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Nitrogen removal performance and microorganism community of an A/O-MBBR system under extreme hydraulic retention time

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ABSTRACT

A lab-scale anoxic-oxic moving bed biofilm reactor (A/O-MBBR) was used to treat simulated nitrogenous wastewater. Transition of nitrogenous compounds and total nitrogen (TN) removal performance were studied under extreme hydraulic retention time of 8 h. The optimal NH_4^+–N and TN removal performance was achieved with dissolved oxygen (DO) concentration of 5.4 mg/L in the oxic reactor, which was 72% and 34% with influent NH_4^+–N and urea of around 30 and 50 mg/L. Polysaccharide concentration, protein concentration and PS/PN fluctuated significantly with the variation of DO concentration in the oxic zone, which achieved around 31.8 mg/g VSS, 7.1 mg/g VSS and 40, respectively. The fluorescence excitation emission matrix showed different distinct peaks under different DO concentration in the oxic zone. *Terrimicrobium*, a kind of strictly anaerobic bacteria, became the main genera when DO concentration was controlled at around 1.9 mg/L in the oxic zone according to the results of high-throughput sequencing.

Keywords: Anoxic/oxic; Moving bed biofilm reactor; Nitrogen removal; Hydraulic retention time

1. Introduction

Anoxic/oxic (A/O) systems have been widely applied in wastewater treatment plants (WWTPs) [1]. However, nitrogen removal performance is not desirable usually, due to the disadvantages of conventional active sludge process (ASP), such as low biomass concentration and short sludge retention time (SRT). The discharge of wastewater containing nitrogenous compounds usually leads to eutrophication in the receiving water [2]. Thus, the upgrading of the A/O system is urgent. Recently, moving bed biofilm reactors (MBBRs) have been studied for improvement of nitrogen removal owing to their advantages of high biomass concentration, long SRT and low excess sludge production [3–6]. If an A/O system is changed into an MBBR process, the cost of physical infrastructure would be saved, by adding bio-carriers into the reactor solely.

Previous researches indicate that hydraulic retention time (HRT) is a key operation parameter in an MBBR system, related with volume loading and effluent wastewater quality, especially with nitrogen removal. HRT directly influences the fraction of ammonium oxidized bacteria in nitrifying bacteria [7]. A biological treatment system operated under suitable HRT not only guarantees pollutant removal performance but also saves operation cost. The studies on HRT are focused on optimizing the parameter, for instance, the optimum HRT for the treatment of rural domestic sewage [1], refinery wastewater [8] and tannery wastewater [9]. However, when HRT deviates from its optimal condition, performance of the MBBR system would

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be affected. Thus, to investigate the threshold of HRT and relevant operation condition in an A/O-MBBR system is meaningful, in favor of providing advices for optimizing performance of the A/O-MBBR system. However, rare studies have been done to investigate the effect of ultimate HRT on biological nitrogen removal in the A/O-MBBR system.

By affecting hydraulic loading, HRT impacts microflora as well. When the reactors are shocked, the dominant bacteria will change, and the bacteria that have better hydraulic shock resistance performance will be enriched by enlarging HRT [10]. Stable and abundant microbial community structure is the basis of good pollutant removal performance in a biological wastewater treatment process. In general, sudden change of pollutant treatment performance in biological wastewater treatment process can be explained by the variation of microbial community structure. In this study, a lab-scale A/O-MBBR system was designed to investigate the effect of ultimate HRT at 8 h on nitrogen removal performance from simulated municipal wastewater, along with chemical oxygen demand (COD) removal performance. The variations of soluble microbial products (SMPs) and fluorescence excitation emission matrix (FEEM) in the effluent of the system were monitored to study the microbial community conditions at different dissolved oxygen (DO) concentrations in the oxic reactor with HRT of 8 h. Moreover, the microbial diversity of the biofilm was assessed at the end of each stage, to understand microbial composition of the system under different operation conditions. Two objectives were intended to be achieved in this study. One was the feasibility and stability of the A/O-MBBR system under extreme HRT, being controlled at around 8 h, which was in favor of optimizing the volume of the A/O-MBBR system. The other was to investigate the effect of DO concentration on performance of the A/O-MBBR system, which would reduce the cost of wastewater treatment. The results obtained from the study would provide technological support for the upgrade of an A/O system by integrating with the MBBR process.

