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PII: S0960-8524(18)31477-9
DOI: https://doi.org/10.1016/j.biortech.2018.10.046
Reference: BITE 20610

To appear in: *Bioresource Technology*

Received Date: 12 September 2018
Revised Date: 17 October 2018
Accepted Date: 19 October 2018

Please cite this article as: Li, D., Zhang, S., Li, S., Zeng, H., Zhang, J., The nitrogen removal of autotrophic and heterotrophic bacteria in aerobic granular reactors with different feast/famine ratio, *Bioresource Technology* (2018), doi: https://doi.org/10.1016/j.biortech.2018.10.046

The nitrogen removal of autotrophic and heterotrophic bacteria in aerobic granular reactors with different feast/famine ratio

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Abstract:

Aerobic granular sludge was cultivated in three column reactors, which had been operated for 120 days under different feast/famine ratio (1:7, 1:11, 1:15). The composition of total bacteria was analyzed by testing oxygen uptake rates of mixed liquor samples taken from the reactors and calculating according to activated sludge model. The results revealed that long famine phase favored the growth of heterotrophic bacteria. The heterotrophic bacteria accounts for 49.80, 53.37, 91.39% of total bacteria respectively in R_1, R_2 and R_3. The heterotrophic nitrification was also observed in all the reactors, which accounts for 58.62, 58.33, 61.54% of total nitrification respectively in R_1, R_2 and R_3. A novel nitrogen-removal pathway involving simultaneous nitrification-denitrification by heterotrophic nitrification bacteria was proposed. The

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results revealed that microbial system consisted of heterotrophic ammonia oxidizing bacteria showed stronger capacity of simultaneous nitrification-denitrification.

Keywords: heterotrophic nitrification; nitrogen-removal pathway; feast/famine time ratio; aerobic granular sludge

1. Introduction

Aerobic granular sludge (AGS) technology was widely applied to wastewater treatment due to its outstanding settling ability and capacity of simultaneous nitrification, denitrification and phosphorus removal. Previous studies (Bassin et al., 2012; He et al., 2018; Wan et al., 2009) have demonstrated the possibility of simultaneous removal of COD, N and P in aerobic granular reactors under an anoxic/oxic (A/O) regime. It has been recognized that simultaneous nitrification-denitrification (SND) under aeration condition is a key characteristic of AGS systems which has advantages over the separated nitrification and denitrification processes (Derlon et al., 2016; Yoo et al., 1999). Based on the studies on biofilms, an aerobic outside zone and an anoxic interior zone of the granule were required for nitrification and denitrification, so that the size of a granule is crucial for SND under a certain dissolved oxygen (DO) concentration, (de Kreuk et al., 2005). If the granule size is not large enough, the SND will be limited because of the diffusion of electron donors and acceptors that determine the anoxic volume within the

granules (Derlon et al., 2016). However, all the mentioned above is based on the hypothesis that ammonia oxidizing bacteria (AOB) and denitrification bacteria play their part separately in the oxic and anoxic zone of the granules (Zeng et al., 2003), but in recent years, it has been demonstrated the existence of some novel strains of heterotrophic AOB with the capacities of aerobic ammonium oxidation and aerobic denitrification (Niel et al., 1992; Padhi et al., 2017), while autotrophic AOB was considered as the dominant bacteria in the nitrification process (Liang et al., 2015). The heterotrophic AOB is generally known as for being well-tolerated in harsh environments (Duan et al., 2015; Huang et al., 2017; Shoda & Ishikawa, 2014; Yao et al., 2013) and having higher nitrogen-removal capacity, compared with autotrophic AOB (Ren et al., 2014; Yang et al., 2015). The studies (He et al., 2018; Khanichaidecha et al., 2018; Neerackal et al., 2016; Padhi et al., 2017; Rout et al., 2017; Zhang et al., 2017) on heterotrophic AOBs mainly focused on isolating pure cultures of heterotrophic nitrification bacteria, yet it is not clear about the SND capacities of AGS systems containing heterotrophic AOB. Commonly, the endogenous respiration rates of heterotrophic bacteria and autotrophic bacteria are different (Ni et al., 2008; Zeng et al., 2015) which indicates the difference in tolerability of heterotrophic and autotrophic bacteria under starvation conditions. While the competition growth also has not been investigated for autotrophic and heterotrophic bacteria surviving in granular reactors under different starvation conditions.

On the whole, this study has three objectives: (i) to start up aerobic granular reactors with same anoxic/oxic (A/O) time ratio and different feast/famine time ratio; (ii) to better understand the growth and competition of autotrophic bacteria and heterotrophic bacteria under different famine duration, and propose a nitrogen-removal pathway in the reactors; (iii) to evaluate the performances of aerobic granular sludge (AGS) system in different operation conditions, the morphology and physical properties of the granules were studied also.

2. Materials and methods

2.1. Reactor set-up and operation

Three lab-scale column reactors (R_1, R_2 R_3) with work volume 6.16L (0.14m of diameter and 0.4m of height) were operated under three different cycle-time with different feast/famine ratios. An operation cycle was consisted of four phases as following: (i) anaerobic feeding phase, (ii) anaerobic reaction phase, (iii) aerobic reaction phase, (iv) settling phase. The operation details can be found in Table 1. Airflow rate of 0.6 L/min was applied on all the three reactors by air diffusers placed in the bottom of the reactors.

The reactors were inoculated with conventional activated sludge from a wastewater treatment plant (WWTP) of Beijing, which had COD, TP and nitrogen removal abilities. The synthetic feeding medium included (per liter): 0.142g NH_4Cl (NH_4^+-N = 30mg/L), 0.022g KH_2PO_4 (PO_4^{3-}-P=5mg/L), 0.257g $C_3H_5O_2Na$ (COD = 300mg/L), 0.040 g $MgSO_4$ and 0.030 g $CaCl_2$. All the medium was dissolved in tap water.

