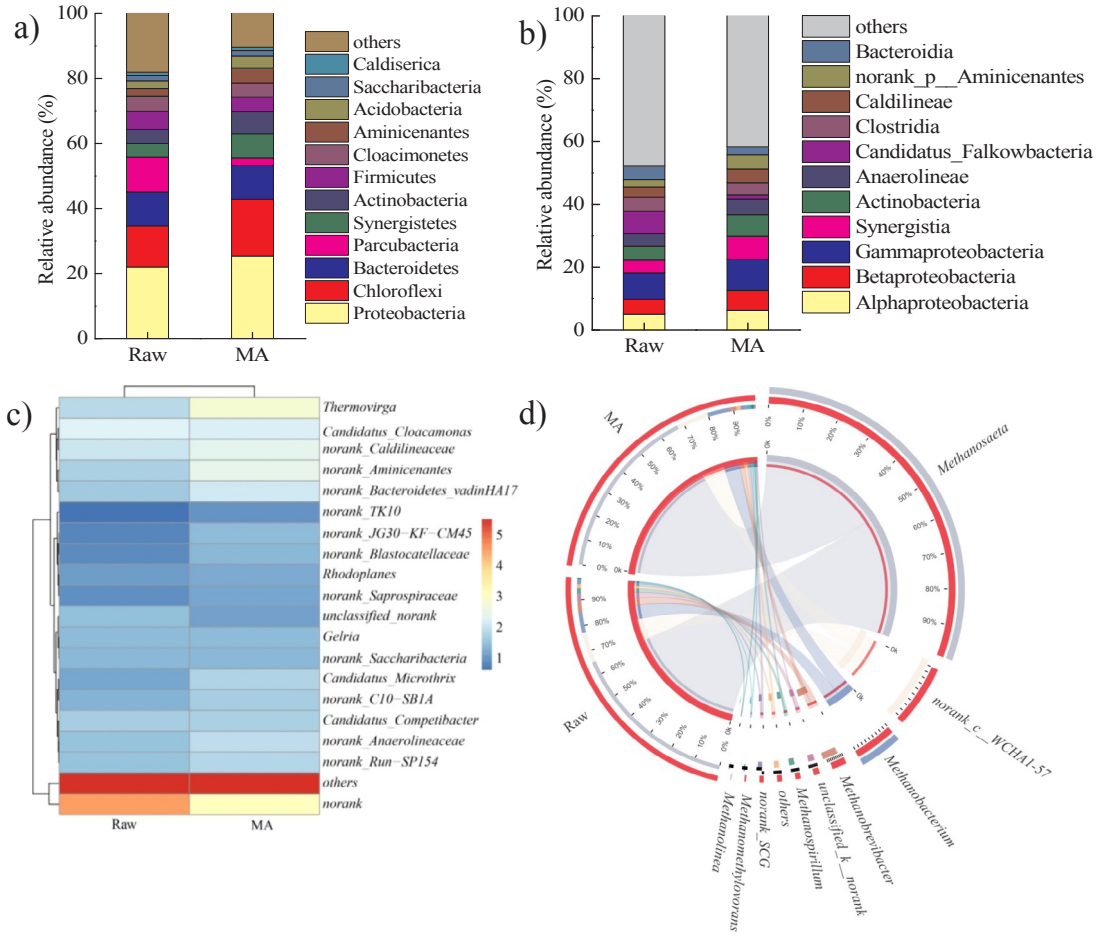

Fig. 5. The relative abundance of bacterial community at the phylum (a), class (b) and genus (c) level and archaeal community at the genus level (d) in two AD reactors with/without microaeration pretreatment.

solids.

4. Conclusions

Microaeration pretreatment enhanced methane production and rate of AD for sewage sludge. Correlation analysis showed that microaeration pretreatment not only enhanced release of DOM and VFAs, but also stimulate activity of hydrolytic enzymes. Semi-continuous experiments showed that microaeration pretreatment accelerated the utilization of organic matters, increased biogas production by 17.8%, enhanced methane content in biogas, and improved sludge dewaterability. Microbial community structure analysis showed that microaeration pretreatment promoted enrichment of hydrolytic and fermentative bacteria in AD reactor rather than methanogenic bacteria, and acetiiclastic methanogenesis was the main methanogenic pathway for methane production.

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