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Raising nutrients removal efficiency by improving the internal recycling strategy in an anoxic/oxic-membrane bioreactor package plant

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ABSTRACT

The effects of two internal mixed liquor recycling strategies on nutrients removal from municipal wastewater were investigated in an anoxic/oxic-membrane bioreactor package plant with a capacity of 100 m^3/d. In the original strategy, in which the mixed liquor was directly pumped back to the anoxic tank from the membrane tank, the removal efficiencies of total nitrogen (TN) and total phosphorus (TP) decreased significantly due to the high dissolved oxygen concentration in the anoxic tank with an increase of the internal recycling rate from 50 to 100%. In contrast, in the improved internal strategy, i.e., the mixed liquor in the membrane tank was returned to the oxic tank with a recycling rate of 150%, and meantime, the mixed liquor in the oxic tank was refluxed to the anoxic tank at a recycling rate of 250%, the average effluent concentration of chemical oxygen demand, NH_4^+-N, TN, and TP were 25.3, 0.55, 28.89, and 1.71 mg/L, respectively. When external acetic acid (2.66 L/d) was added, the effluent concentration of TN decreased to 8.5 mg/L. In addition, a reduction of membrane fouling with the high nutrients removal efficiency was achieved by the improved strategy as compared with that of the original strategy. PCR–DGGE fingerprints showed that bacterial community in the activated sludge from the tanks had a high similarity under the conditions of the improved strategy. The bacteria belong mainly to phyla-*proteobacteria*, *Firmicutes*, and *Bacteroidetes*, which were important for nitrogen and phosphate removal.

Keywords: Anoxic/oxic-membrane bioreactor package plant; Internal mixed liquor recycling strategy; Membrane fouling; Bacterial community

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1. Introduction

A package wastewater treatment plant is a small integrated system that can be carried to any locations for its direct installation after manufacture [1]. Driven by the increasing demands to find a less resource intensive and more environmental-friendly way of sanitation, package plants have become a preferred option for nutrients removal from sewage in remote communities where building a centralized public sewage network is not feasible [2]. Several treatment processes suited for a package plant have been developed and applied widely, such as sequencing batch reactor [3], submerged aerated filter [4], biological aerated filter [5], and Johkasou purification tank [6]. These processes all have specific advantages, whereas most of which are not able to produce stable high-quality effluents [7].

Researches and applications of membrane bioreactor (MBR) for wastewater treatment have increased sharply during the last decade [8,9]. MBR has advantages in comparison with conventional activated sludge processes, including the high concentration of biomass, separation of hydraulic retention time (HRT) and sludge retention time (SRT), small size, and high effluent quality [10]. The conventional MBR system equipped with anoxic, oxic, and membrane tanks is a clear option for the advanced wastewater treatment, due to its efficient nutrients removal without consumption of chemicals. The reduced water purification cost makes it an interesting alternative for the installation as a package plant [10,11].

Previous studies on the optimization of anoxic/oxic-membrane bioreactor (A/O-MBR) systems have indicated that the internal mixed liquor recycling from the oxic zone to the anoxic zone enhances the nutrients removal [12]. The changes in flow are composed of two parts: a spatial change and a temporal change. For the temporal change, the internal recycling changes the velocity of nitrogen compounds, total phosphorus (TP), and dissolved oxygen (DO) concentrations entering anoxic conditions, with their great impact on denitrification and phosphate accumulation [13,14]. Sun et al. [14] found that the total amount of phosphorus removed from the A/O-MBR system could be as high as 64–89%, when the sludge had been discharged only once and the internal rate of recycling was favorable. However, little information is currently available on the spatial changes [15]. More research is needed for a full understanding of the effect of the recycling position and rate on the efficiency of nutrients removal, changes in the membrane fouling and bacterial community compositions in each reaction zone during wastewater treatment in the A/O-MBR system.

Therefore, in our investigation, two internal recycling strategies were compared in an A/O-MBR package plant: an original strategy and an improved strategy, differentiated by their internal recycling positions. By altering the internal recycling positions and rates, the most efficient strategy for nutrient removal and membrane fouling reduction during municipal wastewater treatment was found. In addition, the bacterial community using the polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) was analyzed to investigate the dominant strains under the optimal internal recycling strategy.

2. Methods and materials

2.1. The A/O-MBR package plant

An A/O-MBR package plant with a capacity of 100 m^3/d was set up in the Beijing Golden Source Eco-Tech Development Area Wastewater Treatment Co., Ltd. (GSBDAWWTP), China. The schematic diagram of the package plant is shown in Fig. 1. The total working volume of the A/O-MBR was 56.3 m^3, with baffles installed inside to divide the reactor into four subunits: an anoxic tank (9.87 m^3), an oxic tank (26.08 m^3), a membrane tank (13.55 m^3), and a clean water storage tank (6.8 m^3).

