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Start-up, influence factors, and the microbial characteristics of partial nitrification in membrane bioreactor

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ABSTRACT

The partial nitrification (PN)–Anammox process was considered as one of the most efficient and economical method to remove ammonia from wastewater without organic carbon consumption. In this process, PN is the key step since it should provide stable effluent with suitable NO_2^-/NH_4^+ ratio for the subsequent Anammox. In this study, PN was successfully started-up during 30 d at ambient temperature (25°C) in a continuous flow membrane bioreactor (MBR), by gradually increasing ammonia-loading rate (ALR) under oxygen-limited condition. Finally, the system achieved an ammonia oxidation rate of 0.52 kg m^{-3} d^{-1} and an excellent effluent with appropriate NO_2^-/NH_4^+ ratio as 1.1–1.3. ALR was indicated as the indispensable controlling factor, and the applied alkalinity with the combination of high ALR inhibition and oxygen limitation provided the selective washout of nitrite-oxidizing bacteria (NOB) and an active aerobic ammonia-oxidizing (AerAOB) population. Techniques of fluorescence *in situ* hybridization and clone sequencing were utilized simultaneously to study the microbial characteristics in the stable PN system. The results demonstrated that small amounts of NOB were present and not active, while AerAOB predominated in the MBR–PN system, and Nitrosomonas-related AerAOB were considered to be responsible for the satisfactory PN performance.

Keywords: Ammonia loading rate; Partial nitrification; AerAOB; NOB; FISH

1. Introduction

Nitrogen removal from wastewater is gaining a lot of attention due to its potential threat to the environment. The partial nitrification (PN)–Anammox process was considered to be a substitute nitrogen removal technology for the conventional nitrification–denitrification process since it consumes less oxygen for nitrification and no organic carbon for denitrification [1,2]. In the PN–Anammox process, a long-term stable PN with high nitrite accumulation rate (NAR) is the key step since it should provide effluent with suitable NO_2^-/NH_4^+ ratio for Anammox. Since both aerobic ammonia-oxidizing bacteria (AerAOB) and nitrite-oxidizing bacteria (NOB) existed in the nitrifying sludge, the effective inhibition and washout through sludge discharge on NOB are indispensable.

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Thus, the separate control of HRT and SRT in the reactor is essential to achieve high-rate PN, which was difficult to realize in conventional continuous systems. On the other hand, most studies about PN were conducted in SBR, while few were focused on the continuous systems, in which the sludge loss was the challenge. The newly developed membrane bioreactor (MBR) [3,4] could resolve both the problems, which could also achieve high biomass concentration due to the complete retention by membrane [5,6]. Thus, MBR was used in this study, to achieve rapid start-up and high-rate ammonia oxidation of PN.

Besides of the sludge discharge, other operational conditions should be controlled for inhibiting the bioactivity of NOB to achieve nitrite accumulation. The prevailing strategies to accomplish this encompass manipulation of operational high temperature [7], high pH [8], low DO [9], free ammonia (FA), and free nitrous acid (FNA) inhibition [10]. Among these strategies, pH and temperature control will increase the running cost. DO control is difficult to smoothly implement, and long-term low DO would not only decrease the ammonia oxidation rate but also result in sludge bulking [11]. And the high FA and FNA inhibition would not work out in the long run for the acclimation of NOB [12]. Since each known single factor has their own shortcomings, ammonia-loading rate (ALR) was adopted in this study as a new start-up strategy, which is suggested to be effective due to its combined effects of HRT and ammonia concentration. The adoption of ALR strategy would shorten the start-up period and simplify the process since it could be easily adjusted by changing ammonia concentration or inflow rate. However, until now, little is known about the feasibility of this novel approach for PN.

After the successful start-up, the stability of PN is still a problem due to NOB acclimation to the not favoring conditions during long periods [13–15]. However, few researches focused on the influencing factors of the stable operation. Since ALR was adopted for the start-up in this study, its influence on the PN performance is essential to be detected. On the other hand, several studies have shown that alkalinity could control the effluent NO_2^-/NH_4^+ ratio of PN [16,17], which would become the main means to realize half PN. However, previous research only showed the feasibility of alkalinity on effluent control, it was unclear of influence on the performance of PN. It would contribute to control the process by knowing the organisms affected by different alkalinity, let alone that alkalinity serves as important inorganic carbon for the autotrophic AerAOB and pH modifier in the PN system. Thus, the influence of ALR and alkalinity on the efficiency and stability of PN were investigated in this study.