During the whole operation term, effluent samples were collected every three days for analyzation of COD, ammonia-nitrogen (NH_4^+-N), nitrite-nitrogen (NO_2^--N), nitrite-nitrogen (NO_3^--N) and Phosphate(PO_4^{3-}-P), while mixed liquor samples were collected once a week for analyzation of mixed liquor volatile suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and sludge volumetric index (SVI).

2.2. Cycle tests

Cycle tests were conducted at the end of operation, when the reactors achieved a pseudo-steady-state condition. 11 samples were collected in every cycle test, the first of which was taken 10 seconds after the beginning of aeration. The concentrations of PO_4^{3-}-P, COD, NH_4^+-N, NO_2^--N and NO_3^--N were analyzed, dissolved oxygen (DO) concentrations measured continually (data not shown). The consumption rate of COD, the ammonium uptake rate (AUR) and the nitrite or nitrate production rate (NiPR or NaPR) were obtained from linear regression of their corresponding concentrations. Based on the principle of mass balance shown as equation (1), the difference between the AUR and the sum of the NiPR and NaPR and the nitrogen utilization rate for cell synthesis (α_{SND}) was used for estimating whether SND occurred or not (if α_{SND} was greater than 0 then the SND occurred, otherwise the SND did not occur). The nitrogen utilization rate for cell synthesis was estimated as equation (2) (Derlon et al., 2016). The default bio-kinetic parameters were from literature (Henze et al., 2000).

NH_4^+-N = NO_2^--N + NO_3^--N + nitrogen for utilization (1)

$$r_{N,synthesis} = i_{N,VSS} \cdot i_{VSS,TSS} \cdot P_X \quad (2)$$

where:

$r_{N,synthesis}$: nitrogen utilization rate for cell synthesis, mg/ (min · L);

$i_{N,VSS}$: nitrogen to volatile suspended solids, 0.08 g N/g VSS;

P_X: sludge production, which can be calculated as equation (3), mg/ (min · L).

$$P_X = i_{S_S,BM} \cdot Y_{H,O_2} \cdot v_{COD} \quad (3)$$

where:

$i_{S_S,BM}$: suspended solids to biomass COD ratio, 0.9 g TSS/g COD;

Y_{H,O_2}: Aerobic yield of heterotrophic biomass, 0.64 g COD/g COD;

v_{COD}: consumption rate of COD, mg/ (min · L).

After the whole operation period, all reactors were fed with domestic wastewater (from a residential area of Chaoyang District, Beijing), then series of cycle tests using domestic wastewater (water quality on that day: COD = 253 mg/L, NH_4^+-N = 44mg/L, TP = 7.214mg/L) were applied to investigate the performance of the reactors under different feast/famine conditions. The measurement procedure was the same as that adopted in cycle tests for synthetic wastewater, and The concentrations of PO_4^{3-}-P, COD, NH_4^+-N, NO_2^--N and NO_3^--N were analyzed.

2.3. Analytical measurements

PO_4^{3-}-P, COD, NH_4^+-N, NO_2^--N, NO_3^--N, MLSS, MLVSS and SVI were analyzed according to APHA standard methods(APHA, 1998). Temperature, pH and dissolved oxygen (DO) concentration were determined using the online probes pH/oxi 340i (WTW, Germany). Laser particle size analyzer (Mastersizer 2000, Malvern, UK) was

used to analyze the size and distribution of granules. Optical microscopy images were taken with an Olympus BX 51/52.

2.4. Estimating biomass of autotrophic/heterotrophic bacteria

To quantify the autotrophic and heterotrophic active biomass concentrations as well as their composition of total bacteria, batch aerobic digestion tests (Lee et al., 2006) were applied to the samples. It was assumed that the oxygen was consumed by either heterotrophic or autotrophic bacteria in the reactors during the endogenous respiration phase. The total bacteria oxygen uptake rate (OUR_T) and the heterotrophic bacteria oxygen uptake rate (OUR_H) were measured respectively. The reactors had been maintained in aerobically digested condition for 3 days. Two mixed liquor samples were drawn from the reactor with the volume of 250 mL every 12h (two 300 mL Erlenmeyer flask was used), the supernatant of one sample was replaced with tap water, the other one was replaced with tap water added allylthiourea (ATU, 50 mg/L), this procedure was repeated for 3 times, then the OUR_T and OUR_H responses were measured respectively. The autotrophic bacteria oxygen uptake rate (OUR_A) was determined by the difference between the OUR_T and the OUR_H. The OUR testing apparatus was as follows: the 300 mL Erlenmeyer flask was set on a magnetic stirrer, a magnetic rotor was put into the Erlenmeyer flask to make the liquor well mixed, the DO probe was placed into the mixed liquor, the DO data was recorded every 1 min for 15 min. The OUR was determined by linear regression of DO concentrations over time. According to ASM1 (Henze et al., 2000), the biomass concentration of heterotrophic or autotrophic

bacteria was estimated as equation (4). The specific oxygen uptake rate (SOUR) was determined

$$\mathrm{OUR} = (1 - f_P)bX \quad (4)$$

Where:

f_P: fraction of biomass yielding particulate products, 0.08 (Henze et al., 2000);

X: active biomass concentration of heterotrophic (X_H) or autotrophic (X_A) or total bacteria (X_T)

b: the decay coefficient of heterotrophic or autotrophic bacteria, based on the measurements in this study, which can be calculated by equation (6), h^{-1}.

Based on the ASM1 (Henze et al., 2000), the decay of heterotrophic or autotrophs bacteria can be described as equation (5). Equation (6) can be obtained by integrating equation (5).

$$dX/dt = -bX \quad (5)$$

$$\ln(\mathrm{OUR}) = \ln[(1 - f_P)bX] - bt \quad (6)$$

where:

dX/dt: the derivative of heterotrophic or autotrophic biomass concentration with respect to time, mg/ (min · h).

