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### Post-anoxic denitrification via nitrite driven by PHB in feast–famine sequencing batch reactor



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#### HIGHLIGHTS

• Stable partial nitrification was achieved by controlling aeration time at 2.5 h.

- PHB can be used as a proper carbon source for post-anoxic denitrification.
- The faster growth rate of AOB than NOB was the main reason for achieving nitrite accumulation.

• The secondary SOP release was negligible at low ammonia loading.

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#### ABSTRACT

Recently, it was found that excess phosphorus removal could be induced by aerobic/extended-idle regime. In this study, an anoxic period was introduced after the aeration to realize simultaneous nitrogen and phosphorus removal. The results demonstrated that stable partial nitrification could be achieved by controlling the aeration duration at 2.5 h because it could not only obtain a desirable ammonia oxidation to nitrite but also avoid the extensive aeration converting nitrite to nitrate, and moreover, the accumulated poly-3-hydroxybutyrate still remain in a relative sufficient concentration (1.5 mmol C g<sup>-1</sup> VSS), which could subsequently served as internal carbon source for post-anoxic denitrification. The nitrite accumulation ratio was observed to have relatively high correlation with biological nutrient removal. Over stages with stable high-level nitrite accumulation, the process achieved desirable and stable nitrogen and phosphorus removal efficiencies averaging 95% and 99% respectively. Fluorescence in situ hybridization analysis showed that the faster growth rate of the ammonia oxidizing bacteria than the nitrite oxidizing bacteria was the main reason for achieving nitrite accumulation. In addition, the secondary phosphorus release was negligible and the process maintained excellent nutrient removal under low influent ammonia nitrogen.

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#### 1. Introduction

Excess N and P have long been viewed as major factors causing eutrophication. As to N removal, almost all wastewater treatment plants worldwide achieve N removal by alternately exposing a population of bacteria that includes both nitrifiers and denitrifiers to oxic conditions for nitrification and anoxic conditions for denitrification (Lee et al., 2010). With respect to P removal, enhanced biological phosphorus removal (EBPR) processes which are conventionally conducted by alternating anaerobic and aerobic conditions and exploited the ability of certain microorganisms to

\* Corresponding authors at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, China. Tel.: +86 731 88823967; fax: +86 731 88822829. accumulate P in excess of metabolic requirement and to store it as the intracellular biopolymer polyphosphate (poly-P), are widely applied for real wastewater treatment (Chen et al., 2004; Mullan et al., 2006). In order to achieve simultaneous N and P removal, some wastewater treatment processes such as anaerobic/anoxic/ aerobic process have been developed. The EBPR systems with cyclic changes of anaerobic and aerobic (and/or anoxic) conditions have an economical advantage of lower sludge production and less use of chemicals, and play an increasingly important role in controlling eutrophication from all over the world (Oehmen et al., 2007).

However, there exist some contradictions which limit system efficiency. First of all, the circulating nitrate was reported as an inhibiting factor to anaerobic P release and could lead to reduced efficiency of biological P removal (BPR) (Barker and Dold, 1996). Secondly, the enrichment of nitrifying bacteria needs relatively long sludge retention time (SRT) and exerts negative influence on

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P removal because only relatively short SRT can lead to desirable EBPR performance (van Loosdrecht et al., 1998). On the other hand, denitrifying bacteria tend to compete with phosphate accumulating organisms (PAOs) for the limited dissolved oxygen (DO) in low strength wastewater (Ahn et al., 2002).