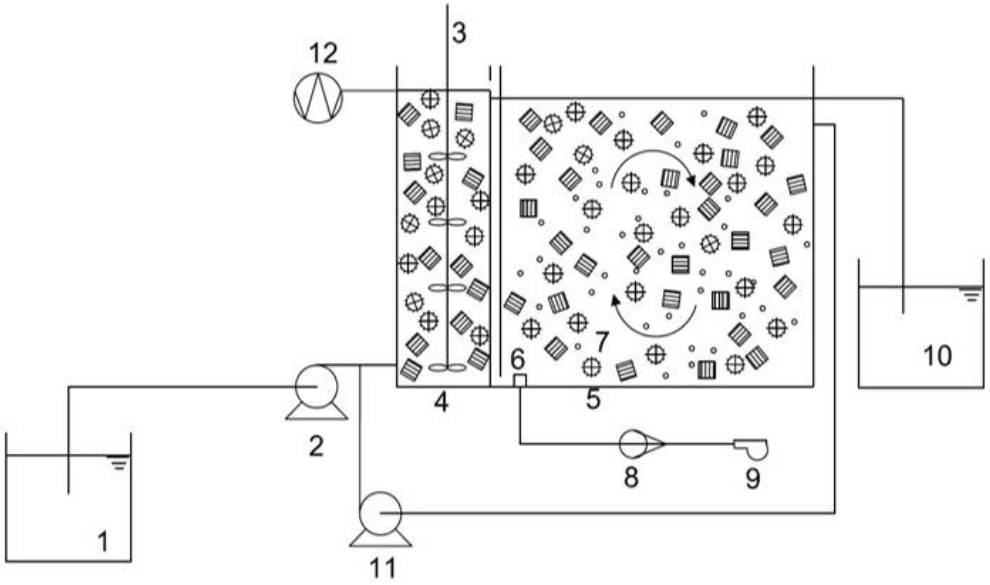
2. Materials and methods

2.1. Lab-scale reactor and bio-carrier

The lab-scale A/O-MBBR system used in the study contained anoxic zone (R_A) and oxic zone (R_O). Effective volume of the reactor was 10.8 L, with length, width and height of 40, 10 and 30 cm, respectively. The anoxic zone and oxic zone were 2.7 and 8.1 L, respectively. The diagram of the A/O MBBR system is shown in Fig. 1. Influent of the system was pumped to R_A by a peristaltic pump. Bio-carrier used in the reactor was made of polyethylene with cylindrical shape and internal support structure. Specific surface area of the carrier was around 1,200 m^2/m^3, and the density was about 0.98 kg/m^3. The filling ratio of the carriers in R_A and R_O was 50% and 40%, because the biofilm growth on the carriers in the anoxic condition was more difficult than in the oxic condition.

2.2. Wastewater characteristics

The wastewater used in this study was prepared according to Chu and Wang [11]. Composition of the wastewater was listed as follows: 300 mg/L of glucose, 50 mg/L of urea, 115 mg/L of NH_4Cl, 20 mg/L of NaH_2PO_4, 2 mg/L of KH_2PO_4, 4 mg/L of $CaCl_2$, 2 mg/L of $MgSO_4$ and 67 mg/L of $NaHCO_3$. The COD, total nitrogen (TN), ammonia nitrogen (NH_4^+–N), and total phosphorus (TP) was 320, 53, 30, and 5 mg/L, respectively.



1. Wastewater storage tank 2. Peristaltic pump 3. Blender 4. Anoxic zone
5. Oxic zone 6. Diffuser 7. Carriers 8. Air flow meter 9. Compressor
10. Effluent 11. Peristaltic pump 12. Thermostat

Fig. 1. Diagram of the A/O MBBR system.

2.3. Start-up and operation conditions

Inoculum used in the MBBR was taken from a WWTP, Chengdu, China. The sludge was acclimated for 5 d with the synthetic wastewater before inoculation. At the beginning of the start-up period, the initial biomass concentration in the R_A and R_O was around 2,000 and 1,500 mg/L, respectively. Carriers were added into the system after the inoculation. Then, the system was operated by intermittent feeding for 10 d, and the synthetic wastewater was fed every 12 h. During the intermittent feeding period, the reaction time and sedimentation time was 11 and 1 h, respectively. After that, continuous influent was adopted with recycle mixed liquor ratio of 50%, and HRT of the system was controlled at 20 h. Specific operation conditions are shown in Table 1. Moreover, DO concentration in the R_O was changed at HRT of 8 h to investigate the feasibility of varying DO concentration on recovery of the A/O MBBR system performance. Temperature of the R_A and R_O was controlled at 25°C ± 2°C throughout the experiments.

Table 1
Operation conditions at different phases

Time (d)	Phase	HRT (h)[a]	*R* (%)	DO[b]	DO[c]
35–68	I	5 + 15	50%	0.2 ± 0.1	4.0 ± 0.4
69–111	II	2 + 6	50%	0.2 ± 0.1	4.0 ± 0.4
112–147	III	2 + 6	50%	0.2 ± 0.1	1.9 ± 0.3
148–186	IV	2 + 6	50%	0.2 ± 0.1	5.4 ± 0.3

[a]HRT of R_A + HRT of R_O.
[b]DO concentration (mg/L) in R_A.
[c]DO concentration (mg/L) in R_O.

