A pilot-scale anaerobic membrane bioreactor under short hydraulic retention time for municipal wastewater treatment: performance and microbial community identification

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ABSTRACT

Anaerobic membrane bioreactor (AnMBR) processes are a promising method of recovering energy from municipal wastewater. In this study, a pilot-scale AnMBR with extremely short hydraulic retention time (HRT = 2.2 h) was operated at a flux of 6 L/(m^2 h) for 340 days without any membrane cleaning. The average value achieved for chemical oxygen demand (COD) removal was 87% and for methane yield was 0.12 L CH$_4$/gCOD$_{removed}$. Based on mass balance analysis, it was found that about 30% of total influent COD was used for methane conversion, 15% of COD for sulfate reduction, 10% for biomass growth and 10–20% of COD remained in the effluent. Microbial community analyses indicated that seasonal changes of feedwater (in terms of organic components and temperature) led to the variations of microbial community structures. Among the bacterial communities, *Chloroflexi*, *Proteobacteria* and *Bacteroidetes* were the three most predominant phyla. In the archaeal consortia, *WCHA1-57* and *Methanobacterium* surpassed *Methanosaeta* and *Methanolinea* to become the predominant methanogens during the long-term operation of short HRT. The sulfate-reducing bacteria, accounting for less than 2% of total abundance of bacteria, might not be the dominant competitor against methanogens.

Key words | anaerobic membrane bioreactor, energy recovery, hydraulic retention time, microbial community, wastewater treatment

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INTRODUCTION

Municipal wastewater is the most abundant and resource-laden type of wastewater, characterized by low organic strength and high particulate organic matter. If chemically bound energy in organic pollutants could be converted to useful energy, such as biological energy or electrical energy, a municipal wastewater treatment plant (WWTP) would become a water reclamation station and renewable energy plant (McCarty *et al.* 2011). Developing an appropriate energy-efficient technology holds the key to achieving resource recycling and energy recovery for future wastewater treatment.

Anaerobic biological treatment, regarded as a possible way to achieve economic sustainability and energy neutrality for WWTPs, has attracted much attention in recent decades (Ketheesan & Stuckey 2015). In addition to the energy that can be recovered from methane-rich biogas, the advantages of anaerobic treatments over aerobic processes include lower energy consumption and reduced

sludge production (Stuckey 2012). Nevertheless, the major concerns with the conventional anaerobic bioreactors are the washout of the slow-growing microorganisms and unsatisfactory effluent quality, especially in the start-up period (Smith *et al.* 2014). Anaerobic membrane bioreactors (AnMBRs) that couple membranes with the anaerobic biological processes not only fully retain the anaerobic microorganisms but also produce a high-quality effluent (Stuckey 2012). Moreover, due to the use of membrane, decoupling of sludge retention time from hydraulic retention time (HRT) allows for high organic loading rates (OLR) and methane productivity (Liao *et al.* 2006). Besides the high effluent quality and high treatment efficiency, the small footprint and the potential agricultural use of the treated effluent could enhance the practical application of AnMBRs for treating municipal wastewater, particularly in water-scarce areas (Stuckey 2012).

Despite AnMBRs being an attractive alternative for wastewater treatment, the low-strength organic matter in real municipal wastewater limits their cost-effectiveness (Ozgun *et al.* 2013). An effective strategy to improve the treatment efficiency is to operate AnMBRs under short HRT. According to the current literature, most HRTs of AnMBRs are longer than 4 h (Gimenez *et al.* 2011; Martinez-Sosa *et al.* 2011), and the performance of AnMBRs under extremely short HRT for processing dilute municipal

wastewater remains unclear. In addition, the microbial communities of AnMBRs under very low HRT need to be clarified to help elucidate their performance.

The objective of this work is therefore to investigate the performance of a pilot-scale AnMBR system operated under extremely short HRT (2.2 h) for treating low-strength municipal wastewater with a particular focus on microbial communities. The reactor performance, including filtration behaviour, pollutant removal rates and biogas production, was monitored and microbial communities were studied during long-term operation. The results are expected to provide a sound understanding of AnMBRs under short HRT and to facilitate the application of AnMBRs for low-strength wastewater treatment.