Fortunately, it is possible to circumvent limits exerted by traditional simultaneous N and P removal theories. Dirck et al. (2001) and Carucci et al. (2001) mentioned that since the bacteria encounter external substrates feast and famine periods. The regime can induce the bacteria to store external substrates as internal storage compounds in the feast period which hereby can take up available substrate very fast and utilize it to gain a more balanced growth. Studies on sequencing batch reactor (SBR) process for wastewater treatment have shown that the build-up of internal electron donors as storage compounds is of great importance for N removal (van Loosdrecht et al., 1997; Beun et al., 2000; Third et al., 2003). Therefore, storage compounds can be served as internal carbon sources for post-anoxic denitrification. Although the endogenous denitrification efficiency is low due to the limited carbon sources (Vocks et al., 2005), the limited internal carbon sources can be used to satisfy the need of denitrification via nitrite. In comparison to the conventional nitrate pathway, the nitrite pathway not only improved the total nitrogen (TN) removal by about 20% but also reduced aeration costs by 24% (Ma et al., 2009). Reports of stable nitritation processes from activated sludge have appeared frequently in the literatures and several process parameters, including DO concentration, temperature, SRT and aeration pattern, have been found to inhibit or wash out nitrite oxidizing bacteria (NOB) selectively to achieve sustainable partial nitrification to nitrite (Pollice et al., 2002; Ruiz et al., 2003). However, reliable termination of nitrification at nitrite (nitritation) has been proved difficult in the treatment of domestic wastewater, and controlling duration of aeration seems to be an ideal option for stable partial nitrification (Blackburne et al., 2008; Guo et al., 2009).

Recently, it has been reported that BPR could be achieved without specific anaerobic phase in activated sludge system if the idle period is extended properly (Wang et al., 2008, 2012). Though BPR can be well achieved during the aerobic period of aerobic/extended-idle regime, the system's denitrification capacity was comparatively weak and the TN removal efficiency was low. Therefore, an anoxic period is needed after the aerobic phase to realize thorough denitrification. The aim of this paper is to develop a process combining BPR with denitrification via nitrite driven by storage compounds to achieve simultaneous N and P removal. In this respect, an anoxic period is performed after the aerobic phase to realize thorough denitrification, and aeration duration control was used to realize sustainable partial nitrification to nitrite. The nitrite accumulation ratio and the ammonia oxidizing bacteria (AOB) and NOB population sizes were monitored. The impact of the nitrite accumulation ratio on P and N removal performance was assessed. Additionally, the mechanism of achieving nitrite accumulation was also discussed.

#### 2. Materials and methods

#### 2.1. Sequencing batch reactor operation

Seed sludge was inoculated into a SBR with a working volume of 42 L. Each cycle consisted of approximately 240 min aerobic period, 150 min anoxic period, 28 min settling, 2 min decanting, and 60 min idle periods. 30 L supernatant was discharged at the end of settling phase, and 30 L refresh wastewater was introduced during the first 2 min of aerobic period. The DO was supplied by an air compressor through an air diffuser inside the reactor during aerobic period and a magnetic stirrer was used to attain sound liquid

mixing during anoxic phase. Temperature inside the reactor was maintained at  $23 \pm 3$  °C with a thermostatic heater and pH was kept about 7.0. The SRT was maintained at approximately 10 d by withdrawing the sludge at the end of the anoxic period.

#### 2.2. Wastewater and sludge

The wastewater was collected from septic tank effluent in a local residential district. It was characterized by 260–350 mg L<sup>-1</sup> COD, 20–30 mg L<sup>-1</sup> NH<sub>4</sub><sup>+</sup>, 8–12 mg L<sup>-1</sup> PO<sub>4</sub><sup>3-</sup>. In addition, the wastewater had typical volatile fatty acid contents of 90–160 mg L<sup>-1</sup> acetic acid, 80–120 mg L<sup>-1</sup> propionic acid and a few other acids. Its pH level was about 7.0.

The seed sludge was obtained from the first wastewater treatment plant in Changsha, PR China. The initial concentration of mixed liquor suspended solids (MLSS) was about 4000 mg  $L^{-1}$ .

#### 2.3. Experiment plans and operational conditions

To understand more clearly how the aeration duration affected N removal and to determine an appropriate aeration time that not only contribute to partial nitrification but also accumulate enough concentration of internal storage compounds, the experiments on the achievement, destruction and recurrence of the nitrite pathway were performed in seven stages. The duration of aeration was shortened from the normal 4 to 2.5 h over the first three stages, and was further shortened to 1.5 h during Stage IV and V. Then in Stage VI, the aeration duration was return to 2.5 h. The variations of aeration duration along with the seven phases are summarized in Table 1. During the whole experimental period, anoxic time was maintained at 2.5 h and each cycle was kept at 8 h. The idle time was correspondingly extended along with the decease of the aeration time.

