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ORIGINAL PAPER

Impact of nitrite on aerobic phosphorus uptake by poly-phosphate accumulating organisms in enhanced biological phosphorus removal sludges

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Abstract Impact of nitrite on aerobic phosphorus (P) uptake of poly-phosphate accumulating organisms (PAOs) in three different enhanced biological phosphorus removal (EBPR) systems was investigated, i.e., the enriched PAOs culture fed with synthetic wastewater, the two lab-scale sequencing batch reactors (SBRs) treating domestic wastewater for nutrient removal through nitrite-pathway nitritation and nitrate-pathway nitrification, respectively. Fluorescence in situ hybridization results showed that PAOs in the three sludges accounted for 72, 7.6 and 6.5 % of bacteria, respectively. In the enriched PAOs culture, at free nitrous acid (FNA) concentration of 0.47×10^{-3} mg HNO_2-N/L, aerobic P-uptake and oxidation of intercellular poly-β-hydroxyalkanoates were both inhibited. Denitrifying phosphorus removal under the aerobic conditions was observed, indicating the existence of PAOs using nitrite as electron acceptor in this culture. When the FNA concentration reached 2.25×10^{-3} mg HNO_2-N/L, denitrifying phosphorus removal was also inhibited. And the inhibition ceased once nitrite was exhausted. Corresponding to both SBRs treating domestic wastewater with nitritation and nitrification pathway, nitrite inhibition on aerobic P-uptake by PAOs did not occur even though FNA concentration reached 3×10^{-3} and 2.13×10^{-3} mg HNO_2-N/L, respectively. Therefore, PAOs taken from different EBPR activated sludges had different tolerance to nitrite.

Keywords Phosphate accumulating organism · Nitrite · Free nitrous acid · Aerobic phosphorus uptake · Enhanced biological phosphorus removal

Introduction

Biological nutrient removal is an economically favorable technology, and widely applied in wastewater treatment plants. Enhanced biological phosphorus removal (EBPR) includes anaerobic phosphorus release and aerobic phosphorus uptake. Under anaerobic conditions, poly-phosphate accumulating organisms (PAOs) take up volatile fatty acids (VFAs) and store them as poly-β-hydroxyalkanoates (PHAs), where energy is generated from hydrolysis of intercellular stored poly-phosphate (poly-P). Under aerobic conditions, the stored PHAs are oxidized to generate energy by PAOs, which is invested to take up excess phosphorus for poly-P synthesis. The net removal of phosphorus can be achieved by discharging waste sludge when rich in poly-P [1]. Biological nitrogen removal is accomplished by nitrification and denitrification. Nitrite as an intermediary compound, in both nitrification and denitrification, can accumulate and be even up to 20 mg/L under certain aerobic condition [2, 3]. Previous studies have verified inhibitory effects of nitrite on microbial activities by inhibiting active transport, aerobic respiration and oxidative phosphorylation, leading to inhibition of anabolism and catabolism [4]. The effect of nitrite on anaerobic P-release by PAOs has been intensively studied. In fact, in the EBPR process, such as anaerobic/anoxic/aerobic process, which is representative of most full-scale

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EBPR plants, nitrite accumulation resulted from nitritation that often occurs in aerobic tank coupled with aerobic P-uptake by PAOs. Therefore, investigating the influence of nitrite on aerobic P-uptake by PAOs is significant to maintain a stable EBPR performance.

Recently, researches on the impact of nitrite on aerobic P-uptake by PAOs have been carried out [5–8]. These studies demonstrated that a certain amount of nitrite could reduce the oxygen uptake rate and P-uptake rate, resulting in inhibition of aerobic growth and P-uptake of PAOs. However, it should be noted that the inhibition levels of nitrite are significantly different in the literature, which may be associated with the differences of seed sludge, sewage types and operational conditions [5, 6, 8–10]. For biological nutrient removal from wastewater, nitrite buildup in system with nitrite-pathway nitritation is much higher than that with nitrate-pathway nitrification. Thus, the tolerance of PAOs to nitrite in these systems will be different. Presently, research on the influence of nitrite on PAOs is mainly focused on enriched PAOs culture fed with synthetic wastewater. Very limited research has been undertaken about the impact of nitrite on PAOs in real domestic wastewater treatment systems with nitrite-pathway nitritation and nitrate-pathway nitrification, especially regarding the comparison of PAOs tolerance to nitrite in different EBPR sludges.