To investigate the existence of anoxic zone exists inside the granules, the penetration depth of the oxygen is calculated according to equation (7) (Henze et al., 2008). The space unavailable for oxygen is considered as anoxic zone.

$$Z_{penetration} = \sqrt{2 \cdot D_F \cdot C_{LF}/(k_{0,O_2,H} \cdot X_{H,F} + K_{0,O_2,AUT} \cdot X_{AUT,F})} \quad (7)$$

where:

$Z_{penetration}$: the penetration depth of the oxygen, m;

D_F: the diffusion coefficient for oxygen, 0.000175 m^2/d (Henze et al., 2008);

C_{LF}: the oxygen concentration at the surface of the granules, 0.65 g/m^3 (in this study);

$k_{0,O_2,H}$: the zero-order constant of the heterotrophic bacteria for oxygen, 7.2 g O_2/ (g COD · d);

$K_{0,O_2,AUT}$: the zero-order constant of the autotrophic bacteria for oxygen, 18.8 g O_2/ (g COD · d);

$X_{H,F}$: the heterotrophic biomass concentrations (based on measurements), g COD/m^3;

$X_{AUT,F}$: the autotrophic biomass concentration, (based on measurements), g COD/m^3.

2.5. Activities of autotrophic/heterotrophic ammonia oxidization bacteria

The autotrophic/heterotrophic specific ammonia uptake rates (SAUR) were used to estimate microbial activities of autotrophic/heterotrophic AOB. Series of 1-h batch tests (Zhang et al., 2017) under fully aeration operation were conducted using two mini-reactors of 1 L and 500 mL mixed liquor samples withdrawn from the three reactors to measure the autotrophic/heterotrophic SAURs individually. The supernatants of the mixed liquor samples were replaced with different kind of nutrient medium. The nutrient medium used for total AOB activity test contained: NH_4^+-N 50 mg/L, $MgSO_4$ 40 mg/L, $CaCl_2$ 30 mg/L and COD 300 mg/L. The nutrient medium used for heterotrophic AOB activity test contained NH_4^+-N 50 mg/L, $MgSO_4$ 40 mg/L, $CaCl_2$ 30 mg/L, COD 300 mg/L and allylthiourea (ATU) 100 mg/L as autotrophic bacteria

inhibitor. The specific ammonia up-take rates were determined by the difference between the NH_4^+-N concentrations at the beginning and at the end divided by the TSS concentration. The autotrophic SAUR was determined by the difference between the total SAUR and the heterotrophic SAUR.

3. Results and discussion

3.1. Start-up and long-term operation

3.1.1. System performances and solid concentrations

The three reactors were operated at different feast/famine time ratio which was achieved by adopting different cycle time of 4, 6, 8 h during 120 days. The cycle tests (fig.2) showed the completely consumption of COD within 30 minutes under all the cycle time with the feast/famine ratios of 1:7, 1:11, 1:15, respectively. Fig.1 illustrated the system performances in the start-up and long-term operation phase. The values of TSS concentrations and the SVI were unstable in start-up phase (the first 59 days), after that, the biomass concentrations in the three reactors (Fig.1) reached stable and the effluent quality was up to standard while the small granules were observed in all of the reactors. Full ammonium removal was established during the whole operation period, the dominating nitrogen compounds in the effluent were NO_3^--N, whose concentrations reached to 11.96, 15.75, 13.30 mg/L in R_1, R_2, R_3, respectively in the end of the operation. In addition, the accumulation of NO_2^--N was almost not observed in the effluent, while the TP and COD removal efficiencies were kept above 95% in all the reactors.

The variations of TSS concentrations in the three reactors showed dissimilar trends. The TSS concentrations of R_1 kept rising up from the beginning until the 50th day, then decreased and stabilized at 5981±408 mg/L. For R_2, the TSS concentration were substantially unchanged and stayed at 4567±883 mg/L during the whole operation period. The TSS concentration of R_3 dropped from 3405 mg/L to 2174 mg/L at the start, then increased step by step and stabilized at 3120±633 mg/L. The ratio of VSS/TSS and the SVIs for all the reactors were kept around 0.85 and below 70 mL/g respectively, which showed favorable settling abilities and biomass content. In addition, it is obviously that the decreasing feast/famine ratio led to reduction of the biomass concentrations. In this study, longer famine time accompanied with longer hydraulic retention time (HRT), stimulated to the decrease in organic loading rate (OLR) (1.125 kg COD/(m^3·day)-0.563 kg COD/(m^3·day))which means less carbon supplying to the microorganisms and growth rates slowing down (Liu & Tay, 2007; Muda et al., 2011). Nevertheless, other studies indicated that feast/famine conditions have influences on physiology and viability of activated sludge culture (Chiesa et al., 1985; Liu & Tay, 2007). It was also demonstrated that aerobic granules of longer famine time contained a lower degree of microbial community diversity and have less dominant strains than that of shorter famine time (Liu & Tay, 2008), from which it can be assumed that feast/famine condition can develop a certain microbial selection pressure, especially for that with long famine phase, which will select microbial population with higher resistance to starvation. Therefore, the recovery of MLSS in R_3 indicated that the

microbial population of the initial seed sludge was inadaptable to the feast/famine condition in R_3 (feast phase of 0.5h, famine phase of 7.5h) at the start, but after the long-term operation the biomass with poor capability of starvation tolerance was eliminated out of the reactor and the microbial population in R_3 exhibited adaptability to its particular feast/famine condition finally.

