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Performance and bacterial community structure of an anoxic/oxic membrane bioreactor treating anaerobically digested piggery wastewater

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ABSTRACT

The performance and bacterial community structure of an anoxic/oxic membrane bioreactor (A/O-MBR) for treating anaerobically digested piggery wastewater were studied. A/O-MBR had excellent performance for the removal of chemical oxygen demand and NH_4^+-N. Denitrifying bacteria were the dominant microbial species in the activated sludge of A/O-MBR. Compared with sequencing batch reactors and single aerobic reactors, the A/O-MBR enhanced nitrogen removal for anaerobically digested piggery wastewater treatment. However, the lack of an organic carbon source inhibited total nitrogen removal. The non-VSS (mixed liquor volatile suspended solids) accumulation led to poor settling capacity and high mixed liquor suspended solids. The A/O-MBR also had smaller particle size flocs, due to the lack of an organic carbon source and the composition of the bacterial community. When precipitate cleaning was halted, the increase in concentration of extracellular polymeric substances and the non-VSS accumulation led to the severe membrane fouling. Additionally, the bacterial community of activated sludge depended on the composition of the anaerobically digested piggery wastewater and the reactor structure.

Keywords: Submerged membrane bioreactor; Anaerobically digested piggery wastewater; Bacterial community; Nitrogen removal

1. Introduction

Piggery wastewater, including piggery waste and flushing wastewater, is a typical livestock farm wastewater with high concentrations of suspended solids, organic matter, nitrogen, and phosphorus [1,2]. As an effective biological technology for the high concentration organic carbon removal and the methane production, the anaerobic digestion has been widely applied for the treatment of piggery wastewater [3]. However, anaerobically digested effluent still contains a high concentration of ammonium (over 500 mg/L;

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ammonia nitrogen (NH_4^+-N): chemical oxygen demand (COD) ≈ 1:1). Discharge of anaerobically digested piggery wastewater is into aquatic systems or municipal wastewater treatment plants, leading to eutrophication or toxic shock to biological system [2,4]. Consequently, anaerobically digested piggery wastewater should be treated before being discharged into aquatic systems or municipal wastewater treatment plants.

Physical-chemical (air stripping, steam stripping, magnesium ammonium phosphate hexahydrate (MAP) precipitation) and biological (sequencing batch reactor (SBR), single reactor system for high ammonium removal over nitrite (SHARON) chemostat, anaerobic ammonium oxidation (ANAMMOX)) processes have been applied for treating anaerobically digested piggery wastewater. Compared with physical-chemical process, biological process is the economical technology, which can be used widely all over the world, including in developing countries. The activated sludge process is a traditional and reliable biological technology. Many studies reported that activated sludge utilizing specific functional bacteria (such as ANAMMOX bacteria, *Cholorophyceae*, *Chlorella* sp., *Scenedesmus obliquus*, etc.) effectively removed NH_4^+-N and COD in the anaerobically digested piggery wastewater [5–9]. Additionally, membrane bioreactor, combining with biological and physical processes, has been used widely in piggery wastewater treatment [1,10]. Recent papers [5–9] have focused on the treatment performance of activated sludge for anaerobically digested piggery wastewater. The properties of activated sludge are still unclear. It is considered that the low nitrogen removal of activated sludge is due to lack of organic carbon [11]. However, whether the bacterial community of activated sludge also plays a significant role in low nitrogen removal is unknown.

This study investigated the properties of the activated sludge in an effort to develop an improved fundamental understanding of the biological process for treating anaerobically digested piggery wastewater. An anoxic/oxic membrane bioreactor (A/O-MBR) was operated for over 350 d, and loaded with anaerobically digested piggery wastewater from 30,000 pig farm. This study focused on the properties of activated sludge, affecting significantly the reactor performance. In addition, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) and 16S rRNA clone library were carried out to characterize the bacterial community and identify the dominant microbial species in the activated sludge.

2. Materials and methods

2.1. Anaerobically digested piggery wastewater

The anaerobically digested piggery wastewater was the effluent of an up-flow anaerobic sludge bed for treating piggery wastewater, which was from a swine farm with 30,000 pigs (Suzhou, Jiangsu Province, China). The solid fraction of the anaerobically digested piggery wastewater was removed through flocculation-sedimentation using polyaluminium chloride, and the liquid fraction was pumped into the anoxic tank. The anaerobically digested piggery wastewater (liquid fraction) contained approximately 540 mg/L COD (biodegradable COD: non biodegradable COD = 10:1), 540 mg/L NH_4^+-N, 560 mg/L total nitrogen (TN), and 20 mg/L total phosphorus (TP) and pH ranged between 7.4 and 7.8.

