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Achieving nitritation for low-strength wastewater at different temperatures in an aerobic cubic sponge reactor under high dissolved oxygen

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ABSTRACT

The application of nitritation in domestic wastewater treatment is very meaningful. In this study, nitritation for low-strength wastewater in a moving bed biofilm reactor was achieved at different temperatures (25°C, 20°C, 15°C and 10°C) under high dissolved oxygen. Partial nitritation was achieved with aerobic flow-separated ball filling cubic sponges through sequencing batch feed mode even at temperature of 10°C. Results indicated that the negative effects of the decrease in temperature on nitritation can be compensated by positive operation conditions, such as reducing hydraulic residence time to improve effluent concentration of ammonia. The contribution of aerobic flow-separated ball to nitritation recovery is due to the enhanced internal oxygen inhibition on nitrite oxidizing bacteria (NOB) without negatively affecting reaction efficiency. Improving effluent concentration of ammonia under continuous mode is also beneficial for internal oxygen repression on NOB meanwhile free ammonia inhibition on NOB is strengthened. Hysteretic growth of NOB compared to ammonia-oxidizing bacteria (AOB) in each cycle should be the main reason for the superiority of sequencing batch feed mode to recover nitritation. The finding that nitritation fails more slowly at lower temperature is good for maintaining stable nitritation at low temperature because we can find the nitrification deterioration in time.

Keywords: Biofilm; Nitritation; Low-strength wastewater; High dissolved oxygen; Different temperatures

1. Introduction

As a novel and promising wastewater treatment process, biological nitrogen removal by nitritation and anaerobic ammonium oxidation (PNA) technology has been proposed as a sustainable method for wastewater treatment [1–3]. PNA can be divided into one-stage or two-stage nitrogen (N)-removal systems for better application. In many studies, the known weak point of one-stage nitrogen removal system is that nitrite oxidizing bacteria (NOB) developing in the long-term operation, which triggering the production of nitrate, and greatly affecting the N-removal performance of anaerobic ammonium oxidation (anammox) [4–6]. Two-stage N-removal system can be thought as an appealing solution for wastewater treatment, of which nitritation reactor can be replaced by activated sludge reactor, granular sludge reactor, biofilm reactors, or other reactors. In comparison, nitritation of granular sludge or biofilm have less influence on the subsequent anammox process than that of activated sludge [7–10].

PNA technology is already successfully used worldwide in side-stream treatment to reduce aeration costs [2,3]. Now

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what the researchers focus on are possible applications of PNA in mainstream treatment. But the nitrate produced from nitritation significantly affected the stable operation in the present autotrophic N-removal [11,12]. So the key process to achieve stable PNA is nitritation process, and it requires the operating conditions to inhibit the growth of NOB effectively. For the domestic sewage with low strength, the repression on NOB is certainly an inevitable and major challenge in nitritation process, especially at low temperature [11,13,14]. Some factors have been identified as influencing the nitritation processes, including dissolved oxygen (DO) [15], free ammonia (FA) [15], pH [16], free nitrous acid (FNA) [17], temperature and sludge retention time [15]. Whereas the ammonia concentration and temperature were relatively low in domestic sewage, so the FA, FNA and temperature could not inhibit the growth of NOB effectively [11].

For low-strength domestic wastewater, most of the studies on nitritation have been carried out in sequencing batch reactors with an activated sludge system [12,18]. One of the major reasons should be that the DO control is more effective [19]. But, it was also reported that NOB can be outcompeted at high DO bulk concentrations, since the oxygen supply to the biofilm can be reduced by a thick external boundary layer [12]. Researchers have confirmed that biofilm processes can accomplish nitrite accumulation, though the mechanism is still unclear [14,20,21]. To date, little attention had been paid to nitritation with biofilm reactors for mainstream because such a process was thought difficult to be maintained in the long term and mainstream contains low-strength ammonia [13,20,22].

To the best of our knowledge, a stable biofilm nitritation reactor at temperatures of 10°C treating low-strength ammonium wastewaters has been rarely reported [7]. Some reactors operated at high strength showed a sudden deterioration of the nitritation at temperatures lower than 15°C [23]. Real-time aeration duration control has been demonstrated as technologically feasible to achieve nitritation when treating low C/N domestic wastewater with sequencing batch reactors [24]. Similarly, for continuous flow reactors, appropriate hydraulic residence time (HRT) should be beneficial to stable nitritation [25].