2.4. Analytical methods

Samples were taken from the influent of the system and the effluent of the R_A and R_O every 2 d, and were analyzed immediately after being filtered by 0.45 μm filter paper. The concentrations of COD, NH_4^+–N, NO_3^-–N, NO_2^-–N and TN were measured according to Monitoring and Analytic Methods of Water and Wastewater [12]. Attached sludge concentration was measured according to Ahmadi et al. [13]. Polysaccharide (PS) and protein (PN) concentrations were measured once a week. PS concentration was measured by the method of anthranone-sulfuric acid. PN concentration was measured by the method of Lowry [14]. FEEM analysis was conducted by fluorospectro photometer (Hitachi, F-7000). Fluorescence regional integration (FRI) was used to analyse the data of FEEM [15]. DO concentration and temperature were measured by the YSI O_2-electrode.

3. Results and discussion

3.1. Nitrogen removal performance in the A/O-MBBR system

With influent COD concentration of 312.5 ± 11.3 mg/L, effluent of the system was around 25 mg/L in each phase. TN removal efficiency of each phase was 19.4% ± 3.1%, 24.7% ± 6.2%, 22.2% ± 4.2%, and 34.8% ± 3.3%, respectively. Fig. 2 shows specific NO_x^-–N (NO_2^-–N and NO_3^-–N), NH_4^+–N and TN concentrations of the R_A and R_O throughout the study. Nitrification performance of the system was significantly different at different phases.

In phase I, average effluent NO_3^-–N, NO_2^-–N and NH_4^+–N concentration of the A/O-MBBR system was 23.4 ± 2.0, 2.5 ± 2.3, 14.0 ± 2.0 mg/L, respectively. Average NH_4^+–N removal performance of the system was 54.5% ± 6.2%.

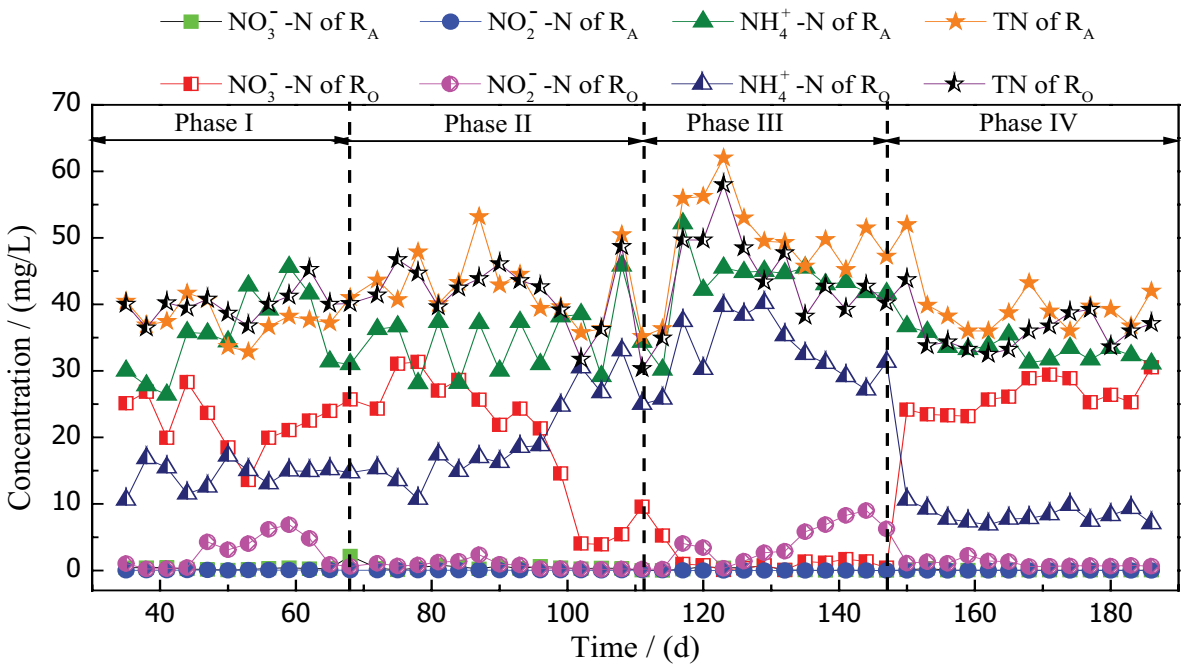


Fig. 2. Total nitrogen, nitrate, nitrite, and ammonium concentrations in the effluent of the R_A (anoxic zone of the reactor) and R_O (oxic zone of the reactor) during the experiments.

Effluent NO_2^-–N and NO_3^-–N concentrations of the R_A were below 1.0 mg/L, which indicated good denitrification performance in the R_A under the recycle ratio of 50%. Thus, it could be speculated that low TN removal efficiency was mainly due to the poor nitrification performance in the R_O. NH_4^+–N removal efficiency reached around 95% with HRT of 12 h in a single aerobic MBBR [16]. Although HRT of the A/O-MBBR system in phase I was longer than the single aerobic MBBR, nitrification performance of the system was poorer than the single aerobic MBBR, which would be due to the performance of the biofilm growth on the carriers was not mature in the A/O-MBBR system.