2.2. A/O-MBR setup and operation

A lab-scale 7.2 L A/O-MBR was used in this study (Fig. 1; anoxic and oxic tank volumes were 2.7 and 4.5 L, respectively). A polyvinylidene fluoride hollow fiber membrane module (pore size 0.4 μm; total surface area 0.01 m^2; Litree Company, China) was installed in the oxic tank and an intermittent suction mode (10 min on/2 min off for each cycle) was operated to maintain a constant fluid flux. Hydraulic retention time (HRT) and solids retention time (SRT) were both maintained at 50 d. The flow rate of recycled mixed liquor from the oxic tank to the anoxic tank was controlled at 400% of the influent flow rate. The influent pH and the reactor temperature ranged between 7.3 and 7.6 (by $NaHCO_3$ adjustion) and 25 and 29 ℃, respectively.

The inoculating sludge was drawn from the return activated sludge stream in the Quyang wastewater treatment plant (Shanghai, China). The A/O-MBR was operated for over 200 d (at first 100 d, SRT was 30 d to fasten bacteria adapting to anaerobically digested piggery wastewater; then SRT was changed into 50 d) for the start-up and acclimatization of activated sludge. Then the inoculated A/O-MBR was operated for 150 d with a new membrane module for the experiments. The precipitate at the reactor bottom was cleaned everyday for the first 100 d (phase I), after which the precipitate was no longer cleaned from the reactor bottom (phase II).

2.3. Extraction and measurement of extracellular polymeric substances (EPS)

Extraction of EPS was performed according to a modified thermal extraction method [12]. Carbohydrate

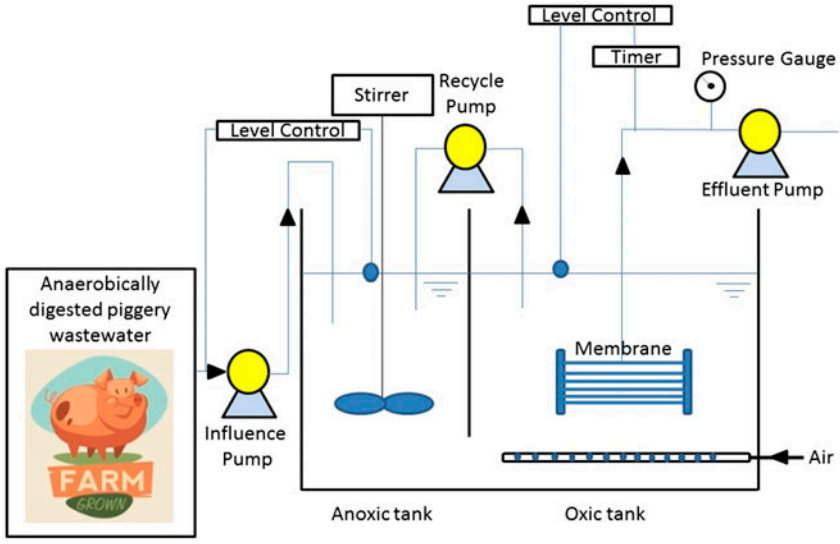

Fig. 1. The schematic of the anoxic/oxic membrane bioreactor (Recycle rate was 400%; Air pump controlled the DO in a range of 4–5 mg/L; the effluent was controlled both with level controller and timer).

and the protein concentrations in EPS were measured by the phenol-sulfuric acid method and Branford method, respectively [13,14].

2.4. PCR-DGGE, 16S rRNA gene-cloning and phylogenetic analysis

To investigate the dynamic changes in the microbial community during operation, PCR-DGGE was carried out to analyze bacterial community shifts. Polymerase chain reaction (PCR) protocol was conducted as described by Xia et al. [15]. The bacterial community composition of activated sludge was measured with cloning library of 16S rRNA genes. The extraction of genomic DNA was performed with the FastDNA Spin Kit (MP Biomedicals, LLC, France). The complete 16S rRNA genes from extracted DNA were amplified with the bacterial universal primers 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) [16]. Triplicate PCR products were pooled to minimize bias. The complete cloning library procedure was described in references [16,17]. One hundred positive clones were selected for sequencing. Chimeric sequences were identified as described and excluded from subsequent analysis software (Bellerophon, Australia). All the sequences were compared to the known sequences for phylogenetic analysis. Operational taxonomic units (OTUs) were defined as groups in which the sequence similarity was more than 97%. Phylogenetic trees were constructed by the neighbor-joining method using the Clustal X software package. Bootstrap resampling analysis for 1,000 replicates was performed to estimate the confidence of tree topologies. The 16S rRNA gene sequences from this study have been deposited in National Center for Biotechnology Information (NCBI) public database (GenBank) under accession numbers KJ651206 to KJ651244. In addition, filamentous microorganisms in the activated sludge were analyzed according to the result from cloning library.