#### 2.4. Batch experiment

To examine the secondary P release during anoxic period under low influent  $NH_4^+$ , batch experimental was conducted in three identical reactors with working volumes of 2 L each. Seed sludge was taken from the parent SBR at the end of the anoxic phase but before settling. Three types of synthetic medium were added to the 3 reactors, all of the medium contained 300 mg L<sup>-1</sup> COD (acetate) and 10 mg L<sup>-1</sup> PO<sub>4</sub><sup>3-</sup>, while  $NH_4^+$  in the reactors were 15, 25 and 40 mg L<sup>-1</sup>, respectively. The duration of aeration in batch experimental was maintained at 1.5 h.

#### 2.5. Analytical methods

Sludge samples from the reactors were immediately filtered through a Whatmann GF/C glass microfiber filter (1.2  $\mu$ m). The filtrate was analyzed for total phosphorus (TP), dissolved organic carbon (DOC), TN, NH<sup>4</sup><sub>4</sub>, NO<sup>-</sup><sub>2</sub>, NO<sup>-</sup><sub>3</sub> and the filter was assayed for MLSS, mix liquor volatile suspended solids (MLVSS), poly-3-hydroxybutyrate (PHB) and sludge TN content.

TN,  $NH_4^+$ ,  $NO_2^-$ ,  $NO_3^-$ , soluble orthophosphate (SOP), MLSS and MLVSS were measured according to Standard methods (APHA, 1998). DO was measured by WTW Oxi 3210 SET 3 with DO probes (WTW company, Germany). DOC was determined by using a TOC analyzer (Shimadzu TOC-500, Japan) after membrane filtration (0.45 mm cellulose nitrate filter). In addition, Energy Dispersive Spectrometer (EDS, QUANTA 200, USA) has also been used to determine nitrogen content of dried activated sludge. 10 mL of activated sludge has been collected for freeze drying over 24 h and the samples were tested by EDS afterwards.

PHB was measured according to Oehmen et al. (2005) in a GC system operated with a Hewlett Packard 5890 column (30 m

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experiment plans and operational conditions.								
Parameter	Stage I	Stage II	Stage III	Stage IV	Stage V	Stage VI	Stage VII	
Time (d)	1-60	61-90	91-120	121-180	181-210	211-240	241-300	
Aeration time (h)	4	3.5	3	2.5	2	1.5	2.5	

length  $\times$  0.53 mm id  $\times$  1.00 µm film). Fluorescence in situ hybridisation (FISH) detailed in Ma et al. (2009) was used to analyze the microbial composition of the sludge during the steady state operation. Sludge samples taken from the aerobic period at various times were analyzed by FISH for both AOB and NOB. FISH quantification was performed by Image-pro plus 7.0 Software, the relative abundance of interested bacteria was determined as the mean percentage of all bacteria.

#### 3. Results and discussion

Table 1

#### 3.1. Achievement, destruction and recurrence of the nitrite pathway

Fig. 1a shows the profiles of influent and effluent  $NH_4^+$ , and effluent  $NO_2^-$  and  $NO_3^-$  concentration during the long-term operation. Fig. 1b demonstrates the nitrite accumulation ratio (the ratio of nitrite to the sum of nitrate and nitrite), NOB population size, sludge volume index (SVI) and aeration time during the whole experimental period. Fig. 1c shows the profiles of  $NH_4^+$ , TN and SOP removal efficiencies during the entire course of the experimental study.

