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Formation and performance of partial nitrification granular sludge treating domestic sewage

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ABSTRACT

Partial nitrification granular sludge (PNGS) treating domestic sewage was successfully cultivated in a sequencing batch reactor through shortening settling time gradually based on total suspended solids in the effluent. The granulation process contained the washout of a part of flocculent sludge, the gathering of the remaining flocculent sludge, and the growth of granules. Depending on the measurement of extracellular polymer substance (EPS), including polysaccharide (PS) and protein during different periods, it was concluded that EPS was the key factor for PNGS formation and PS was the main component of EPS in the PNGS biomass. PS was mainly from the secretion of heterotrophic bacteria during endogenous respiration period. The treatment ability of PNGS was improved effectively by high ammonia concentration in a short time (18 d), and then, PNGS achieved an efficient and stable partial nitrification for domestic sewage at 20˚C. The ammonia removal rate reached 0.7 $kg\,N\,m^{-3}\,d^{-1}$, and the nitrite accumulation rate was maintained above 95%. The granule structure improved the partial nitrification process, mainly due to the low DO concentration in the inner layer of the granules surface and the large amount of aeration in the bulk liquid.

Keywords: Domestic sewage; Partial nitrification; Granular sludge; EPS; Dissolved oxygen

1. Introduction

Compared with traditional nitrification–denitrification process, partial nitrification–Anammox process has many advantages, such as higher treatment load, less oxygen consumption, less sludge production, and no requirement for external organic carbon [1]. However, there are some problems about partial nitrification, which include a slow growth rate of nitrifying bacteria, an excessive loss of sludge in

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sequencing batch reactor (SBR), strict operation conditions, and difficulties in maintaining a long-time stable nitrite accumulation [2]. All of these reasons make it difficult to realize efficient and stable partial nitrification.

The characteristics of granular sludge could solve the problems about partial nitrification. Compared with traditional flocculent sludge, granular sludge has good settling property as its big size and high density. Therefore, granular sludge process could prevent sludge running off the reactor. Meanwhile, there is an obvious concentration gradient of substrate and dissolved oxygen (DO) from outside to inside of the granular sludge, which could improve the stability of treatment [3]. Thus, it is worthwhile to study the efficient and stable operation of partial nitrification using granular sludge.

Some researchers have been done about the cultivation and performance of partial nitrification granular sludge (PNGS) in recent years, as shown in Table 1. PNGS is a specific aerobic granule sludge which mainly aims at oxidizing ammonia to nitrite. Almost all the researches are about wastewaters with high ammonia concentration, and few has been done on wastewaters with low ammonia concentration, such as domestic sewage. Partial nitrification is difficult to be applied to domestic sewage treatment because of the characteristics of water quality: ammonia concentration of 30–90 mg L^{-1}, temperature of 10–25℃, and pH of 7.2–7.8. Although partial nitrification for treating domestic sewage has been successfully achieved using limited oxygen supply and real-time control strategy, both the stability of partial nitrification and the ability of ammonia oxidation are at low levels [2,3,11]. Thus, it is valuable to study the performance of PNGS treating domestic sewage.

Moreover, most researches of PNGS focus on cultivation method, performance, and microbiologic characteristics of PNGS, but the detailed information on the formation of PNGS has not been obtained, as shown in Table 1. So, exploring the granulation process and the key factor for PNGS is valuable to the application of PNGS. In view of the formation of common aerobic granular sludge, there have been many hypotheses in recent years, such as the nucleation hypothesis that inorganic salt or inert substance is used as a nucleus to form granular sludge [12], the self-cohesion hypothesis that external condition stimulates microbe to get together with each other to form granular sludge [13], the filamentous bacteria hypothesis that filamentous bacteria overlap to constitute a skeleton that is conducive for microbe adhering to form granular sludge [14], the extracellular polymer substance (EPS) hypothesis that adhesion and bridging of EPS contribute to the formation of granular sludge [15,16], and so on. However, there are conspicuous differences between PNGS and common aerobic granule sludge. The main bacteria of PNGS are autotrophic bacteria: nitrifying bacteria, while the main bacteria of common granule sludge are heterotrophic bacteria. So, the formation of PNGS needs further research.

Table 1
Researches on partial nitrification granular sludge

Year	References	Substrate	Main contents
2010	[4]	Synthetic wastewater with NH_4^+-N concentration from 380 to 1,520 mg/L	Long-term partial nitrification by nitrifying granules at room temperature and the reason for the stable partial nitrification
2011	[5]	Supernatant of anaerobic digestion of sludge with NH_4^+-N concentration 800 mg/L	Treatment performance for the supernatant of anaerobic digestion of sludge and biomass characterization
2012	[6]	Fosfomycin pharmaceutical wastewater with NH_4^+-N concentration from 200 to 550 mg/L	Partial nitrifying granule stimulated by struvite carrier in treating pharmaceutical wastewater
2012	[7]	Synthetic wastewater with NH_4^+-N concentration of 300 mg/L	Effects of step feed on granulation processes and nitrogen removal performances of partial nitrifying granules
2013	[8]	Synthetic wastewater with NH_4^+-N concentration of 125 mg/L	Rapid startup strategy of partial nitrification in continuous-flow reactor using aerobic granule
2013	[9]	Synthetic wastewater with NH_4^+-N concentration of 600 mg/L	Cultivation, performance, and microbial population structure of partial nitrification aerobic granule
2013	[10]	Synthetic wastewater with NH_4^+-N concentration of 400 mg/L	Activated carbon facilitated sludge granulation and microbial population dynamics

