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Operational strategies for nitrogen removal in granular sequencing batch reactor

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ABSTRACT

This study investigated the effects of different operational strategies for nitrogen removal by aerobic granules with mean granule sizes of 1.5 mm and 0.7 mm in a sequencing batch reactor (SBR). With an alternating anoxic/oxic (AO) operation mode without control of dissolve oxygen (DO), the granular sludge with different size achieved the total inorganic nitrogen (TIN) removal efficiencies of 67.8–71.5%. While under the AO condition with DO controlled at 2 mg/l at the oxic phase, the TIN removal efficiency was improved up to 75.0–80.4%. A novel operational strategy of alternating anoxic/oxic combined with the step-feeding mode was developed for nitrogen removal by aerobic granules. It was found that nitrogen removal efficiencies could be further improved to 93.0–95.9% with the novel strategy. Obviously, the alternating anoxic/oxic strategy combined with step-feeding is the optimal way for TIN removal by granular sludge, which is independent of granule size.

1. Introduction

Aerobic granule technology has been studied for over 10 years for wastewater treatment. Compared to conventional activated sludge, aerobic granule has regular and compact physical structure, diverse microbial species, good settling property, high biomass retention, and great ability to withstand shock load or shock of toxic compound. Therefore, aerobic granule is becoming a very promising technology for wastewater treatment [1–9].

Nutrient removal is the basic requirement for wastewater treatment with the implementation of high discharge standard of treated water. Nitrogen is one of the key nutrients causing eutrophication in water body, which is required to be removed from water resources in many countries. Generally, biological nitrogen removal consists of two steps, i.e., nitrification, with which ammonia is converted into nitrite and finally nitrate aerobically, and denitrification, with which nitrite or nitrate is converted into gaseous nitrogen anoxically. Therefore, anoxic and oxic phases are normally exerted in different compartments for nitrification and denitrification with traditional activated sludge, which thus compromises the compactness of the whole system. However, the presence of anaerobic zone in dense aerobic activated sludge due to low aeration makes it possible for the integrated nitrogen removal in single aeration basins, i.e., simultaneous occurrence of nitrification and denitrification (SND). Since an anoxic zone exists in aerobic granules due to mass transfer resistance from compact structure and big size [4,10,11], it is generally believed that nitrogen could be removed efficiently in single granules. As the nitrogen removal depending on aerobic and anoxic zones of granules, DO concentration in the bulk liquid is usually required to be precisely controlled. In detail, aerobic and anoxic volume created by DO penetration in the single granules may influence nitrification and denitrification rates and further total nitrogen removal rate. In addition, the practically uncontrollable factors, such as granule size, granule density, biomass spatial distribution and activity of diverse bacteria species, are associated with DO diffusion in granules, which may also influence nitrogen removal of single granules [12]. Among these factors, granule size has been reported to determine the volume of anoxic zones in the granules at certain DO concentrations in the bulk liquid, which is thus closely correlated with nutrient removal [12–15]. However, there is little report on the effects of granule size on nitrogen removal under different operating modes.

So far, a few operating modes of granular sludge systems for nitrogen removal have been reported on the basis of lab-scale experiments, such as alternating oxic–anoxic mode (OA) [16,17], continuous or on/off aeration with controlled DO for simultaneous nitrification and denitrification (SND) [13,18], anaerobicly-controlled oxic mode (AO) [19] and alternating anaerobic–oxic or anaerobic–oxic–anoxic mode (AO or AOA) using denitrifying polyphosphate accumulating organisms (DNPAOs) [11,14,15,20]. During granular N-removal process, nitrification is easy to be achieved when aeration is sufficiently supplied, while denitrification is normally a rate-limiting step due to deficient anoxic condition and/or carbon source supply. As for these modes,

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denitrification occurs either in anoxic zone inside single granules or anoxic condition (phase) provided in some parts of an entire cycle. For OA process, extra carbon addition is normally needed because of insufficient carbon source after oxic period. For SND at controlled DO, long retention time is normally required due to low reaction rate at low DO. In addition, the variable granule size or density will affect aerobic and anoxic volumes in granules, which could probably result in unstable nitrogen removal. For SND and AO or AOA (using DNPAOs) mode, it is difficult to achieve optimal microbial community only by regulating operation conditions. Ammonia or oxidized nitrogen may thus be present in the effluent [19].