In Stage I, when the aeration time was controlled at 4 h, the effluent  $NH_4^+$  concentration was as high as  $10 \text{ mg L}^{-1}$ , the nitrite accumulation ratio was just 11% and the NOB population was about 2.8%. During this stage, the accumulation of nitrite was less than that of nitrate. Then in Stage II, the aeration time was adjusted to 3.5 h, the nitrite accumulation ratio gradually increased to 23% at day 50, 3% higher than the nitrate accumulation ratio. In the meantime, the NOB population reduced to 2.1% at the end of Stage II. During Stage III, the aeration time was further shorten to 3 h, at day 120 the nitrite accumulation ratio was increased to 30% and the NOB population was reduced to 1.4%.

During Stage IV, the aeration time was reduced to 2.5 h, nitrite accumulation ratio increased slowly from the initial 30% to approximately 66% in the first 20 d (2 SRTs), and then increased rapidly reaching over 90% on day 140 and stabilized at an average value of 97% subsequently. The NOB population reduced to 0.8% at day 140, and further reduced to 0.4% at the end of Stage IV. As shown in Fig. 1b, the NOB population decreased from 2.8% to 0.3% from day 1 to 140.

The aeration time was gradually decreased to 1.5 h during day 181–240. Nitrite accumulation ratio maintained at above 60% in the first 10 d, and then gradually decreased to about 5% in the following 50 d. The corresponding NOB population increased from 0.2% (day 180) to 2.8% by the end of Stage V.

To resume high nitrite accumulation, the aeration time was resumed to 2.5 h during day 241–300. As shown in Fig. 1b, nitrite accumulation ratio maintained at 15–30% in the first 20 d (2 SRTs) before rapidly increased to above 90%, confirming the lag phase in Stage IV.

The experimental results demonstrated that N removal via nitrite can be achieved in activated sludge system by controlling the aeration time at 2.5 h. Under the above aeration duration, the nitrite pathway can be established in 2 SRTs through the elimination or substantial reduction of the NOB population. The nitrite pathway can be destructed by applying duration of aeration more than 3.5 h or less than 1.5 h, and 2 SRTs is required for the recurrence of the nitrate pathway.

#### 3.2. Impact of nitrite accumulation on nutrient removal performance

A summary of the performance parameters in the reactor during Stage IV is shown in Table 2. As shown in Table 2, over stages with stable high-level nitrite accumulation, the average  $NH_4^+$  removal efficiency was over 95% under the influent N load of 0.067 ± 0.015 g  $NH_4^+$  g<sup>-1</sup> VSS d<sup>-1</sup>. The effluent  $NH_4^+$  concentration was lower than 1 mg L<sup>-1</sup>, and for the end of stages IV and VI lower than 0.5 mg L<sup>-1</sup>.

Fig. 1 shows a clear correlation between the nitrite accumulation ratio and the TN removal efficiency. The TN removal efficiency in aerobic period increased with the increase of the nitrite accumulation ratio. During Stage IV, the TN removal efficiency increased from 77% to 83% when nitrite accumulation ratio rose from 61% to 96%, representing a relative increase of 6%. During Stage IV and VII, when the level of nitrite accumulation ratio was the highest, so was the TN removal efficiency. In contrast, in Stage VI when the nitrite pathway was destructed, the worst N removal was detected despite a similar wastewater composition. This clearly demonstrates the benefits of the nitrite pathway in enhancing the N removal performance.

In addition, the nitrite accumulation ratio was also associated with SOP removal. This treatment process without anaerobic phase which was necessary for EBPR could achieve a desirable SOP removal. As shown in Fig. 1 and Table 2, during high-level nitrite accumulation stages, the SOP removal efficiency was maintained over 99%. The effluent SOP concentration maintained at low level (lower than 0.5 mg L<sup>-1</sup>) under an influent SOP concentration of  $10 \pm 2 \text{ mg L}^{-1}$ . When nitrite accumulation ratio decreased quickly in Stage V and VII, the SOP removal efficiency was correspondingly decreased from 99% to 75%. The performance of SOP removal finally deteriorated with the decrease of nitrite accumulation ratio.