Previous studies proposed that the protonated species of nitrite, free nitrous acid (FNA) rather than nitrite is likely the actual inhibitor of microbial metabolism. A series of studies by Zhou et al. [11–15] have demonstrated that FNA inhibits the anoxic metabolism of PAOs and denitrifying poly-phosphate accumulating organisms (DPAOs), the nitrous oxide reduction by DPAOs and anoxic/aerobic P-uptake of FNA-adapted PAOs. Pijuan et al. [16] investigated the inhibitory effect of nitrite/free nitrous acid (FNA) on the aerobic metabolism of highly enriched PAOs and the sludge treating abattoir wastewater. In another study, Ye et al. [17] also observed that FNA has a negative impact on anabolic and catabolic processes of glycogen accumulating organisms (GAOs). Most studies focused on the enriched PAOs culture and the sludge treating industrial wastewater. Very limited researches were undertaken regarding "real" sludge treating real wastewater. Even though several of them were taken from wastewater treatment plants, seed sludges had been acclimated with synthetic wastewater for a long time to enrich PAOs, and then were used for nitrite/FNA inhibition tests. Particularly, the studies on "real" sludge treating domestic wastewater with nitrite or nitrate-pathway nitrification, namely with different tolerance to nitrite, were very few. Since FNA concentration was significantly affected by nitrite concentration and pH value, further research regarding FNA effect on aerobic P-uptake under different nitrite levels in real domestic wastewater treatment systems with nitrite-pathway nitritation and nitrate-pathway nitrification would be valuable.

In this study, three activated sludges from different EBPR systems were used, i.e., the enriched PAOs culture fed with synthetic wastewater, the lab-scale sequencing batch reactors (SBRs) treating domestic wastewater for nutrient removal through nitrite-pathway nitritation and nitrate-pathway nitrification. To analyze and find the inhibition mechanism of nitrite, the impact of nitrite on aerobic metabolism of PAOs in the three systems was investigated and compared under different FNA levels by controlling pH and nitrite concentration.

Materials and methods

Seed sludge

Three activated sludges were used in this study. The first activated sludge was taken from lab-scale SBR1 fed with synthetic wastewater for 3 months. SBR1 with a working volume of 10 L was operated two cycles everyday under anaerobic/aerobic conditions to enrich PAOs. The duration of each cycle was 290 min consisting of 2 h anaerobic condition, 160 min aerobic condition and 10 min settling and decanting. The SBR was only operated during the day and was idle at night. The synthetic wastewater of 2.5 L was added during each cycle. Sludge retention time (SRT) was about 12 days. The dissolved oxygen (DO) concentration during aerobic period was controlled at 2.0 ± 0.5 mg/L. The pH values in influent, anaerobic period and aerobic period were controlled at 7.5, 7.4–7.6 and 7.3–7.6, respectively, by adding 1 M HCl and 1 M NaOH. Nitrite accumulation did not occur during the aerobic period.

The second activated sludge was taken from lab-scale SBR2 fed with real domestic wastewater for 4 months. SBR2 with a working volume of 11 L was operated three cycles everyday under anaerobic/aerobic conditions. The real domestic wastewater of 5.5 L was added during each cycle. The SRT was about 18 days. The duration of each cycle was 5–7 h consisting of 2 h anaerobic condition, 2–4 h aerobic condition according to influent ammonia concentrations and 1 h settling and decanting. The DO concentration during aerobic period was controlled at 0.5–1.0 mg/L. Operational pH in SBR2 was 7.0–7.6. The system performed nitrite-pathway nitritation, and the ratio of nitrite accumulation per NO_x reached above 95 % with 60 mg/L of the highest nitrite nitrogen (NO_2^--N) concentration at the end of aerobic period.