Step-feed mode in activated sludge system has been reported to be effective in making good use of carbon source in influent, which increases denitrification rate and further total nitrogen removal efficiency. In addition, it allows nitrification to occur at a lower organic loading in the aerobic phase, which accelerates nitrification rate and saves aeration consumption to oxidize organic matters in influent. Some studies have been documented on alternating aerobic/anoxic and step-feed mode in activated sludge system [21–26], which shows that it is both technologically and economically effective in enhancing nitrogen removal efficiency. With more compact structure and bigger size than activated sludge, whether granular sludge can achieve effective nitrogen removal under this operational strategy is to be investigated.

In this study, nitrogen removal rates and efficiencies in granular sequencing batch reactor with AO mode with or without DO control were compared on the basis of two different granule sizes. In addition, a novel operational strategy with alternating anoxic/oxic combined with step-feed was developed for nitrogen removal by aerobic granules.

2. Materials and methods

2.1. Reactor set-up and operation

Two columns (a diameter of 5 cm and a height/diameter ratio of 20) with a working volume of 2 l were inoculated by granules with a mean size of 1.5 mm (R1) and 0.7 mm (R2). Since the inoculated granular sludge was stored in a refrigerator for months, revival of granule activity was conducted in the two reactors under a condition of non-aeration followed by aeration. For the both reactors, cycle time of 4 h was used which consisted of 10 min influent filling phase, 10 min of non-aerobic phase, 213 min of aerobic phase, 2 min of settling phase and 5 min of effluent discharging phase. Effluent was discharged from the middle part of the reactors with a volumetric exchange ratio of 50%. The non-aeration phase was realized by stopping aeration supply, during which water recycling from the upper to the bottom of the reactor was conducted by a peristaltic pump. The aeration phase without DO control was carried out by supplying fine air bubbles through an air spargar at the bottom of the reactor with an airflow rate of 3 l/min. In the meantime, DO may be controlled at 2 mg/l by reducing aeration rate during the aeration phase.

The compositions of the synthetic wastewater were COD 600 mg/l, NH_4^+–N 60 mg/l, K_2HPO_4 22.5 mg/l, $CaCl_2$ 15 mg/l, $MgSO_4{\cdot}7H_2O$ 12.5 mg/l, $FeSO_4{\cdot}7H_2O$ 10 mg/l, and trace element solution. Micronutrients in the trace element solution contained: $MnCl_2{\cdot}4H_2O$ 0.12 mg/l, $ZnSO_4{\cdot}7H_2O$ 0.12 mg/l, $CuSO_4{\cdot}5H_2O$ 0.03 mg/l, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.05 mg/l, $NiCl_2{\cdot}6H_2O$ 0.1 mg/l, $CoCl_2{\cdot}6H_2O$ 0.1 mg/l, $AlCl_3{\cdot}6H_2O$ 0.05 mg/l, H_3BO_3 0.05 mg/l.

2.2. Reactor operation description

The two reactors were seeded with 500 ml aerobic granules treating real wastewater in granular SBR with size of 1.5 and 0.7, respectively, which had been stored in the refrigerator for several months. The seeded granules had low activity and poor settleability with a lot of black fragments inside. By reducing settling time stepwise from 10 min to 2 min, wash-out of the fragments while good retention of the granules were achieved in the both reactors. In the meantime, substrate removal efficiency and settling ability increased in the both reactors. After the physical characteristics and performance of the granules in the both rectors were stable for about two weeks, it was assumed that the granules were stable enough for the test of operational strategies for nitrogen removal. Then, batch experiments were carried out under different operational strategies to examine nitrogen removal efficiency. The three operational strategies for nitrogen removal in R1 and R2 were as follows (Table 1): (1) non-aeration followed by aeration (AO) mode without DO control. DO during COD decreasing period was around 2–4 mg/l and increased to 8 mg/l after COD depletion; (2) non-aeration followed by aeration (AO) mode with DO controlled at around 2 mg/l during the entire aeration period; (3) combination of alternating anoxic/oxic mode with step-feeding. Samples taken from all the batch experiments were filtered immediately after collection and kept in 4 °C refrigerator (not more than one day) before test.