It is possible to achieve efficient and stable partial nitrification for domestic sewage by PNGS, so study about both the granulation process and key factor for PNGS are necessary. In this experiment, PNGS treating domestic sewage was cultivated in a SBR at 30℃, by reducing the settling time gradually, and the key factor for PNGS formation was indicated by the analysis of EPS in sludge during different periods. The treatment ability of PNGS was strengthened by high ammonia in a short period. Finally, the PNGS was operated at room temperature. The stability of partial nitrification under high DO was also discussed.

2. Materials and methods

2.1. Experimental setup and wastewater

PNGS was cultured in a SBR. Its working volume was 4 L, height was 100 cm, and inner diameter was 8 cm. Oxygen was supplied by an air compressor through air diffusers installed in the reactor. Temperature was controlled by a thermostatic bath. The aeration rate was always kept at 2.0 L min^{-1}, corresponding to a superficial air velocity of 0.66 cm s^{-1}. DO and pH were monitored online.

There were 4 operation cycles per day, which were realized by an automatic controlling system. Each cycle consisted of feeding (1 min), aeration (control aeration time by measuring ammonia concentration in inflow and effluent every day to maintain ammonia oxidation rate at 80–90%), sedimentation (reduce gradually from 15 to 4 min), and decanting (2 min). The volumetric exchange ratio in the reactor was 62.5%. After 4 operation cycles, the reactor was in idle per day. Sludge was cleaned twice with tap water at the end of the fourth cycle in order to prevent denitrification in idle time.

Domestic sewage (main characteristic described in Table 2) was collected each day from a residential district sewer. It was firstly pumped into an intermediate tank before being induced into the reactor.

Table 2
Characteristics of the domestic sewage

Contents	Range	Average
COD (mg L^{-1})	223.5–348.2	293.4
NH_4^+-N (mg L^{-1})	5	69.45
NO_2^--N (mg L^{-1})	0.00–0.94	0.21
NO_3^--N (mg L^{-1})	0.00–2.49	0.61
pH	7.32–7.71	7.51
Alkalinity ($CaCO_3$ mg L^{-1})	492–614	534
SS (mg L^{-1})	83.4–143.5	108.4

2.2. Seeding sludge and operational strategy

Seeding sludge, taken from the Gaobeidian wastewater treatment plant located in Beijing, was cultured in the reactor using wastewater with high concentration of ammonia until its nitrite accumulation rate (NAR) reached 95%. And the concentration of seeding sludge was 3.40 g VSS L^{-1}.

As shown in Table 3, the experiment was divided into three main phases, including granule cultivating stage at 30℃ (I), load improving stage (II), and room temperature operation stage (III). In the stage I, domestic wastewater was treated and the settling time was gradually shortened from 15 to 4 min based on total suspended solids (TSS) in the effluent. In stage II, $(NH_4)_2SO_4$ and $NaHCO_3$ were added into domestic sewage until the concentration of ammonia and alkalinity ($CaCO_3$) reached 175 ± 10 mg L^{-1} and $1,500 \pm 80$ mg L^{-1}, respectively. Temperature was 30 ± 1℃ to maintain partial nitrification of sludge in stage I and II and was changed to 20 ± 1℃ to investigate the performance of PNGS for domestic sewage at room temperature in stage III.

2.3. Analytical methods

Ammonia, nitrite, nitrate, COD, alkalinity, TSS, and VSS were measured according to the APHA standard methods [17]. DO and pH were monitored online by DO/pH determinator (Multi 340i, WTW, Germany). The morphological characteristic of sludge was observed and recorded by optical microscope (BX51, Olympus, Japan) and digital camera (C-4040 zoom, Olympus, Japan); structure of the granular sludge was observed and recorded by scanning electron microscope (SEM) (S-4300, Hitachi, Japan). Average size, size distribution, and specific surface area of sludge were measured by laser granule size analyzer (Mastersizer 2000, Malvern, Britain). EPS was extracted by heat extract method [18], polysaccharide (PS) was measured by phenol–sulfate acid method, and protein was measured by coomassie brilliant blue method. All of the samples were obtained in the first cycle of every day.