When HRT was reduced to 8 h in phase II, the quality of effluent wastewater did not change immediately. After 98th d, NH_4^+–N concentration in the effluent of the R_O suddenly increased and NO_3^-–N concentration decreased. NO_2^-–N accumulation was not observed in the effluent of the R_O. It was indicated that the relative low nitrification performance caused the low NH_4^+–N removal efficiency. It was reported that nitrification and denitrification need at least 6 and 2 h in theory [17], and a good nitrification performance was exhibited when HRT was longer than 6 h. Besides reaction time, hydraulic shock must be considered in a continuous flow system. As HRT reduced from 20 to 8 h, attached sludge concentration decreased from 1.9 ± 0.3 to 1.4 ± 0.2 g/L in the R_A and dropped from 2.5 ± 0.5 to 2.1 ± 0.2 g/L in the R_O. Biomass concentration decreased with the enhancement of hydraulic shock. Low NH_4^+–N removal rate showed stress of nitrification performance in the R_O under HRT of 6 h. Thus, 8 h of HRT for the A/O-MBBR system was not enough at the operation condition for nitrogen removal, and 6 h of HRT for the R_O was insufficient for good nitrification performance.

Reducing DO concentration in the R_O in phase III had no obvious effects on increase of nitrogen removal performance compared with phase II. TN removal rate decreased by around 2% from phase II to III. Effluent NO_3^-–N concentration of the R_A and R_O was maintained at a low level. Effluent NO_2^-–N concentration of the system decreased from 5.6 to 0.3 mg/L and then increased to 9.0 mg/L, while the variation of effluent NH_4^+–N concentration showed an opposite trend. NO_2^-–N accumulation indicated recovery of nitrification performance. Moreover, the potential of short-cut simultaneous nitrification and denitrification (SSND) was observed in the R_O. A response surface model of the MBBR achieving SSND suggested the optimum condition was at HRT of 8 h and DO concentration of 3 mg/L [5]. The relative HRT and DO concentration in the R_O caused the possibility of SSND. Therefore, HRT of 6 h and DO concentration of around 1.9 mg/L in the R_O was not a suitable operation condition for nitrification, and enhancement of DO concentration in the R_O would promote nitrification performance of the reactor.

Increasing DO concentration in the R_O improved nitrification and TN removal performance promptly and significantly. At phase IV, average NH_4^+–N removal rate was 72.5% ± 4.2%, with average effluent concentration of 8.3 ± 1.1 mg/L. Effluent NO_3^-–N and NO_2^-–N concentrations of the R_O were 25.9 ± 2.4 mg/L and 1.1 ± 0.5 mg/L. From phase III to IV, attached sludge concentration increased from 2.8 ± 0.5 to 3.0 ± 0.3 g/L in the R_A, and increased from 1.6 ± 0.2 to 2.2 ± 0.2 g/L in the R_O. The improvement of attached sludge concentration was one of the reasons why NH_4^+–N removal efficiency increased. The A/O-MBBR system achieved NH_4^+–N rate of 98.4% under HRT of 9 h and DO concentration higher than 2.0 mg/L [18]. Moreover, TN removal rate achieved around 72% in an MBBR-MBR process at HRT of 9.5 h [19]. Moreover, DO concentration of 5.4 mg/L was another reason for improving nitrification performance in the R_O, although the NH_4^+–N removal rate was still lower than the previous study. It could be speculated that the difference of HRT was the reason why NH_4^+–N removal efficiency of the two systems was different. Only 1 h more in the HRT, the A/O-MBBR system got more than around 26% of NH_4^+–N removal rate and higher than 40% of TN removal percentage, which indicated that 8 h of HRT was a limitation for the system to achieve good nitrification performance even though DO concentration was high. The A/O-MBBR system exhibited good COD and TN removal performance at relative low HRT, which would reduce the cost on municipal wastewater treatment. For a large-scale wastewater treatment process, comparing with the ASP-based A/O system, the carriers added in the MBBR process and the transformation of wastewater outlet would induce higher cost (around 10%). However, the relative high hydraulic load could reduce the volume of the WWTP, which would compensate the cost for the alteration of the MBBR process to a large extent.

3.2. SMP and FRI analysis

SMPs were produced by the decay of active biomass and the hydrolysis of bound extracellular polymeric substances (EPS) [20]. In the wastewater treatment system, SMPs may affect effluent quality and bring potential pollutant in the received water [15]. As a protective mechanism, abnormal situation of SMPs would indicate abnormity of running conditions [21]. PS and PN were the main components of SMP [22]. By FRI analysis, main substances would be distinguished. Variations of PS and PS concentrations and FEEM analysis would be helpful in checking the statement of the system. Fig. 3 shows the PS and PN concentrations of the R_A and R_O throughout all phases. Fig. 4 shows the FEEM images of eight samples taken from the R_A and R_O in the four different phases, respectively.