2.3. Analytical methods

Chemical oxygen demand (COD), NH_4^+–N, sludge volume index (SVI), biomass dry weight (MLSS) and volatile solids (MLVSS) were analyzed in accordance to the standard methods [17]. Nitrate and nitrite were measured by an ionic chromatography (IC, Dionex, USA). The determination of biomass density followed the method described by Beun et al. [2,13]. Dissolved oxygen concentration in the bulk liquid was measured with a DO-electrode (Pisco DO 400, Japan). Average particle size was measured by a laser particle size analysis system (Malvern MasterSizer Series 2600, Malvern instruments Ltd., Malvern, UK) when the size is smaller than 2000 μm, and an image analysis system (Image-Pro Plus, V4.0, Media Cybernetics) with an Olympus SZX9 microscope (Japan) when the size is larger than 2000 μm.

3. Results

After about one-month acclimation, granules seeded to the reactors became stable in terms of physical characteristics and COD and ammonia removal. MLVSS maintained at 5–8 g/l with sludge SVI of 41–48 ml/g in R1 and 5–6.4 g/l with sludge SVI of 20–30 ml/g in R2. At the steady state, different operating modes were applied in the both reactors. The COD and nitrogen removal rates were monitored in the batch cycles.

3.1. Reactor performances under AO (non-aeration + aeration) mode without DO control

Fig. 1 shows COD and nitrogen removal profiles in one batch cycle in R1 and R2 under AO condition without DO control. It was found that during 20-min anoxic phase, nitrate from the previous cycle can be totally removed with denitrification rates of 0.178 d^{-1} and 0.179 d^{-1} in R1 and R2, respectively. During the subsequent aeration phase, COD was effectively depleted, leading to corresponding specific removal rates of 3.940 d^{-1} and 5.470 d^{-1} in R1 and R2, respectively. After COD removal, ammonia was fully oxidized to NO_3^- within 100 min in R1 and R2, during which little NO_2^- accumulation was observed. While total inorganic nitrogen (TIN) removal in the aeration phase was only observed in the first 30 min in R1 and 15 min in R2, leading to 7.4 mg N/l removal by the 1.5 mm-size granules and 2.9 mg N/l by the 0.7 mm-size granules. TIN removal is generally due to assimilation of biomass or denitrification. Deducting the nitrogen assimilation of ammonia

Table 1
Detailed operational conditions of three strategies for nitrogen removal.

Phase (min)	Cycle time	Non-A	A	Non-A	A	Non-A	Low-A	Settling	Discharging
Mode 1	240	20	213					2	5
Mode 2	360	30					323	2	5
Mode 3	360	30	60	30	60	30	143	2	5

Note: Non-A, non-aeration phase; A, aeration phase; Low-A, low aeration phase. Mode 1, AO condition without DO control; Mode 2, AO condition with DO controlled at 2 mg/l; Mode 3, alternating anoxic/oxic condition combined with multi-feeding.

during COD consumption period, 5.3 mg N/l was removed in R1 by denitrification and almost no TIN was removed in R2 by denitrification. This indicated that the nitrogen removal process was limited mainly by denitrification process. Since the influent wastewater quality and all the other operational conditions were same, the difference of TIN removal in the two reactors during aeration phase was proposed from the different physical properties of the granules. The less DO penetration depth in the granules with big size in R1 might create bigger anoxic zones for denitrification during COD degradation period and the subsequent initial nitrification period, while the smaller size of granules in R2 was unfavorable to denitrification due to limited anoxic zones inside.

Table 2 shows nitrogen removal characteristics of both types of granules under AO conditions without DO control. Nitrogen removal efficiency in R1 was a little higher than that in R2, e.g., 71.5% for the 1.5 mm-size granules and 67.9% for the 0.7 mm-size granules. The higher specific denitrification rate of the 1.5 mm-size granules than 0.7 mm-size granules (0.108 d^{-1} vs. 0.066 d^{-1}) was due to the relatively favorable properties (more anoxic zone inside) of the big size granules for denitrification. It is generally accepted that granules with a smaller size are supposed to be more effective in degrading substrate due to their bigger specific surface area [5,19,20]. However, in this study, it was noticed that specific ammonia oxidation rate of the 1.5 mm-size granules was higher than that of the 0.7 mm-size granules. By comparing granule density, it was found that the density of the 0.7 mm-size granules was two times as high as that of the 1.5 mm-size granules. Possibly, the low specific nitrification rate caused by the high density of the small size granules may be attributed to the bigger diffusion limitation of oxygen and substrate.