Stable nitritation in biofilm reactor under high DO concentration is appealing due to no need of strict control for low DO concentration, but it cannot be ignored that the inhibition on nitrifying bacteria activity caused by low DO concentration. Here, based on the above discussion, the study aims to investigate the nitritation performance in a biofilm reactor under high DO concentration, in which stable nitritation at different temperatures (approximately 25°C, 20°C, 15°C and 10°C) for low-strength wastewater (approximately 60 mg NH_4^+-N/L) was successfully achieved. Especially, the conditions and strategies for achieving stable nitritation at different temperatures were investigated through measuring the biofilm activity and microbiological analyses.

2. Materials and methods

2.1. Reactor setup and inoculum

The working volume of the reactor was 5.0 L with a height of 30 cm and an internal diameter of 15 cm (Fig. 1).

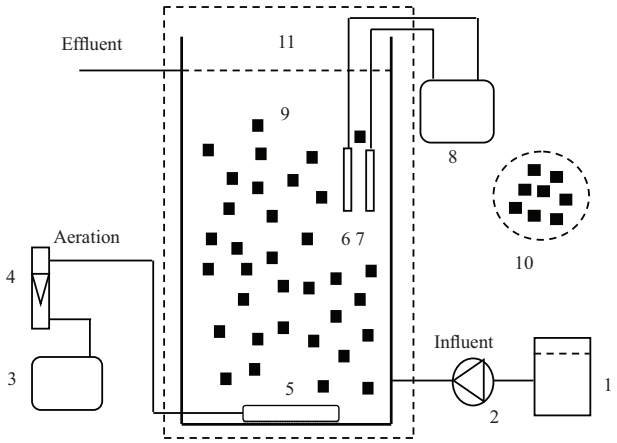

Fig. 1. Schematic diagram of the biofilm reactor showing the peripheral instrumentation: (1) influent tank; (2) influent pump; (3) air pump; (4) air flowmeter; (5) air diffuser; (6) dissolved oxygen electrode; (7) pH electrode; (8) WTW; (9) cubic sponges; (10) plastic flow-separated ball; (11) temperature control cabinet.

The reactor was placed in a temperature-controlled cabinet in which water temperature could be controlled as expected (25°C, 20°C, 15°C and 10°C). Compressed air was supplied via a diffuser at the bottom of the reactor at a flux of 36 L/h. HRT was adjusted by means of a peristaltic pump, and the initial HRT was set to 5 h, corresponding to the initial influent flow rate of 1 L/h. No pH adjustment was carried out in the reactor and in the effluent it was about 7.3–7.4. Cubic sponges (approximately 10 × 10 × 10 mm) were used as biomass carriers. The volume of the carriers was about 20% of the working volume of the reactor. It was noteworthy that cubic sponges were singly suspending in the reactor before day 76, and after that they were gathered in the plastic flow-separated ball filler (diameter: approximately 60 mm) suspended in the reactor. Inoculated activated sludge used for biofilm culturing was taken from aeration tank of a WWTP, which has a good nitrification performance, and its f (MLVSS/MLSS) and SVI were about 0.75 and 90, respectively.

2.2. Wastewater and operational conditions

The biofilm reactor was fed with a synthetic influent, which contained, in average, 10 mg COD/L, added as CH_3COONa; 2.5 mg P/L, added as KH_2PO_4; 60 mg N/L, added as NH_4Cl. $NaHCO_3$ was added for alkalinity, its concentration was 720 mg/L. The pH was about 7.8–8.0. The synthetic wastewater also contained 1 mL of trace elements solution per litre of influent [26].

The reactor ran for 210 d, which could be divided into four phases according to different set temperatures. The set temperatures of phases 1 (days 1–60), 2 (days 61–100), 3 (days 101–150) and 4 (days 151–210) were 25°C, 20°C, 15°C and 10°C, respectively. The DO concentration was above 5.0 mg/L continuously during whole phases. During days 1–179, continuous influent mode was carried out and during which the HRT was adjusted to obtain appropriate ammonia (NH_4^+-N) concentration in the bulk volume. During days

180–210, the reactor was operated in sequencing batch feed mode with a volumetric exchange ratio of approximately 80%. Each cycle included 5 min for feeding, 8.5 h for aeration, 10 min for settling, 10 min for decanting and left undisturbed for 5 min. No additional sludge was discharged from the reactor. The conditions about operation are shown in Table 1.