In addition, since the stable PN was significantly depended on both the effective inhibition on NOB and the enrichment of AerAOB, the information related with microbial ecology and population in PN reactor is essential for a better understanding about the performance of the system. Some molecular techniques, such as fluorescence *in situ* hybridization (FISH), denaturing gradient gel electrophoresis, and clone library, had enabled monitoring of the growth of nitrifiers and other microbial populations. However, most of the PN processes focused on treating wastewater with high temperature and high ammonia [18]. Little attention was paid on PN process at ambient temperature, let alone the corresponding microbial characteristics. Moreover, due to the different ways in the operation of MBR system, the microbial community and the distribution of functional bacteria could possibly different from the other systems. Understanding the microbial characteristics could be valuable to a widely used PN process in MBR.

In this study, ALR was adopted as a new strategy for the start-up of PN at ambient temperature in MBR. The influence factors, including ALR and alkalinity, on the efficiency and stability of PN were studied. The microbial characteristics, including community biodiversity and the distribution of AerAOB and NOB in the PN system, were detected by gene clone sequencing and FISH.

2. Material and methods

2.1. Wastewater and seed sludge

The synthetic wastewater consisting of $(NH_4)_2SO_4$ and $NaHCO_3$ as the main substrates along with other minerals was adopted as the feeding for the reactor. The composition of the synthetic wastewater was as follows: 0.330–0.942 g $(NH_4)_2SO_4$, 0.939–2.685 g $NaHCO_3$, 0.136 g KH_2PO_4, 0.136 g $CaCl_2$, 0.3 g $MgSO_4{\cdot}7H_2O$ per liter and 1 mL L^{-1} of trace element solution [19]. Three liters of conventional activated sludge from a municipal sewage plant was seeded to MBR after washed by distilled water for three times to eliminate the dissolved impurities. The MLSS and mixed liquor volatile suspended solids of the seed sludge were 12.9 and 10.6 g L^{-1}, respectively.

2.2. Design and operational parameters of MBR

A submerged MBR (Fig. 1) with a hollow fiber membrane module (material: PVDF; pore size: 0.1 μm; effective area: 0.2 m^2; water permeability: 36 L h^{-1};

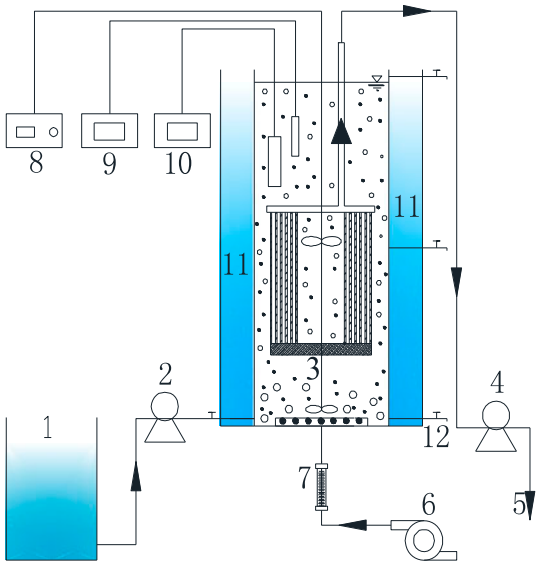

Fig. 1. Schematic diagram of the MBR. (1) Influent tank; (2) influent pump; (3) membrane module; (4) effluent pump; (5) effluent; (6) blower; (7) aeration flow meter; (8) mixer; (9) online pH; (10) online DO; (11) water bath; (12) sludge discharge.