The concentration variations of SOP,  $NH_4^+$ ,  $NO_2^-$ ,  $NO_3^-$ , PHB, Glycogen, DO and DOC in a cycle of Stage I (a, b), IV (c, d) and VI (e, f) are illustrated in Fig. 2. As shown in Fig. 2c, SOP concentration rose to 30 mg L<sup>-1</sup> rapidly during the first 15 min of the operation, indicating that the start-up of the aerobic zone might serve as anaerobic phase because during this period the DO level remained very low. These results were consistent with those gained by Wang et al. (2012). Moreover, most of the SOP was removed along with the accumulation of N, and SOP concentration decreased from 30 to 0.4 mg L<sup>-1</sup> from 15 to 300 min.

The possible approach of SOP removal was similar to the removal of organic matters and part of N. During the extended-idle period, when external substrates were no longer available, poly-P and PHB became the main energy sources for the subsistence of microorganisms. Therefore, in the feast period, after experiencing the famine period, microorganisms excessively took up SOP into cells and transformed it to poly-P. Therefore, SOP concentration rapidly decreased after a moderate rise at the beginning of the cycle and finally maintained a low level at the end of the anoxic period. The gradual decrease of SOP concentration in the anoxic period was partly attribute to the SOP required for the growth of microorganisms but mainly due to denitrifying phosphorus removal, which occurs due to the capacity of PAOs to use nitrate and/or nitrite as an electron acceptor for SOP removal instead of oxygen under anoxic conditions (Oehmen et al., 2007). This indicated that the degree of denitrification decided SOP removal in anoxic period and further affected SOP removal efficiency of the system.

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**Fig. 1.** (a) Profiles of influent and effluent NH<sup>4</sup><sub>4</sub>, effluent NO<sup>-</sup><sub>2</sub> and NO<sup>-</sup><sub>3</sub> concentration during the long-term operation. (b) Profiles of nitrite accumulation ratio, aeration time, SVI and NOB population size. (c) Profiles of NH<sup>4</sup><sub>4</sub>, TN and SOP removal efficiencies.

3.3. Nitrogen removal via nitrite pathway

# 3.3.1. The partial nitrification achieved by controlling aeration duration

The nitrite accumulation under low DO condition is usually explained by the difference in oxygen saturation constant between AOB and NOB (Ma et al., 2009). The oxygen saturation constant values of AOB and NOB are 0.03 and 0.4 mg  $L^{-1}$ , respectively according to Blackburne et al. (2008). In other words, the

oxygen-binding capacity of NOB is weaker than that of AOB, and oxygen limitation therefore influences the activity of NOB more enormously than that of AOB.

It is observed from Table 3 that at the beginning of the Stage I, the community structure of the activated sludge was that the number of NOB was nearly equal to that of AOB. However, 140 d later, AOB became dominant bacteria, while the number of NOB gradually decreased. Additionally, on the 180th d, NOB had been almost washed out from the system.

Table 2
Summary of the performance parameters in the reactor during Stage IV.

Item	Max	Min	Average
N influent concentration (mg L <sup>-1</sup> )	41	38	40
N effluent concentration (mg L <sup>-1</sup> )	1.5	0.3	0.6
N removal efficiency (%)	99	96	98
TN influent concentration (mg $L^{-1}$ )	49	41	45
TN effluent concentration (mg $L^{-1}$ )	3.0	0.9	1.4
TN removal efficiency (%)	84	77	81
SOP influent concentration (mg L <sup>-1</sup> )	12	8	10
SOP effluent concentration (mg L <sup>-1</sup> )	0.7	0.2	0.4
SOP removal efficiency (%)	99	98	99
MLSS (mg $L^{-1}$ )	4490	3785	4120
MLVSS (mg $L^{-1}$ )	3217	2796	2966

Fig. 2 shows a strong dependency of the nitrite accumulation on aeration duration. As shown in Fig. 2d, DO inside the reactor experienced a rapid decline in the initial 15 min (from 3.6 to 0.75 mg  $L^{-1}$ ) and then rose gradually to 3.7 mg  $L^{-1}$  during the remainder of the aerobic period.