The third activated sludge was taken from lab-scale SBR3 fed with real domestic wastewater for 4 months. SBR3 with a working volume of 7 L was operated three cycles everyday under anaerobic/aerobic conditions. The real domestic wastewater of 3.5 L was added during each cycle. The SRT was about 20 days. The duration of each cycle was 7 h consisting of 2 h anaerobic condition, 4 h aerobic condition and 1 h settling and decanting. The DO concentration during aerobic period was controlled at 2.0–3.0 mg/L. Operational pH in SBR3 was 7.0–7.6. The SBR3 performed nitrate-pathway nitrification without nitrite accumulation at the end of aerobic period.

Wastewater

Domestic wastewater and synthetic wastewater were used in this study. Domestic wastewater from a campus sewer line was pumped into a storing tank for sedimentation, and then fed into the SBR2 and SBR3. The influent characteristics are presented in Table 1.

The composition of synthetic wastewater (per liter) used for PAOs enrichment in the SBR1 was shown in Table 2. The COD concentration was controlled at 800 mg/L, NH_4^+-N concentration was 40 mg/L and the P-concentration was 20 mg/L.

The synthetic wastewater used for the batch experiments regarding aerobic P-uptake of the three activated sludges mentioned above contained, per liter: 180 mg $MgSO_4{\cdot}7H_2O$, 21 mg $CaCl_2{\cdot}2H_2O$, 3 mg peptone, 0.6 ml nutrient solution and without carbon source. The P-concentration in synthetic wastewater was controlled at the range of 25–85 mg/L by adding K_2HPO_4 and KH_2PO_4 according to the different experimental conditions. The NO_2^--N concentration in the synthetic wastewater was controlled at different levels by adding different amounts of sodium nitrite.

The nutrient solution contained, per liter: 1.5 g $FeCl_3{\cdot}6H_2O$, 0.15 g H_3BO_3, 0.03 g $CuSO_4{\cdot}5H_2O$, 0.18 g KI, 0.12 g $MnCl_2{\cdot}4H_2O$, 0.06 g $Na_2MoO_4{\cdot}2H_2O$, 0.12 g $ZnSO_4{\cdot}7H_2O$, 0.15 g $CoCl_2{\cdot}6H_2O$ and 10 g ethylenediamine tetra-acetic acid (EDTA) [18].

Table 1 Characteristics of the domestic wastewater

Contents	Average
COD (mg/L)	195 ± 28
NH_4^+-N (mg/L)	69 ± 10
NO_2^--N (mg/L)	0
TN (mg/L)	72 ± 11
C/N	2.6 ± 0.4
PO_4^{3-}-P (mg/L)	6.3 ± 2.0

Table 2 Composition of the synthetic wastewater (per liter)

Contents	Quantity	Contents	Quantity (mg)
NaAc	1.176 g	KH_2PO_4	87.55
NH_4Cl	0.153 g	K_2HPO_4	112.2
$MgSO_4{\cdot}7H_2O$	0.18 g	ATU	1.2
$CaCl_2{\cdot}2H_2O$	0.02 g	Peptone	1.5
Nutrient solution	0.6 mL		

Batch experiments

Tested sludges were taken from the three SBRs at the end of anaerobic stage. After washing, the sludge was divided into four parts and each part was put into a 1-L batch reactor. Mixed liquid suspended solids (MLSS) concentration in each batch reactor was controlled at 2,500 ± 100 mg/L. Four batch reactors were supplied with synthetic wastewater. The initial NO_2^--N concentration in the four batch reactors was controlled at different levels by adding different amounts of sodium nitrite. During the tests, pH was on-line monitored and controlled at 7.5 ± 0.05 by adding 0.5 M HCl or 0.5 M NaOH. The DO level was maintained at 2–3 mg/L for the aerobic P-uptake tests. The triplicate tests at each nitrite level were carried out.

Experimental conditions used in the batch tests of aerobic and anoxic P-uptake of activated sludge 1 with enriched PAOs culture, aerobic P-uptake of activated sludge 2 treating domestic wastewater through nitrite-pathway nitritation and activated sludge 3 treating domestic wastewater through nitrate-pathway nitrification were presented in Table 3.