2.3. Analytical methods

All the samples were filtered with a 0.45 μm filter before analyzing. NH_4^+-N, NO_2^--N, NO_3^--N, MLSS, MLVSS and alkalinity were measured according to the standard methods [27]. For MLSS and MLVSS measurement, biofilms were, respectively detached from sponges by ultrasound (45 kHz, 120 W, 2–3 min) and then centrifuged (10,000 g, 15 min). DO, pH, and temperature were monitored by WTW Multi 3420i meter (WTW Company, Germany).

The nitrite accumulation rate (NAR, %) was calculated as Eq. (1):

$$\mathrm{NAR} = \frac{C(\mathrm{nitrite})}{C(\mathrm{nitrite}) + C(\mathrm{nitrate})} \quad (1)$$

where nitrite (NO_2^--N) and nitrate (NO_3^--N) all refers the produced, excluding influent.

The FA and FNA concentrations were calculated by the following Eqs. (2) and (3) [28]:

$$\mathrm{FA} = \frac{17}{14} \times \frac{c(\mathrm{ammonia}) \times 10^{\mathrm{pH}}}{e^{\left(\frac{6344}{T}\right)} + 10^{\mathrm{pH}}} \quad (2)$$

$$\mathrm{FNA} = \frac{46}{14} \times \frac{c(\mathrm{nitrite})}{e^{\left(\frac{-2300}{T}\right)} \times 10^{\mathrm{pH}}} \quad (3)$$

where T is temperature in K.

The actual oxygen uptake rate (OUR) was calculated from the measured TNN (r_{nitrite}) and nitrate (r_{nitrate}) production rates.

$$\mathrm{OUR} = 3.43 \times r_{\mathrm{nitrite}} + 4.57 \times r_{\mathrm{nitrate}} \quad (4)$$

Then specific oxygen uptake rate (SOUR) was calculated as OUR/MLVSS.

2.4. Microbial sampling and phylogenetic assignment

The biofilm samples used for phylogenetic assignment were, respectively, detached from sponges by ultrasound (45 kHz, 120 W, 2–3 min) and then centrifugation (10,000 g, 15 min). And then according to the methods [29], the DNA was extracted from each sample. The extracted DNA was used for high-throughput pyrosequencing and phylogenetic assignment (Sangon, China). In this study, as Fig. 2 shows, single cubic sponge samples were divided into internal (SA) and external (SB) portions for phylogenetic assignment, cutting approximately 2 mm from surface. In the same way, cubic sponges in single aerobic flow-separated ball were divided into internal (SC) and external (SD) cubic sponge samples, cutting approximately 10 mm from surface. The dimensions of those assigned volumes are artificially determined.

3. Results and discussion

3.1. Performance of reactor

The performance of the biofilm reactor is described in Figs. 3 and 4. In phase 1 (days 1–60), biofilm had been cultivated successfully and the performance of nitritation remained stable. Fig. 3 shows that MLVSS (biomass) increased

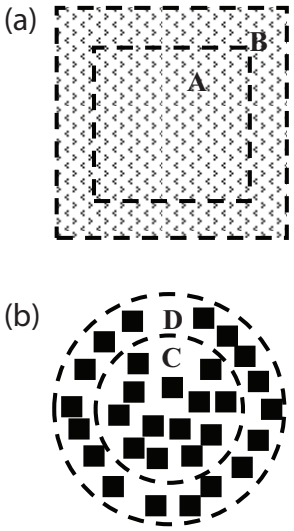

Fig. 2. Schematic diagram of dividing the biofilm samples for phylogenetic assignment: (A: internal portions of single cubic sponge sample; B: external portions of single cubic sponge sample; C: internal cubic sponge samples of in single aerobic flow-separated ball; D: external cubic sponge samples of in single aerobic flow-separated ball).

Table 1
Conditions about operation

	Temperature (°C)	DO (mg O_2/L)	NH_4^+-N (mg N/L)	Duration (d)
Phase1 (1–60)	25±1	5±0.2	6±0.5	60
Phase 2 (61–100)	20±1	5±0.2	6±0.5	40
Phase 3 (101–150)	15±1	5±0.2	6±0.5/30±2[a]	50
Phase 4 (151–210)	10±1	5±0.2	30±2	60

[a]In Phase 3, the first 20 d the NH_4^+-N concentration of the effluent is 6±0.5 mg N/L, and the other 30 d is 30±2 mg N/L.