According to Guo et al. (2009), partial nitrification could be achieved by controlling aeration duration, which avoided the extensive aeration converting nitrite to nitrate. In this study, partial nitrification was achieved when aeration duration was controlled at 2.5 h because it fully met the requirement for achieving a desirable ammonia oxidation rate while accumulating nitrite other than nitrate. The results presented in Fig. 2 show that nitrite accumulation occurred mainly from 60 to 150 min, while nitrate



Fig. 2. Profiles of SOP, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PHB, glycogen, DO and DOC concentration in a cycle of Stage I (a, b), IV (c, d) and VI (e, f).

Table 3           Shift of nitrifuing bostorial community structure in the reactor
Shift of nitrifying bacterial community structure in the reactor.

Time (day)	AOB (%)	NOB (%)		
	(probe-NSO1225)	Nitrobacter (probe-NIT3)	Nitrospira (probe-NTSPA662)	
Seed inoculum	$2.9 \pm 0.4$	$0.9 \pm 0.3$	1.9 ± 0.4	
58	3.3 ± 0.6	$0.7 \pm 0.2$	$2.2 \pm 0.4$	
100	4.1 ± 1.1	<0.5	1.6 ± 0.3	
116	5.7 ± 1.3	N.D	1.1 ± 0.2	
180	6.7 ± 1.6	N.D	<0.5	
206	5.8 ± 1.4	N.D	<0.5	
240	$1.2 \pm 0.3$	$1.0 \pm 0.3$	2.6 ± 0.5	
298	3.8 ± 0.7	N.D	<0.5	

N.D: No detect.

accumulation was detected mainly during 30–60 min and after 150 min. The results suggest that in the initial 150 min (especially from 60 to 150 min) AOB was the dominant bacteria and the activity of NOB was inhibited, but after 150 min aeration the activity of NOB became more stronger than that of AOB and NOB turned into the dominant bacteria. This means that aeration less than 2.5 h will lead to an insufficiency ammonia oxidation, while the extensive aeration exceeding this duration will convert the accumulated nitrite to nitrate. Therefore, the aeration duration of 2.5 h is the optimum option because it could not only obtain a desirable ammonia oxidation to nitrite but also avoid the extensive aeration converting nitrite to nitrate.

#### 3.3.2. Storage compounds driving denitrification via nitrite

As shown in Fig. 2a, in Stage I almost all  $NH_4^+$  and SOP were removed. SOP concentration decreased from 12 to 0.4 mg L<sup>-1</sup>. By the end of the aerobic period (240 min), PHB declined to a low concentration (0.8 mmol C g<sup>-1</sup> VSS), while glycogen maintained a high level (3.78 mmol C g<sup>-1</sup> VSS). Due to the low PHB concentration, nitrite and nitrate accumulated during aeration time could not be largely denitrificated despite the high glycogen level. Besides, it was observed from Fig. 2a that the extensive aeration gradually converted

nitrite to nitrate after 150 min aeration, and nitrate increased by more than 5.5 mg L<sup>-1</sup> while nitrite decreased from 8.1 to 2.3 mg L<sup>-1</sup> at the end of the aerobic period, which implies that partial nitrification could not be achieved through 240 min aeration.

The concentration variations of SOP, NH<sub>4</sub><sup>+</sup>, NO<sub>2</sub><sup>-</sup>, NO<sub>3</sub><sup>-</sup>, PHB, glycogen, DO and DOC during a cycle in Stage IV were illustrated in Fig. 2c and d. It was observed that by the end of the aerobic phase, about 98% of NH<sup>+</sup><sub>4</sub> was depleted, and a considerable nitrite accumulation (4.2 mg  $L^{-1}$ ) and a relatively small accumulation of nitrate  $(1.8 \text{ mg L}^{-1})$  were detected. Along with the consumption of DO and DOC, PHB was rapidly accumulated during the first 30 min (feast period) of the operation, and gradual decrease afterwards accompanied with the nitrification and denitrification. About 0.66 mmol C g<sup>-1</sup> VSS PHB was consumed in the anoxic phase, while just a little glycogen consumption was detected. Dirck et al. (2001), Carucci et al. (2001) and Carta et al. (2001) reported the similar phenomenon that acetate was consumed fast at the beginning of the process and simultaneously stored as internal storage compounds. During the anoxic phase, the concentration of PHB continued decreasing to the initial level by the end of this process, and correspondingly nitrate and nitrite were almost completely removed. This phenomenon indicated that PHB driven denitrification of nitrate and nitrite.