2.5. Additional analysis

A focused beam reflectance measurement (Eyetech particle size and shape analyzer, Ankersmid, Holland) was used to identify the particle size of activated sludge. The standard methods were used to measure the concentrations of NH_4^+-N, COD, TN, TP, mixed liquor suspended solid (MLSS), and mixed liquor volatile suspended solids (MLVSS) as well as the sludge settling volume after 30 min (SV30 (%)) and the sludge volume index (SVI) [18]. The membrane surface was characterized by SEM (XL30, Philips, Netherlands). Three replicate samples (with liquid-nitrogen pretreatment), and eight spots on each replicate were visualized by SEM.

During operation of the A/O-MBR, activated sludge samples were collected from anoxic and oxic tanks, analyzed as described above. Preliminary results indicated that activated sludge samples in both tanks were similar, due to the small volume of A/O-MBR and the high recycled mixed liquor flow rate

(400%). Consequently, the activated sludge results reported in this paper were measured in the activated sludge from the oxic tank.

3. Results

3.1. A/O-MBR performance

A/O-MBR performance metrics for the phase I are presented in Table 1. The average removal efficiencies of COD (loading rate: 68 mg/d) and NH_4^+-N (loading rate: 75 mg/d) were approximately 89 and 97%, respectively. COD and NH_4^+-N results indicated that the A/O-MBR had better COD and NH_4^+-N removal from anaerobically digested piggery wastewater than other physical-chemical methods, such as struvite crystallization, chemical precipitation, and air stripping [19–21]. However, the removal efficiencies of TN (28%) and TP (40%) were similar to other activated sludge processes [8]. TN loading rate (22 mg/d) was approximately a third of the NH_4^+-N loading rate. Compared with previous studies [2,8,19,21,22], low TP removal efficiency in A/O-MBR was because PO_4^{3-} showed a high concentration in the anaerobically digested piggery wastewater, and it was more easily removed by physical-chemical methods. The removal efficiencies of COD, NH_4^+-N, TN, and TP had only decreased slightly in phase II (Precipitate cleaning was halted in phase II; the final precipitation cleaning was in day 100). In addition, the TN/TP ratios in the influent and effluent were much higher than optimum values, indicating that nitrogen concentrations were greater than those that can be tolerated by bacteria. The reactor pH decreased to 5.5, and $NaHCO_3$ was added to the reactor continuously to maintain pH between 7.3 and 7.6.

3.2. MLSS and MLVSS

MLSS and MLVSS are important properties of activated sludge, and used as the indicators of wastewater treatment performance (Fig. 2(a)). The MLSS concentration was between 8 and 10 g/L in phase I, which was higher than that in SBR for treating anaerobically digested piggery wastewater [8]. In phase II, MLSS increased to 26 g/L at the rate of approximately 1.5 g/(L d). Incomplete denitrification and nitrate accumulation should lead to a decrease in MLSS [11]. The MLSS/MLVSS ratio remained approximately 0.002/d decrease rate from 0.66 to 0.25.

MLVSS had a slight increase throughout the experiment, and the average of MLVSS in the activated sludge was 5 ± 2.5 g/L. SV30 increased gradually (about 0.9% per day) from 20 to 70%, while SVI were 28–64 mL/g (Fig. 2(b)). Additionally, non VSS (NVSS) increased in phase II.

3.3. EPS of activated sludge

EPS played a significant role in bacterial cells aggregation in activated sludge [23]. Although EPS is a mixture of different organic and inorganic compounds, proteins and polysaccharides were found to be the dominant compound. Protein concentration increased from 20 to 58 mg/g VSS in the phase II (Fig. 3). Conversely, the polysaccharide concentration in EPS remained stably around 25 mg/g VSS throughout the experiment.

3.4. Particle size of activated sludge flocs

Fig. 3 presents the particle size distribution of activated sludge flocs in A/O-MBR on days 20th, 50th,

Table 1
Average characteristics of the influent and effluent for A/O-MBR (all the values represent mean ± SD)

		Phase I[a]		Phase II[b]	
Parameters	Influent (mg/L)	Effluent (mg/L)	Removal (%)	Effluent (mg/L)	Removal (%)
COD	540 ± 170	60 ± 10	89 ± 3	92 ± 8	83 ± 4
NH_4^+-N	540 ± 150	15 ± 10	97 ± 5	16 ± 5	97 ± 5
NO_3^--N	18 ± 10	220 ± 30	–	250 ± 10	–
TN	560 ± 140	410 ± 60	28 ± 8	420 ± 30	25 ± 3
TP	20 ± 5	12 ± 6	40 ± 5	13 ± 3	34 ± 2
TN/TP[c]	28 ± 1	34 ± 8		32 ± 4	

[a]Phase I: The reactor was with the precipitate cleaning, $n = 100$.

[b]Phase II: The reactor was without the precipitate cleaning, $n = 50$.

[c]Optimum TN/TP = 5: TN/TP ratios of the influent and effluent were much higher than optimum value, indicating that nitrogen concentration overflowed the bacteria tolerance.