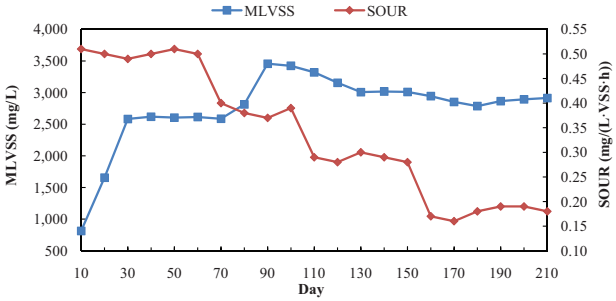

Fig. 3. Biomass and activity performances of the biofilm reactor treating low-strength wastewater at different temperatures.

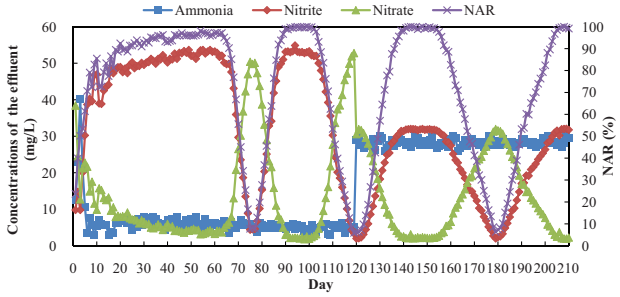

Fig. 4. Nitritation performance of the biofilm reactor treating low-strength wastewater at different temperatures.

up to day 30 and then remained nearly stable. Fig. 4 shows NAR remained above 90% and both of ammonia, nitrite and nitrate concentrations were nearly stable in the effluent during days 30–60. These results reveals that biofilm of stable nitritation can be considered as obtained on day 30. During the startup of this aerobic cubic sponges reactor, it took about 30 d (days 1–30) to achieve stable nitritation (25°C).

Anthonisen et al. [28] reported that the threshold FA concentration for NOB inhibition was between 0.1 and 1 mg/L. Similarly, Park and Bae [30] pointed out that the threshold FA concentration for NOB inhibition was 0.7 mg/L, while the activity of NOB was inhibited by 50% at FNA concentrations between 0.02 and 0.1 mg/L. Vadivelu et al. [31] also found that the biosynthesis of *Nitrobacter* was totally stopped at the FNA concentration of 0.023 mg/L. The inhibition of on NOB was not significant during days 30–60, while the concentrations of FA and FNA were 0.05–0.14 and 0.014–0.016 mg/L, respectively. Therefore, apart from the advantage of the maximum specific growth rate of ammonia-oxidizing bacteria (AOB) was higher than that of NOB at temperature of 25°C, appropriate HRT control could not be ignored for the direct occurrence of nitritation biofilm. To confirm the necessity of that, during days 30–45, contrast experiment was carried out in which only major difference compared with this experiment was that the HRT was extended an hour, resulting in the effluent concentration of ammonia decreasing to zero. The NAR decreased from 91.2% to 35.8% from day 30 to 45 in contrast experiment, but it remained stable in this experiment (Fig. 4).

In phase 2 (days 61–100), temperature was switched to 20°C and other conditions remained as before. As shown in Fig. 4, stable nitritation failed resulting from decreased temperature during days 61–76. From the 77th day, cubic sponges were gathered in the plastic flow-separated ball (Fig. 1). Nitritation performance went well along with NAR increased from 4.8% to nearly 100% during days 77–90 and remained stable during days 91–100 (Fig. 4). Also, the biomass increased (MLVSS) sharply between day 70 and 90 which might due to the function of interception, as Fig. 3 shown. As temperature further decreased to 15°C in phase 3 (days 101–150), nitritation failed again with NAR decreased from nearly 100% to 5.9% during days 101–119 (Fig. 4). When improving the effluent ammonia concentration via shortening HRT from the 120th day, nitritation recovered again and NAR was up to 98.3% on day 139. Stable partial nitritation was achieved during days 139–150, in which ratio of ammonia and nitrite concentrations was 1:1.08–1:1.17 and NAR was nearly 100% (Fig. 4). In phase 3 (days 101–150), MLVSS decreased slightly and then remained stable (Fig. 3) which might be due to some microbes could not survive under low temperature. In phase 4 (days 151–210), the temperature was decreased to 10°C, nitritation failed under lower temperature with NAR decreased to 6.3% on day 179 (Fig. 4). From the 180th day until the end, sequencing batch feed mode was adopted and then nitritation recovered. Stable partial nitritation was achieved in the last 5 d as Fig. 4 shows. In Fig. 3, it could be seen that in phase 4 (days 151–210) MLVSS decreased slightly during days 150–180 which might be caused by lower temperature. However, it increased slightly and remained stable during days 181–210. This should be related to sequencing batch feed mode. Furthermore, the reactor activity performance (SOUR) is also described in Fig. 3, significant result was that SOUR was largely affected by temperature. The lower the temperature, the lower the SOUR.