As can be seen from Fig. 3, PS and PN concentrations varied as operation conditions changed. PN concentration of both R_A and R_O changed a little, while PS concentration changed a lot. The variation of PS and PN concentrations was because PS was the main component of EPS when temperature was controlled between 20°C and 30°C [23]. From phase I to IV, average PS concentration of the R_A was 7.0 ± 2.3 mg/g VSS, 1.7 ± 0.4 mg/g VSS, 3.9 ± 2.4 mg/g VSS, and 3.3 ± 1.2 mg/g VSS, respectively. Average PS concentration of R_O was 17.4 ± 4.1 mg/g VSS, 7.4 ± 8.2 mg/g VSS, 16.7 ± 10.0 mg/g VSS and 17.7 ± 3.7 mg/g VSS, respectively. PS concentration of R_A and R_O decreased from phase I to II. SMP concentration would decrease when the biomass was formed. Before day 103, the range of PS concentration was 1.2–5.8 mg/g VSS. At day 103, PS concentration sharply increased to 25.3 mg/g VSS, which could be explained by the

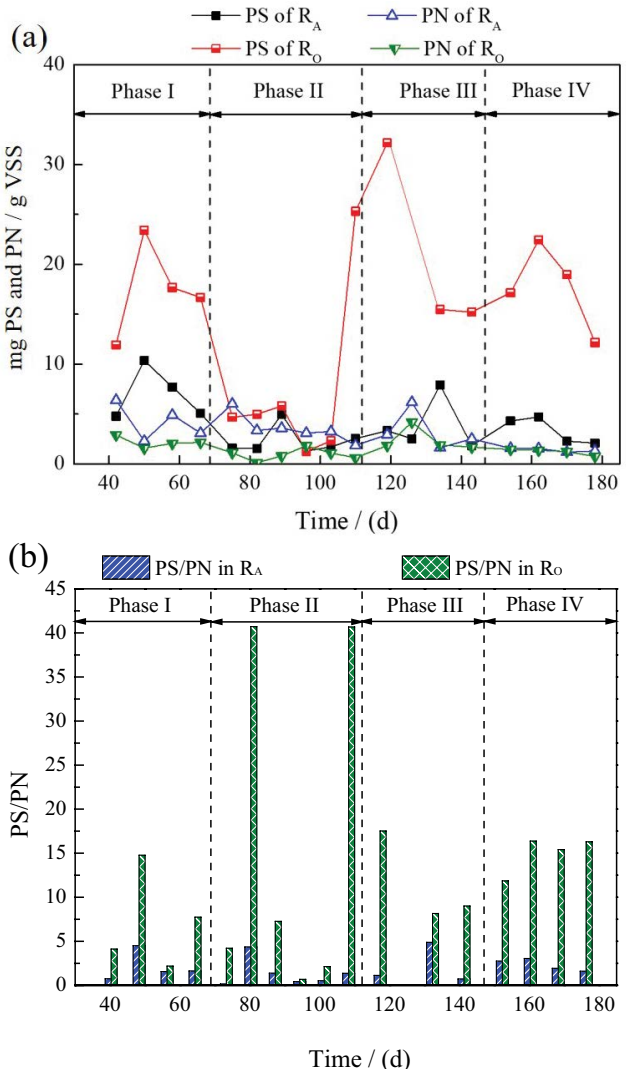


Fig. 3. SMP results in the R_A (anoxic zone of the reactor) and R_O (oxic zone of the reactor) throughout the process. (a): PS and PN concentrations in the R_A and R_O throughout the process; (b): PS/PN in R_A and R_O throughout the process.

sudden decrease of effluent NO_3^-–N concentration of the R_O, since shortage of NO_3^-–N would lead to increase of SMPs production [22]. Although NO_3^-–N concentration increased at phase IV, PS concentration was still higher than that at early phase II. The results indicated the decay of active biomass [20]. Thus, the operation condition may not be suitable for the activity of microflora in the system. The variation of PS concentration may be a signal for the biological nitrogen removal system. The change of PS concentration showed that HRT of 8 h was not applicable for the A/O-MBBR system to remove TN. When PS concentration suddenly increased, it was necessary to change the operation condition of the A/O-MBBR system to keep TN removal performance.