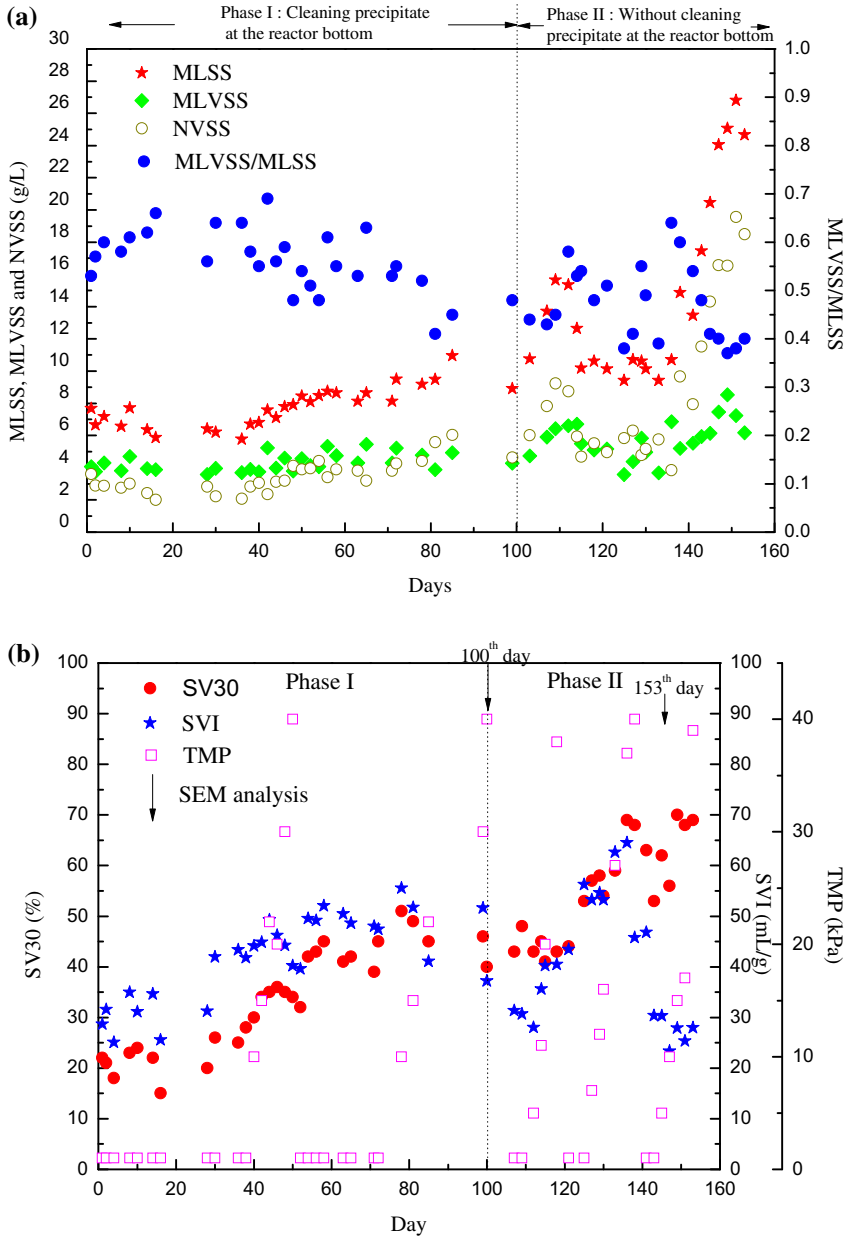

Fig. 2. Variations of (a) MLSS, MLVSS, and MLVSS/MLSS ratio and (b) TMP, SVI and SV30 during A/O-MBR operation (The reactor was with the precipitate clean in phase I, and without the clean in phase II; MLSS was increased with NVSS advance, but MLVSS had a slight variation. Compared with during phase I, TMP increased more rapidly during phase II).

70th, 105th, and 140th. The mean activated sludge size particle ranged from 8 to 20 μm. Additionally, when MLSS and NVSS increased, the mean particle size was enhanced slightly.

3.5. Membrane fouling

TMP is an important parameter for evaluating membrane fouling. TMP in the A/O-MBR increased at a low rate (0.51 kPa/d) during the first 100 d (with the precipitate clean), then had a higher increase rate (2.64 kPa/d) during the phase II (Fig. 2(b)). Meanwhile, MLSS and NVSS increased suddenly at day 130, but MLVSS remained at a similar range (around 7.5 g/L). The fouling resistances when TMP = 40 kPa (Table 2) were calculated on the basis of permeation data and resistance-in-series model [24,25]. Total resistance and cake resistance were enhanced in phase II,