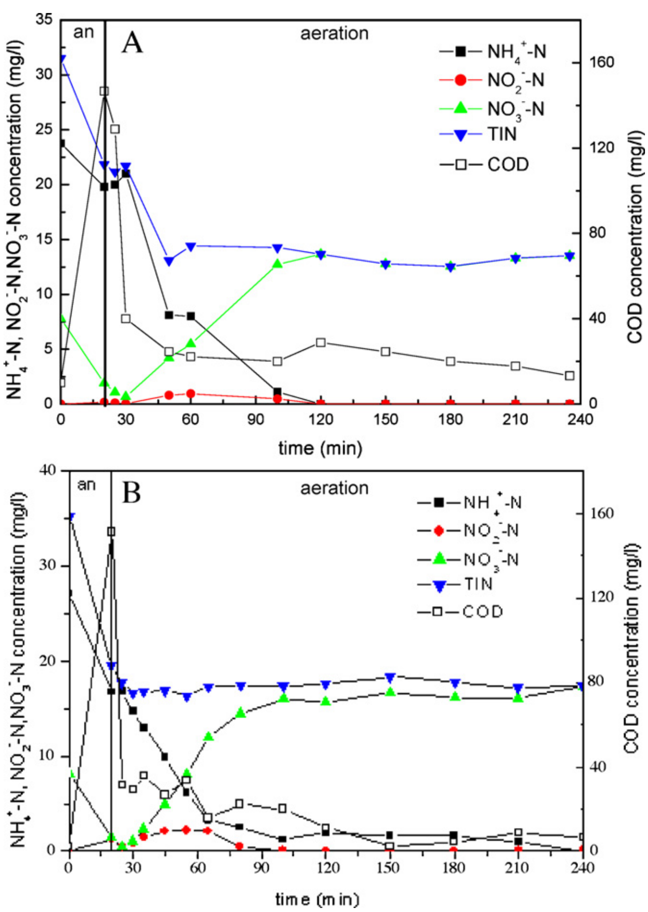

Fig. 1. Cycle profiles of the granules under AO condition without DO control. (A) Cycle for the 1.5 mm-size granules in R1. (B) Cycle for the 0.7 mm-size granules in R2.

3.2. Reactor performance under AO mode with DO of 2 mg/l

To improve nitrogen removal efficiency of both types of granules, denitrification has to be enhanced. In this section, DO was controlled at 2 mg/l by reducing aeration rate during the aeration phase in order to limit oxygen penetration depth in the granules for better denitrification. COD consuming rate may thus be slowed down, favorable to carbon source preservation for denitrification process. In addition, cycle time was prolonged to 6 h to compensate slower nitrification rate in the both reactors. However, it was found that most of the biomass was accumulated at the lower part of the reactors due to the high biomass density with the limited aeration rate and mixing of three phases was very poor. To generate a well-mixing situation, nitrogen gas was supplied to increase the overall gas rate in the reactors. Fig. 2 shows the performance profiles of the two types of granules. It is obvious that both COD degradation rate and nitrification rate declined with the decrease of DO in the bulk liquid. COD degradation rate in R1 and R2 dropped to 2.092 d^{-1} and 2.925 d^{-1}, respectively, leading to over two fold increase of the feast period in aeration phase than that without DO control. Ammonia oxidation almost lasted for the entire cycle time in the both reactors, and denitrification lasted much longer than the previous cycle. There was around 50-min NO_3^- depletion in the controlled aeration phase of the both reactors, indicating that the nitrification and denitrification rates were almost the same at that time. TIN removal by denitrification was 6.8 mg N/l for the 1.5 mm-size granules and 10.6 mg N/l for the 0.7 mm-size granules, which were higher than those under the mode without DO control.