Kunyang, China) was adopted for PN process. The entire reactor with an effective volume of 13.2 L (diameter: 200 mm, height: 500 mm) was placed in a water bath (diameter: 300 mm, height: 500 mm) to ensure a constant reaction temperature (25˚C). While the synthetic wastewater was fed into the reactor, the effluent was continuously drawn out from the reactor using a peristaltic pump. A mixer was set in the system to assure homogeneous condition. Oxygen was supplied continuously from an aeration ring located at the bottom of the reactor which was connected to an air blower. For constant membrane flux, the membrane module was back washed when the transmembrane pressure increased to −80 kPa or cleaned using tap water before soaking in 8‰ sodium hypochlorite solution for 24 h. Backwashing and cleaning were performed every 25 and 90 d, respectively.

For the start-up of PN, ALR was gradually increased by increasing ammonia concentration from 70 to 200 mg L^{-1} with slight fluctuation of inflow rate under oxygen-limited condition. The whole experiment was conducted as five phases based on each particular function with different ALR. The temperature in the reactor was maintained at 25 ± 0.5˚C, pH was around 7.6, DO was about 0.15 mg L^{-1}, SRT was 100 d, and MLSS was 4.0 g L^{-1}. When the reactor got stable operation on day 130, the sludge sample was obtained from the reactor, for FISH and clone-sequencing analysis.

2.3. Analytical methods

Concentrations of NH_4^+–N, NO_2–N, and NO_3^-–N were daily measured according to standard methods [20]. The temperature, DO, and pH were detected using online instruments with DO and pH probes, respectively (WTW, Germany). Alkalinity was measured by potentiometric titration (ZDJ-2D). ALR, NAR, specific ammonia oxidation rate (SAOR), and specific nitrite oxidation rate (SNOR) were calculated using Eqs. (1)–(3), respectively.

$$\mathrm{ALR} = \frac{[\mathrm{NH_4^+ - N}]_{\mathrm{in}} \times Q}{1000 \times V} \times 24 \quad (1)$$

$$\mathrm{NAR} = \frac{[\mathrm{NO_2^- - N}]_{\mathrm{out}}}{[\mathrm{NO_2^- - N}]_{\mathrm{out}} + [\mathrm{NO_3^- - N}]_{\mathrm{out}}} \times 100\% \quad (2)$$

$$\mathrm{SAOR} = \frac{\left([\mathrm{NH_4^+ - N}]_{\mathrm{in}} - [\mathrm{NH_4^+ - N}]_{\mathrm{out}}\right) \times Q}{\mathrm{MLSS} \times V} \quad (3)$$

$$\mathrm{SNOR} = \frac{\left([\mathrm{NO_2^- - N}]_{\mathrm{in}} - [\mathrm{NO_2^- - N}]_{\mathrm{out}}\right) \times Q}{\mathrm{MLSS} \times V} \quad (4)$$

2.4. FISH analysis

Sludge sample was obtained from MBR on day 130, when the reactor was operated as stable PN process. The SNOR reached 1.8 from 0.3 mg g^{-1} SS h^{-1} when the alkalinity ratio went down from 10 to 4. The samples were then suspended in a 1:1 mixture of phosphate buffer saline and 100% ethanol. Hybridization was performed according to the method as previously described [21]. All the oligonucleotide probes were shown in Table 1, which were purchased from Sangon Company (China). Image acquisition was achieved by an Olympus BX-51 fluorescence microscope with DP72 camera (Japan). Using the CellSens Dimension software, the abundance of the AerAOB and NOB populations was determined as the ratio of the areas covered by biomass stained with both the specific and the EUB_{MIX} probes to the area covered by EUB_{MIX} probes stained biomass in at least 10 randomly distributed microscope fields.

2.5. DNA extraction and clone sequencing

The partial nitrifying sludge was collected from the reactor on day 130 for the DNA extraction. The genomic DNA was extracted using a bacterial genomic mini extraction kit (Sangon, China) according to the manufacturer's manual and was detected by 0.8% (w/v) agarose gel electrophoresis.