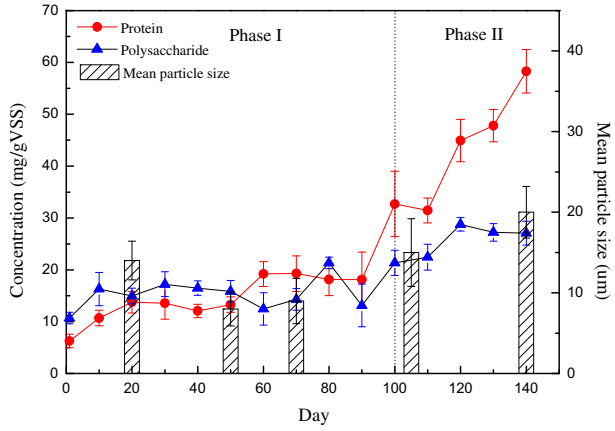

Fig. 3. Variations of EPS and mean particle size during A/O-MBR operation (Mean particle size was measured at day 20th, 50th, 70th, 105th, and 140th).

and pore blocking resistance increased obviously. In addition, Fig. 4 showed the membrane surface SEM image (1,000×) at TMP = 40 kPa.

3.6. Bacterial community of activated sludge

The activated sludge bacteria community played a key role in the pollutant removal. As shown in Fig. 5, the bacterial community was similar on days 50, 70, 105, and 140. The bacterial community on day 140 was further analyzed. The phylogenetic tree (100 randomly selected clones) of activated sludge on day 140 is presented in Fig. 6. Thirty-nine OTUs were obtained from the activated sludge. *Comamonas denitrificans* strain (19%), *Thauera* sp. (10%), *Candidatus aquirestis calciphila* (8%), and *Chitinophaga* sp. (7%) were the dominant microbial species in activated sludge. Some of the species found in this study, such as *Chitinophaga* sp., *Thauera* sp., were also found by others anaerobically biological treatment of anaerobically digested piggery wastewater [7,26]. The activated sludge also contained other minor microbial species, such as *Aeromonas* sp. (5%), *Sphingobacteriales* bacterium (4%), *Stenotrophomonas* sp. (2%), *Afipia feils* (2%), *Steroidobacter* (1%),

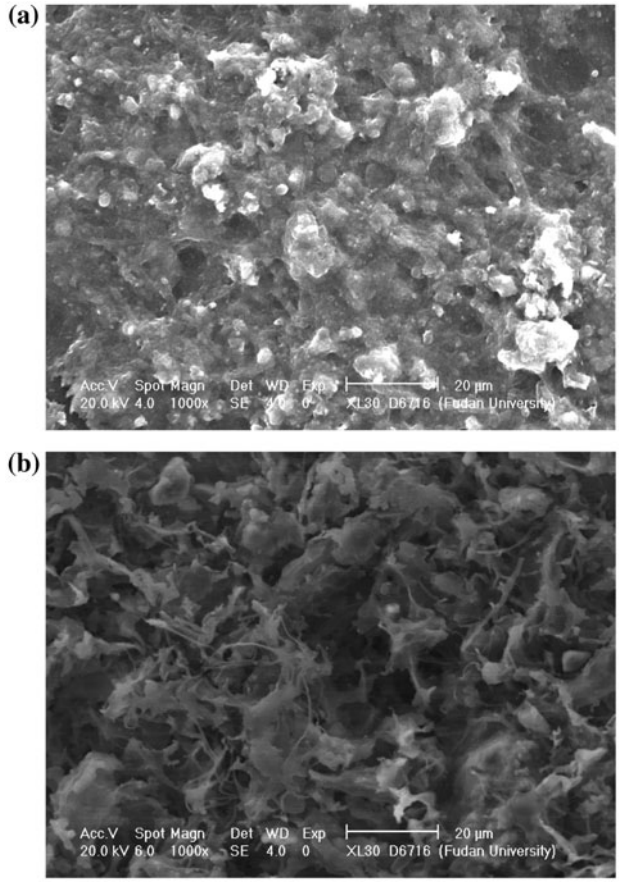

Fig. 4. SEM image of the membrane surface in (a) phase I and (b) phase II (The Sample was collected from the middle section of membrane fiber at 40 kPa TMP. With liquid nitrogen pretreatment, the samples was measured with SEM in 1,000×. The lightspot indicated the crystal particles, and the filament predicted the filamentous organism. Membrane surface was full of lightspot in the Fig. 4(a), and membrane surface was covered with the filament in the Fig. 4(b)).

Rhodobacter sp. (1%), *Aquimonas* sp. (1%), *Thermomonas koreensis* (1%), *Piscinibbacter* sp. (1%), and *Acidovorax* sp. (1%). Additionally, 41% of bacteria identified in activated sludge were denitrifying bacteria (*C. denitrificans* strain (19%), *Thauera* sp. (10%), *Flavobacteria*

Table 2
Analysis results of membrane resistance (R_m), pore-blocking resistance (R_p), cake resistance (R_c), and total resistance (R_t)

Item	50th day	103rd day	118th day	138th day	153rd day
R_t[a]	10.2	12.4	21.8	22.8	23.5
R_c	8.4	11.6	15.8	16	17.1
R_p	1.52	0.51	5.7	6.5	6.09
R_m	0.28	0.29	0.3	0.3	0.31

[a]$R_t = R_c + R_p + R_m$.