3. Results and discussion

3.1. Reactor performance and granulation process

3.1.1. Reactor performance

Treatment performance of the reactor was shown in Fig. 1 Due to the high temperature, NAR $(\Delta NO_2^- - N/(\Delta NO_2^- - N + \Delta NO_3^- - N))$, where Δ represents the difference between influent and

Table 3
Operational conditions in different stages

Stage	Cycles	Settling time (min)	Temperature (℃)	Ammonia concentration (mg L^{-1})
I-1	1–20	15	30 ± 1	70 ± 10
I-2	21–64	13	30 ± 1	70 ± 10
I-3	65–100	10	30 ± 1	70 ± 10
I-4	101–148	8	30 ± 1	70 ± 10
I-5	149–184	6	30 ± 1	70 ± 10
I-6	185–360	4	30 ± 1	70 ± 10
II-1	361–432	4	30 ± 1	175 ± 10
II-2	433–476	4	30 ± 1	70 ± 10
III	477–636	4	20 ± 1	70 ± 10

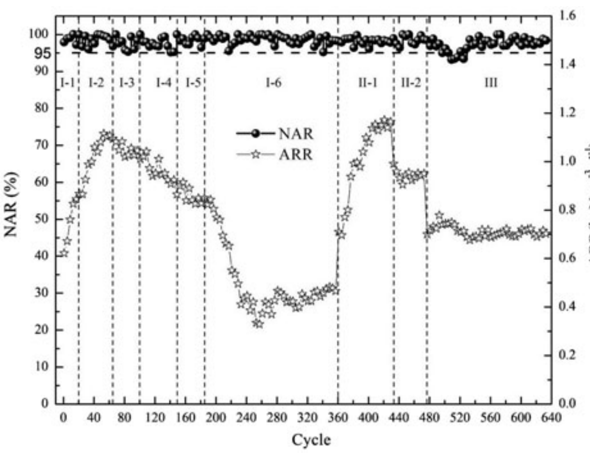

Fig. 1. Variations of NAR and ARR during the experiment.

effluent) remained above 95% in the whole stage I and II. In the stage I, ammonia removal rate (ARR, mass of ammonia consumed in unit volume of reactor per day) firstly increased, reaching 1.107 kg N m^{-3} d^{-1}, and then reduced gradually during the formation of PNGS, which was caused by the adaptation of microbe to the domestic sewage and the change of sludge concentration. ARR held steady at 0.46 kg N m^{-3} d^{-1} at the end of the stage I and did not increased with the growth of granules (Fig. 3). In order to improve the treatment ability of PNGS, additional ammonia had been added into the domestic sewage since 361st cycle (Stage II-1). After that the ARR increased to 1.150 kg N m^{-3} d^{-1} in 405th cycle, it remained stable for seven days. Then, domestic sewage without additional ammonia was introduced to the system. The ARR reduced slightly and then remained stable at 0.940 kg N m^{-3} d^{-1} after 440th cycle. By operating under high ammonia concentration, the ammonia-oxidizing ability of PNGS could be strongly improved with an ARR doubling in a short time (18 d) and could still be remained.

In order to investigate the partial nitrification stability of PNGS for domestic sewage at room temperature, the system was operated under 20℃ after 477th cycle (Stage III), and NAR decreased to 93.5% from 509th cycle to 525th cycle. After a short time adaptation, NAR recovered to above 95% again in 530th cycle. Until 633rd cycle, ARR was 0.700 kg N m^{-3} d^{-1}. Being operated under 20℃, the system remained stable for over 40 d, which was about twice longer than sludge retention time of the reactor in this study.

3.1.2. Granulation process

Considering a too short settling time could lead to an excess loss of sludge in SBR and the sludge concentration in partial nitrification process for domestic sewage increased slowly, the settling time was gradually shortened from 15 to 4 min based on TSS in the effluent. The change of the TSS concentration in the effluent became smaller over the time, showing that the sludge had adapted to this settling time and it is time to shorten to the next settling time.

The morphological characteristic of the sludge in typical periods is shown in Fig. 2. Average size of sludge and distributions of granule size at the end of each stage are shown in Fig. 3. Stage I-6 was described in three stages, including I-6-1 (185–229 cycle), I-6-2 (229–292 cycle), and I-6-3 (293–360 cycle) due to the great variations of granule size and EPS.

The seeding sludge was completely flocculent (Fig. 2(a)), the average size was only 0.083 mm, and granules less than 0.2 mm accounted for 95.6%. When settling time was above 10 min (stage I-1 & I-2), there were no obvious variations in the morphological characteristic of sludge and granular size. However, when settling time was shortened to 10 min (stage I-3), some of them grew up to tiny granules which were observed at the end of this stage (Fig. 2(b)). After that settling time was gradually shortened from 10 to

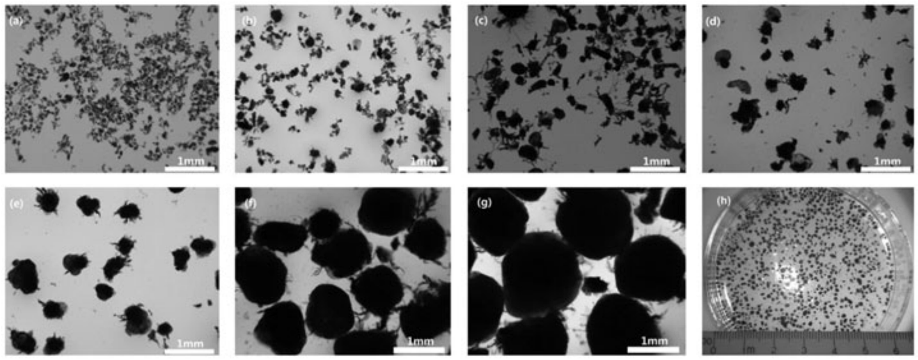

Fig. 2. The morphological characteristic of sludge in typical periods. (a) Seeding sludge (I-1), (b) 100th cycle (I-3), (c) 148th cycle (I-4), (d) 184th cycle (I-5), (e) 228th cycle (I-6), (f) 360th cycle (I-6), (g) and (h) 636th cycle (III).