Fig. 4 shows EEM results among different phases in R_A and R_O. From phase I to II, in R_A (Fig. 4a and c), peak 1 was narrowed and peak 4 disappeared, while peaks 2 and 5 appeared. According to FRI, peak 1 represented tyrosine-, protein-like, protein-like and tryptophan, while peak 2 represented tyrosine and BOD. The main substances of peak 4 and 5 were polyaromatic-type humic acid and humus, respectively. In R_O, the intensity of peak 1, 3 and 4 receded. Main substance of peak 3 was PS, in accordance with FRI. At phase III, only peak 1 left both in R_A and R_O, and the range and intensity of peak 1 significantly reduced. Nitrification performance and TN removal performance was poor in phase III, especially, thus electronic accepter (NO_x^-–N) recycled from R_O to R_A was lack in R_A. To resist the shock, SMPs would be biodegraded by bacteria flora [24]. Once nitrification performance was recovered, peaks 1 and 2 reappeared in R_A, and peaks 2 and 4 reformed in R_O. In view of nitrogen removal performance described above, TN removal performance improved at phase II and IV and peak 2 appeared. It could be speculated that tyrosine contributed to the removal of nitrogen. Previous studies reported that growth of certain bacteria and decomposition of dead cells and macromolecular organics also led to changes of peaks [25,26].

3.3. Variation of microbial construction

High-throughput sequencing was used to understand microbial construction under different conditions. The values of chao 1 and the quantity of phyla and genera of each sample are shown in Table 2. Biodiversity of the R_A and R_O in phase I was less than other phases, which indicated that shortening HRT of the system had no obvious effect on the biodiversity of the R_A and R_O. Compared with phase I, the values of Chao 1 in the R_A and R_O increased by about 185 times and 100 times in phase II, respectively. Quantities of phyla and genera also increased sharply. It indicated that as the system running, the microbial community became more and more abundant. However, the low DO concentration made the quantities of phyla and genera in the R_A and R_O decreased. When DO concentration rose to 5.4 mg/L in phase IV, the values of Chao1 in the R_A and R_O increased by around two times, and the quantity of phyla and genera recovered. Fig. 5 shows specific phyla and genera of each phase. Proteobacteria, Bacteroidetes, and Verrucomicrobia were the main phyla. When HRT was reduced from 20 h to 8 h, the ratio of main phyla changed. In the R_A, the percentage of Proteobacteria increased from 36.9% to 74.8%, as percentage of Bacteroidetes decreased from 52.7% to 12.5%. In the R_O, the percentage of Proteobacteria decreased from 66.3% to 44.8%, and Verrucomicrobia became one of the dominate phyla, accounting for 14.2%, and the main genus was *Terrimicrobium* (5.4% in all genera). *Terrimicrobium* is a strict anaerobic, mesophilic, carbohydrate-fermenting bacterium and oxygen, nitrate, sulfate, sulfite, thiosulfate, elemental sulfur and Fe (III) nitrilotriacetate do not serve as electron acceptors for growth [27].

The change of DO concentration in the R_O affected microbial community structure in the R_A and R_O. When DO concentration was controlled at around 1.9 mg/L, Verrucomicrobia accounted for 39.4% in the R_O, in which *Terrimicrobium* played an important role of the phyla (34.5% in all genera). As strict anaerobic bacteria, *Terrimicrobium*