The feed sewage passed through 50-mm coarse screens and 20-mm fine screens and a hair filter, and then was pumped into the anoxic tank, where it was mixed with activated sludge by a top-mounted mechanical stirrer. A sieve was installed at the end of the anoxic tank to prevent the pass of small solids from damaging the membrane. After biological treatment in the anoxic, oxic, and membrane tanks, the effluent was withdrawn by a suction pump and was partially stored in a clean water storage tank for membrane backwash. The effluent withdrawal was controlled by a water level sensor. Two submerged hollow fiber membrane modules (polypropylene, pore size: 0.2 μm, Lisheng Membrane Technology Co. Ltd., Hainan, China) were attached to the membrane tank, providing a total surface area of 416 m^2. Coarse bubble diffusers were installed in the oxic zone as well as in the membrane tank for the purpose of oxygen supply and mixing [16]. The hydrodynamic condition is one of the most important factors affecting the membrane fouling [17]. Therefore, additional cross-flow bubble diffusers were placed below the membrane modules to enhance the removal of cake deposition on the membrane surfaces. According to Gui et al. [18], an intermittent suction was used (8 min on and 2 min off) to reduce the fouling of the membranes. The trans-membrane pressure (TMP) was monitored by an

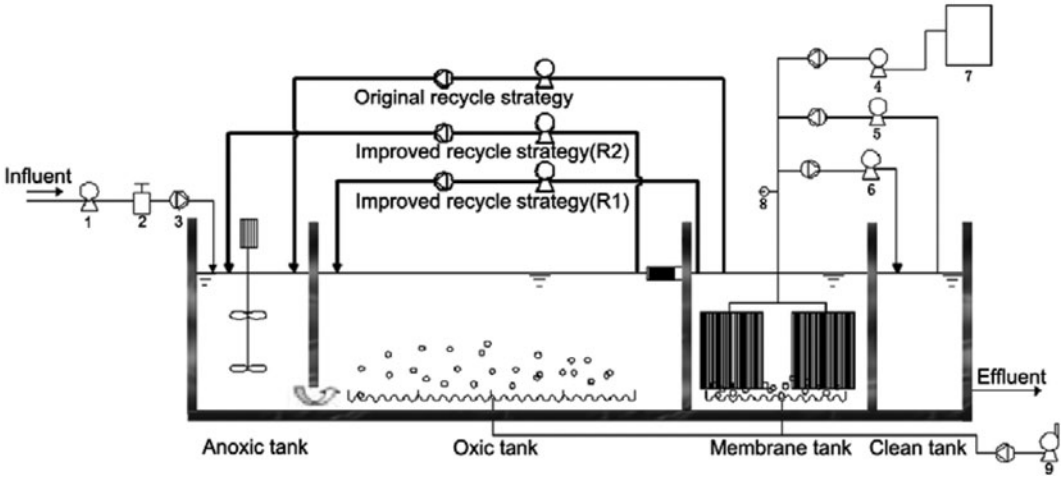

Fig. 1. Schematic diagram of an A/O-MBR package plant. (1) Feed pump, (2) hair filter, (3) flow meter, (4) chemical washing pump, (5) back washing pump, (6) suck pump, (7) container, (8) pressure sensor and (9) air blower.

online pressure meter to check membrane fouling. The membranes were chemically cleaned with 5.5% sodium hypochlorite at a flux of 0.84 m^3/h for 21 min and then were immersed in sodium hypochlorite for 30 min before the start of the next experiment.

2.2. Wastewater characteristics

The sewage entering GSBDAWWTP was used as the feed wastewater for the experiment. The concentrations of chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total nitrogen (TN), ammonia (NH_4^+-N), nitrate (NO_3^--N), TP, and suspended solids (SS) in the influent were 244–536 mg/L, 123–143 mg/L, 42.7–69.2 mg/L, 14.6–40.6 mg/L, 28.4–38.1 mg/L, 6.31–12.25 mg/L, and 110–460 mg/L, respectively. The values were from the regular wastewater quality monitoring by the GSBDAWWTP.

2.3. Operating conditions

The operating conditions of the package plant are listed in Table 1. The reactor was inoculated with the sludge from the GSBDAWWTP. The initial quantity of mixed liquor suspended solids (MLSS) was about 1,300 mg/L. No sludge was discharged during the entire study, except for sampling purposes.

To investigate the effect of mixed liquor recycling on nutrient removal, two different internal recycling strategies were compared: the original strategy and the improved strategy (Fig. 1; Table 2). The changes in the internal mixed liquor recycling rates of each strategy separate the entire study into five runs. In run 1 to run 3 of the original strategy, the mixed liquor was directly pumped into the anoxic tank from the membrane tank at internal recycling rates of 50, 100, and 50%, respectively. After 55 d of operation, the recycling system was changed to the improved strategy (runs 4 and 5), in which the mixed liquor in the membrane tank was pumped to the oxic tank (R1), and meanwhile, the mixed liquor of the oxic tank was pumped to the anoxic tank (R2). In run 4, the recycling rates were 100% (R1) and 150% (R2), and in run 5, the rates were 150% (R1) and 250% (R2). Recirculation of the mixed liquor provided an adequate amount of NO_3^- for nitrogen removal in the anoxic zone.