3.1.2. Aerobic granular sludge morphology

At the end of the operation the average granule sizes reached to 873, 931, 612 μm in R_1, R_2, R_3 respectively (Table.2). The results showed that larger granules were easier to be formed under an appropriate feast/famine ratio (in this study the appropriate feast/famine ratio means 1:11, apparently). Furthermore, the $d_{0.9}/d_{0.1}$ ratio in the R_2 reached its minimum value (3.09), which meant that the size of granules in R_2 was the most even one in the three reactors. Also, the $d_{0.9}/d_{0.1}$ ratio in R_3 was the largest (7.09), it could be surmised that long-time famine phase might contribute to the development of irregular shaped granules. In the middle of the whole operation period, nearly all the microbial aggregates in R_1 and R_2 were regular compact granules while large amounts of flocculent sludge were observed in R_3. The same results were reported by Corsino et al. (2017), but in the whole operation phases of this study the granules under different feast/famine ratio were all stable and the granules disaggregation or filamentous structures were not observed during the whole operation, which indicated that the length of famine time was not a prerequisite for the granules stability. Moreover, previews studies (de Kreuk & van Loosdrecht, 2004; Liu et al., 2004; Wan et al., 2009) reported

that aerobic granules with low growth rates showed strong structure and Liu et al. (2004) deemed that enriching autotrophic nitrifying population in aerobic granules could markedly lowered growth rate of aerobic granules. Base on the fact that the aerobic granules from R_1 and R_2 were evidently larger than that from R_3 in sizes, it can be presumed that aerobic granules from R_1 and R_2 contained more autotrophic nitrifying bacteria.

3.2. COD, N, TP removal performances and SND capacities

The cycle testes results were illustrated in Fig. 2. As shown in Fig. 2. a), b) c), to begin with, the microbial systems of R_1, R_2, R_3 were under feast condition with abundant substrates. After the first 30 minutes of the cycles, COD concentrations in R_1, R_2, R_3 were 15.75, 25.19, 15.75 mg/L respectively, almost all the easily biodegradable COD were consumed (COD concentrations in the effluent of R_1, R_2, R_3 were 7.87, 9.45, 7.87 respectively), which made the reactor in famine conditions, meanwhile a number of suspended solids which is not easy to be biodegraded were produced due to metabolic degradation (Henze et al., 2000). The peak phosphorus release concentrations were 30.52, 27.39, 30.52 mg/L respectively, and TP concentrations in the effluent of R_1, R_2, R_3 were all nearly 0 mg/L. In this study, the TP removal efficiency was not influenced by the variation of feast/famine conditions.

Figs. 2. d), e), f) illustrated the changes of AUR, NiPR, NaPR, $r_{N,synthesis}$ and α_{SND} during the cycle. The peak AURs of R_1, R_2, R_3 were 0.389, 0.754, 0.725 mg NH_4^+-N/ (L · min) and the peak SAURs of R_1, R_2, R_3 were 0.722, 2.174, 3.196 mg NH_4^+-N/ (mg

MLSS · L · min) respectively, the SAUR of R_3 was almost 5 times as that of R_1. It is obviously that the microbial system in R_3 showed the most outstanding nitrification ability. Although the TN concentrations in the effluents of R_1, R_2, R_3 were very similar (16.86, 14.63, 14.41 mg/L respectively), the nitrifying bacteria (autotrophic and heterotrophic) showed different activities, namely the activity of nitrifying bacteria got higher with the decreasing of feast/famine ratio (1:7 to 1:15) which indicated the different populations of the nitrifying bacteria under different feast/famine conditions. The details will be discussed further below. Besides, the abnormal increase of NH_4^+-N concentrations was also observed in the anoxic phase of R_3, which is consistence with the conclusion of Zhao et al. (2010), who found that dissimilatory nitrite reduction to ammonium (Smith, 1982) occurred during the denitrification achieved by heterotrophic nitrification-aerobic denitrification bacteria *Bacillus* sp. LY. And Seenivasagan et al. (2014) had revealed that generally most isolated dissimilatory nitrite/nitrate reducing bacteria belonged to the genera *Bacillus*, which indicated that *Bacillus* sp. was likely to exist in R_3 in this study. Based on the conclusions of Zhao et al. (2010), a possible pathway for nitrogen removal by heterotrophic SND can be proposed: the NH_4^+-N was firstly transformed to NH_2OH, which was transformed to N_2 subsequently.

Furthermore, as the trends of α_{SND} in R_1, R_2, R_3 showed by Figs. 2. d), e), f), the SND occurred respectively between the 40th minute and the 140th minute in R_1, between the 90th minute and the 150th minute in R_2, between the 120th minute and the 210th minute in R_3. The peak values of α_{SND} in R_1, R_2, R_3 were 0.242, 0.581, 0.662. The peak values

of α_{SND} obtained in R_2 and R_3 were significantly higher than that in R_1, which also suggests the microbial system in R_2 and R_3 had a higher SND capacity than that in R_1. Other authors (Chen et al., 2011; Derlon et al., 2016) reported SND capacities correlated with granules sizes which were directly linked to the anoxic volume within the granules. However, the granules sizes in R_1 and R_2 were approximate while distinct SND capacities were observed, so it can be speculated that there is great difference in the microbial populations for R_1 and R_2 which accordingly led to the different SND capacities. And considering the feast/famine conditions in R_1, R_2, R_3, the microbial populations in R_2 and R_3 may have higher ability to resist starvation.

Besides, the results of cycle tests using real wastewater is shown in Fig.3. It is can be seen that the effluent of the three reactors all achieved good quality. However, as we all know, the carbon resource in domestic wastewater is more difficult to be used than that in synthetic wastewater, so some NH_4^+-N (0.486 mg/L in R_1, 0.619 mg/L in R_2, 0.085 mg/L in R_3) and NO_2^--N (0.207 mg/L in R_1, 0.148 mg/L in R_2, 0.000 mg/L in R_3) were found in the effluent, which influenced the denitrification process.