MATERIALS AND METHODS

AnMBR setup and operation

Figure 1 shows the submerged pilot-scale AnMBR setup comprised of a 25 L anaerobic bioreactor and a 35 L membrane tank (MT). A curtain-type hollow-fibre membrane module (mean pore size 0.2 μm, total membrane area of 5.4 $m^{2;}$ RF-I, OriginWater, China) was installed in the MT. Four peristaltic pumps (BT600-2J, Longer, China) were
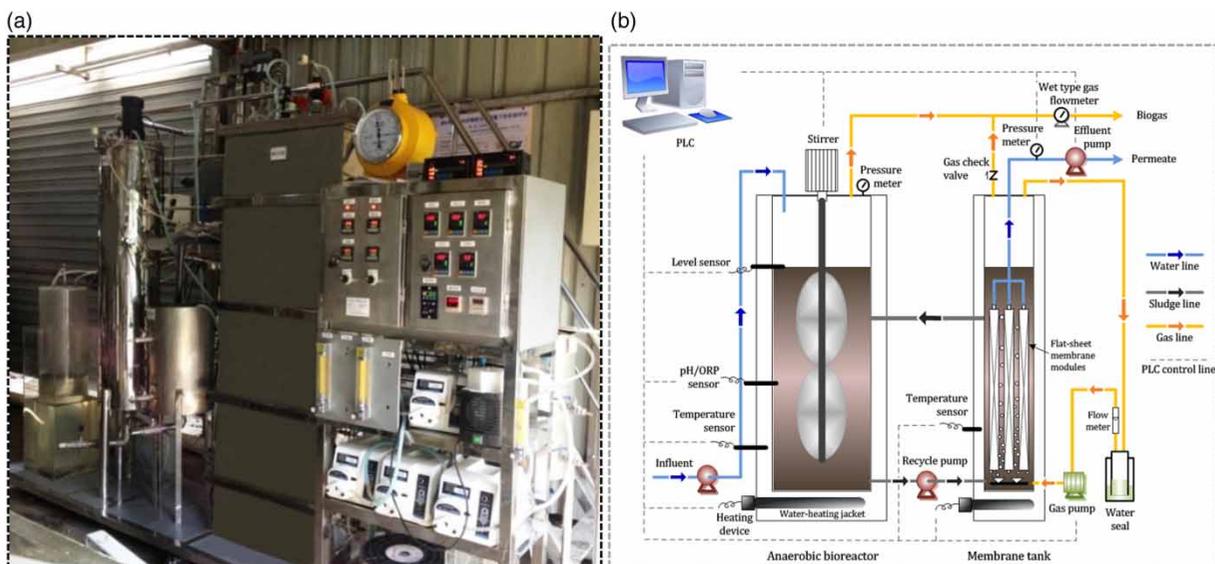

Figure 1 | (a) Photograph and (b) flow schematic of the pilot-scale AnMBR.

used to feed wastewater into the bioreactor, recycle mixed liquor from the bioreactor to the MT, extract permeate from the membranes and to pump backwashing solution (effluent) to clean the membranes. A diaphragm gas pump (N840 FT.18, KNF, Germany) was used to recirculate biogas from the head space to scour the membranes and a gas flow meter was used to control the flow rate at 28 L/min. A water-seal container was installed between the MT and the gas pump to ensure stable operation of the system. A gas check valve (70/70 U, WITT, Germany) and a wet-type gas flowmeter (LML-1, DETAIR, China) were connected to the MT headspace to monitor daily biogas production. Both tanks were equipped with water-heating jackets to control the temperature at 35°C. A programmable logic controller (PLC) system was installed to monitor and record the real-time temperature, pH, oxidation reduction potential (ORP), sludge level, water-jacket level and head-space pressure. The liquid level was controlled by pressure transducers and the PLC.

The AnMBR was seeded with digested anaerobic sludge (concentration of raw sludge, 35 g/L) from a digestion tank in Bailonggang WWTP in Shanghai. The bioreactor was fed with real municipal wastewater from Quyang WWTP in Shanghai, China, which was pretreated by a 0.18-mm micromesh screen. The characteristics of the feedwater are shown in Table S1 and Figure S1 in Supporting Information (available with the online version of this paper). The pilot-scale AnMBR was continuously operated for 340 days. The HRT and sludge retention time were 2.2 h and 60 d, respectively (calculated based on the total volume including bioreactor and MT), and the membrane flux was maintained at 6 L/(m^2 h). The backwash flux was fixed at 12 L/(m^2 h). The filtration/relaxation/backwashing duration was set as 5 min/30 s/30 s.

Chemical oxygen demand mass balance

For the chemical oxygen demand (COD) mass balance analysis, the main fractions of COD conversion included: gaseous biogas generation (CH_4 and CO_2), dissolved methane loss, biomass growth, sulfate reduction, effluent residuals and others (e.g. membrane fouling). The dissolved methane was measured by the method described by Souza *et al.* (2011) and calculated based on Henry's law. It was assumed that sulfate was entirely reduced to sulfide and

the COD equivalent of biomass was 1.42 gCOD/gVSS (volatile suspended solids).

Organic matters analysis

The distribution of organic fractions in mixed liquor was analysed to determine the difference between the supernatant and the effluent. The sludge sample was centrifuged at 6,000 rpm for 10 min and the supernatant was defined as total organic fractions (presented as tCOD), which were further divided into three categories by using 0.45-μm and 0.2-μm filter papers. Organic matter that could be retained by 0.45-μm filter paper was colloidal, and matter that could pass through 0.45-μm filter paper was the soluble fraction (also termed dissolved organic matter, DOM). The organic matter passing through 0.45-μm filter paper but retained on 0.2-μm filter paper was defined as the 0.2–0.45 μm fraction and the residual after 0.2-μm filtration was termed as <0.2 μm fraction.