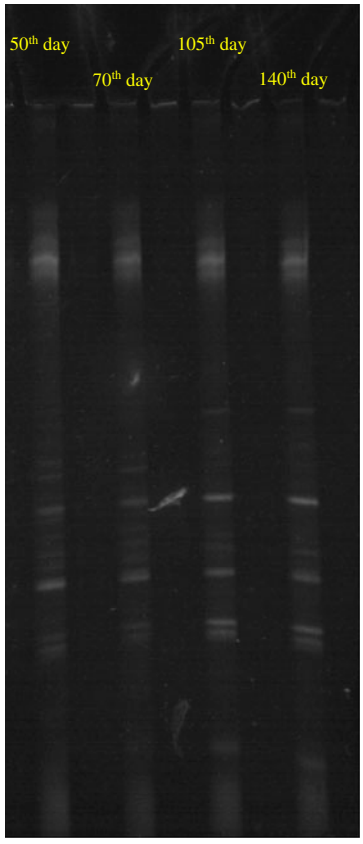

Fig. 5. DGGE profile of bacterial communities of activated sludge (Activated sludge samples at 50th, 70th, 105th, and 140th were measured with DGGE for investigate the dynamic changes of bacterial community).

bacterium (1%), *Aidovorax* sp. (1%), *Aeromonas* sp. (5%), *Rhodobacter* sp. (1%), *Hyphomonas* sp. (1%), *Steroidobacter* sp. (1%), *Denitratisoma* sp. (2%)). Other biological reactors treating anaerobically digested piggery wastewater contained fewer denitrifying bacteria in the activated sludge [26,27]. In addition, only *Hyphomonas* sp. (1%) and *Thiothrix eikelboomii* (1%) were filamentous micro-organisms.

4. Discussion

During the entire 150 d of reactor operation, the TN removal efficiency of the A/O-MBR treating anaerobically digested piggery wastewater was only 27 ± 8%, although NH_4^+-N removal efficiency was 97 ± 5%. During the activated sludge process, nitrogen removal occurred through nitrification and denitrification [28]. Nitrification was the biological conversion of NH_4^+-N to NO_3^--N by nitrifying bacteria (autotrophic bacteria; CO_2 works as the carbon source) under aerobic condition. The stoichiometric equation for nitrification was the following [28]:

$$NH_4^+ + 2O_2 = NO_3^- + 2H^+ + H_2O \quad (1)$$

Excess O_2 was supplied to the activated sludge in A/O-MBR. Additionally, 11% of bacteria (*Aquimonas* sp. (1%); *Candidatus aquirestis calciphila* (8%); *Afipha felis* (2%)) in the activated sludge were nitrifying bacteria (Fig. 6). Consequently, the activated sludge effectively transformed NH_4^+-N to NO_3^--N, which was accompanied by a rapid decrease in pH due to H^+ production during the nitrification reaction.

Denitrification was the dissimilatory reduction of NO_3^- to N_2 gas under anoxic condition (NO_3^- was the electron acceptor; organic carbon is the carbon source). The half reaction per mole e^- transferred for denitrification is as follows [29]:

$$0.20\,NO_3^- + 1.2\,H^+ + e^- = 0.1\,N_2 + 6H_2O \quad (2)$$

The COD consuming of denitrification was 2.86 g COD/g NO_3-N. As shown in Table 1, the removable COD of influent was approximately 480 mg/L, which was only enough for the 167 mg/L NO_3-N reduction without considering biomass growth (A/O-MBR removed 150 mg NO_3-N/L during operation). Denitrifying bacteria constituted 41% of the bacteria identified in the activated sludge, which made them the dominant species in activated sludge. It is therefore predicted that enough denitrifying bacteria were present in the A/O-MBR for denitrification to occur. Yang et al. [30] and Zheng et al. [31] both reported that the organic carbon source lack could reduce obviously the TN removal. Xia et al. [32] reported that carbon source could induce the variation of bacterial community, changing the TN removal. Based on the DGGE result, the activity sludge maintained the stable community during operation. Therefore, the low TN removal efficiency was due to the lack of an organic carbon source, not the bacterial community structure.