Analytical methods

NO_3^--N, NO_2^--N, PO_4^{3-}-P and MLSS were measured according to APHA Standard Methods [19]. Analysis of PHA, consisting of poly-β-hydroxybutyrate (PHB) and poly-β-hydroxyvalerate (PHV), was performed using gas chromatography (6890N, Agilent, American) according to the method described by Lemos et al. [20]. DO and pH was measured on-line using DO/pH meters (MultiLine 340i, WTW, Germany).

FNA concentration was calculated as formula (1) [21]:

$$\mathrm{FNA} = \frac{\mathrm{NO_2^-N}}{K_a \times 10^{\mathrm{pH}}} \quad (1)$$

where K_a was calculated by fitting the temperature T (°C) to the formula $K_a = e^{-2300/(273+T)}$.

Fluorescence in situ hybridization (FISH) was carried out to quantify PAOs in the three sludges according to Amann [22], i.e., the enriched PAOs culture fed with

Table 3 Experimental conditions used in batch tests of three activated sludges

	Aerobic P-uptake of sludge 1				Anoxic P-uptake of sludge 1			
NO_2^--N (mg/L)	0	2	5	10	5	10	20	30
pH	7	7	7	7	7.5	7.5	7.5	7.5
FNA × 10^{-3} (mg HNO_2-N/L)	0	0.47	1.18	2.37	0.37	0.75	1.50	2.25

	Aerobic P-uptake of sludge 2							Aerobic P-uptake of sludge 3			
NO_2^--N (mg/L)	0	3	6	9	20	30	40	0	3	6	9
pH	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7	7	7	7
FNA × 10^{-3} (mg HNO_2-N/L)	0	0.22	0.45	0.67	1.50	2.25	3.00	0	0.71	1.42	2.13