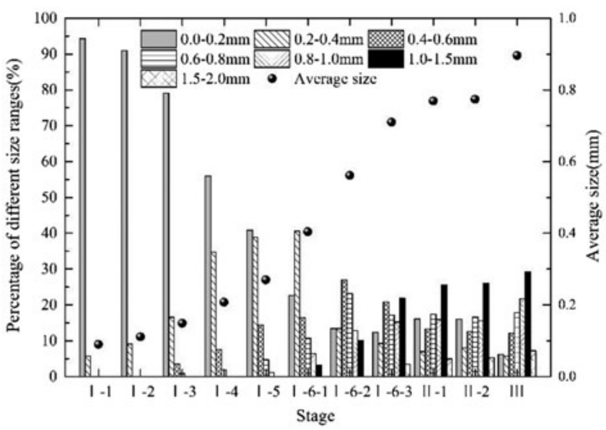

Fig. 3. Average size of sludge and distributions of granule size at the end of each stage.

4 min (stage I-4–I-6-1), average size increased rapidly with more and more flocculent sludge being washed out of the reactor, and the remaining flocculent sludge gathered to be small granules (Fig. 2(c)–(e)). From stage I-6-1, granules could be observed significantly by naked eyes (Fig. 2), average size of sludge reached at 0.404 mm and granules less than 0.2 mm only accounted for 22.62% (Fig. 3). At the end of stage I-6-1, the PNGS had formed. The sludge concentration reduced from 3.40 to 1.60 g VSS L^{-1}. From then on, settling time was kept in 4 min (stage I-6-2–III), flocculent sludge was eliminated sufficiently, granules grew up rapidly with the size becoming bigger and the shape becoming more regular (Fig. 2(f)). At the end of stage III, the average size reached 0.896 mm and granules less than 0.2 mm only accounted for 7.80% (Fig. 3). The sludge concentration also increased to 3.12 g VSS L^{-1}. PNGS was close to sphere (Fig. 2(g) and (h)) and the sludge whose roundness over 0.7 accounted for more than 80%.

Distributions of granule size at the end of each stage are shown in Fig. 3. From stage I-3 to stage I-6-1, the percentage of granules less than 0.2 mm reduced quickly and the percentage of other size ranges all increased gradually, especially for 0.2–0.6 mm. Until stage I-6-1, flocculent sludge was either eliminated or gathered. From stage I-6-2 to stage III, granule size distribution transferred from small range to large range, with the gradual growth of granules. Most granules reached 0.6–1.2 mm at last. The distribution of granule size tended to be stable and approached to normal distribution.

The granulation process contained the washout of a part of flocculent sludge, the gather of the remaining flocculent sludge and the growth of granules. Granules formed from stage I-3 to stage I-6-1; in this period, a part of flocculent sludge was washed out and the others gathered to be small granules. Then, granules grew up from stage I-6-2 to stage III; in this period, the size became bigger and the shape became more regular.

3.2. Key factor for PNGS formation

3.2.1. Effects of EPS

It was effective to cultivate PNGS by shortening settling time gradually based on TSS in the effluent. Appropriate settling time could eliminate sludge with worse settling property by hydraulic filtration and led the granular sludge to be the main part of the reactor. However, the gathering of flocculent sludge and the growth of granules could not be explained clearly by the hydraulic filtration of settling time. So, there must be something that contributes to the formation of PNGS.

Previous studies [15,16] have shown the effect of EPS on the formation of common aerobic granular sludge. So, in this study, EPS was measured every three days to help explain the formation of PNGS. Relationships between average EPS and velocity of size increasing in each stage are shown in Fig. 4.

From stage I-1 to I-6-1, with settling time being shortened, EPS increased and plenty of sludge gathered to be granules, so that the granule size increased quickly. The increase of granule was faster with higher concentration of EPS. Then, EPS decreased in stage I-6-2 and I-6-3. This could be explained as the EPS was extracted from the surface of sludge and the specific surface area of sludge reduced from 0.0583 $m^2\,g^{-1}$ at the beginning of stage I-6-1 to 0.0210 $m^2\,g^{-1}$ at the end of I-6-3. Velocity of size increasing reduced with the decrease of EPS. So, velocity of size increasing and EPS were positive correlation during granule cultivating stage. In stage II, PNGS tended to stability, velocity of size increasing reduced significantly and EPS increased slowly. Probably, in order to adapt to the lower temperature, bacteria secreted more EPS in stage III. EPS increased a lot and velocity of size increase also increased significantly. So, it was inferred that EPS was the key factor for PNGS formation.