The ratio of chemical oxygen demand to nitrogen (C/N) is a significant parameter affecting the rate of

Table 1
Operational conditions of the A/O-MBR package plant

Parameter	Value (Average)	Parameter	Value
Temperature (℃)	17.7–28.2 (23.8)	HRT (h)	11.47
pH	6.5–7.9 (7.1)	SRT (d)	∞
Flux (LMH, L/m^2 h)	10.1	TMP[a] (kPa)	6.8–28.3 (11.6)

[a]Temperature correction to 25℃.

Table 2
Operational conditions of the two internal recycle strategies

Stage	Operational time (d)	Internal mixed liquor recycling rate (%)	DO in anoxic tank (mg/L)	DO in membrane tank (mg/L)	Aeration rate in membrane tank (m^3 air/h)	MLSS (mg/L)
The original strategy (runs 1–3)	Run 1: 1–20	50	0.44–0.51	4.32–5.17	42	1,300–2,970
	Run 2: 21–30	100	0.79–1.97	4.89–6.59	42	2,970–4,840
	Run 3: 31–55	50	0.48–0.56	2.36–3.29	29	4,840–5,790
The improved strategy (runs 4–5)	Run 4: 56–70	R1: 100 R2: 150	0.38–0.45	4.76–6.71	42	5,790–7,120
	Run 5: 71–89	R1: 150 R2: 250	0.39–0.49	4.81–5.22	42	7,070–8,310

denitrification as well. On the basis of the chemical equilibrium suggested by Matějů et al. [19], the theoretical optimal C/N ratio is 3.74. External carbon sources should be utilized when the COD contained in the influent is inadequate. Therefore, the need for the external carbon was calculated according the equation as follows:

$$C_{\text{extra}} = \frac{R_{\text{theo}} \times (N_{\text{ef}} - N_{\text{standard}}) - \text{COD}_{\text{ef}}}{\text{COD}_{\text{acid}}} \times Q_{\text{cap}} \quad (1)$$

where C_{extra} is the acetic acid dosage (kg/d), R_{theo} is the theoretical optimal C/N ratio (3.74), N_{ef} is the TN concentration in the effluent (mg/L), N_{standard} is the required TN effluent concentration (mg/L) according to the regional wastewater discharge standard [20], COD_{ef} is the COD concentration of the effluent (mg/L), Q_{cap} is the capacity of the system (m^3/d), and COD_{acid} corresponds to the actual COD equivalent of acetic acid set as 0.76 (g/g), as obtained by Yang et al. [21]. To adjust the C/N ratio, acetic acid was added as the external carbon source for 10 d at the end of run 5.

2.4. Analytical methods

Influent and effluent were sampled daily from the input and output pipes of the package plant. Samples of supernatant liquor from the anoxic, oxic, and membrane zones were obtained by filtering the mixed liquor through filter paper with a pore size of 0.45 μm. The concentrations of NH_4^+-N, TN, TP, SS, mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS) were analyzed according to the Chinese Water and Wastewater Monitoring Methods [22]. The COD was monitored using a spectrophotometer (DR220, HACH, USA), DO was gauged by a portable parameter meter (HQ30d, HACH, USA) and TMP values were measured using an online pressure sensor (ACD 201, ANCN Intelligent Instrument Co., Ltd., Xi'an, China).

The TP content of the sludge was determined by an analysis method according to Zhang et al. [23]. The sludge mixture was taken from the anoxic and oxic tanks, respectively, during each run, to analyze the TP concentration of supernatant and that of sludge mixture compound. MLVSS was determined simultaneously. The TP content of the sludge was calculated based on the following equation:

$$\text{TP content of sludge } [\text{mg } (\text{g VSS})^{-1}] = \frac{[\text{TP}]_{\text{M}} - [\text{TP}]_{\text{S}}}{\text{MLVSS}} \quad (2)$$

where $[\text{TP}]_{\text{M}}$ is the TP concentration of sludge mixture compound. $[\text{TP}]_{\text{S}}$ is the TP concentration of supernatant.

2.5. Microbial community analysis

2.5.1. DNA extraction

Biomass samples were taken from each tank of the A/O-MBR under the improved strategy at the end of run 5. Each sample was pre-treated with a method described earlier by Qiu et al. [24]. Total DNA was extracted with a soil DNA isolation kit (Q-BIOgene, United Kingdom) and then purified using a DNA purification kit (Biowatson Biotechnology, China).

2.5.2. PCR–DGGE

PCR amplification of Eubacterial 16S rRNA gene fragments was carried out using the primers EUB338F (5′-GAC TCC TAC GGG AGG CAG CAG-3′) and EUB534R (5′-ATT ACC GCG GCT GCT GG-3′) with a GC clamp (CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG) attached to the 5′

end of the forward primer to restrain the melting of the DNA fragments. The PCR amplification reaction was conducted in a DNA dyad Peltier thermal cycler (BIO-RAD, Hercules, CA, USA). To enhance the specificity of the amplification and avoid the formation of invalid by-products [25], the PCR was carried out under the following conditions: the initial denaturation at 94℃ for 5 min, 35 cycles of denaturation at 94℃ for 1 min, the annealing temperature was initially set at 65℃ and then decreased by 0.5℃ per cycle until reaching 58℃, followed by primer elongation at 72℃ for 2 min, and a final extension at 72℃ for 10 min. The PCR products were then separated by DGGE, using the DcodeTM, Universal Mutation Detection System (BIO-RAD, Hercules, CA, USA). DGGE was applied to 8% (m/v) polyacrylamide gels in a 1 × TAE buffer with a denaturing gradient ranging from 35 to 65%. The 100% denaturant contained 7 M urea and 40% (w/v) formamide. Electrophoresis was performed at 60 V and 60℃ for 16 h. The DNA was stained with SYBR Green I dye (Sigma, Oakville, Ontario, Canada) and then visualized on a UV transilluminator.