The concentration and components of DOM and bound extracellular polymeric substances (EPS) were examined along with the operation time, since DOM and EPS play an important role in membrane fouling. DOM was extracted by centrifugation coupled with filtration by a 0.45-μm filter paper and EPS was extracted by a thermal method based on our previous study (Wang *et al.* 2013). Three main components of DOM and EPS, i.e. polysaccharides, proteins and humic substances, were detected in triplicate. Polysaccharides were analysed using the anthrone-sulfuric acid method (Gaudy 1962), and proteins and humic substances were measured by the corrected Lowry method (Frolund *et al.* 1995). Glucose, bovine serum albumin and humic acid (Fluka 53680), respectively, were used as standards.

Microbiology analysis

Illumina MiSeq was used in this study to analyse the 16S rRNA gene of bacteria and archaea to investigate the seasonal effects of feedwater on microbial communities. Sludge samples from the bioreactor and MT were collected and analysed on Day 9 (March 2015), Day 122 (July 2015) and Day 305 (January 2016). The detailed procedures for microbial analyses can be found in our previous publication (Yu *et al.* 2014).

Wastewater characteristics analysis

Samples were filtered with 0.45-μm filter papers. COD, NH_4^+-N, total nitrogen (TN) and total phosphate (TP) in influent and effluent, total mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) in the MT were tested according to *Standard Methods* (APHA 2012). A gas chromatography system (6890N, Agilent, USA) was used to measure volatile fatty acids (VFA) with a flame ionization detector and biogas composition (CH_4 and CO_2) with a thermal conductivity detector. Sulfate was measured by an ion chromatography system (ICS-3000, Dionex, USA) and sulfide was tested by *Standard Methods* (APHA 2012), which were used to conduct COD mass balance analysis.

RESULTS AND DISCUSSION

Filtration performance and pollutant removal

The pilot-scale AnMBR was continuously operated for 340 days without any chemical cleaning, with an initial 28 days for reactor start-up and acclimatization. The evolution of trans-membrane pressure (TMP) is shown in Figure 2(a), indicating that the reactor ran stably with low TMP increase rate. COD removal performance is shown in Figure 2(b). The average COD removal rate reached 87% (Table S1, available with the online version of this paper) at an average OLR of 3.0 kgCOD/(m³·d). Despite the fluctuations in influent COD, the effluent COD could achieve a low concentration (<60 mg/L), indicating that AnMBR could resist shock organic loads. Approximately 30% of TN and 35% of TP were also removed via utilization for biomass growth or interception by membrane (see Table S1). These results show that the AnMBR could achieve stable operation for low-strength municipal wastewater treatment, which was also reflected by the pH and ORP profile (see Figure S2, available online).

As shown in Figure 2(b), the supernatant concentration of COD (average = 223 ± 111 mg/L) in the reactor was much higher than that of the effluent (average = 50 ± 22 mg/L). However, the VFA concentrations were very low both in the reactor and effluent (Figure S3(a)), suggesting that the high COD concentration in the supernatant was not caused by the accumulation of VFA in the reactor. In order to identify
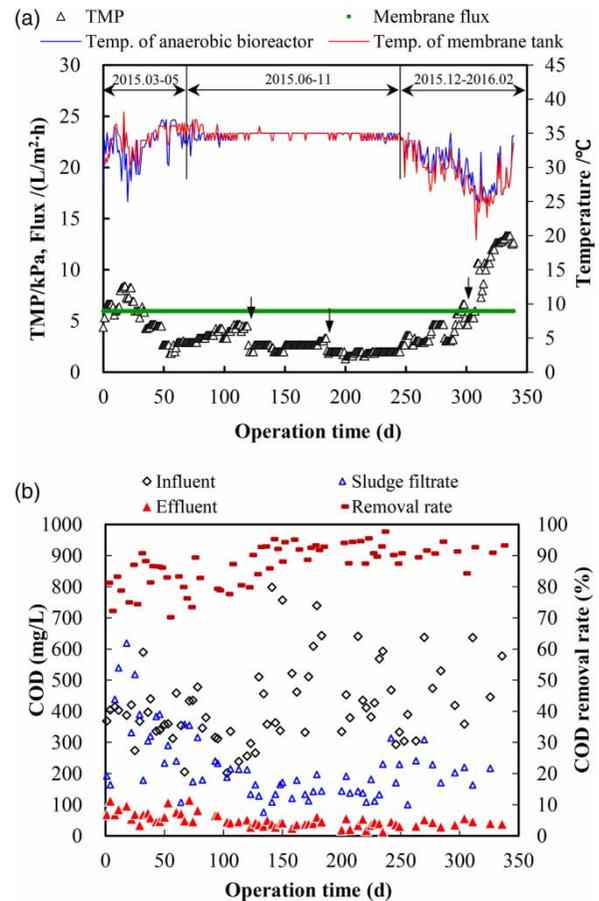