EPS could make microbes gather by electrostatic interaction and bridging action of divalent ions. Meanwhile, the entanglement of polymer chains in EPS could also make a big contribution to adsorption among microbe [19]. So, the sludge with higher EPS was more likely to gather and could be held in the reactor. With settling time being shortened, the sludge with less EPS was washed out gradually, so that the concentration of EPS in the reactor became higher. Sludge was gathered by EPS, which lead to the formation and growth of PNGS.

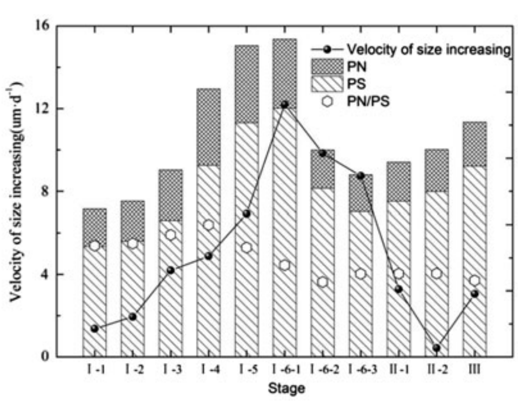

Fig. 4. Relationship between average EPS and velocity of size increasing in each stage. EPS consists of PS and PN. Velocity of size increasing was calculated by the average granule sizes at the end of each stage.

3.2.2. Main component of EPS

As shown in Fig. 4, PS and protein (PN) that are components of EPS, were also measured during the experiment. Many studies have shown that PN is the main component of EPS during the formation process of common aerobic granule sludge [15,16,20]. Most components of PN are hydrophobic amino acids with positive charge which can neutralize negative charge on sludge surface, and most components of PS are hydrophilic groups with a negative charge. The increase of PN/PS can reduce the negative charge on sludge surface and improves hydrophobic property of sludge surface, which can promote the gathering of sludge and maintaining of granular structure [15,20]. However, in this research, PS was the main component of EPS during the formation process of PNGS, and on the contrary, the increase of PN was always little. From stage I-4 to I-6-2 with the sludge gathering quickly, PS increased largely which led to the decrease of PN/PS. PS could promote the formation of granule, and the strength of gel that formed by PS in aerobic granular sludge was apparently higher than that in flocculent sludge [21]. The bridging of PS could promote the gather and conglutination among microbes [22]. Thus, it was inferred that PS played a decisive role in the formation of PNGS.

The standards for stopping aeration of common aerobic granule sludge reactor and PNGS reactor were different. During the cultivation of PNGS, the oxidation of ammonia was considered as the standard of stopping aeration, while the oxidation of COD was considered as the standard of stopping aeration for common aerobic granule sludge. In this study, variations of main substrates in a typical SBR cycle were monitored. In the stage III, when COD was completely oxidized (after 60 min), ammonia oxidation rate just reached 60%, and heterotrophic bacteria would be in starvation without substrate from then on. The high DO in the reactor (more than 5 $mg\,L^{-1}$) further accelerated endogenous respiration of heterotrophic bacteria. The system was in a state of "extended aeration of heterotrophic bacteria." In an endogenous respiration period, bacteria secrete the capsules which are extracellular storage material [23], whose major component is exactly PS.

In order to prove this, EPS in typical cycle was also measured, as shown in Fig. 5. The values of EPS were the averages of the first cycles in three consecutive days. In order to have an ammonia removal efficiency of 80%, the reaction should have been stopped at 90 min in normal operation cycle. However, to further study the variations of EPS, aeration time was extended to 150 min. There was no obvious variation

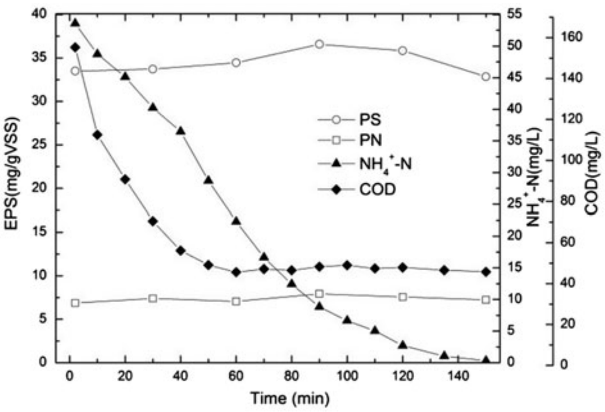

Fig. 5. Variations of EPS and main substrates in a typical cycle of stage III.

in PN, while PS changed significantly. During the first 60 min of the reaction time, COD had been consumed completely and the concentration kept stable for 30 min, which resulted in the secreting of PS by heterotrophic bacteria in the period of endogenous respiration state. The concentration of PS got its maximum at 90 min, and at the same time, ammonia oxidation rate achieved to 83.5% which exactly met the standard of stopping aeration in each cycle. All these demonstrated the results and discussions mentioned above. PS was mainly from the secretion of heterotrophic bacteria in the period of endogenous respiration state. With the aeration time extending to 150 min, PS gradually decreased because heterotrophic bacteria used it as the organic carbon source.