During the degradation period, interestingly, ammonia oxidation process was significantly different between the two types of granules. Little nitrite was accumulated during the whole nitrification process in R1, while the nitrite concentration increased lineally at a slightly lower increasing rate than that of nitrate in R2, leading to almost the same effluent concentration of nitrate and nitrite at the end of the cycle. This demonstrated that ammonia oxidation by ammonia oxidizing bacteria (AOB) is a limiting step for the big size granules during the nitrification process under a low aeration rate, while the nitrite oxidation by nitrite oxidizing bacteria (NOB) is a limiting step for the small size granules, which is completely different from the case under AO mode without DO control. The lower DO may impact the activity of microorganisms inside granules significantly.

TIN removal efficiency at controlled DO increased up to 80.4% by using the 0.7 mm-size granules and 75.0% by using the 1.5 mm-

Table 2
Physical characteristics and nitrogen removal by both types of granules under AO mode without DO control.

Items	Characteristics	Cycle without DO control		
		Nitrification rate (d^{-1})	Denitrification rate (d^{-1})	Removal efficiency (%)
Big size granules	Diameter 1.5 mm SVI 47.2 ml/g Density 21.2 g/l	0.084	0.108	71.5
Small size granules	Diameter 0.7 mm SVI 24.9 ml/g Density 40.2 g/l	0.059	0.066	67.9

Table 3
Physical characteristics and nitrogen removal by both types of granules under AO mode with DO controlled at 2 mg/l.

Items	Characteristics	Cycle with DO of 2 mg/l		
		Nitrification rate (d^{-1})	Denitrification rate (d^{-1})	Removal efficiency (%)
Big size granules	Diameter 1.5 mm SVI 46.8 ml/g Density 21.4 g/l	0.035	0.019	75.0
Small size granules	Diameter 0.7 mm SVI 35.3 ml/g Density 28.3 g/l	0.036	0.037	80.4

size granules, which indicated that low DO had positive effect on denitrification, further to TIN removal of both types of granules (Table 3). However, both specific ammonia oxidation rates and denitrifying rates of two types of granules decreased obviously at low DO. The controlled low DO concentration in the bulk liquid decreased not only specific ammonia oxidation rates but also specific denitrification rates. Specific ammonia oxidation rates by using the 1.5 mm-size and 0.7 mm-size granules decreased to 0.033 d^{-1} and 0.034 d^{-1}, respectively. Their specific denitrification rates decreased to 0.019 d^{-1} and 0.037 d^{-1}, respectively. TIN removal, however, was improved at low DO, which was at the expense of treating capacity of the reactors. In addition, it was noted that decreasing trend of the specific ammonia oxidation rates was associated with granular size. Since granules density could not be controlled during the operation, it was observed that granule density was almost not changed for the 1.5 mm-size granules while it decreased from 40.2 to 28.3 g l^{-1} for the 0.7 mm-size granules. The decreased density of the 0.7 mm-size granules was beneficial to DO penetration, further to ammonia oxidation rate at low DO concentration in the bulk liquid. This was probably related with the phenomenon on the lower decreasing trend of the ammonia oxidation rate in the 0.7 mm-size granules. The decrease of the specific denitrification rate of both types of granules may be attributed to the deficient substrate availability under low aeration condition.