Table 1
Oligonucleotide probes used in this study

Probes	Sequence(5′–3′)	Target	Fluorescent dye	Reference
EUB338	GCTGCCTCCCGTAGGAGT	Total bacteria	FITC	[21]
EUB338II	GCAGCCACCCGTAGGTGT			
EUB338III	GCTGCCACCCGTAGGTGT			
NSO190	CGATCCCCTGCTTTTCTCC	AerAOB	CY3	[22]
NIT3	CCTGTGCTCCATGCTCCG	NOB	HEX	[23]

The amplification of the 16S rRNA gene was performed using the following primers: 27 F (forward primer: 5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (reverse primer: 5′-GGTTACCTTGTTACGACTT-3′). The PCR conditions were as follows: an initial denaturation at 98℃ for 1.5 min; 25 cycles of denaturation (10 s at 98℃), annealing (45 s at 55℃) and elongation (80 s at 72℃); a final elongation at 72℃ for seven minutes. Then, the PCR products were purified with the purification kit (Sangon, China) and cloned by pMD19-T plasmid vector system (TaKaRa, Japan). Sequencing was performed on an ABI3730 DNA sequencer by a commercial service (Sangon, China). Twenty-four clones were randomly selected for sequencing and further analysis, and eight different sequences were obtained. All sequences obtained were compared with the reference microorganisms available in Gentbank by BLAST tool and submitted to GenBank database with accession numbers of KF973215–KF973222 related to the 16S rRNA gene of total bacteria.

3. Results and discussion

3.1. Start-up of PN in MBR

ALR was adopted as the primary control parameter for the start-up of PN in this study, which was gradually increased by rising up the influent ammonia concentration. The corresponding performance of the reactor with the variation of ALR during the experiment was shown in Fig. 2. A transient nitrite accumulation with a rate of nearly 10% was observed in the reactor at the first day after seeded, indicating the existence of AerAOB, which was consistent with the FISH results (Fig. 5(a)). However, NAR reduced sharply to zero in the next several days. Little nitrite could be detected in the reactor because of the immediate consumption by NOB. It was speculated that the initial nitrite accumulation was due to the higher bioactivity of AerAOB than that of NOB when enters into a new situation [24]. Since the ALR in this phase was relative low, which was not effective for the inhibition of NOB, the bioactivity of NOB was gradually

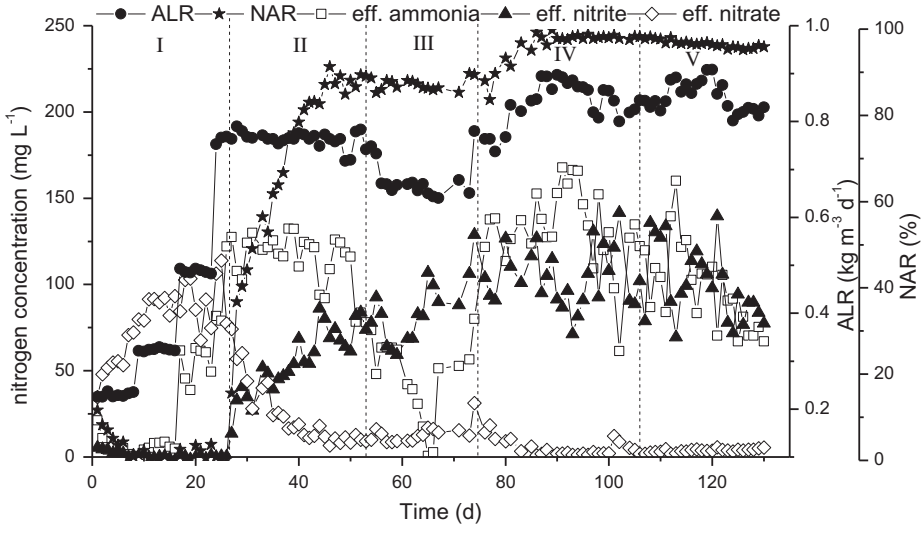

Fig. 2. Performance of the reactor during the experiment.

elevated. The nitrite produced by AerAOB was immediately oxidized to nitrate by NOB, resulting in both the decrease of nitrite and increase of nitrate in effluent. As a result, nitrite accumulation disappeared. In phase II, ALR was increased to more than 0.7 kg m^{-3} d^{-1}, nitrite began to accumulate on day 27 after a short "lag time" with the NAR increased rapidly from 0.8 to 89.1% during only 15 d. The nitrite accumulation showed a significant dependence on the efficient inhibition of NOB by ALR, which led to the successful start-up of PN.