From the above, the reason of PNGS formation could be explained. Since the oxidation of ammonia was as the standard of stopping aeration in partial nitrification SBR, heterotrophic bacteria were in the period of endogenous respiration state at the end of the aeration phase. They secreted plenty of PS, which led to the increasing of EPS, in this period. EPS could make sludge gather and make a contribution to adsorption among microbe. So, granule sludge formed and grew. Meanwhile, with settling time being shortened gradually, sludge which did not gather by EPS was washed out of the reactor, so that the concentration of EPS in the sludge was further improved. With the gathering and the elimination of sludge, PNGS became the main part of the reactor. The contribution of EPS to the formation of PNGS was similar to the related research of aerobic granular sludge. However, the main component and source of EPS was different.

3.2.3. SEM results

In order to further study the function of EPS, the structure of PNGS was observed by SEM. In 636th cycle, granular sludge was cut into halves. It was observed by naked eyes that the color of the core of PNGS was black, which meant the existence of anaerobic zone. The cross section of PNGS was observed using SEM, as shown in Fig. 6.

PNGS was regularly round in good integrity (Fig. 6(a)). After pretreatment of SEM, granule was dewatered. The dehydration of inside and outside was different, so that the core of granule was bulgy. The bulgy core was identical to the black core observed by naked eyes. It was inferred that the core was the anaerobic zone and DO could arrive at the distance only about 100 μm away from the surface of granules. According to the further observation, it was discovered that there were plenty of substances which were not bacteria in the core, as shown in Fig. 6(b). It was guessed that the substances were EPS, especially PS, whose adhesion and bridging contributed to the formation of granule.

In the research of Linlin et al. [12], the results of fluorescence *in situ* hybridization (FISH) of PNGS cross section indicated that most abundant population located in the outside of the granule belong to ammonia-oxidizing bacteria (AOB). Nitrite-oxidizing bacteria (NOB) were also present but in very low amounts widespread in the outside of the granule. As shown in Fig. 6(c) and (d), the shape of microbe in both surface area and inside of the aerobic zone was similar. Short rod-shaped bacteria and round-shaped bacteria were in mixed distribution, and the population of the former was much more than that of the latter. It could infer that short rod-shaped bacteria were AOB and round-shaped bacteria were nitrite-oxidizing bacteria (NOB).

3.3. *Influencing factors for partial nitrification of PNGS*

In this research, an efficient and stable partial nitrification for domestic sewage treatment at room temperature was obtained by PNGS. Being operated under 20℃, the reactor remained stable for over 40 d with ARR of 0.7 kg N m^{-3} d^{-1} and NAR of above 95%.

Some researchers have realized partial nitrification on the similar conditions by flocculent sludge, but both the NAR and ARR were at low levels. Zeng et al. has gotten NAR of 80% and ARR of 0.160 kg N m^{-3} d^{-1} [2] using residential sewage at 20℃; Guo et al. has gotten NAR of 88% and ARR of 0.224 kg N m^{-3} d^{-1} [3] using campus sewage at 18–25℃. Thus, both the stability of

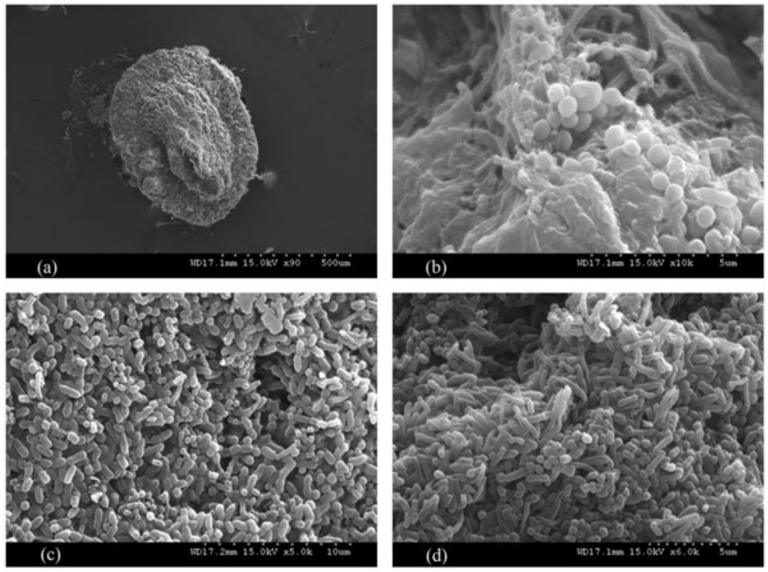

Fig. 6. Scanning electron microscope images of PNGS cross section. (a) Complete cross section, (b) the core, (c) surface area, and (d) inside of the aerobic zone.

partial nitrification and the treatment ability in this experiment were higher than these reports.