Figure 2 | (a) TMP profile and (b) removal performance for COD of the pilot-scale AnMBR. The black downward arrows in (a) indicate the accidental cut-off of wastewater supply, resulting in a slight recovery of TMP. The operating temperature was controlled at 35°C, while the actual temperature seasonally fluctuated due to the cold wastewater and the insufficient power of heating devices, especially in winter and early spring.

the difference between the effluent and supernatant, the distribution of organic fractions in the mixed liquor was analysed (as shown in Figure S3(b)) (Figure S3 is available online). The 0.2–0.45 μm fractions made a major contribution, accounting for 61% of the total organic fractions. Colloidal fractions accounted for 19% of the total organic fractions. These two fractions could be intercepted by the 0.2 μm pore-size membrane. However, the <0.2-μm fraction only contributed to 20% of the total organic matter, leading to the high-quality effluent of the AnMBR.

Biogas production and COD mass balance

Daily methane production profile is shown in Figure 3(a). The average production volume was 13.2 L/d with methane
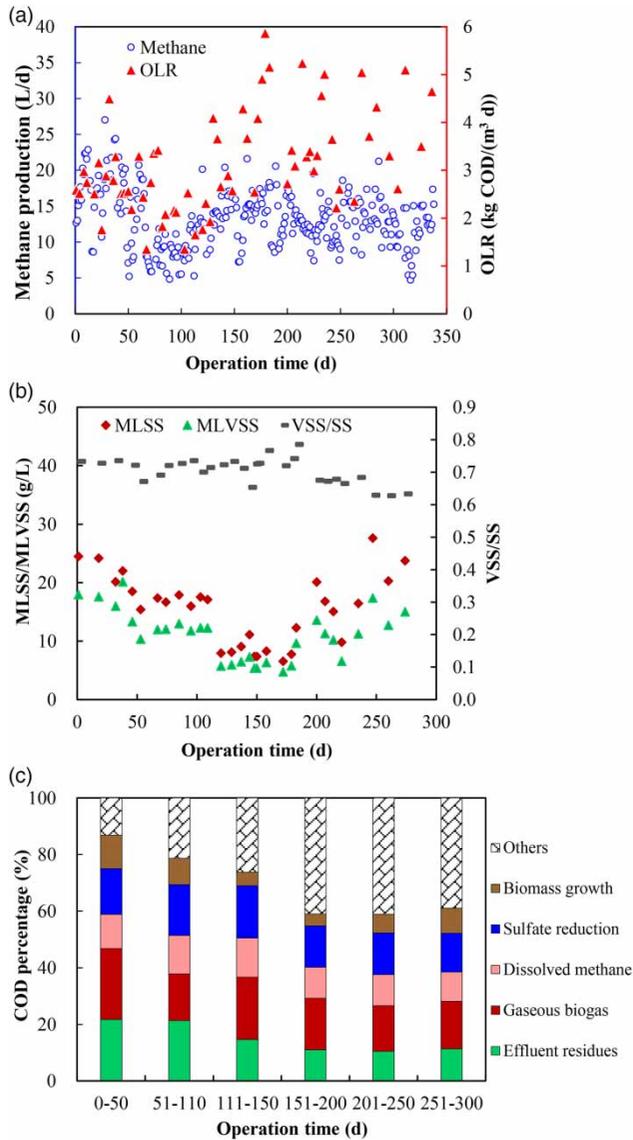
Figure 3 │ (a) Methane production profiles during the operation time, (b) variation of sludge concentration and (c) COD mass balance in different periods.

contents in biogas around 50%–70%. Correspondingly, the methane yield was 0.07 L CH_4/gCOD, lower than the reported values for AnMBR processes under mesophilic conditions (HRT of 6–24 h) (Gimenez *et al.* 2011; Martinez-Sosa *et al.* 2011). It has been reported that 30%–50% of methane is lost in the effluent (dissolved methane) (Martinez-Sosa *et al.* 2011; Smith *et al.* 2013). Therefore, attention should be paid to the recovery of dissolved methane in the effluent in order to avoid greenhouse gas emission and increase methane gas recovery efficiency. Other researchers

reported that the low carbon conversion rate was due to utilization by sulfate-reducing bacteria (SRB) (Gimenez *et al.* 2011). In order to identify the carbon conversion pathway in the AnMBR system, COD mass balance analysis was conducted as follows.