2.5.3. Cloning and sequencing

Prominent DGGE bands were excised from the gel with a sterile razor and incubated overnight at 4℃ in sterile Eppendorf tubes containing a 40 μL TAE buffer. Individual DNA fragments were re-amplified with corresponding primers without the GC clamp. The amplicons products were purified using a D6492 Cycle-Pure Kit according to the instructions (Omega Bio-Tek Inc., Norcross, Georgia, USA), followed by ligation into the pGEM-T Easy Vector Systems (Promega, Madison, Wisconsin, USA) and transformed into competent *Escherichia coli* DH5α cells for blue/white screening. The positive clones selected were sequenced by the Taihe Biotechnology Co., Ltd. (Beijing, China).

2.5.4. Phylogenetic analysis

The sequences obtained were analyzed by comparing them with the 16S rDNA sequences in the National Centre for Biotechnology Information (NCBI) database (USA), using a basic local alignment search tool (BLAST). A neighbor-joining method was applied using the Molecular Evolutionary Genetics Analysis program (MEGA, version 3, Tempe, AZ, USA) to construct a phylogenetic tree. The confidence of the phylogenetic tree topologies was analyzed by a bootstrap (1,000 replicates). Quantity One 4.6.2 (BIO-RAD, Hercules, CA, USA) was introduced to analyze the similarity of the bacteria populations in the activated sludge and the Shannon diversity index for their diversity.

3. Results and discussion

3.1. Nutrient removal with two internal recycling strategies

3.1.1. TN removal

The daily variation of the influent, anoxic, aerobic, and effluent qualities was studied in five runs of the original and improved strategies (Figs. 1 and 2(a)). During run 1 of the original strategy, the average TN removal efficiency remained at 32.4% with an average DO concentration of 0.43 mg/L in the anoxic tank. However, when the recycling rate increased from 50% of run 1 to 100% of run 2, the DO concentration of the anoxic tank jumped to 1.97 mg/L, with the TN concentration of the effluent rising dramatically to an average of 58.34 mg/L, showing a removal efficiency of only 12.4%. In run 3, the average TN removal efficiency recovered to about 32%, approximating that in run 1. The recycling rate of mixed liquor is a crucial operating parameter in controlling the amount of nitrate for denitrification. However, this only holds true on the assumption that the DO concentration can be maintained at a given level in the anoxic tank during the recycling of the mixed liquor. The DO concentration is usually high of 4–5 mg/L in the mixed liquor of the membrane tank, due to the high aeration rate to prevent membrane fouling [26]. If the mixed liquor is pumped directly back into the anoxic tank with an increasing recycling rate, the DO concentration increases and deteriorates the effectiveness of denitrification process, as can be seen from the results of run 2 of the original strategy.

In contrast, the TN removal efficiency increased in the improved strategy. During run 4, the DO concentration of the anoxic tank continued to be maintained at an average of 0.41 mg/L, causing the average TN removal efficiency to increase to 48.5%. The system was stable, and the TN removal efficiency was remarkably better than that in runs 1–3. During run 5, with an increase in the recycling rate, the concentration of TN in the effluent dropped gradually to 28.89 mg/L while the removal efficiency became 71.1% on day 80. The results showed that in the improved strategy, a low DO concentration can be maintained at a high recycling rate due to the double-stage recycling position. The intensive air flow of the membrane tank did not disturb the low DO concentration of the anoxic tank, and the denitrification process was enhanced under the recycling conditions of the improved strategy in comparison with the original strategy.