However, NAR could not be further improved in phase III, which was possibly attributed to the reduction of ALR. Thus, ALR was increased again to be more than 0.7 kg m^{-3} d^{-1} in phase IV. As a result, NAR showed a second "jump" increase and finally got a stable rate of nearly 100%, PN was successfully started-up. The result in this phase indicated the effective inhibition on NOB by keeping ALR to be more than 0.7 kg m^{-3} d^{-1}. Then, in phase V, influent ammonia was kept around 200 mg L^{-1} without change of ALR, NAR remained at nearly 100% for a long period. Moreover, an excellent effluent with appropriate NO_2^-/NH_4^+ ratio as 1.1–1.3 was obtained by slightly adjusting inflow rate, which was suitable for the subsequent Anammox reactor. Temperature, pH, and DO could not be considered as the key factors for the establishment of PN since they were kept at around 25℃, 7.6, 0.15 mg L^{-1}, respectively, without big fluctuation except in phase I. The start-up results suggested the feasibility of ALR as PN start-up strategy, which was also proved in our another study [25]. That study was aimed to buildup CANON process in MBR, and PN was started-up firstly by decreasing HRT without changing ammonia for ALR increasing. When ALR was increased to 0.5 kg m^{-3} d^{-1}, nitrite began to accumulate. However, NAR was limited at 60%. Then, HRT was further decreased for an ALR of 0.7 kg m^{-3} d^{-1}, NAR rose up rapidly to more than 95% and sustained for a long-term. Thus, ALR was an effective strategy for PN start-up in 30 d, which can be simply realized by either changing ammonia or adjusting the inflow rate.

3.2. Factors influencing the reactor performance

3.2.1. ALR influence

SAOR was used to measure the bioactivity of AerAOB in this study, which showed a good correlation with the variation of ALR. As shown in Fig. 3, when ALR was less than 0.5 kg m^{-3} d^{-1}, SAOR went up almost linearly with the increase in ALR and achieved to the maximum when ALR was increased to 0.6 kg m^{-3} d^{-1}. SAOR was kept in a flat range when ALR was between 0.5 and 0.7 kg m^{-3} d^{-1} and then decreased sharply to be 70% of the maximum when ALR was increased to 0.8 kg m^{-3} d^{-1}. A quadratic curve was fitted as an equation of $y = -29.70575x^2 + 34.25745x - 2.94958$ with the R^2 of 0.987. One

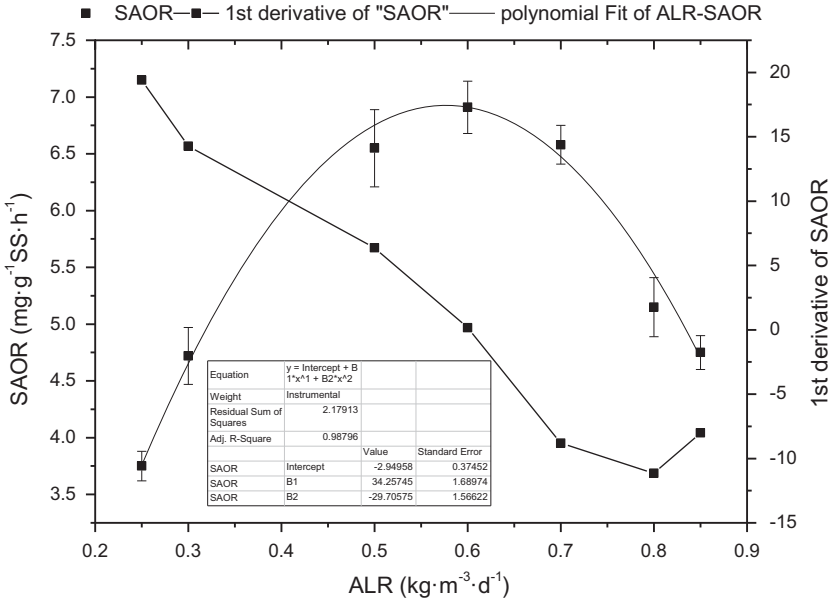

Fig. 3. Variation of SAOR with different ALR.

explanation of this phenomenon is that when ALR was not too high, the increase in ALR provided sufficient ammonia for AerAOB who prefers high ammonia load, and the generated nitrite from ammonia was consumed immediately by NOB who had not been inhibited. As a result, little nitrite remained in the reactor and then accelerated the oxidation of ammonia. NOB was effectively inhibited when ALR was more than $0.7\,kg\,m^{-3}\,d^{-1}$, leading to the nitrite accumulation in the reactor. Plenty of nitrite remained in the reactor possibly slowed down the nitrite generation, which constrained the ammonia oxidation rate [26]. On the other hand, AerAOB was also inhibited by ALR due to the high load imposed on the microbial cell and thus led to the lower bioactivity of AerAOB.