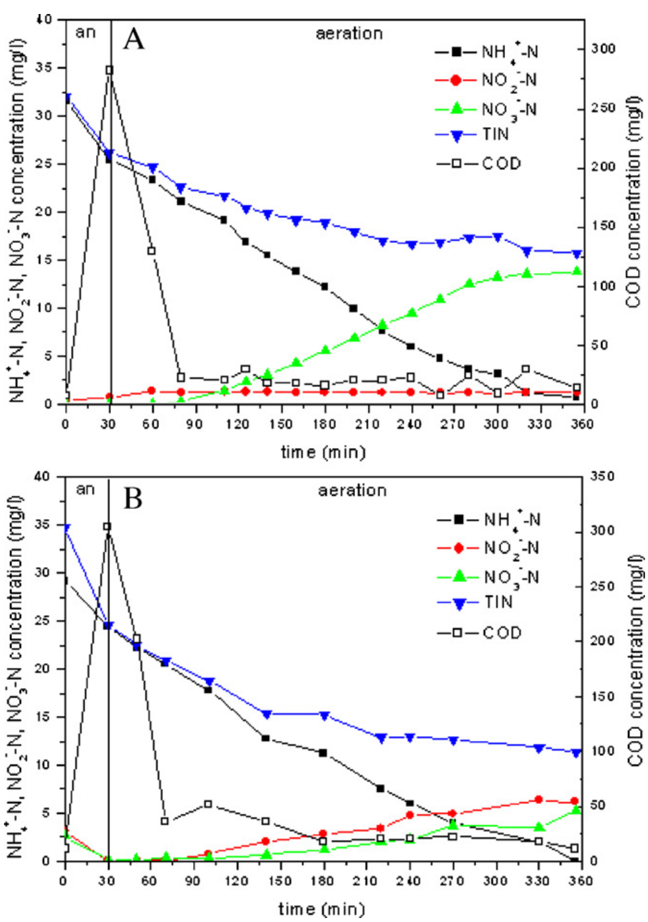

Fig. 2. Cycle profiles of the granules under AO mode with DO controlled at 2 mg/l. (A) Cycle for the 1.5 mm-size granules in R1. (B) Cycle for the 0.7 mm-size granules in R2.

Obviously, control of DO concentration to a low value is effective to increase denitrification efficiency and further improve TIN removal efficiency to some extend. However, this was realized at the expense of both specific nitrification and denitrification rates. Reactor treating capacity was thus decreased. Furthermore, the low aeration rate in granule sludge system may easily cause poor-mixing situation due to the big size and high density of granular sludge.

3.3. *Reactor performance under alternating anoxic/oxic mode combined with step-feeding*

An alternating anoxic/oxic mode combined with step-feeding was applied in R1 and R2 for nitrogen removal. The influent was divided into 3 streams and fed into 3 non-aeration phases sequentially with respective volume of 60%, 20% and 20% of the total feed. As shown in Fig. 3, NH_4^+–N and NO_X^- concentrations in the reactors were reduced to low values after 6-h operation, which lead to a much higher total nitrogen removal efficiency, i.e., 93.0% for the 1.5 mm-size granules and 95.9% for the 0.7 mm-size granules, respectively. In the two aeration phases without DO control, ammonia was totally oxidized without NO_2^- accumulation. In the three non-aeration phases, 18.9 mg N/l of NO_X^- in R1 and 19.6 mg N/l

Table 4
Nitrifying and denitrifying rates of both types of granules under alternating anoxic/oxic mode combined with step-feeding.

	Nitrification rate (d^{-1})		Denitrification rate (d^{-1})	
	Big size	Small size	Big size	Small size
Non-aeration phase				
1 (0–30 min)			0.041	0.065
2 (90–120 min)			0.078	0.094
3 (180–210 min)			0.037	0.057
Aeration phase				
1 (30–90 min)	0.060	0.079		
2 (120–180 min)	0.040	0.061		
Low aeration phase				
(210–353 min)	0.008	0.009		

in R2 were removed, which was much higher compared with the operational strategies 1 and 2. This indicated that the anoxic condition with sufficient carbon source by feeding influent wastewater improved denitrification dramatically. The remained nitrogen was partially removed in the low aeration phase with DO controlled at around 2 mg/l through simultaneous nitrification and denitrification in granules. Obviously, both nitrification and denitrification were well carried out under the respective aerobic and anoxic conditions. In each aerobic phase of the cycle, nitrification after COD depletion occurred with a high rate due to less oxygen limitation at the beginning of the aeration phases. In each subsequent non-aeration phase with substrate feeding, denitrification benefited from sufficient COD addition by the stepwise feeding and NO_X^- accumulated from the previous aeration phase. In the low aeration phase of the cycle, simultaneous nitrification and denitrification (SND) took place due to the coexistence of aerobic and anoxic zones in the granules under low DO condition for further nitrogen removal, and sufficient COD addition from the separated feeding ensured the removal of the remaining nitrogen. The optimal conditions for nitrification and denitrification in each aerobic, anoxic and low DO controlled phase of the cycle led to greatly enhanced total nitrogen removal efficiency.