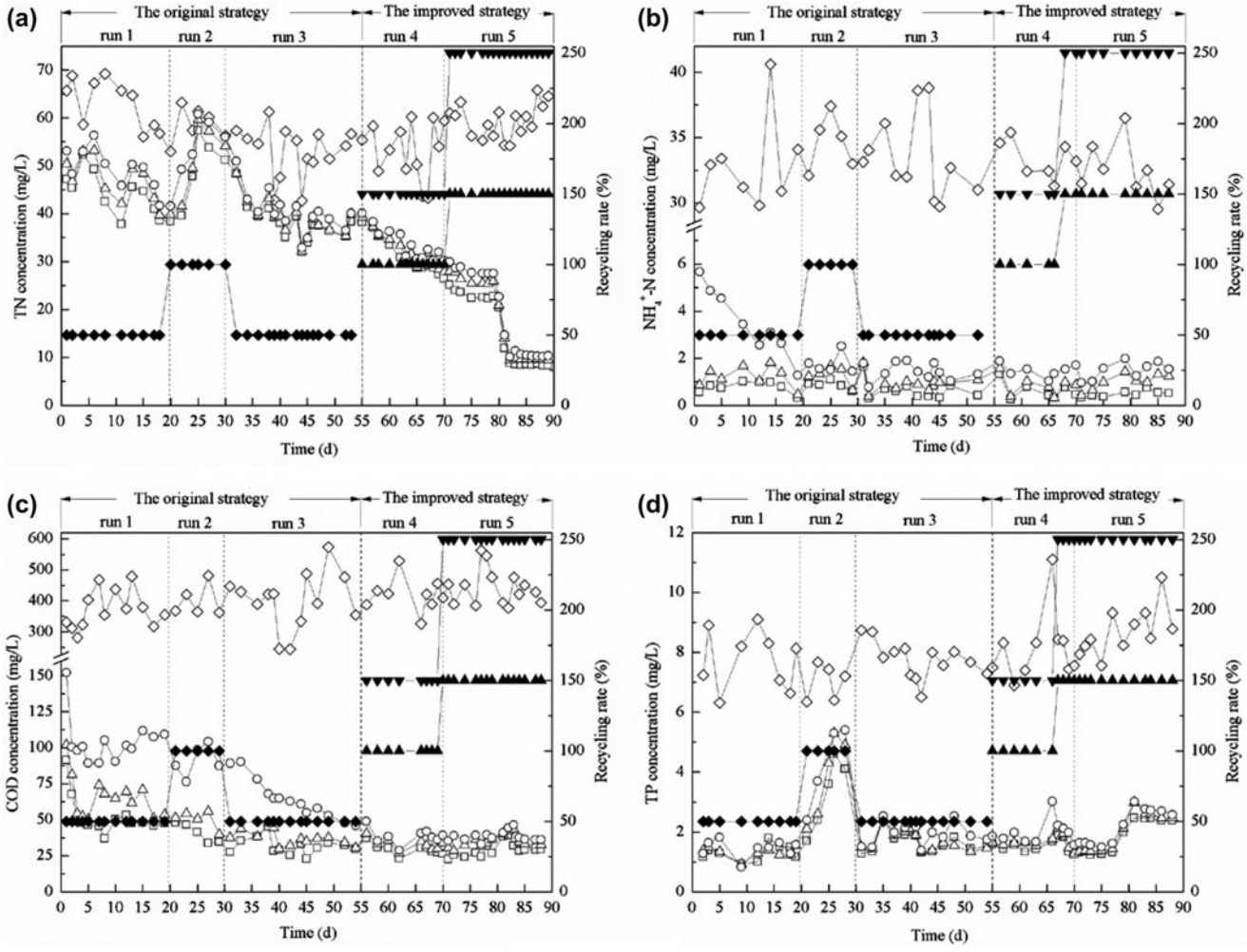

Fig. 2. Changes in nutrient concentrations in the A/O-MBR at various internal recycling strategies and different rates. (a) TN (b) NH_4^+-N (c) COD (d) TP (-◇- influent -□-effluent -△- oxic supernatant -○- anoxic supernatant -◆- original strategy recycling rate -▼- improved strategy recycling rate 2 -▲- improved strategy recycling rate 1).

The municipal wastewater used in this study had too low C/N ratio for the efficient denitrification. Therefore, Eq. (1) was used to calculate that 2.66 kg/d of acetic acid was needed as the external carbon source. Acetic acid was supplemented for 10 d at the end of run 5. The experimental results showed that the apparent TN concentration decreased to 8.5 mg/L (with a removal efficiency of 86.7%) within 3 d. This level was maintained until the end of the experiment.

3.1.2. Ammonia conversion

Fig. 2(b) presents the performance of ammonia concentration throughout the entire experimental period. Ammonia conversion efficiency is the principal indicator for the nitrification process, which greatly impacts the TN removal from the system. DO is the primary contributor controlling the ammonia conversion. Ammonia conversion efficiency decreases when the DO concentration is below 2.5 mg/L [11]. In order to achieve complete nitrification, the DO concentration in the oxic tank was maintained around 3.5 mg/L. It can be seen from Fig. 2(b) that in run 1 of the original strategy, the ammonia concentration in each tank decreased gradually at a recycling rate of 50% became stable and remained below 2 mg/L. Changes in the recycling rate had no obvious effect on the removal of ammonia. While adjusting the recycle mode to the improved strategy, the concentration of effluent NH_4^+-N remained stable. Xia et al. [16] explained that slow growing microorganisms, such as nitrobacteria, enhance the ammonia conversion due to the increase in biomass (MLSS was as high as 7,450 mg/L). Nitrate concentrations in the effluent varied between 8.3 and

45.9 mg/L, which corresponded with the amounts of TN, suggesting that the nitrification process was complete and ammonia had been completely oxidized to nitrate [12].

3.1.3. COD removal

Fig. 2(c) illustrates the results of COD concentrations in the influent, supernatant, and effluent. The effluent concentration of COD decreased rapidly from 96.3 to 46.4 mg/L within 5 d in run 1, resulting in an increase of COD removal from 69.35 to 85.23%. Microorganisms seemed adapt well to the DO concentration varying between 3.2 and 3.9 mg/L in the mixed liquor of the oxic tank. When the rate increased to 100% in run 2, the effluent COD concentration reduced continuously to 25.3 mg/L at the end of run 2, which corresponded to 91.9% reduction in COD from the initial value of the influent. The results indicated that the sufficient portion of recycled mixed liquor enabled the efficient consumption of organic matter by biomass. And at the beginning of run 3, the effluent COD increased slightly, but then it decreased again gradually (Fig. 2(c)).