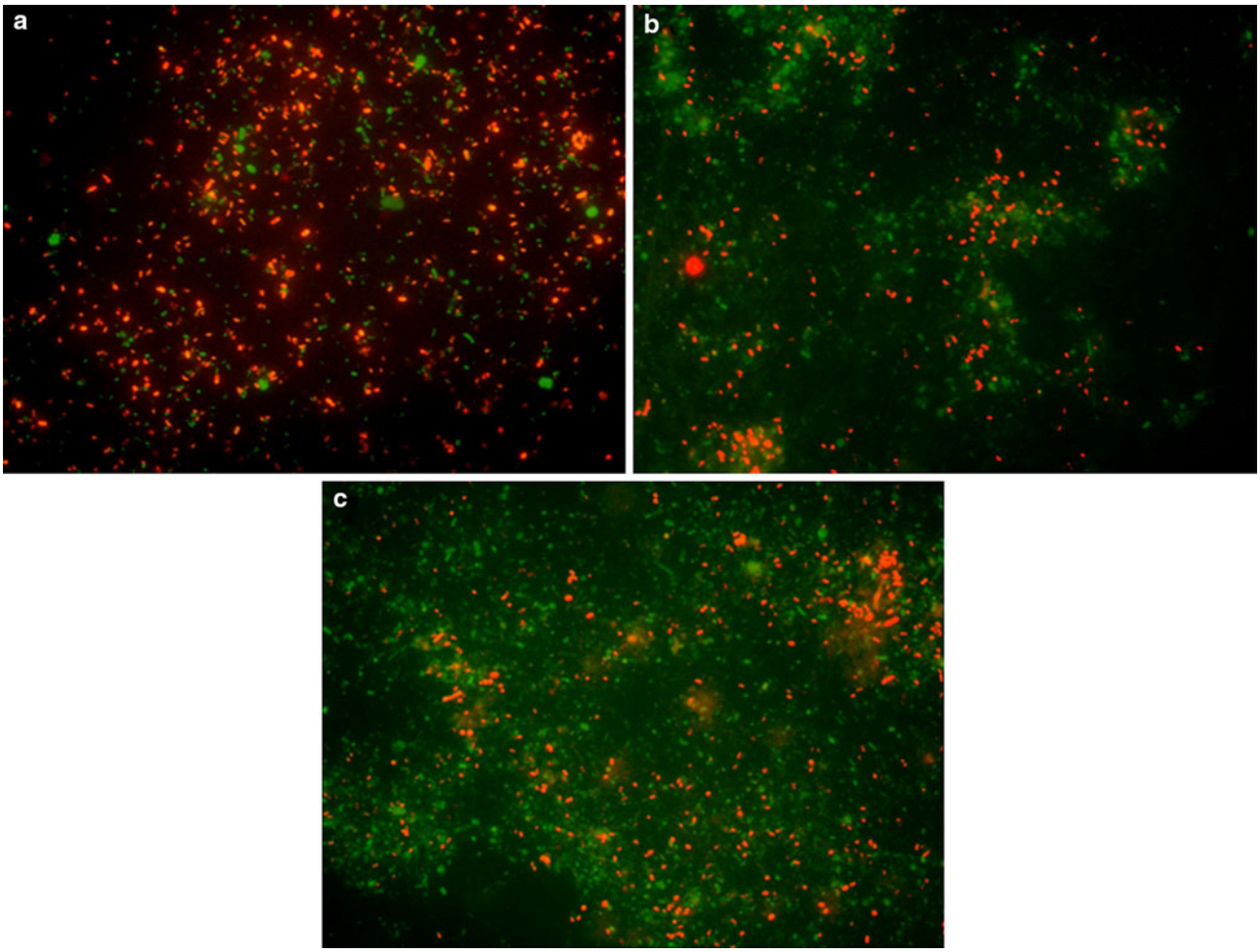

Fig. 1 FISH image for PAOs in three activated sludges (**a** activated sludge 1; **b** activated sludge 2; **c** activated sludge 3). EUB_{mix} target for Eubacteria labeled by FITC with *green* and PAO_{mix} target for PAOs labeled by Cy3 with *red* (color figure online)

synthetic wastewater, the two lab-scale SBRs treating domestic wastewater through nitrite-pathway nitritation and nitrate-pathway nitrification, respectively. The 16S rRNA-targeted oligonucleotide probes employed for FISH analyses were EUB_{mix} (an equimolar mixture of probes EUB338, EUB338-II and EUB338-III) target for Eubacteria and PAO_{mix} (an equimolar mixture of probes PAO462, PAO651 and PAO846) target for PAOs [23–25]. FISH samples were observed and PAOs in the FISH images were quantified using an Olympus BX61 fluorescence microscope with Image-Pro Plus 6.0 software, following the method described in Crocetti et al. [24]. In order to decrease the error of FISH quantification, both cell-counting procedures and area measurement were used. For the randomly acquired fields with efficient