In order to study the reason for the efficient and stable partial nitrification of PNGS, variations of the main factors for partial nitrification in a typical cycle were measured, as shown in Fig. 7. Each value was the average of the first cycle in three consecutive days. The growth rates of AOB and NOB were basically the same at 20˚C [24]. The best pH levels for AOB and NOB growth were 7.7–8.1 and 7.0–7.8, respectively [25]. During the reaction phase, only in the first 15 min, pH was above 7.7, and most time, it was beneficial for NOB. The lowest inhibition concentrations of free ammonia (FA) and free nitrite acid (FNA) for NOB were 1.0 and 0.011 mg L^{-1}, respectively [26,27]. Only in the first 20 min, FA was more than 1.0 mg L^{-1}, while for the most of time, it did not reach the FA inhibition concentration for NOB. FNA could not reach the inhibition concentration throughout the reaction phase. The oxygen saturation constant of AOB and NOB was 0.2–0.4 and 1.2–1.5 mg L^{-1}, respectively [28]. DO in the bulk liquid increased rapidly to 7.3 mg L^{-1} in 15 min and then increased slowly to 9.2 mg L^{-1}. It is completely enough for both AOB and NOB. There was no conspicuous washout of sludge after the formation of PNGS that led to a long SRT which was also not benefit to NOB. Therefore, all the factors mentioned above could not maintain stable partial nitrification.

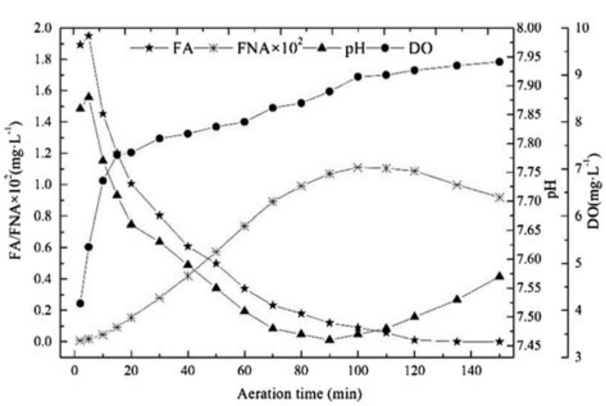

Fig. 7. Variations of the main factors for partial nitrification in a typical cycle of stage III.

Compared with flocculent sludge, the biggest characteristic of the granular sludge was the structure, which might result in the stable operation of partial nitrification. According to analysis, there was a great difference between the concentration of DO in liquid and DO arrives on the surface of microbe. Firstly, oxygen transmitted from the bulk liquid to the surface of granule sludge, so that the value of the transmitting area had a great impact on the oxygen transfer. In this study, the ratios of total surface area of sludge to liquid volume were 2,430 and 225 $m^2 m^{-3}$ for seeding sludge and PNGS, respectively. Actually, the transmitting area reduced largely, which limited the concentration of transferred DO. Secondly, the granules were very dense, which resisted DO from diffusing into the inner of granules. DO could arrive at the distance only about 0.1 mm away from the surface of granules, which was proved by the SEM result. Only the surface of granules was exposed at relatively high DO, while most microbes of PNGS were at low DO, which was beneficial for AOB. Furthermore, the oxidation of COD was always quicker than that of ammonia, which indicated that heterotrophic bacteria had an advantage over nitrifying bacteria in DO using. The striving of heterotrophic bacteria further reduced the DO on the surface of nitrifying bacteria. The low concentration of DO had a stronger inhibition of NOB than AOB. So, the stable partial nitrification could be maintained.

Although the reduction of transmitting area and the striving of heterotrophic bacteria could decrease the concentration of DO on the surface of nitrifying bacteria, they would not reduce the speed of DO transmission. The shell of granules could resist substrates including DO and ammonia diffusing, but the speed of substrates transmission would be enhanced by the large concentration gradient. What is more, the flow pattern of substrates would be strengthened by large amount of aeration, so that both of the speed of substrates transmission and the probability of contact between substrates and bacteria were enhanced. So, the treatment load was kept at a relatively high level.

4. Conclusions

It was effective to cultivate PNGS by shortening settling time gradually. The average granular size reached 0.863 mm and granules less than 0.2 mm only accounted for 7.8%. During the granulation process, a part of flocculent sludge was washed out and the others gathered to be small granules, and then, granules became bigger and more regular. EPS was the key factor for PNGS formation and PS was the main component of EPS in the PNGS. PS was mainly from the secretion of heterotrophic bacteria during endogenous respiration period. This was different from common aerobic granule sludge that mainly aims at oxidizing organic. The system maintained good performance of partial nitrification for domestic sewage with ARR of $0.70\,kg\,N\,m^{-3}\,d^{-1}$ and NAR of above 95% under high DO concentration at 20˚C. The good performance was achieved thanks to the structure of granules and large amount of aeration.