The activated sludge had a high MLSS for treating anaerobically digested piggery wastewater (Fig. 3), which could have led to the poor settling and sludge bulking observed in the system [29]. However, the poor settling capacity (high SV30) of activated sludge in the A/O-MBR was not due to the sludge bulking, because of low filamentous micro-organisms content (2%) and low SVI (28–64 mL/g SVI indicated the lack of carbon source [29]). Both MLSS and NVSS obviously increase during operation but the MLVSS slight increase indicated that the high MLSS was mainly due to NVSS accumulation, not bacterial growth. Consequently, the activated sludge for the anaerobically digested piggery wastewater treatment

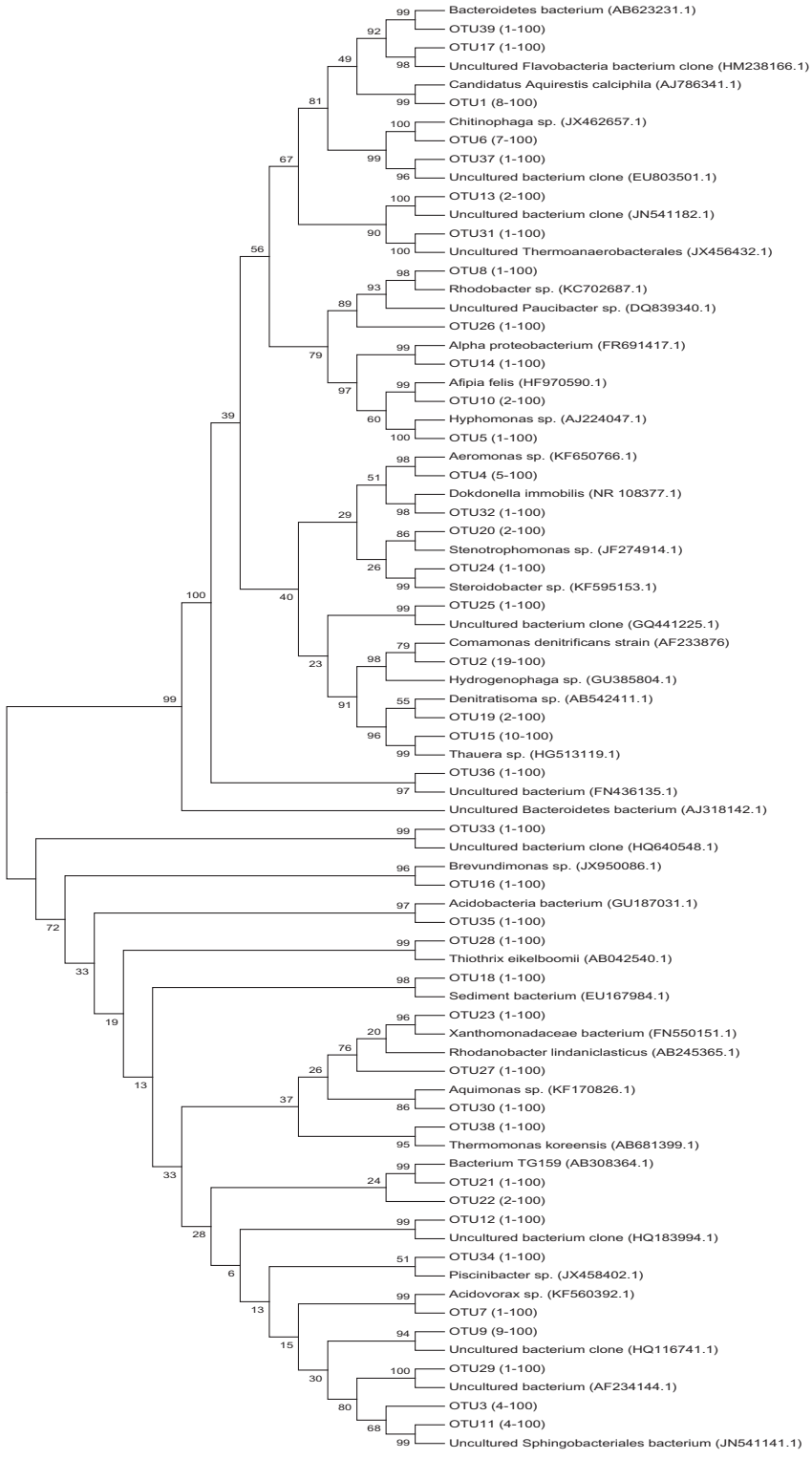

Fig. 6. Phylogenetic tree of activated sludge at 140th day (DGGE result showed the similar community of activated sludge during operation, sample at 140th day was selected for clone library analysis.

had a poor settling capacity due to NVSS accumulation, though SVI indicated that the bacterial properties of activated sludge were good for settling. This result indicated that the activated sludge process with settling section (such as SBR) was not suitable for the long-term treatment of anaerobically digested piggery wastewater without pretreatment. Pretreatment (mainly coagulation and flocculation [22]) was important for inhibiting NVSS accumulation and ensuring long-term performance of SBR.