3.3. Estimating biomass of autotrophic/heterotrophic bacteria

The results of OUR tests of R_1, R_2, R_3 is shown in Fig. 4 and Table 3. A shift of bacterial community was observed accompanied under different feast/famine conditions. The estimated autotrophic bacteria proportion of total bacteria in R_1, R_2, R_3 (Table 3) were 33.64, 23.09, 3.26% respectively, and the SOUR of total bacteria in R_1, R_2, R_3 were 3.913, 3.520, 2.067 mg O_2/ (g MLVSS · h). Generally, AOB and nitrite oxidation

bacteria (NOB) were considered as autotrophic bacteria (Henze et al., 2008). In theory the lack of autotrophic bacteria should have negative effect on nitrification. However, as discussed above, the microbial system in R_3 showed excellent nitrification and SND capacities. Therefore, the heterotrophic bacteria may have taken part in the nitrification or the nitrogen removal pathway. Previously, plenty of researchers (Fitzgerald et al., 2015; Niel et al., 1992; Qing et al., 2018; Zhang et al., 2018) have demonstrated the existences of heterotrophic AOB, by which aerobic heterotrophic ammonium oxidation and aerobic denitrification could happened simultaneously (Padhi et al., 2017). To verify the occurrence of heterotrophic nitrification in the reactors, series of additional cycle tests were applied to R_1, R_2, R_3 under fully aeration. In the beginning of cycle tests, NH_4^+-N concentration was increased to almost as twice as that in the normal operation. The results showed (Table 4) that heterotrophic nitrification accounted for 58.62, 58.33, 61.54% in total nitrification of R_1, R_2, R_3 respectively, which confirmed that heterotrophic bacteria contributed to nitrification or SND process in all the reactors. Besides that, the composition of autotrophic/heterotrophic nitrification in R_1 and R_2 were similar, while the proportion of heterotrophic AOB and the heterotrophic SAUR in R_3 was slightly higher than that in R_1 and R_2, which indicated that heterotrophic AOB may have better adaptability than autotrophic AOB under the conditions of low feast/famine ratio based on the fact that R_3 had longer starvation phase than R_1 and R_2. Combined with previous analysis of cycle tests under normal operation, it can be concluded that microbial system consisted of more heterotrophic AOB might showed

greater ability of SND.

In addition, Satoh et al. (2003) had demonstrated that SND could occur as long as an anoxic zone exists inside the granule. Based on the calculation, that the theoretical oxygen penetration depth of granules from R_1, R_2, R_3 were 122, 134, 167 μm respectively while the average granule sizes were 873, 931, 612 μm led to the anoxic zone volume accounts for 52.1, 50.6, 20.8% of total volume. The existence of anoxic zones built an environment where nitrite/nitrate produced in outside aerobic zones of the granules could be denitrified inside, which contributed to a certain amount of total ammonia removal. Above all, as shown in Fig. 5, there were mainly two SND pathways in this study: one is the traditional autotrophic nitrification-heterotrophic denitrification; the other is the heterotrophic SND.

4. Conclusions

Aerobic granules were successfully developed under different feast/famine ratios. It is more easily to form larger granules under an appropriate feast/famine ratio, which was 1:11 in this study. Disaggregation was not observed during the whole operation; the length of famine time is not a prerequisite for stability of the granules. The nitrifying bacteria showed better nitrification activities with the extending of famine phase. Besides, the high feast/famine ratio favored the development of heterotrophic AOBs which had higher abilities to resist starvation and may have potential to achieve higher SND. The mechanism of heterotrophic SND should be further investigated.

Acknowledgements

This work was supported by Fund for the Beijing Excellent Talents by the Beijing Municipal Education Commission, Beijing Youth Top Team Project (2014000026833TD02)

Appendix A. Supplementary data

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FIGURE CAPTIONS

Fig. 1. Concentrations in the effluent and removal efficiency of TN, COD, TP; TSS, VSS and SVI of aerobic granular sludge. a) d) R_1, b) e) R_2, c) f) R_3.

Fig. 2. Cycle tests under normal operation: profiles of NH_4^+-N, NO_2^--N, NO_3^--N, COD, TP, DO concentrations in a) R_1, b) R_2, c) R_3, variations of AUR, NiPR, NaPR, $r_{N,synthesis}$ and α_{SND} in d) R_1, e) R_2, f) R_3.

Fig. 3. Cycle tests using domestic wastewater: profiles of NH_4^+-N, NO_2^--N, NO_3^--N, COD, TP concentrations in a) R_1, b) R_2, c) R_3.

Fig. 4. OUR tests result of a) R_1, b) R_2, c) R_3.

Fig. 5. Nitrogen removal pathways in this study.