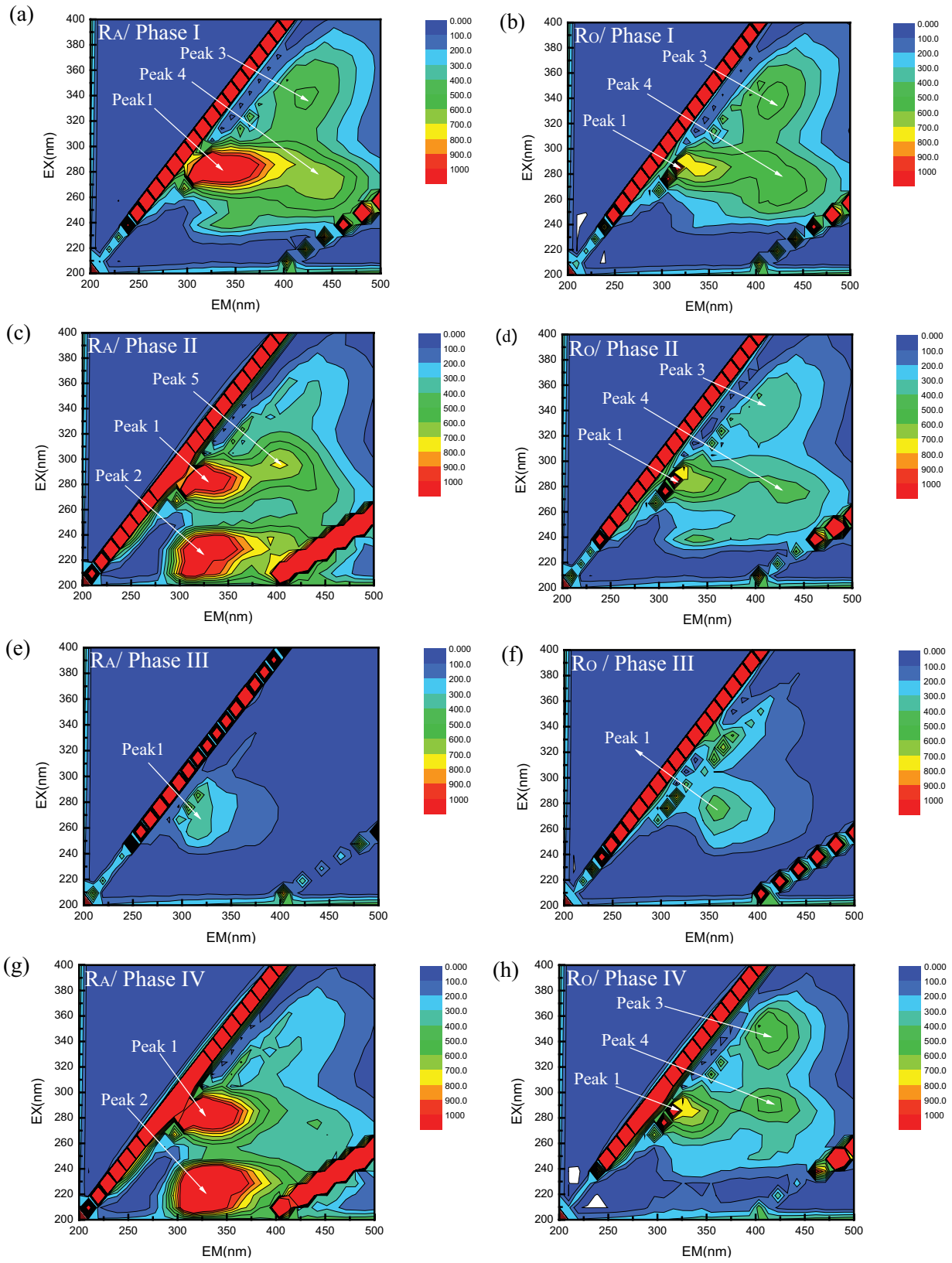


Fig. 4. FEEM results in the R_A (anoxic zone of the reactor) and R_O (oxic zone of the reactor) throughout the process. (a): FEEM results in the R_A at phase I; (b): FEEM results in the R_O at phase I; (c): FEEM results in the R_A at phase II; (d): FEEM results in the R_O at phase II; (e): FEEM results in the R_A at phase III; (f): FEEM results in the R_O at phase III; (g): FEEM results in the R_A at phase IV; and (h): FEEM results in the R_O at phase IV.

Table 2
Species diversity of samples in each phase

Sample	Chao 1	Phyla	Genera
R_A of I	97.6	9	66
R_A of II	17,955.5	28	173
R_A of III	17,928.3	22	159
R_A of IV	34,474.1	27	220
R_O of I	120.1	11	80
R_O of II	12,075.6	30	330
R_O of III	18,849.3	22	250
R_O of IV	37,593.3	20	291

became dominant bacteria in the oxic reactor, which was traceable in phase II. According to the poor nitrogen removal performance at phase II and III, Terrimicrobium was an indicator for the deterioration of nitrogen removal performance. As DO concentration in the R_O decreased, the environment was more suitable for the growth of *Terrimicrobium*. Growth of the aerobic bacteria was inhibited significantly. The Terrimicrobium had no impacts on nitrification and denitrification performance. Moreover, poor nitrification performance of the R_O meant little NO_x^-–N was recycled to R_A. Thus the denitrification bacteria lost superiority compared with other bacteria in the R_A. Tolumonas was the dominant genera of R_A at phase III, accounting for 34.5%, by which NO_x^-–N would not be removed. As DO concentration