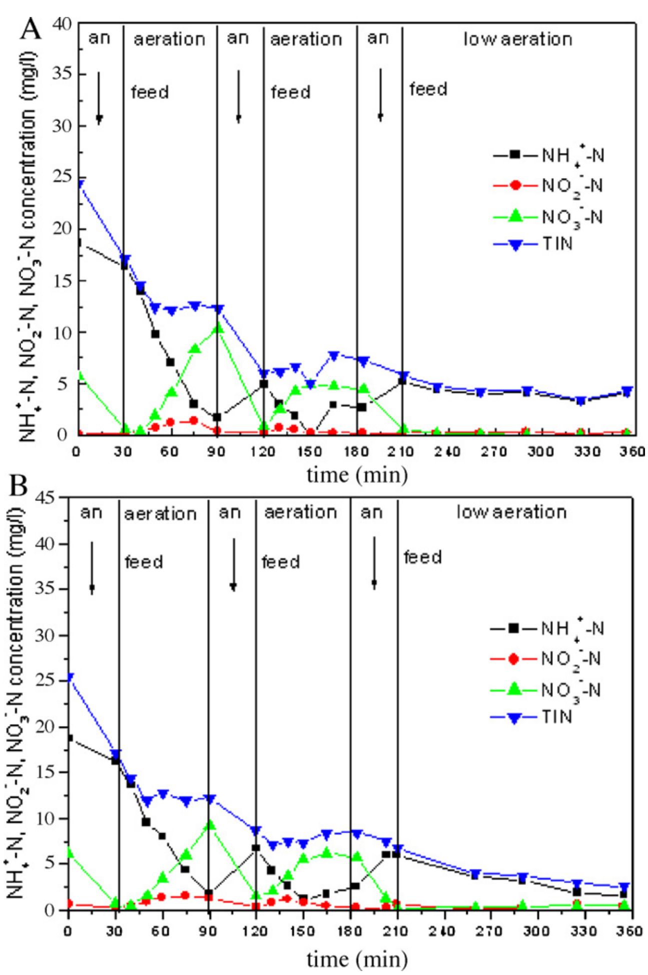

Fig. 3. Cycle profiles of the granules under alternating anoxic/oxic mode combined with step-feeding. (A) Cycle for the 1.5 mm-size granules in R1. (B) Cycle for the 0.7 mm-size granules in R2.

Compared with AO mode with DO controlled at 2 mg/l, both nitrification and denitrification rates of the 1.5 mm- and 0.7 mm-size granules greatly increased as shown in Table 4. With similar densities (32.6 g/l for the 1.5 mm-size granules and 32.9 g/l for the 0.7 mm-size granules), the 0.7 mm-size granules exhibited higher ammonia oxidation rate and denitrification rates than the 1.5 mm-size granules, which was consistent with the previous study. The high denitrification rates of the both granules under this mode indicated that anoxic phase with substrate supplement is more efficient than that only relying on anoxic zone inside granules under low DO concentration.

4. Discussion

4.1. Influence of size and density of granules on reaction rates

Aerobic granules display a wide range of sizes, approximately 0.3–5.0 mm in diameter, which causes very different morphological and physical characteristics of granules [27]. Liu et al. reported that large-size aerobic granules would not favor biological removal of substrate due to potential mass transfer limitation. The diffusion resistance would thus be a limiting factor to the reaction in aerobic granules with a mean size larger than 0.7 mm [28]. Therefore, small-size granules are more efficient in terms of mass transfer and substrate conversion. However, in this study, ammonia oxidation rate of the granules with size of 1.5 mm was higher than that of granules with size of 0.7 mm under AO mode without DO control, which showed that granule size was not the sole factor affecting mass transfer and substrate conversion rate. Chiu et al. found that both high biomass density and large size of granules limited the transfer of dissolved oxygen (DO) in aerobic granules [29]. In this study, it was noted that the density of the 0.7 mm-size granules was almost 2 times of that of the 1.5 mm-size granules under the AO mode without DO control, which was related with the lower ammonia oxidation rate in the 0.7 mm-size granules. The density of the 0.7 mm-size granules fluctuated during the operational period. When density of the 0.7 mm-size granules was almost the same as that of the 1.5 mm-size granules, nitrification and denitrification rates of the 0.7 mm-size granules became higher than that of the 1.5 mm-size granules. Therefore, it is reasonable to speculate that density is another un-negligible rate-limiting factor on substrate conversion rate in granular sludge systems, which was usually neglected in the previous studies.