3.2. Nitritation performance of single cubic sponge at temperature of 25°C

On the 60th, 75th, 77th, 100th, 119th, 150th, 179th and 210th day, phylogenetic assignment has been carried out as Fig. 2(b) describes. The results are listed in Table 2.

Only the share of AOB in external portion (SB) was much higher compared with the share of AOB and NOB in internal portions (SA) on day 60. These results reveal that both of AOB and NOB activities in SA and NOB activity in SB were inhibited. Zheng et al. [12] pointed that due to the substrate mass transfer resistance, the ammonium in the inner part of the biofilm might be far less than 1 mg/L when the ammonia concentration was below 7 mg/L in the reactor. As a result, the AOB activity would be limited inside the biofilm. During days 31–60 of this study, effluent concentration of ammonia was 3.1–7.8 mg/L, meanwhile the reactor was continuous and completely mixed, which indicates that effluent concentration was just the concentration in bulk liquid. Therefore, the activity of AOB in the SA would be limited during days 31–60. At this time, even nitrite was adequate for NOB in SA, but oxygen was limited due to oxygen transfer resistance. In consequence, both the activities of AOB and NOB in SA were inhibited during days 31–60.

As referred above, the achievement of stable nitritation in phase 1 should be related to the temperature of 25°C at which the maximum specific growth rate of AOB was higher than that of NOB. According to reports of Hellinga et al. [32], the maximum specific growth rates of AOB and NOB with different temperature could be calculated using Eq. (5).

Table 2
Shares of AOB and NOB in total bacteria (unit: %)

Day	60		75		77		100	
	SA	SB	SA	SB	SC	SD	SC	SD
AOB	0.08	0.33	0.07	0.29	0.18	0.19	0.09	0.34
NOB	0.07	0.05	0.27	0.09	0.19	0.18	0.08	0.06
AOB+NOB	0.15	0.38	0.34	0.38	0.37	0.37	0.17	0.40
AOB/NOB	114	660	26	322	95	106	138	567
Day	119		150		179		210	
	SC	SD	SC	SD	SC	SD	SC	SD
AOB	0.08	0.25	0.20	0.32	0.17	0.22	0.26	0.36
NOB	0.23	0.16	0.10	0.08	0.19	0.21	0.07	0.09
AOB+NOB	0.31	0.41	0.30	0.40	0.36	0.43	0.33	0.45
AOB/NOB	35	156	200	400	89	105	371	400

$$\mu^{T}_{\max,\mathrm{AOB(NOB)}} = \mu^{T_0}_{\max,\mathrm{AOB(NOB)}} \cdot \exp\left[\frac{E_{a,\mathrm{AOB(NOB)}} \cdot (T - T_0)}{R \cdot T \cdot T_0}\right] \quad (5)$$

where $\mu^{T}_{\max,\mathrm{AOB(NOB)}}$ was the maximum specific growth rate of AOB (NOB) when temperature was T, d^{-1}; T was the actual temperature in K; T_0 was the temperature with which the maximum specific growth rate of AOB (NOB) has been reported in 308 K; $\mu^{T_0}_{\max,\mathrm{AOB(NOB)}}$ was the maximum specific growth rate of AOB (NOB) at temperature of T_0, 2.1 (1.05) d^{-1}; $E_{a,\mathrm{AOB(NOB)}}$ was the free energy of AOB (NOB), 68 (44) kJ/(mol·K); R is the ideal gas constant, 8.31 J/(mol·K).

Calculated with Eq. (5), the maximum specific growth rates of AOB at temperatures of 25°C, 20°C, 15°C and 10°C are 0.86, 0.54, 0.33 and 0.20 d^{-1}, respectively. Similarly, that of NOB at temperatures of 25°C, 20°C, 15°C and 10°C are 0.59, 0.44, 0.32 and 0.23 d^{-1}. It is clear that both of AOB and NOB in the external portion (SB) were not limited by substance in phase 1. Fast-growing organisms were located towards to the outer part of the biofilm [12,33]. Therefore, with the higher maximum specific growth rate and no substance inhibition, more AOB would appear in SB, which was identical to the results of day 60 in Table 1.