Considering the seasonal fluctuation of sludge concentration (MLSS range 4.7–20.1 g/L [average, 10.9 g/L], as shown in Figure 3(b)), the overall COD mass balance was divided into six periods (see Figure 3(c)); the calculation details are presented in Table S2 (available online). As described in Figure 3(c), approximately 30% of total influent COD was converted into methane (including gaseous and dissolved methane, the concentration of dissolved methane was 8 mg/L in the effluent). Thus, the overall methane yields were 0.12 L CH_4/gCOD. About 15% of COD was utilized by SRB for sulfate reduction, less than 10% was utilized for biomass production, and about 10%–20% remained in effluent. In addition, there were 25%–40% COD fractions unknown (denoted as Others), which may be converted to membrane foulants in the cake layer, or be adsorbed on the sludge which was discharged and/or be transformed by other metabolic pathways. Development of countermeasures to efficiently use this part for methane production is worth investigating further.

Organic matter properties

DOM and EPS, mainly composed of protein, polysaccharide and humic acid, were measured during the operation time to determine the distribution of organic matter types in the reactor. As shown in Figure 4(a) and 4(b), DOM concentration kept increasing at a rate of 3.4 mg/(L·d) until it exceeded 1,000 mg/L and then decreased dramatically, while EPS concentration increased slightly at a rate of 2.1 mg/(L·d) and surged to a peak of 1,300 mg/L. A short HRT leads to a large OLR and a high MLSS concentration in the AnMBR. This could be a major reason causing a high concentration of soluble microbial products (SMP) since the substrate-utilization associated products and biomass-associated products, being SMP components, are positively correlated to the available substrate and biomass concentration in the reactor, respectively (Wang *et al.* 2013). The increase of DOM concentration during the operation suggested an accumulation of SMP and consequently
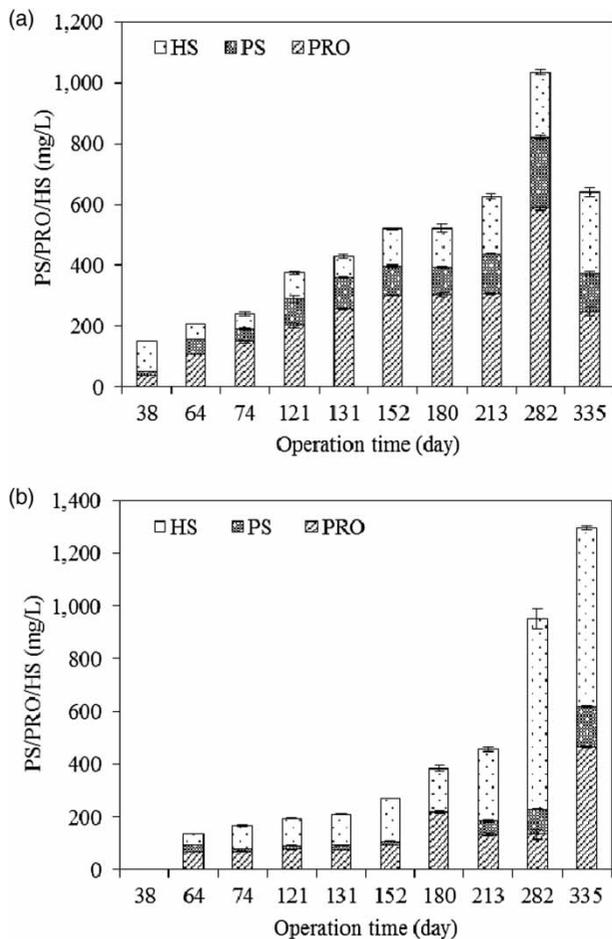
Figure 4 │ Variations of (a) DOM and (b) EPS during the operation time.

might cause an increased membrane fouling rate, which has been also reported by Huang *et al.* (2011).

Microbial community analysis

In order to discern the changes in microbial communities in the AnMBR, the Illumina MiSeq platform was employed to identify the bacteria and archaeal domains for anaerobic sludge sampled on Day 9 (March 2015), Day 122 (July 2015) and Day 305 (January 2016). The rarefaction curves of the six samples are shown in Figure S4 and the statistical indices related to the richness and diversity of the microbial communities are listed in Table S3 (Figure S4 and Table S3 are available online). The coverage values of sludge samples were close to 1 in both bacterial and archaeal communities, implying that almost all common phylogenetic groups were

detected in the constructed libraries (Ma *et al.* 2013a). Chao and Shannon indices indicated a decrease in bacterial diversities and an increase in archaeal diversities in July but an opposite trend in March and January.

In bacterial communities, the microbial structures varied from season to season. In the March and January samples (Day 9 and Day 305), there was a difference in microbial communities between bioreactor and MT, while they were quite similar in summer samples (Day 122) (see Venn analyses in Figure 5(a)), red and green circles represent bioreactor and MT, respectively). Among the communities, *Chloroflexi*, *Proteobacteria* and *Bacteroidetes* were the three most predominant phyla in the anaerobic sludge samples (Figure 5(a)), as has been observed in other anaerobic processes (Ma *et al.* 2013b). Members of *Chloroflexi*, as reported by Miura *et al.* (2007), are responsible for degradation of SMP including carbohydrates and cellular materials, which consequently reduces membrane fouling potential. *Proteobacteria* are able to degrade a wide range of macromolecules. *Bacteroidetes*, known as proteolytic bacteria, are involved in protein degradation and able to ferment amino acids to acetate (Yu *et al.* 2016). These three main phyla accounted for a large proportion (51%–63%) in the AnMBR, leading to enhanced organic matter degradation.