Correspondingly, the 1st derivative curve, which indicated the increment speed of SAOR, presented a positive value with reduction trend at first and finally decreased to below zero. SAOR achieved the highest value when the ALR was kept between 0.5 and $0.7\,kg\,m^{-3}\,d^{-1}$. However, a high-rate PN process depends on not only the high bioactivity of AerAOB, but also the thoroughly bioactivity inhibition of NOB. Given that the ALR should be maintained be not less than $0.7\,kg\,m^{-3}\,d^{-1}$ for the effective inhibition on NOB, the ALR in PN reactor should be controlled at around $0.7\,kg\,m^{-3}\,d^{-1}$, to realize both excellent NAR and SAOR.

3.2.3. Alkalinity influence

Alkalinity served as one of the most important substrates and the only inorganic carbon source for AOB in this study. SAOR and SNOR under different alkalinity conditions were detected to measure the bioactivity of AerAOB and NOB, which respectively reflected the efficiency and stability of PN. The alkalinity in influent was varied to achieve the ratio of alkalinity to ammonia (A/a) as 10, 8, 6, 4, correspondingly led to the of pH variation in the reactor, as shown in Fig. 4. The results indicated that alkalinity greatly affected the reaction rate of both ammonia and nitrite oxidations. The SAOR was found to be around $7.0\,mg\,g^{-1}$ SS h^{-1} at high alkalinity levels (A/a = 10 or 8); however, it decreased to 5.5 and 3.9 $mg\,g^{-1}$ SS h^{-1} at the A/a ratio of 6 and 4, respectively. This demonstrated that the bioactivity of AerAOB was limited with decreasing alkalinity. Moreover, decrease in alkalinity caused the recovery of NOB bioactivity, which was adverse for the stability of PN. The SNOR reached 1.8 from $0.3\,mg\,g^{-1}$ SS h^{-1} when the alkalinity ratio went up from 4 to 10. On the other hand, the alkalinity concentration

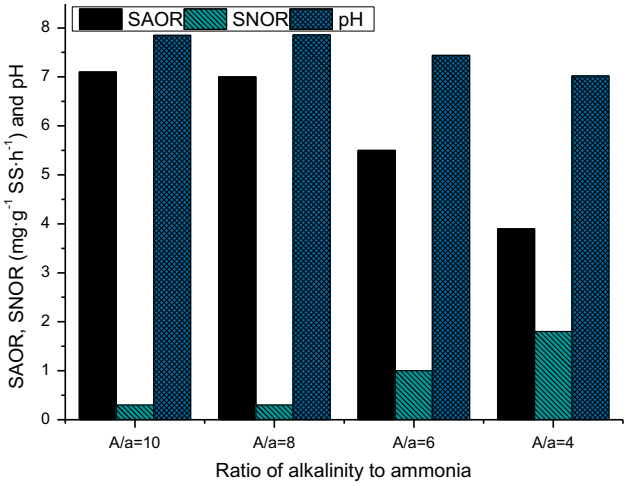

Fig. 4. Ammonia and nitrite oxidation rate with the variation of alkalinity.

would affect the pH in the reactor, which would also affect the bioactivity of AerAOB and NOB. Thus, the high alkalinity concentration was profitable for both the efficiency and the stability of PN. Given that both the SAOR and SNOR showed little difference under the alkalinity ratio of 10 and 8, the ratio of 8 was selected as the preferred alkalinity for cost savings. So the applied alkalinity, in this study, was suitable and indispensable for the stability of PN.