It was reported that granule possesses aerobic zone at the outer layer and anoxic zone in the interior [10]. It is thus reasonable to expect nitrification in the aerobic zone and denitrification in anaerobic zone of granules. Indeed, simultaneous nitrification and denitrification (SND) by granular sludge under controlled DO was observed and reported [13,18,30]. SND greatly depends on the ratio of aerobic zone to anaerobic zone, which relies on a few of factors such as granule size, granule density, and DO in the aqueous solutions [9]. However, there is no strategy to control granule size and density in the reactors so far, although DO in the aqueous solutions could be controlled precisely. Furthermore, granule size and density may fluctuate even at the steady state of reactor operation, which may cause variable ratios of aerobic to anaerobic volume inside granules at the fixed DO concentration [12]. The change of granule density and resulted change of nitrification and denitrification rate were also observed in this study. Therefore, simply depending on single granules and their aerobic and anoxic zone inside for nitrogen removal may not be reliable and could result in unstable nitrogen removal efficiency, especially when granules are used to treat real wastewater with wild fluctuations on COD and ammonia nitrogen.

4.2. Influence of different modes on nitrogen removal in granular SBR

From the two strategies performed under AO condition with and without DO control in R1 and R2, it shows that denitrification process was the key rate limiting step for nitrogen removal in granular sludge systems. For denitrification process, carbon source supply and anoxic condition are two major factors, i.e., carbon needs to be provided as electron donors and NO_X^- needs to be present in non-aeration condition as electron acceptors. Under AO mode without DO control, there was little denitrification in R1 and R2 during aeration phase due to fast consuming rate of carbon source and the lack of anoxic condition. The accumulation of PHB during short COD depletion period in aerobic phase may not be enough for the subsequent denitrification [11]. It has been reported in a few studies that a high DO concentration created by a high rate aeration was favorable to the conversion of flocs to granular sludge and the long-term stability of granular system [31,32]. However, the results in this study demonstrated that high aeration rate is not suitable to efficiently remove nitrogen. Under AO mode with DO controlled at 2 mg/l, due to more areas of the anoxic zone inside, denitrification was improved in R1 and R2, but still gradually reduced by the exhaustion of carbon source in the middle of the cycle. Carbon source from feed was not fully utilized by denitrification due to growth and maintenance of the microorganisms, which also led to insufficient denitrification and thus incomplete nitrogen removal. In addition, controlling DO at a low value generally leads to decreased nitrification rate, denitrification rate, and sometimes poor mixing situations of three phases (gas, liquid and biomass solid) in reactors. Cycle time thus has to be extended and extra mixing power probably needs to be introduced to ensure high effluent quality, which lead to low reactor treating capacity and high operation cost.

Under an alternating anoxic/oxic strategy combined with step-feeding, extra non-aeration phases with separated feeding were set exclusively for denitrification after ammonia was totally oxidized, which created an optimal condition for denitrification without solely depending on anoxic zone inside granules. With influent COD/N (COD and nitrogen ratio) of 10, even in the second and third non-aeration phases, COD/NO_X^- ratio can still be kept at 5.6 and 12.1, respectively, which guaranteed higher denitrification rate and N-removal efficiency in the whole cycle.

5. Conclusions

Apart from granule size, granule density is another important factor to influence nitrification rate and denitrification rate, which would further affect nitrogen removal efficiency. However, granule size and density may fluctuate even at the steady state of the reactor operation, which may cause shifting volumes of aerobic and anoxic zone inside granules and thus unstable nitrogen removal. Therefore, simply depending on single granules and their aerobic and anoxic zone inside for nitrogen removal is not reliable. Without depending on single granules, extra anoxic phases and substrate supplement were set under alternating anoxic/oxic mode combined with step-feeding. Compared to 67.9–71.5% nitrogen removal efficiency under the alternating anoxic/oxic mode and 75.0–80.4% under the alternating anoxic/oxic mode with DO controlled at 2 mg/l, nitrogen removal efficiency reached to 93.0–95.9% under alternating anoxic/oxic mode combined with step-feeding. Obviously, alternating anoxic/oxic mode combined with step-feeding was the optimal nitrogen removal mode for granular sludge, independent of density and size of granules.

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