Further, *Proteobacteria*, the highest-ranked phylum, could be divided into five subdivisions (i.e. *alpha-*, *beta-*, *gamma-*, *delta-* and *epsilon-*). As shown in Figure 5(b), *Deltaproteobacteria* and *Betaproteobacteria* were the most predominant class in samples of Day 9 (71%–84%), while *Deltaproteobacteria* alone was the dominant class in samples of Day 122 and Day 305 (82%–84%). The presence of abundant *Betaproteobacteria* on Day 9 might be related to the high-concentration of organic matter (Figure 2(b)) in the reactor since higher relative abundance of *Betaproteobacteria* in anaerobic processes might contribute to its improved degradation performance (Yu *et al.* 2016). Furthermore, *Deltaproteobacteria*, the major member of *Proteobacteria*, were mostly affiliated with syntrophic substrate-degrading bacteria, related to methanogenic hydrocarbon degradation, and partially involved in sulfate respiration (Liu *et al.* 2010). Figure 5(c) depicts the subdivision of *Deltaproteobacteria* group. The detected SRB in the AnMBR consisted of *Desulfovibrionales*, *Desulfuromonadales*, *Desulfobacterales* and
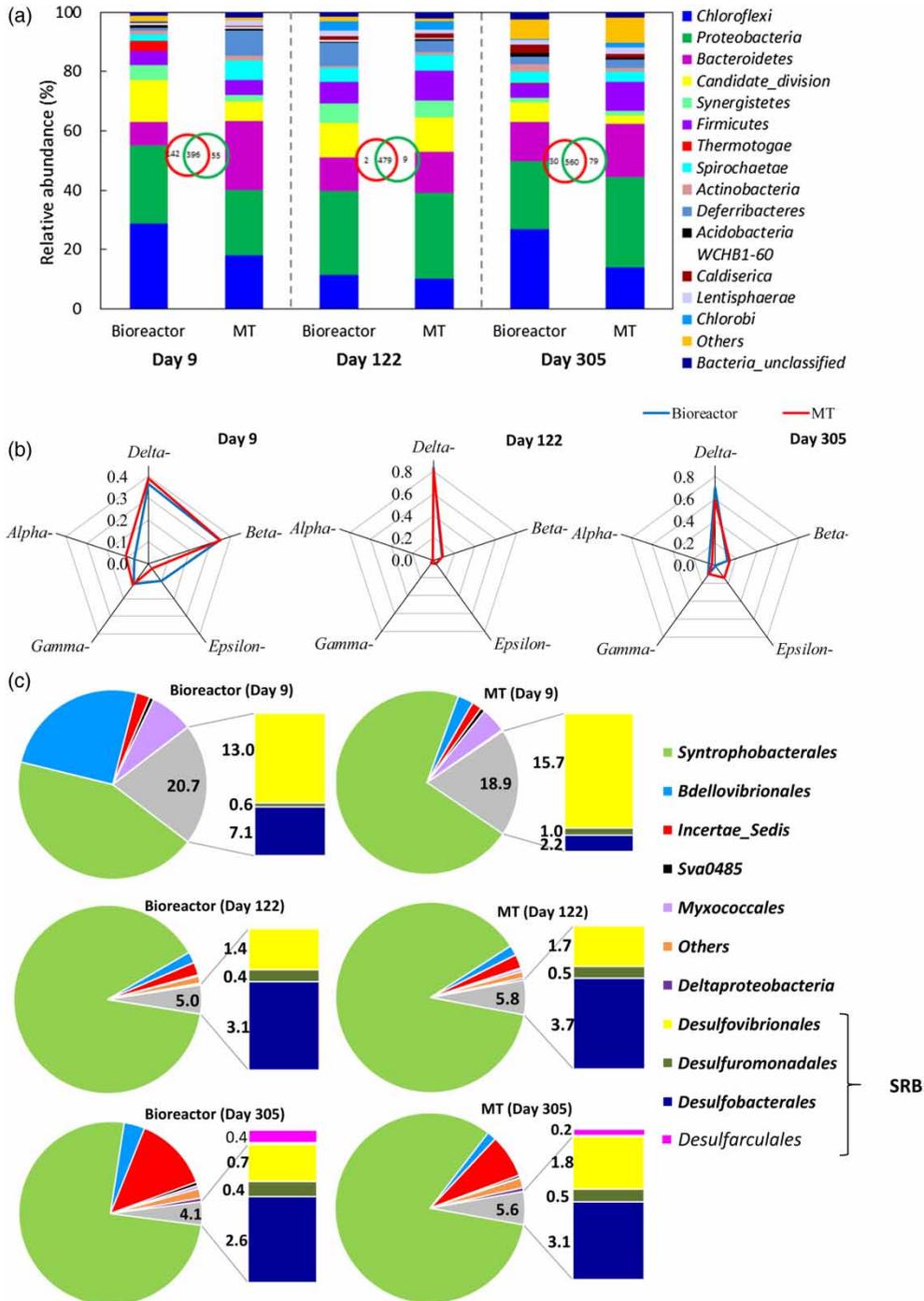
Figure 5 | Bacterial communities: (a) phylum level, (b) subdivisions of *Proteobacteria* and (c) subdivisions of *Deltaproteobacteria*. Relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%). Phyla accounting for less than 1% of relative abundance are regarded as others. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/wrd.2017.164.