In runs 4 and 5, the total COD removal efficiency was maintained at the same level as at the end of run 3. When the acetic acid was supplemented during last 10 d to increase C/N ratio, there was no obvious increase in COD concentration of the system, which indicated that the extra carbon source was consumed as the electric donor for improving denitrification.

3.1.4. TP removal

Fig. 2(d) illustrates the results of TP removal, showing that the process performed well with an efficiency of 76.4% at the beginning of the experiment in run 1, and the average TP concentration of the effluent was maintained at 1.84 mg/L. However, when the recycling rate increased to 100%, considerable variation of TP concentration was observed in the effluent because of the lifted DO concentration in the anoxic zone. While in run 3, when the recycling rate reduced to 50%, the TP removal efficiency returned to an average of 75.9%, suggesting that the original strategy might disturb the stability of the anoxic condition.

In run 4 and the first 10 d of run 5, the average TP concentration in the effluent was 1.71 mg/L, which was more stable compared with the concentration in run 2. However, during the last 10 d of run 5, the average TP concentration of the anoxic tank and effluent increased to 2.67 and 2.43 mg/L, respectively, with the addition of acetic acid.

The TP accumulation has been observed in A/O-MBR under a long SRT in several studies without circulation [12,14]. In this study, an efficient TP removal was achieved using developed double-stage recycling

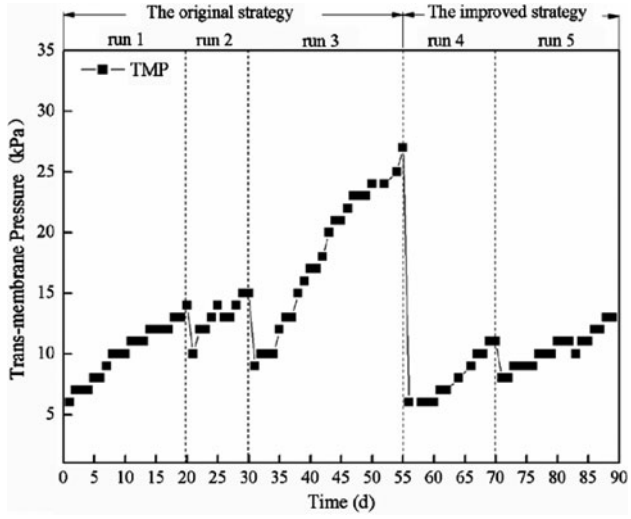

Fig. 3. Variation of the TMP at different internal recycling strategies.

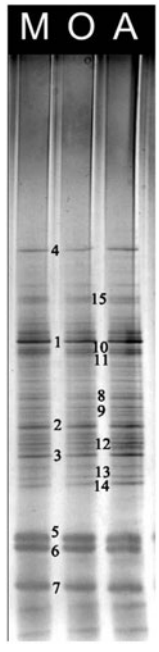

Fig. 4. DGGE profile of PCR products of activated sludge in the different A/O-MBR tanks at the end of run 5 under the improved internal recycling strategy (A: activated sludge at the anoxic tank; O: activated sludge at the oxic tank; M: activated sludge at the membrane tank).

strategies. Based on Eq. (2), it was calculated that the average TP contents in sludge samples from anoxic and oxic tanks were 42 mg/g VSS and 44 mg/g VSS, respectively. Although it was relatively lower than the value of 53 mg/g VSS, which was reported by Guglielmi and Andreottola [27], it indicated that the phosphorus uptake occurred in the anoxic and oxic tanks.

Current studies suggest that phosphorus-accumulating organisms (PAOs) and denitrifying phosphorus-accumulating organisms (DNPAOs) are important in TP removal process [28,29]. PAOs store the poly-*β-hydroxybutyrate* (PHB) in the absence of external electron acceptor, such as oxygen. Under such anaerobic or anoxic conditions, PAOs generate energy from internally stored polyphosphate, which leads to the release of phosphate. Besides, PAOs use PHB for growth and phosphorus uptake under aerobic condition, resulting in a net phosphorus removal [28]. In contrast, the DNPAOs oxidize the PHB by means of nitrate as an electron acceptor to provide energy for phosphorus uptake under anoxic condition [29]. Meanwhile, in this study, the addition of the acetic acid caused a rapid release of TP and consumption of NO_3^- in the anoxic tank. It is in agreement with the observation reported by Akin and Ugurlu [30], which indicates that the acetic acid can inhibit the denitrifying phosphorus accumulation. Consequently, simultaneous nitrogen and phosphorus removal might occur by DNPAOs in the A/O-MBR system, leading to the low phosphorus concentration under the anoxic condition.