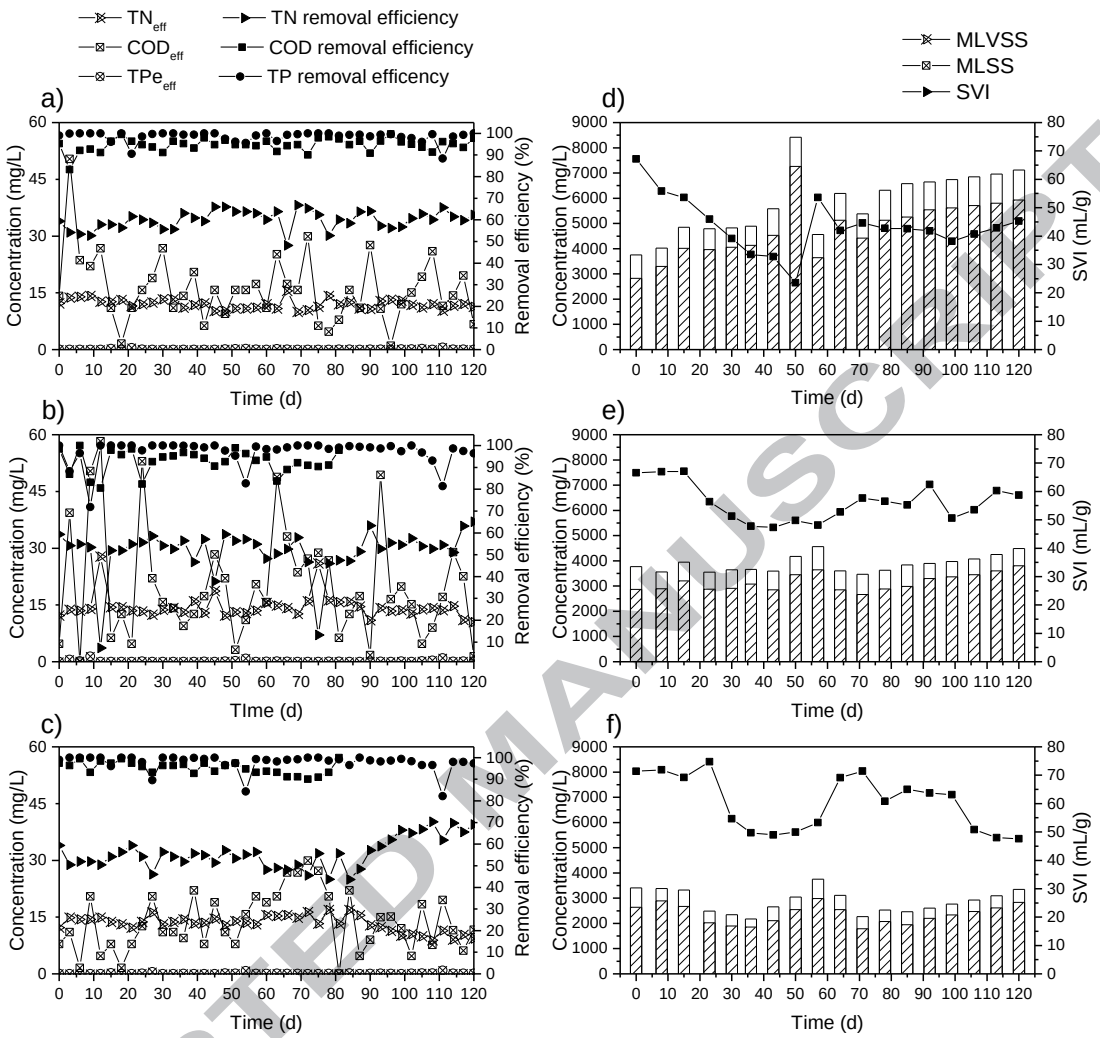

Fig. 1.

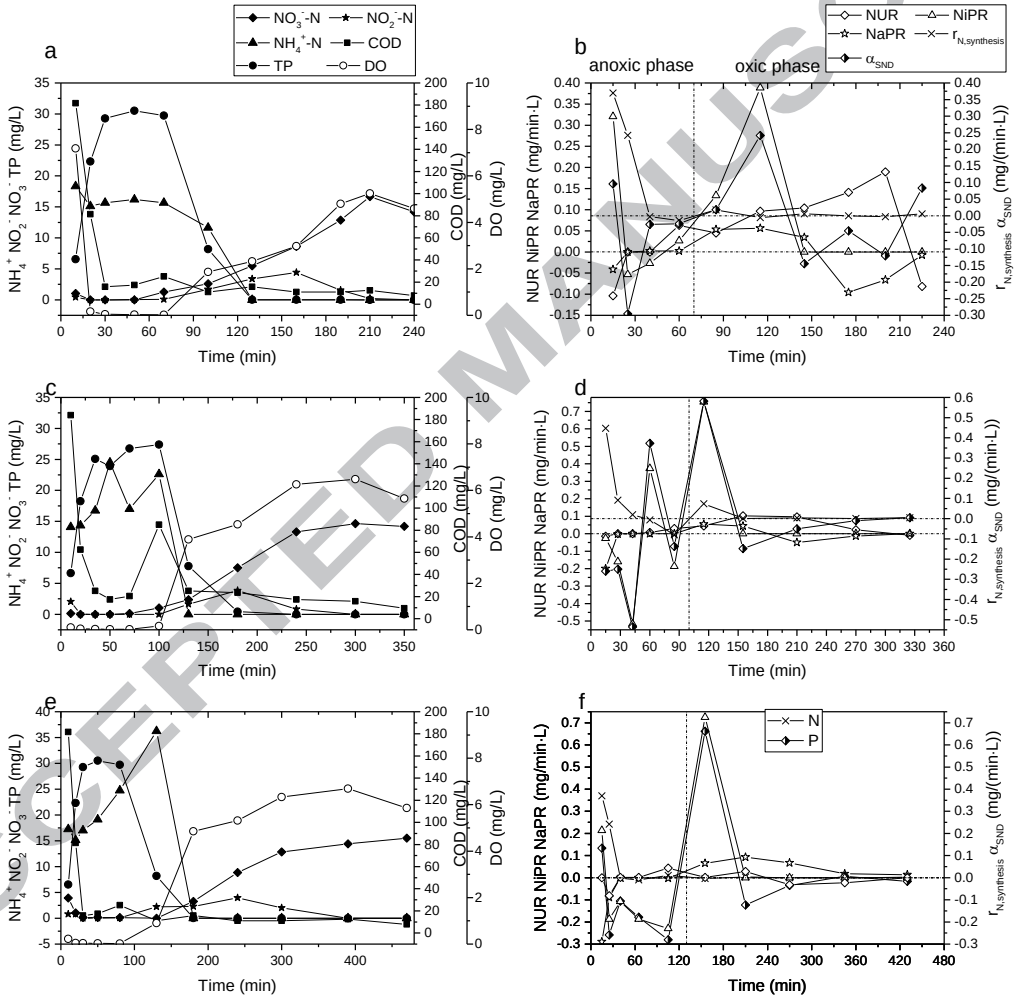

Fig. 2.