3.3. Nitritation performance of aerobic flow-separated ball

As Fig. 4 demonstrates, in phase 2 (days 61–100) nitritation failed as the temperature being adjusted to 20°C, and it recovered after taking single cubic sponges into the plastic flow-separated ball filler. Apparently aerobic flow-separated ball was beneficial for achievement and stable operation of biofilm nitritation.

During days 64–75, nitritation failed clearly which should be caused by temperature reduction from 25°C to 20°C on day 61. It can be found in Fig. 3 that when temperature decreased, SOUR reduced from 0.50 to 0.40 mg O_2/(L·VSS·h) from phase 1 to phase 2. And then the reduction of SOUR would result in oxygen consumption reduced in the SB of cubic sponge which made more oxygen transfer into the SA of cubic sponge. Afterwards NOB could not be inhibited effectively in the SA which might be the main reason for failure of nitritation here. Also this can be seen from the results of SA and SB on days 60 and 75 in Table 2. From day 60 to 75, the share of AOB was almost invariant in SA, whereas that of NOB increased sharply. The share of nitrifying bacteria (AOB+NOB) of SA on day 75 increased sharply compared with that on day 60, to which the share of NOB made great contribution. The large multiplication of NOB in SA from day 60 to 75 could not stand away from adequate oxygen, so the results of day 75 indicates that oxygen was not limited in SA during days 61–75. While the invariant share of AOB should be due to the ammonia concentration limited for AOB caused by mass transfer resistance in SA. The share of AOB was still 3.22 times of the share of NOB on day 75 in SB, which should be related to the higher maximum specific growth rate of AOB and no substance limited for AOB.

Delightfully, nitritation recovered with aerobic flow-separated ball during days 77–100, resulting from the large oxygen concentration gradient in the internal cubic sponges of aerobic flow-separated ball (SC). Where NOB in SC could be inhibited imposed by limited oxygen. As can be seen from Table 2, the share of nitrifying bacteria in SC decreased sharply from day 77 to 100, to which both shares of AOB and NOB made contribution. Reasonable explanation was that NOB was inhibited effectively by limited oxygen, which should be one of the main reasons for recovery of nitritation. And AOB was inhibited by the limited ammonia concentration. However, although the share of AOB in SC decreased largely, that in SD increased largely from day 77 to 100. On day 100, AOB/NOB was up to 5.67 and superiority of AOB was prominent. As discussed above, that was because of higher maximum specific growth rate at temperature of 20°C and no substance limited. In addition, it was generally accepted that AOB have higher oxygen affinity than NOB [7,34], resulting in more AOB multiplied in SD. The large multiplication of AOB in SD should also be the other main reason for recovery of nitritation.

The SOUR reduced slightly from 0.40 to average 0.38 mg O_2/(L·VSS·h) when using aerobic flow-separated ball (Fig. 3). It is acceptable that the effect of aerobic flow-separated ball was only about 5% reduction. No obvious inhibition on activity might be due to that the reactor was operated under high oxygen. Moreover, MLVSS increased from 2,590 to

average 3,220 mg/L, and about 24% increment was benefit for improving efficiency. Here SOUR was not affected negatively with aerobic flow-separated ball. It can be concluded that the contribution of aerobic flow-separated ball to recovery of nitritation under high oxygen results from enhanced internal oxygen inhibition on NOB and large external growth of AOB without negative effect on reactor efficiency.

3.4. Achieving partial nitritation through improving effluent concentration of ammonia

Temperature was further decreased to 15°C in phase 3 (days 101–150). During days 101–119, nitritation failed even using aerobic flow-separated ball, as variation of NAR being described in Fig. 4. In phase 3, average SOUR was 0.29 mg O_2/(L·VSS·h) which was about 76% of that in phase 2 (Fig. 3). Same with days 61–75 of phase 2, from day 100 to 119 in SC the share of NOB which increased significantly resulted from no oxygen limited and adequate nitrite and the stable share of AOB was resulted from limited ammonia concentration. Something different was that in SD the AOB/NOB decreased more extent from day 100 to 119 compared with that from day 60 to 75 (Table 2). Because there was no difference between the maximum specific growth rates of AOB and NOB at temperature of 15°C, leading to more NOB growth in SD.