The particle size of activated sludge was an important factor for solid–liquid separation and membrane fouling during activated sludge process [29,33–35]. Marcato et al. [36] reported that the solid size in the raw anaerobically digested piggery wastewater was approximately 53 μm in diameter, which was much larger than the particle sizes (8–20 μm) of activated sludge in this study. This result was due to the following reasons. First, there was more biodegradable organic carbon for bacteria during the piggery wastewater treatment than during the anaerobically digested piggery wastewater treatment, leading to the worse bacterial growth during the anaerobically digested piggery wastewater treatment. Second, filamentous micro-organisms acted as the backbone of a stable structure in the activated sludge [37]. During digestion, the activated sludge contained a large concentration of filamentous micro-organisms, which formed granules [38]. However, the filamentous micro-organisms made up only 2% of the bacterial community of the activated sludge in this study. Therefore, the small particle size of activated sludge for treating anaerobically digested piggery wastewater was due to the joint effect of the lack of an organic carbon source and the composition of the bacterial community.

Membrane fouling is the major obstacle for the long-term operation of MBRs. The TMP increase rate in phase II was about five times less than that in phase I (Fig. 2(b)), indicating that precipitate removal mitigated membrane fouling. In addition, total resistance, cake layer resistance, and pore-blocking resistance increased greatly in phase II, indicating that precipitates promoted membrane fouling, especially in the way of pore blocking. Although the mean size of activated sludge was enlarged, the protein concentration in EPS increased in phase II. A previous review [39] reported that the molecular weight of protein ranged from 67 to 200 kDa, and these proteins easily blocked membrane pores. Additionally, crystals and filamentous organism were observed in Fig. 4, indicating the accumulation of crystals and filamentous organism onto membrane surface during membrane fouling. The variations between Fig. 4(a) and (b) predicted that the precipitate cleaning could reduce the fouling process of filamentous organisms. Therefore, cleaning precipitate could reduce effectively the membrane process in the anaerobically digested piggery wastewater treatment.

The bacterial community of activated sludge presented the special properties. As mentioned above, the nitrogen removal was depended on the denitrifying bacteria, which was the dominant species in the bacterial community. In previous studies [6,22,27], SBR or aerobic reactors were used to treat anaerobically digested piggery wastewater. These reactors supplied high concentration of oxygen in a single tank, causing aerobic bacteria to quickly consume biodegradable carbon, thus leading to a shortage of carbon available for denitrification and the decrease in denitrifying bacteria. Consequently, the bacterial community was related to the nitrogen removal in the anaerobically digested piggery wastewater. In this study, the membrane module was able to successfully retain each bacterial species in the reactor. In addition, the biodegradable carbon source was first offered for denitrification due to the preposed anoxic tank. Therefore, the bacterial community of activated sludge in the A/O-MBR was able to maintain a higher percentage of denitrifying bacteria. Compared with SBR or a single aerobic reactor, MBR overcame the "low denitrifying bacteria concentration" barrier to enhance nitrogen removal from anaerobically digested piggery wastewater. *Acidobacteria* bacterium (1%), which was considered to be a common bacteria in piggery wastewater, was reported to be involved in degrading and hydrolyzing some organic compounds under microaerophilic conditions to produce acids and form syntrophic association with hydrogenotrophic methanogens [40,41]. Due to the high NH_4^+-N concentration and low organic carbon concentration, the bacterial community contained some microbial species, which could live in extreme conditions (*T. eikelboomii* (1%) and Uncultured *Thermoanaerobacterales* (1%)). Consequently, the bacterial community of the activated sludge depended on the composition of the anaerobically digested piggery wastewater and the reactor structure.

In conclusion, A/O-MBR worked as a promising biological technology for treating anaerobically digested piggery wastewater, and its properties (including activated sludge and reactor) had been identified in this study. A/O-MBR had the excellent removal performance of COD and NH_4^+-N. Although denitrifying bacteria were the dominant microbial species in the activated sludge, A/O-MBR maintained a low TN removal due to the lack of an organic carbon source. During the operation, the non-VSS

accumulation caused the poor settling capacity and high MLSS. In addition, the lack of an organic carbon source and the bacterial community structure led to smaller particle flocs. When precipitate cleaning was halted, the EPS increase and the non-VSS accumulation led to severe membrane fouling, indicating that precipitate cleaning could mitigate effectively the membrane fouling. Additionally, precipitate cleaning could reduce the fouling process of filamentous organisms.

Acknowledgments

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Nomenclature

A/O-MBR	—	anoxic/oixc membrane bioreactor
COD	—	chemical oxygen demand
MAP	—	magnesium ammonium phosphate hexahydrate
SBR	—	sequencing batch reactor
SHARON	—	single reactor system for high ammonium removal over nitrite
ANAMMOX	—	anaerobic ammonium oxidation
EPS	—	extracellular polymeric substances
TMP	—	trans-membrane pressure (kPa)
TN	—	total nitrogen
TP	—	total phosphorus
HRT	—	hydraulic retention time
SRT	—	solids retention time
PCR	—	polymerase chain reaction
OTUs	—	operational taxonomic units
NCBI	—	national center for biotechnology information
MLSS	—	mixed liquor suspended solids
MLVSS	—	mixed liquor volatile suspended solids
NVSS	—	non mixed liquor volatile suspended solids
SV30	—	sludge settling volume after 30 min
SVI	—	sludge volume index

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