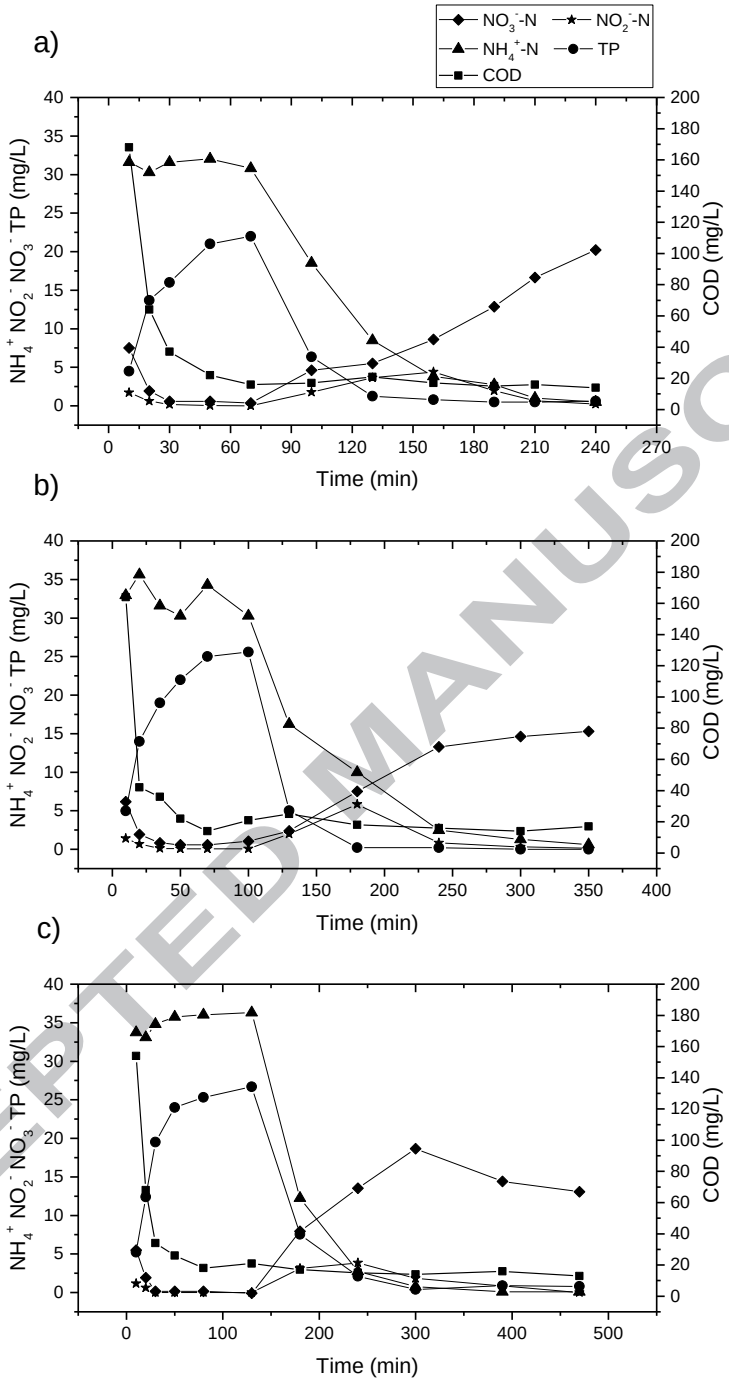

Fig. 3.

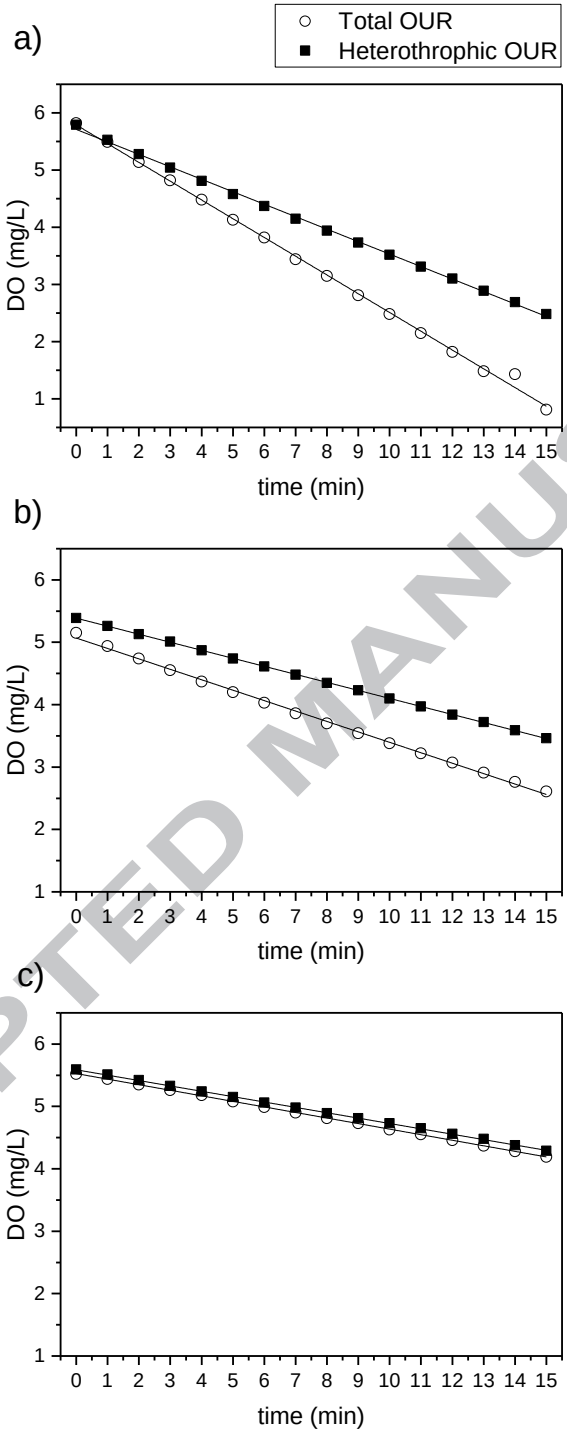

Fig. 4.