Moreover, it can be seen from Table 2 that the MLSS increased steadily during the experiment, giving rise to biosynthesis of the biomacromolecules, which extracellular polymeric substances (EPS) are mainly included [31]. Cloete and Oosthuizen [32] found that the percentage of phosphorus bound to EPS accounts for 27–30%. The results suggest that TP removal in A/O-MBR is not only due to the microbial process, but also because of the EPS as TP containers. Therefore, two possible mechanisms for the TP removal in the A/O-MBR were likely involved, including microbial metabolic activity and adsorption on sludge particles [12,14].

Table 3
Dice coefficients (C_s, %) comparing similarities of bacterial community compositions in sludge samples collected from anoxic (A), oxic (O), and membrane (M) tanks

	A	O	M
A	100	95.74	94.63
O	95.74	100	90.17
M	94.63	90.17	100

3.2. Membrane performance

The A/O-MBR is operated under high MLSS concentration, which easily increases the TMP and causes problems in membrane fouling. Therefore, the high aeration rate should be utilized, not only to provide oxygen for biomass growth, but also to suppress the membrane fouling by scouring the membrane surface [13]. In this study, the air flow rate was fixed to approximately 10.1 liters per square meter per hour

Table 4
16S rRNA gene sequence-based phylogenetic affiliations of DGGE bands using a BLAST search of the NCBI database

DGGE band	Phylogenetic affiliation (NCBI accession number)	Similarity (%)
1	*Methylobacterium radiotolerans* (HF558417)	98
2	Uncultured bacterium (JN104776)	97
3	*Eubacterium* sp. (JF709903)	93
4	*Sphingopyxis* sp.(KC160718)	99
5	*Syntrophomonas* sp. (NR044008)	96
6	Uncultured *Saprospiraceae* bacterium (JN802687)	99
7	*Sedimentibacter* sp. (GU64504)	97
8	Uncultured *Ruminococcus* sp. (JX548125)	100
9	Uncultured *Sphingobacteriales* bacterium (JQ723651)	94
10	Uncultured *Crocinitomix* sp. (FM175783)	99
11	*Bacteroidetes* bacterium SCGC AAA206-I05 (JF488139)	99
12	Uncultured *Desulfovibrio* sp. (JX505317)	98
13	*Sorangiineae* bacterium SBNa008 (GU249616)	99
14	Uncultured *Nitrosomonas* sp. (GQ255606)	100
15	Uncultured *Crocinitomix* sp. (FM175783)	99

(LMH). TMP changes in different runs are shown in Fig. 3.

In runs 1 and 2 of the original strategy, the aeration rate in the membrane tank was initially set to 42 m^3 air/h. It can be observed that the TMP slowly increased in each run with the similar specific TMP growth rate of 0.4 kPa/d and 0.5 kPa/d, respectively. While in order to recover the TN and TP removal efficiency under run 2 as a result of the higher recycling rate (100%) carrying excessive DO into the anoxic tank from the membrane tank, the aeration rate was declined to 29 m^3 air/h in run 3, in which the specific TMP growth rate jumped dramatically to 0.72 kPa/d. After the chemical cleaning at the beginning of run 4, the deceleration of TMP growth rate was achieved simultaneously with the stable and efficient nutrient removal under the aeration rate of 42 m^3 air/h. Moreover, the TMP growth rate was maintained at 0.4 kPa/d and was no longer accelerated with the increasing recycling rates of R1 and R2, indicating that the improved recycling strategy was capable of eliminating the adverse effects of aeration in membrane tank on the DO concentration in the anoxic tank.

3.3. Microbial community analysis

As discussed above, the improved strategy has a higher efficiency of nutrients removal compared to the original strategy. To investigate the dominant strains under the optimal strategy, the bacterial community was analyzed by the PCR–DGGE. Fig. 4 illustrates the DGGE profiles of all bacterial communities in the V3 region of the 16S rDNA collected in the following order from the anoxic tank (A), the oxic tank (O), and