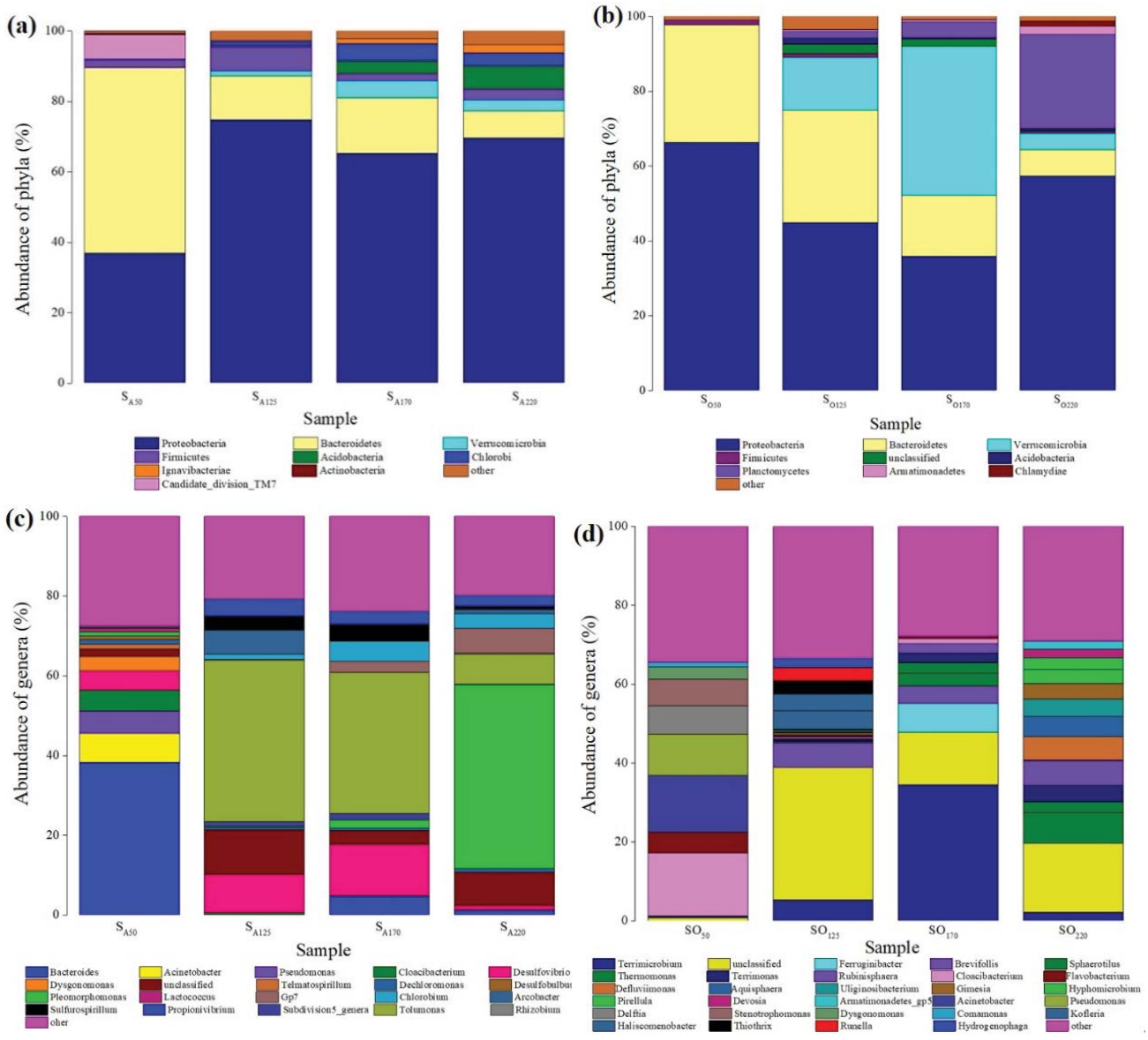


Fig. 5. Taxonomic classification of the major species based on 16S rRNA gene sequences at phylum and genus levels in the R_A (anoxic zone of the reactor) and R_O (oxic zone of the reactor). (a): Taxonomic classification at phylum levels in the R_A; (b): Taxonomic classification at phylum levels in the R_O; (c): Taxonomic classification at genus levels in the R_A; and (d): Taxonomic classification at genus levels in the R_O.

of R_O increased, the percentage of Proteobacteria in the R_A slightly increased from 65.3% to 69.4%, while Bacteroidetes decreased from 15.7% to 7.7%. At the meanwhile, the percentages of Proteobacteria and Planctomycetes increased from 36.0% to 57.4% and from 4.2% to 25.1% in the R_O, respectively, while Verrucomicrobia sharply decreased from 39.7% to 4.3%. In genus level, percentage of nitrifying bacteria in the R_O increased from 0.6% to 1.9%. However, nitrifying bacteria was still not the dominant bacteria in the R_O. It explained that although nitrogen removal performance improved, NH_4^+–N removal efficiency was not high. DO concentration of 5.4 mg/L was sufficient for nitrifying bacteria, but nitrifying bacteria did not gain advantages over other bacteria. It indicated that HRT of 6 h in the aerobic MBBR was adverse to the growth of nitrifying bacteria.

4. Conclusion

The A/O-MBBR system was used to treat simulated municipal wastewater with HRT of 8 h. With average influent COD concentration of 312.5 mg/L, effluent COD concentration of the system was around 25 mg/L in each phase. When HRT of the system was maintained at 8 h and DO concentration in the oxic reactor was controlled at around 5.4 mg/L, average NH_4^+–N removal rate achieved about 72.5% and average TN removal efficiency was around 34.8% at reflux ratio of 50%. The results of SMP and FEEM indicated that microorganisms were under self-protection conditions with HRT of 8 h, especially when DO concentration in the R_O was 1.9 mg/L. According to the results of high-throughput sequencing, HRT of 6 h in the R_O was adverse to the growth of nitrifying bacteria.

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