Desulfarculales, which accounted for less than 2% of the total abundance of bacteria (1.6%–2.0% for Day 9 sample, 1.2%–1.4% for Day 122 sample and 0.7%–1.0%

for Day 305 sample). The decreasing trend inferred that SRB may not be the dominant competitor against methanogens.

As for the phylum *Bacteroidetes*, in addition to the main classes *Sphingobacteria* and *Bacteroidia*, the class *VadinHA17* had a relative high abundance (see Figure S5(a), available online), 3.2%–3.5% for Day 9 samples, 2.0%–2.5% for Day 122 samples and 5.0%–7.9% for Day 305 samples), and could degrade recalcitrant organic matters as reported by Baldwin *et al.* (2015) and thus enhance the anaerobic digestion process. Moreover, it is worth noting that some minor classes, such as *Clostridia* (phylum *Firmucutes*), were able to carry out bio-hydrogen production, as well as *Alphaproteobacteria* (Kapdan & Kargi 2006). In this study, the relative abundance of *Alphaproteobacteria* plus *Firmucutes* increased from 5.0% to 5.3% in the bioreactor and from 6.0% to 8.5% in the MT, respectively (Figure S5(a)), implying that the pathway of methanogenesis might be affected by the increase of bio-hydrogen products
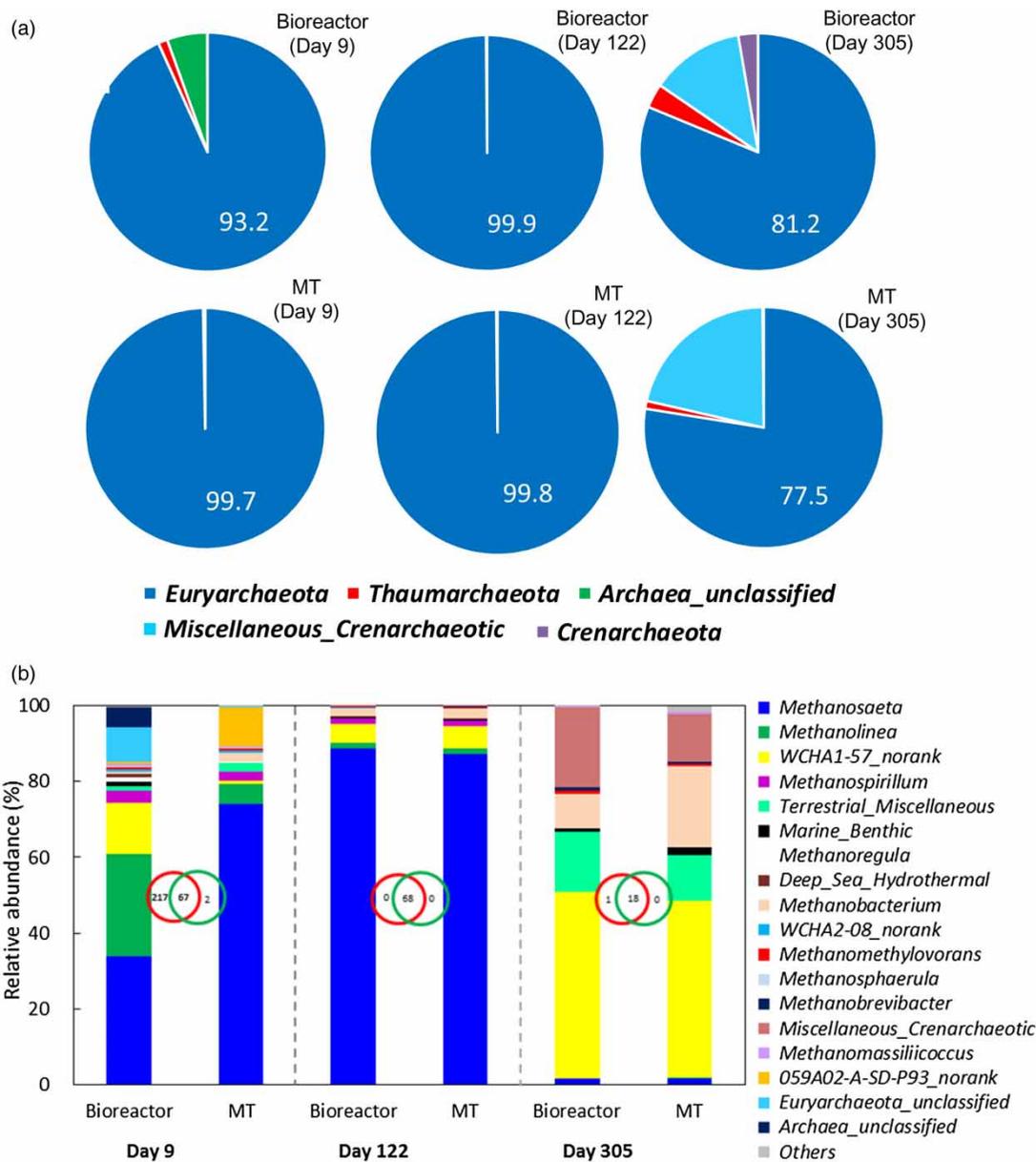
Figure 6 | Archaeal communities at (a) the phylum level and (b) at the genus level. Relative abundance is defined as the number of sequences affiliated with that taxon divided by the total number of sequences per sample (%).