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References

[1] M. Strous, E. Van Gerven, P. Zheng, J.G. Kuenen, M.S. Jetten, Ammonium removal from concentrated waste streams with the anaerobic ammonium oxidation (anammox) process in different reactor configurations, Water Res. 31 (1997) 1955–1962.
[2] B. Sinha, A.P. Annachhatre, Partial nitrification—Operational parameters and microorganisms involved, Rev. Environ. Sci. Biotechnol. 6 (2007) 285–313.
[3] J. Grotenhuis, M. Smit, C. Plugge, Y. Xu, A. Van Lammeren, A. Stams, A. Zehnder, Bacteriological composition and structure of granular sludge adapted to different substrates, Appl. Environ. Microbiol. 57 (1991) 1942–1949.
[4] J. Vázquez-Padín, M. Figueroa, J. Campos, A. Mosquera-Corral, R. Méndez, Nitrifying granular systems: A suitable technology to obtain stable partial nitrification at room temperature, Sep. Purif. Technol. 74 (2010) 178–186.
[5] S. Lopez-Palau, A. Pericas, J. Dosta, J. Mata-Alvarez, Partial nitrification of sludge reject water by means of aerobic granulation, Water Sci. Technol. 64 (2011) 1906–1912.
[6] G. Wang, D. Wang, X. Xu, F. Yang, Partial nitrifying granule stimulated by struvite carrier in treating pharmaceutical wastewater, Appl. Microbiol. Biotechnol. 97 (19) (2013) 8757–8765.
[7] X.-H. Wang, L.-X. Jiang, Y.-J. Shi, M.-M. Gao, S. Yang, S.-G. Wang, Effects of step-feed on granulation processes and nitrogen removal performances of partial nitrifying granules, Bioresour. Technol. 123 (2012) 375–381.
[8] C.L. Wan, S.P. Sun, D.J. Lee, X. Liu, L. Wang, X. Yang, X.L. Pan, Partial nitrification using aerobic granules in continuous-flow reactor: Rapid startup, Bioresour. Technol. 142 (2013) 517–522.
[9] Y. Song, S. Ishii, L. Rathnayake, T. Ito, H. Satoh, S. Okabe, Development and characterization of the partial nitrification aerobic granules in a sequencing batch airlift reactor, Bioresour. Technol. 139 (2013) 285–291.
[10] A.-J. Li, X.-Y. Li, H.-Q. Yu, Aerobic sludge granulation facilitated by activated carbon for partial nitrification treatment of ammonia-rich wastewater, Chem. Eng. J. 218 (2013) 253–259.
[11] S.Y. Wang, S.F. Zhang, C.Y. Peng, A. Takigawa, Intercross real-time control strategy in alternating activated sludge process for short-cut biological nitrogen removal treating domestic wastewater, J. Environ. Sci. (China) 20 (2008) 957–963.
[12] H. Linlin, W. Jianlong, W. Xianghua, Q. Yi, The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules, Process Biochem. 40 (2005) 5–11.
[13] S.S. Adav, D.-J. Lee, K.-Y. Show, J.-H. Tay, Aerobic granular sludge: Recent advances, Biotechnol. Adv. 26 (2008) 411–423.

[14] J. Beun, A. Hendriks, M. Van Loosdrecht, E. Morgenroth, P. Wilderer, J. Heijnen, Aerobic granulation in a sequencing batch reactor, Water Res. 33 (1999) 2283–2290.
[15] B. McSwain, R. Irvine, M. Hausner, P. Wilderer, Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge, Appl. Environ. Microbiol. 71 (2005) 1051–1057.
[16] S.S. Adav, D.-J. Lee, J.-H. Tay, Extracellular polymeric substances and structural stability of aerobic granule, Water Res. 42 (2008) 1644–1650.
[17] J.H. Tay, Q.S. Liu, Y. Liu, The role of cellular polysaccharides in the formation and stability of aerobic granules, Lett. Appl. Microbiol. 33 (2001) 222–226.
[18] G.-P. Sheng, H.-Q. Yu, X.-Y. Li, Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review, Biotechnol. Adv. 28 (2010) 882–894.
[19] S.-F. Yang, X.-Y. Li, Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions, Process Biochem. 44 (2009) 91–96.
[20] L. Zhang, X. Feng, N. Zhu, J. Chen, Role of extracellular protein in the formation and stability of aerobic granules, Enzyme Microbiol. Technol. 41 (2007) 551–557.
[21] T. Seviour, M. Pijuan, T. Nicholson, J. Keller, Z. Yuan, Gel-forming exopolysaccharides explain basic differences between structures of aerobic sludge granules and floccular sludges, Water Res. 43 (2009) 4469–4478.
[22] Y.-Q. Liu, Y. Liu, J.-H. Tay, The effects of extracellular polymeric substances on the formation and stability of biogranules, Appl. Microbiol. Biotechnol. 65 (2004) 143–148.
[23] B.E. Rittmann, P.L. McCarty, Environmental Biotechnology, McGraw-Hill, New York, NY, 2001.
[24] B. Balmelle, K. Nguyen, B. Capdeville, J. Cornier, A. Deguin, Study of factors controlling nitrite build-up in biological processes for water nitrification, Water Sci. Technol. 26 (1992) 1017–1025.
[25] D.S. Hagopian, J.G. Riley, A closer look at the bacteriology of nitrification, Aquacult. Eng. 18 (1998) 223–244.
[26] O. Turk, D.S. Mavinic, Maintaining nitrite build-up in a system acclimated to free ammonia, Water Res. 23 (1989) 1383–1388.
[27] V.M. Vadivelu, Z. Yuan, C. Fux, J. Keller, The inhibitory effects of free nitrous acid on the energy generation and growth processes of an enriched Nitrobacter culture, Environ. Sci. Technol. 40 (2006) 4442–4448.
[28] H.J. Laanbroek, S. Gerards, Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed continuous cultures, Arch. Microbiol. 159 (1993) 453–459.