From day 120, HRT was shortened to improve effluent concentration of ammonia which was 25.9–30.1 mg/L during days 120–150 as described in Fig. 4. Nitritation recovered gradually and NAR reached to 100% on day 140. During days 141–150, stable partial nitritation was achieved. Because the reactor was continuous and completely mixed, so improving effluent concentration of ammonia resulted in ammonia nitrogen loading of SD increased approximately six times of that during days 101–120. More oxygen was consumed in the SD, therefore, NOB in the SC was inhibited by limited oxygen, the share of NOB in SC decreased from day 120 to 150. But the share of nitrifying bacteria in SC on day 120 was similar to that on day 150, resulting from large increase of the share of AOB from day 120 to 150 because of the adequate ammonia. Despite the maximum specific growth rates of AOB and NOB were similar, nitritation performance still went well from day 120 to 150 (AOB/NOB increased in Table 1). On the hand, improving effluent concentration of ammonia made the actual specific growth rate of AOB higher than that of NOB. Through simulation, Isanta et al. [7] pointed that a lower specific growth rate of NOB can be maintained at any point in the biofilm (even at 12.5°C) due to the bulk concentration of ammonium imposed which was in full agreement with the results in this study. On the other hand, FA inhibition on NOB might play a role, which was 0.19–0.22 mg/L and in the range of inhibition values for NOB [28,30]. Nevertheless, FA inhibition should be slight because the FA concentration here was not much greater than the low value of the range. What's more, improving effluent concentration of ammonia made SOUR increase slightly (Fig. 3) resulting from increased substance concentration for AOB.

In the study of Sun et al. [35] nitritation process can be achieved rapidly at low temperatures (13.0°C–17.6°C) in 77 d and was maintained stably for as long as 233 d by applying FA inhibition on NOB coupled with a real-time control. In order to investigate the effect of improving ammonia concentration on the recoverability of nitritation in biofilm reactor, Zheng et al. [12] shortened HRT from 7 to 4.6 h. But nitritation was not recovered in their study, which was not in agreement with this study's results. It should be related to the aerobic flow-separated ball inside where oxygen limiting condition could be built.

3.5. Recovery of nitritation through sequencing batch feed mode

In phase 4 (days 151–210), temperature was decreased to 10°C. Nitritation failed same with phases 2 and 3. Reasons should also be similar with phases 2 and 3. Additionally, at temperature of 10°C the maximum specific growth rate of NOB was higher than that of AOB which was not benefit for nitritation. Such explanations can also be verified by the results on day 150 and 179 in Table 2, same with phase 2 and 3. It was remarkable that both in SC and SD nitritation performance went bad obviously from day 150 to 179, especially in SD on day 179 AOB/NOB was only 1.05 (Table 2). This was due to lower specific growth rate of AOB.

In theory and practice, nitritation with batch feed mode can easily be achieved compared with continuous feed mode [12,36,37]. In this study, sequencing batch feed mode was used to recover the nitritation performance. As shown in Fig. 4, NAR increased gradually during days 180–205 and then it reached to 100% during days 206–210 where stable partial nitritation was achieved. Here the contributions of sequencing batch feed mode to recovery of nitritation should include three aspects as follows.

First, due to in one cycle ammonia was varied from 60 to 27.1–30.2 mg/L the ammonia nitrogen loading of SD was higher than that in continuous mode of days 150–180. So stronger oxygen limited was built in the SC, same with phases 2 and 3, which can also be seen from results on day 179 and 210 in Table 2. Second, FA inhibition on NOB under sequencing batch feed mode was stronger than that under continuous mode. That was because the FA concentration was varied from 0.29 to 0.14–0.15 mg/L in one cycle, while under continuous mode FA concentration was 0.14–0.15 mg/L. Last but not least, growth of NOB compared with AOB was hysteretic in each cycle under sequencing batch feed mode. Because the needed nitrite for NOB was produced gradually in each cycle, while under continuous feed mode in completely mixed reactor effluent nitrite was the substance for NOB. In addition, only about 50% ammonia was converted to nitrite in this study for partial nitritation, resulting in for sequencing batch feed mode in each one cycle, the maximum of nitrite concentration was approximately equal to the minimum of ammonia concentration. Therefore specific growth rate of AOB could be kept higher than that of NOB which was also good for nitritation.

Contrast experiment was carried out during days 180–210 to state the importance of hysteretic nitrite for effective NOB inhibition. Between this study and contrast experiment, only difference was that additional 30 mg/L nitrite was added into the influent of contrast experiment. Nitritation had not been recovered in contrast experiment. And sequencing batch feed mode was carried out in contrast experiment, but from the beginning of one cycle nitrite for NOB was adequate. It could be concluded that hysteretic growth of NOB compared with AOB was required to recover nitritation performance during

days 180–210 of this study, which might be the most main reason for recovery of nitritation.