during the long-term operation of the AnMBR. This will be further elaborated in combination with the following archaeal community analysis.

Archaeal consortia retrieved from the sludge samples showed low phylogenetic diversity (Figure 6). *Euryarchaeota* was the predominant phylum in the AnMBR system (relative abundance of 77.5%–99.7%), which included the major methanogens. However, other phyla, e.g. *Thaumarchaeota* and *Crenarchaeota* were much lower in relative abundance, especially in samples of Day 122 (Figure 6(a)). Further, the Venn analyses between bioreactor and MT samples showed that the archaeal communities were closely similar in July (Day 122) but showed some differences in March (Day 9) and January (Day 305) (Figure S5(b), available online). Additionally, *Thermotogae*, a kind of thermophile (phylum *Crenarchaeota*), was detected in samples of Day 305 (3.7%). It can be inferred that though the operating temperature was constant, seasonal changes of feedwater (organic components and temperature) could vary the distribution of microorganisms in the AnMBR system.

To further investigate the functional groups related to methanogenesis, archaeal communities were analysed at the genus level. As shown in Figure 6(b), *Methanosaeta* and *Methanolinea* were the two major methanogenic genera in Day 9 samples; these genera are affiliated to acetoclastic methanogens and hydrogenotrophic methanogens, respectively (Garcia *et al.* 2000). This is different from a previous study reporting that *Methanosarcina* is the dominant genus in an AnMBR for landfill leachate treatment (Xie *et al.* 2014), suggesting that the influent wastewater might significantly affect the microbial communities. In samples of Day 122, *Methanosaeta* surpassed *Methanolinea* to become the predominant genus (87.2%–88.7%). It is reported that the high abundance of *Methanosaeta* was in accordance with low acetate concentration (Yu *et al.* 2016), which is in agreement with the VFA analysis above (Figure S3(a)). In samples of Day 305, *WCHA1-57* was the most abundant archaeal genus (46.8%–49.2%), followed by the genus of *Methanobacterium* (9.0%–21.6%), which are both reported as hydrogenotrophic methanogens (Saito *et al.* 2015). Combined with the bacterial community results, it can be inferred that the methanogenesis might be changed from a acetoclastic to hydrogenotrophic

pathway during long-term operation, as supported by previous studies showing that the operational condition with short HRT (e.g. 2 h) could inhibit the activity of methanogens (Braga *et al.* 2016) and achieve enhanced hydrogen production compared to the longer-HRT operations in a UASB reactor (Braga *et al.* 2016). Thus, in our study, it may be inferred that the short HRT (2.2 h) could decrease the relative abundance of methanogens, strengthen the bio-hydrogen production and enable the hydrogenotrophic methanogenesis to be the predominant pathway; this possibility needs further investigation.

CONCLUSIONS

The pilot-scale AnMBR was continuously operated for 340 days with the average OLR of 3.0 kgCOD/(m^3·d). COD removal efficiency could reach over 87% and the methane yield was 0.12 L CH_4/gCOD$_{removed}$. According to COD mass balance, approximately 30% of total influent COD was converted into methane, 15% was utilized by SRB for sulfate reduction, less than 10% was used for biomass production and 10%–20% remained in the effluent. Microbial analyses showed that seasonal changes of feedwater might vary the distribution of microorganisms in the system. For the bacterial communities, *Chloroflexi*, *Proteobacteria* and *Bacteroidetes* were the three most predominant phyla. In the archaeal consortia, during the long-term operation under short HRT, *WCHA1-57* and *Methanobacterium*, which are affiliated to hydrogenotrophic methanogens, surpassed *Methanosaeta* and *Methanolinea* to become the predominant methanogens. The SRB, accounting for less than 2% of total abundance of bacteria, might not be the dominant competitor against methanogens.

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