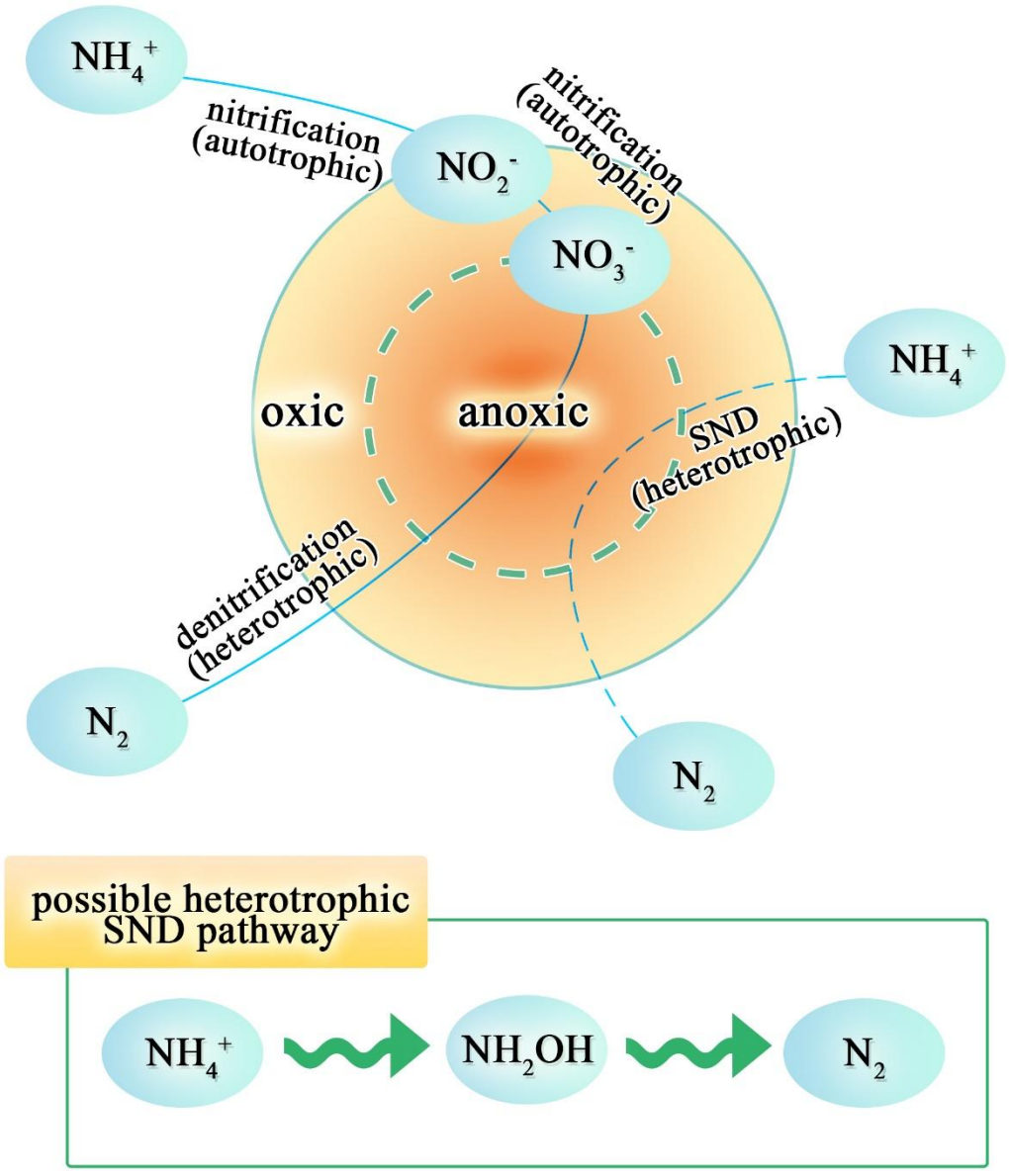

Fig. 5.

Table 1 Operation conditions of the reactors

Operation parameters	R_1	R_2	R_3
Cycle time (h)	4	6	8
Anaerobic reaction time (min)	50	80	110
Aerobic reaction time (min)	170	260	350
Settling time (min)	15~3	15~3	15~3
Airflow rate(L/min)	0.6	0.6	0.6
Volume exchange rate (%)	62.5	62.5	62.5

Table 2 Granules sizes in R1, R2, R3

Reactors	Average granules size (μm)	$d_{0.1}$ (μm)	$d_{0.9}$ (μm)	$d_{0.9}$/ $d_{0.1}$
R_1	873.74	426.63	1438.66	3.37
R_2	931.42	479.79	1486.13	3.09
R_3	612.16	167.18	1184.96	7.09

Table 3 The oxygen uptake rates (OUR) of heterotrophic bacteria (OUR_H), autotrophic bacteria (OUR_A) and specific oxygen uptake rates (SOUR) of total bacteria ($SOUR_T$) and corresponding biomass concentration and relative community composition in three reactors in the end of the operation.

		R_1	R_2	R_3
	b_H (d^{-1})	0.348	0.342	0.158
	b_A (d^{-1})	0.167	0.118	0.056
OUR_H	Rate (mg/(min · L))	0.335	0.129	0.086
	Composition (%)	67.36	76.91	96.74
OUR_A	Rate (mg/(min · L))	0.109	0.039	0.003
	Composition (%)	33.64	23.09	3.26
Heterotrophs	concentration (mg/L)	2534	1471	2135
	Composition (%)	49.80	53.37	91.39
Autotrophs	concentration (mg/L)	2554	1285	201
	Composition (%)	50.20	46.63	8.61
Biomass concentrations based on calculation (mg/L)		5088	2757	2336
Actual biomass concentrations (mg/L)		5131	2850	2546
$SOUR_T$ (mg O_2/(g MLVSS · h))		3.913	3.520	2.067

Table 4 Compositions of heterotrophic and autotrophic nitrification in R_1, R_2, R_3

Reactors	Specific ammonia uptake rates (mg NH_4^+-N/ (mg MLSS · L · h))			Composition (%)	
	Total	Heterotrophic	Autotrophic	Heterotrophic	Autotrophic
R_1	2.879	1.687	1.191	58.62	41.38
R_2	2.775	1.619	1.156	58.33	41.67
R_3	3.063	1.885	1.178	61.54	38.46

- Aerobic granules were cultivated successfully with different feast/famine ratio;
- Microbial system consisted of heterotrophic AOB showed stronger capacity of SND;
- A novel N-removal pathway was proposed which involving SND by heterotrophic AOB.