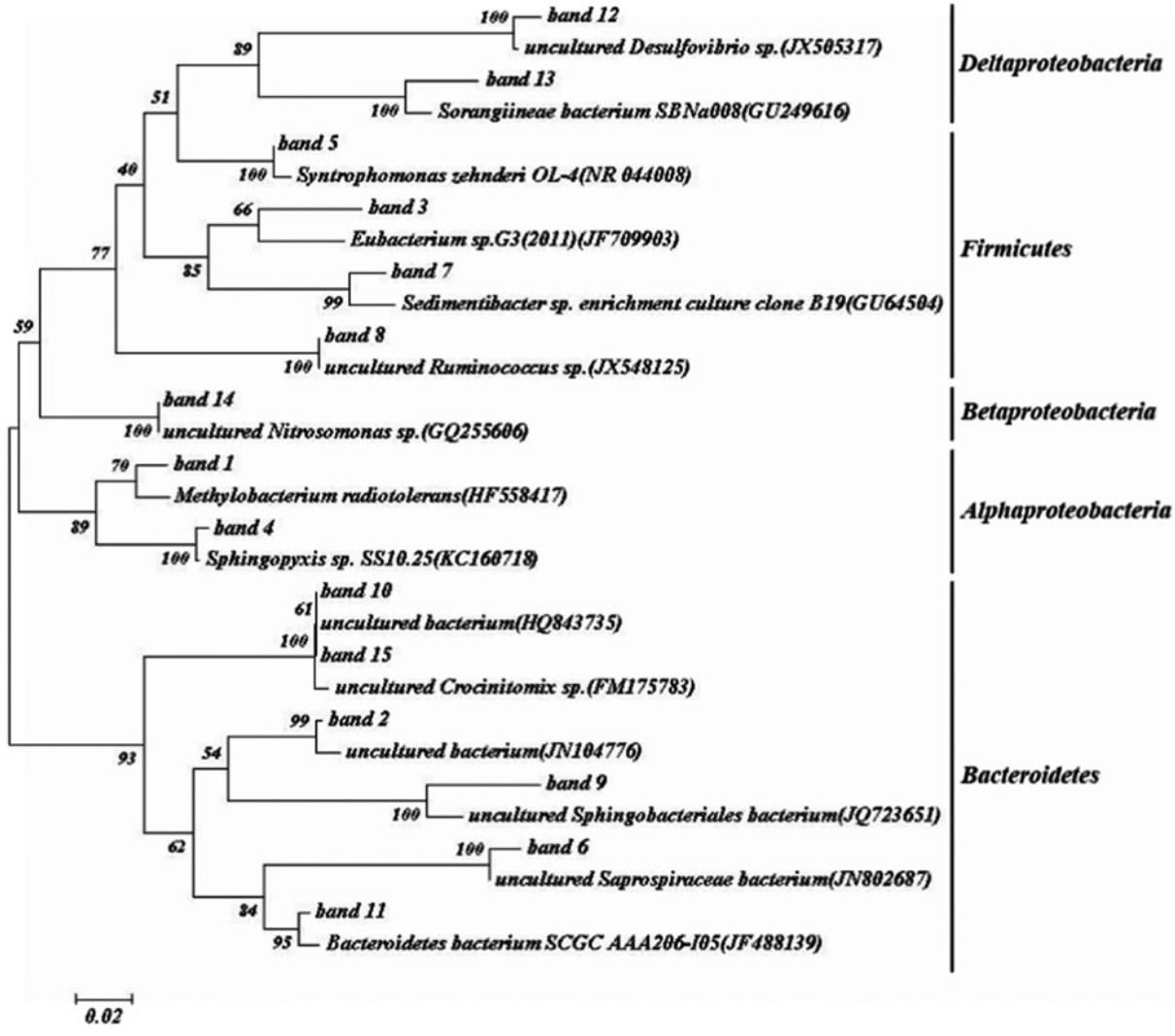

Fig. 5. Phylogenetic tree of 16SrRNA gene sequences from DGGE bands.

the membrane tank (M), at the end of run 5 of the improved strategy.

As can be seen from DGGE profiles (Fig. 4) and Dice coefficients (C_s, %) (Table 3), the bacterial community compositions of the sludge samples showed a high degree of similarity, owing largely to adequate internal recycling of the mixed sludge liquor, despite the different conditions prevailing in each tank. The Shannon–Wiener indices showed that the diversity of bacterial species presented in the anoxic tank (2.38) was the greatest, intermediate in the membrane tank (2.27), and the lowest in the oxic tank (2.24), even though the differences were generally small.

The results of the phylogenetic analysis reveals that most of the bacterial populations in each tank were affiliated with the phyla-*proteobacteria*, *Firmicutes*, and *Bacteroidetes* (Table 4; Fig. 5), commonly found in wastewater treatment [33]. However, there were some specific microorganisms that contributed to nutrient removal. The dominant population represented by band 1 was closest to *Methylobacterium radiotolerans* (HF558417), which belongs to a group of *α-proteobacteria*. These bacteria are capable of producing PHB while utilizing substitution and oxidized methane derivatives, as well as more sophisticated organic compounds to enhance biological phosphorus removal [34,35]. The strain of band 4 was a *Sphingopyxis* sp. (KC160718), also belonging to the group of *α-proteobacteria*. This particular species has been identified as a type of strictly aerobic, chemoheterotrophic, gram-negative bacterium that purifies contaminated water by degrading various xenobiotic substances with other organic compounds [36]. The bacterium shown in band 14 was identified as a *β-proteobacterium* with a 100% similarity to an uncultured *Nitrosomonas* sp. (GQ255606). Mota et al. [37] found that this chemolithotrophic ammonium-oxidizing bacterium is important in wastewater nitrification process.

4. Conclusions

Compared with the original strategy, the removal efficiencies of COD, NH_4^+-N, TN, and TP under the improved strategy reached, respectively, 93.27, 98.84, 86.7, and 71.1%, with a recycling rate of 150% for R1 and 250% for R2, which was more efficient in nutrients removal since it enhanced the control of DO concentrations in the anoxic tank. Meanwhile, the improved strategy could efficiently reduce the membrane fouling by the high aeration rate without the DO interference to the anoxic tank. The bacterial communities in the activated sludge in each tank had high similarity and diversity in the improved strategy. The *M. radiotolerans* (HF558417) and an uncultured *Nitrosomonas* sp. (GQ255606) were found to be the two critical contributors to the removal of TN and TP.

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