In conclusion, by investigating the bioactivity of AerAOB and NOB in MBR, the results demonstrated that ALR and alkalinity played the most important roles in the stability and high-rate ammonia oxidation of PN. The decrease and increase in ALR level or alkalinity resulted in the reduction either in ammonia oxidation efficiency or in nitrite accumulation. The optimal operation conditions favoring ammonia oxidation and nitrite accumulation were found at $0.7\,kg\,m^{-3}\,d^{-1}$ of ALR and 8 times of alkalinity to ammonia.

3.3. Microbial characteristics

3.3.1. AerAOB and NOB distribution

Long-term operation could either lead to the acclimation of the existing bacteria to the new conditions or a significant shift in the microbial community. Thus, the changes in the bacterial populations were monitored by FISH, which provided the necessary biological information for the realization of PN. In addition to the seed sludge, partial nitrifying sludge sample was taken from the reactor on day 130 for

the analysis of AerAOB and NOB distribution. Oligonucleotide probe of Nso190 was carried out to target AerAOB while Nit3 was utilized to detect Nitrobacter. The proportion of AerAOB or NOB in the sludge samples was determined as the ratio of the area covered by biomass stained with both the specific and the EUB_{MIX} probes to the area covered by EUB_{MIX} probes stained biomass.

Fig. 5(a) presented the hybridizing result of AerAOB and total bacteria in seeding sludge, in which the relative abundance of AerAOB was only 3.8 ± 1.2%. This result suggested that the AerAOB numbers were not competitive at the beginning of the reactor operation. However, cells hybridized with Nso190 for AerAOB increased to 31.2 ± 3.3% of the total bacteria detected by Eub338mix probes in phase V (Fig. 5(b)). When Fig. 5(a) is compared with Fig. 5(b), FISH results suggested that the operational conditions in MBR did profitable effect on the enrichment of AerAOB. AerAOB became the predominant bacteria in the stable PN system, which contributed to improving the ammonia oxidation and nitrite accumulation efficiency of the MBR.

On the other hand, the fraction of NOB in PN sludge was only 1.8 ± 0.9% (Fig. 5(c)), which was far less than that of AerAOB. Compared with NOB, AerAOB could survive better with high ALR because of their lower inhibition constants. In the present study, seeding sludge was derived from a municipal plant, which was operated as conventional nitrification–denitrification process. Thus, AerAOB and NOB were both functional microbial populations in seeding sludge. During the experiment, high ALR combining with low DO was used to suppress NOB growth, which had little adverse effect on AerAOB growth. So the proportion of AerAOB increased to 31.2%, whereas NOB only amounted to 1.8% in the stable operation phase of MBR. It was clearly demonstrated that NOB was thoroughly washed out from the reactor in the stable operation. The AerAOB and NOB population showed a direct correlation with the performance of the reactor. NAR was nearly 100% in phase V, when AerAOB became the dominant bacteria among microbial compositions. FISH results also proved that ALR was a crucial factor, which can inhibit the growth of NOB intensively.

3.3.2. Microbial community

Evaluation of the bacteria existing in the microbial community of the stable PN system is helpful for considering the implementation of a long-term and stable PN process under high ALR and limited DO. Analysis of the microbial community was based on 16S rRNA gene sequences to investigate the species that existed in the PN reactor. The results were interesting because only three phyla were involved, including Betaproteobacteria, Actinobacteridae, and Anaerolineae (Table 2). This result indicated the low biodiversity and stable community of the PN system in MBR. The clone 1 and 8 were closely related to *Nitrosomonas europaea* (AerAOB), both of which had a high similarity of 99%. The clone 3 and 6 were related to *Nitrosomonas* sp. (AerAOB) with the similarity of 98% and 97%, respectively. The other four clones were related to the microorganism irrelevant for the ammonia oxidation in the reactor. The Nitrosomonas-related AerAOB amounted 50% in the PN sludge from MBR, indicating the predominant role of AerAOB in the system. It was notable that no NOB members were detected in the gene clone sequencing results. This result indicated the thoroughly washout of NOB since the clone sequencing could only detect the bacteria with a certain amount. It could be concluded that in the MBR-PN system, the main functional microorganisms were Nitrosomonas-related AerAOB, which was responsible for the stable nitrite accumulation.