3.6. Effect of temperature on nitritation performance

To test the application of nitritation in low-strength wastewater treatment, temperature was the necessary investigated factor. Therefore, four temperature levels (25°C, 20°C, 15°C and 10°C) were set in this study. As demonstrated in Fig. 4 and discussed above, reduction of temperature was not beneficial to keep the specific AOB growth rate higher than NOB, and it made SOUR decreasing as well as the weakening of oxygen limited conditions. Hence, nitritation at lower temperature needs more strict control conditions. Drew from this study, adoption of aerobic flow-separated ball, improving effluent concentration of ammonia and sequencing batch feed mode were beneficial for recovery of nitritation in completely mixed and continuous biofilm reactor under high oxygen. Stable partial nitritation for low-strength wastewater at temperature of 10°C could be achieved in aerobic flow-separated ball reactor under high oxygen. Thankfully, even low temperature was the recognized difficulty to apply nitritation in domestic wastewater, many solutions were feasible, such as strengthening oxygen limiting condition and FA inhibition and keeping NOB hysteretic grow. Conclusions derived from this study would provide other biofilm reactor with reference to solve nitritation at low temperature. Perez et al. [36] has pointed by simulation that the effect of temperature on nitritation in biofilm reactor was slighter than competition for oxygen. In the study of Bian et al. [37], regardless of temperature, nitritation could be kept stable with different oxygen limiting conditions through adjusting the ratio of DO/TAN. Some results of this study were in agreement with the previous researches.

As Fig. 4 demonstrates, NAR decreased more slowly at lower temperature which indicated that failure of nitritation was related to temperature and lower temperature resulted in slower failure. In this study, failure of nitritation in phases 2, 3 and 4 spent approximately 12, 16 and 24 d, respectively. The actual failure rate at temperature of 15°C was about 75% of that at temperature of 20°C. Similarly, the actual failure rate at temperature of 10°C was about 50% of that at temperature of 20°C. As we all know, the failure of nitritation was directly resulted from growth of NOB. The maximum specific growth rate of NOB at temperatures of 20, 15 and 10°C were 0.44, 0.32 and 0.23 d^{-1}, respectively. Here the theoretic failure rate at temperature of 15°C and 10°C were about 73% and 52% of that at temperature of 20°C. The ratio of theoretic failure rates was similar to that of actual failure rates, which indicated that slower growth of NOB at lower temperature was the main reason for slower failure of nitritation. Such a find was very beneficial for stable nitritation in biofilm reactor under low temperature, because in which nitritation failed slowly, and then we had enough time to discover the failure of nitritation and recover it.

Obviously, SOUR was largely affected by temperature in this study as Fig. 3 describes. Adoption of aerobic flow-separated ball, improving effluent concentration of ammonia and sequencing batch feed mode all had effect on SOUR, but their effects could be neglected when considering the effect of temperature in this study. The average SOURs in phases 1, 2, 3 and 4 were 0.50, 0.39, 0.29 and 0.18 mg O_2/(L·VSS·h), respectively. Linear fitting of SOUR and temperature (T, °C) could be expressed as: SOUR = 0.0212 × T – 0.031, R^2 = 0.9996. It was useful that the effect of temperature on activity could be considered as linear when temperature changed several or ten several °C in actual application. Besides, the SOURs at temperature of 15°C and 10°C were about 74% and 46% of that at temperature of 20°C, whose ratio was also similar to that of actual failure rates. The nitritation failure rate should be related to the SOUR.

4. Conclusions

Nitritation for low-strength wastewater at different temperatures (25°C, 20°C, 15°C and 10°C) in an MBBR was achieved through different operation conditions under high DO. Negative effect of decreased temperature on nitritation can be compensated by positive operation conditions, even at temperature of 10°C partial nitritation can be achieved with aerobic flow-separated ball through sequencing batch feed mode. Contribution of aerobic flow-separated ball to nitritation recovery under high DO results from enhanced internal oxygen inhibition on NOB without negative effect on the efficiency of reactor. Improving effluent concentration of ammonia under continuous mode is also beneficial for internal oxygen repression on NOB meanwhile FA inhibition on NOB is strengthened. Hysteretic growth of NOB compared with AOB in each cycle should be the main reason for the superiority of sequencing batch feed mode to recover nitritation. The finding that nitritation fails more slowly at lower temperature is good for stable nitritation at low temperature due to no need of strict inspection for failure of nitritation. In actual, the effect of temperature on activity can be considered as linear when temperature changes several or ten several °C.

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