It was observed from Fig. 2e that in the aerobic period, with the decrease of  $NH_4^+$ , nitrite increased by 2.2 mg L<sup>-1</sup> and nitrate slightly increase to 1.5 mg L<sup>-1</sup>. Along with the rapid consumption of DOC during the first 30 min, PHB reached its peak value of 2.66 mmol C g<sup>-1</sup> VSS and then declined. After 90 min aeration, PHB still remained relatively high level of 1.75 mmol C g<sup>-1</sup> VSS. In anoxic zone, nitrite and nitrate accumulated during aeration phase were largely removed which justified that this PHB concentration was desirable for the treatment process. Fig. 2e shows that at the end of the aeration phase, the concentrations of SOP and NH<sub>4</sub><sup>+</sup> were 6.1 and 6.8 mg L<sup>-1</sup> respectively, suggesting that aeration time was not long enough for nutrient removal. Therefore, the operational procedure of Stage VI was proven to be inappropriate for nitrite accumulation.



Fig. 3. Profiles of  $NO_3^-$ ,  $NO_2^-$ ,  $NH_4^+$ , SOP concentration in a cycle under different influent  $NH_4^+$  concentration.

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Aeration time is a key factor in controlling this treatment process. The criterion for an applicable aeration time was that it should not only maintain partial nitrification but also help bacterial to remain desirable PHB concentration. 2.5 h of aeration is an appropriate duration for the process because when the system achieved an almost complete ammonia oxidization, the accumulated PHB still remain in a relative sufficient concentration (1.5 mmol C g<sup>-1</sup> VSS), which could subsequently served as an internal carbon source for denitrification via nitrite.

#### 3.3.3. Nutrient removal under low ammonia nitrogen concentration

The influent  $NH_4^+$  concentration is particularly important to post-anoxic processes because it determines anoxic nitrite and nitrate availability. Low influent  $NH_4^+$  can lead to the untimely depletion of nitrate during anoxic period and then cause secondary P release (Barnard et al., 1998).

Fig. 3 illustrates the concentration profiles of NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, SOP in a cycle under different influent NH<sup>+</sup> concentration (15, 25 and 40 mg  $L^{-1}$ , respectively). As presented in Fig. 3, the process maintained excellent TN and SOP removal even under low influent  $NH_{4}^{+}$  (15 mg L<sup>-1</sup>), the TN and SOP removal efficiencies were 95% and 97%, respectively. The accumulations of nitrite and nitrate both experienced the ultimate value at 180 min (30 min after the anoxic phase was began) and then gradually decreased to the initial level. Though incomplete aerobic SOP uptake was observed, denitrifying P removal in the anoxic period was detected. The secondary SOP release during the idle period was negligible because nitrate was denitrified along with the time and no untimely depletion of nitrate was happened. It was noted that the denitrification was end at the same time despite of the difference of nitrite and nitrate accumulation. Therefore, by controlling aeration time at 2.5 h can not only achieve thoroughgoing denitrification but also avoid the secondary SOP release.

#### 4. Conclusions

Post-anoxic denitrification via nitrite driven by storage compounds (PHB) was successfully achieved in a SBR operated as aerobic/anoxic/extended-idle regime by controlling aeration time at 2.5 h. The aeration duration of 2.5 h could not only obtain a desirable ammonia oxidation to nitrite but also avoid the extensive aeration converting nitrite to nitrate. The results indicated that this treatment process could achieve desirable and stable TN and SOP removal efficiencies averaging 95% and 99% respectively. Microbial analysis demonstrated that the nitrite pathway is established through the substantial reduction of the NOB population in the system. In addition, the process maintained excellent nutrient removal even under low influent ammonia nitrogen of 15 mg L<sup>-1</sup>.

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