These results suggested that high ALR and limited DO offer Nitrosomonas a competitive advantage

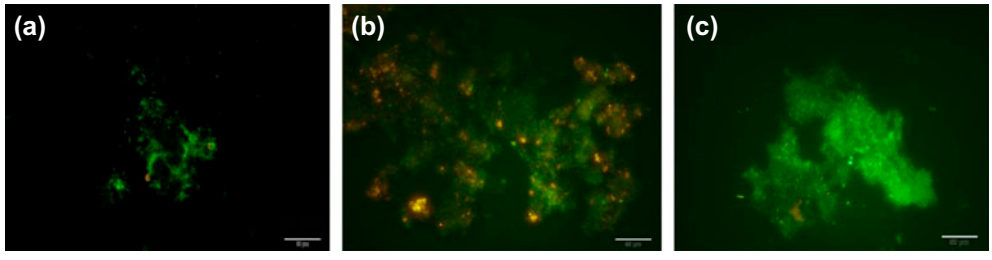

Fig. 5. Distribution of AerAOB and NOB in FISH images (400×) ((a) seed sludge, total bacteria (green), and AerAOB (orange); (b) sludge on day 130, total bacteria (green) and AerAOB (orange); (c) sludge on day 130, total bacteria (green) and NOB (orange)).

Table 2
Clone sequencing results of 16S rRNA in PN reactor

Clone	Closest relative	Identity/%	Accession numbers	Phylum (classifier)	Accession numbers
1	*Nitrosomonas europaea*	99	AL954747	Betaproteobacteria	KF973215
2	*Mycobacterium* sp.	92	JX096500	Actinobacteridae	KF973216
3	*Nitrosomonas* sp.	98	AL954747	Betaproteobacteria	KF973217
4	*Levilinea* sp.	86	JQ292916	Anaerolineae	KF973218
5	*Bellilinea* sp.	88	JQ515490	Anaerolineae	KF973219
6	*Nitrosomonas* sp.	97	AB079053	Betaproteobacteria	KF973220
7	*Bellilinea* sp.	88	JF828760	Anaerolineae	KF973221
8	*Nitrosomonas europaea*	99	NR_074774	Betaproteobacteria	KF973222

over other microorganisms. Nitrosomonas was considered to be the functional bacteria for the ammonia oxidation and the high-rate PN in MBR. This result is consistent with the previous reports that Nitrosomonas-like species were the only detected AerAOB, while few Nitrosospira-like sequences could be detected in a PN reactor with high ammonia loading [25,27].

The predominance of AerAOB possibly was due to the following two reasons: First, the AerAOB population that is identified here could have adapted to the high ALR condition, which then resulted in the population growth of AerAOB. This has been proved by the FISH results. The second reason could be the effective inhibition of high ALR on NOB in the reactor, which constrained the multiplication of NOB but had no adverse effect on AerAOB, and then resulted in the high-rate nitrite accumulation. It could be speculated that high ALR under the oxygen-limited condition was effective to improve the AerAOB population, as well as decrease the NOB population, and the symbiotic relationship among the existing bacteria in the PN community could be maintained.

4. Conclusions

Increasing ALR to be $0.7\,kg\,m^{-3}\,d^{-1}$ was proved to be an effective strategy for PN start-up, through which a high-rate PN was obtained at ambient temperature in MBR. The stability of PN was significantly depended on the combined effects of the applied ALR and alkalinity. For both high-rate and stable PN, ALR should be controlled around $0.7\,kg\,m^{-3}\,d^{-1}$ and the ratio of alkalinity to ammonia should be controlled as 8. FISH results indicated that AerAOB became the dominate bacteria in the PN system, while NOB was barely detectable. Microbial community result showed that Nitrosomonas-related AerAOB was the functional bacteria for the high-rate PN in MBR.

Acknowledgements

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Abbreviations

PN	—	partial nitrification
Anammox	—	anaerobic ammonia-oxidizing process
NAR	—	nitrite accumulation rate
NOB	—	nitrite-oxidizing bacteria
FA	—	free ammonia
FNA	—	free nitrous acid
AerAOB	—	aerobic ammonia-oxidizing bacteria
ALR	—	ammonia loading rate
MBR	—	membrane bioreactor
MLSS	—	mixed liquid suspended solids
SRT	—	sludge retention time
SAOR	—	specific ammonia oxidation rate

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