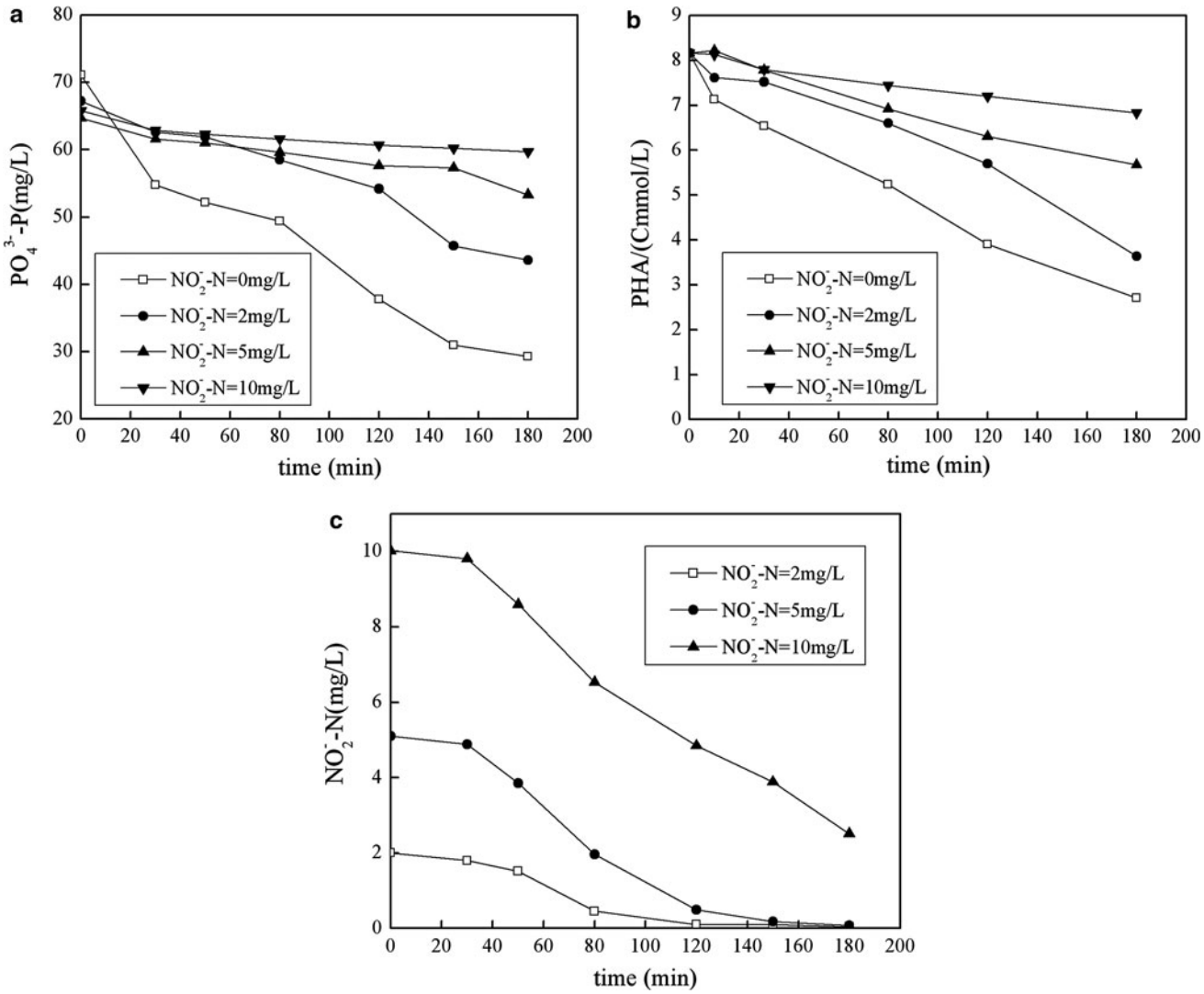

Fig. 2 Variations of PO_4^{3-}-P, PHA and NO_2^--N during aerobic P-uptake batch tests of enriched PAOs culture

dispersion of cells, the relative abundance of PAOs was determined by comparison of the obtained numbers with counts of all bacterial cells. For the fields with dense clusters, the areas of hybridized PAOs were measured. The abundance of PAOs was then expressed as fraction of the area occupied by all bacteria [26].

Results and discussion

Impact of nitrite on aerobic P-uptake of enriched PAOs culture fed with synthetic wastewater

Impact of nitrite on aerobic P-uptake of PAOs

The activated sludge 1, i.e., the enriched PAOs culture was used to investigate the impact of nitrite on aerobic P-uptake. PAOs in enriched culture (activated sludge 1) were quantified by FISH analysis. Figure 1a shows the typical FISH image using probe EUB_{mix} target for Eubacteria and PAO_{mix} target for PAOs. In the enriched culture, PAOs account for 72 % of the total bacterial population.

Figure 2 presents the variations of PO_4^{3-}-P, PHA and NO_2^--N during aerobic P-uptake batch tests of enriched PAOs culture. As shown in Fig. 2a, the amount of aerobic P-uptake decreased along with the increase of initial nitrite dosage. When the calculated FNA level as in formula (1) was 0, 0.47×10^{-3}, 1.18×10^{-3} and 2.37×10^{-3} mg HNO_2-N/L corresponding to the nitrite concentration at 0, 2, 5 and 10 mg/L (Table 3), the amount of P-uptake was 41.8, 23.6, 11.4, 6.1 mg/L, respectively. Figure 2b presents the consumption of intercellular PHA at four initial levels of 0, 2, 5 and 10 mg/L. The amount of PHA consumed in

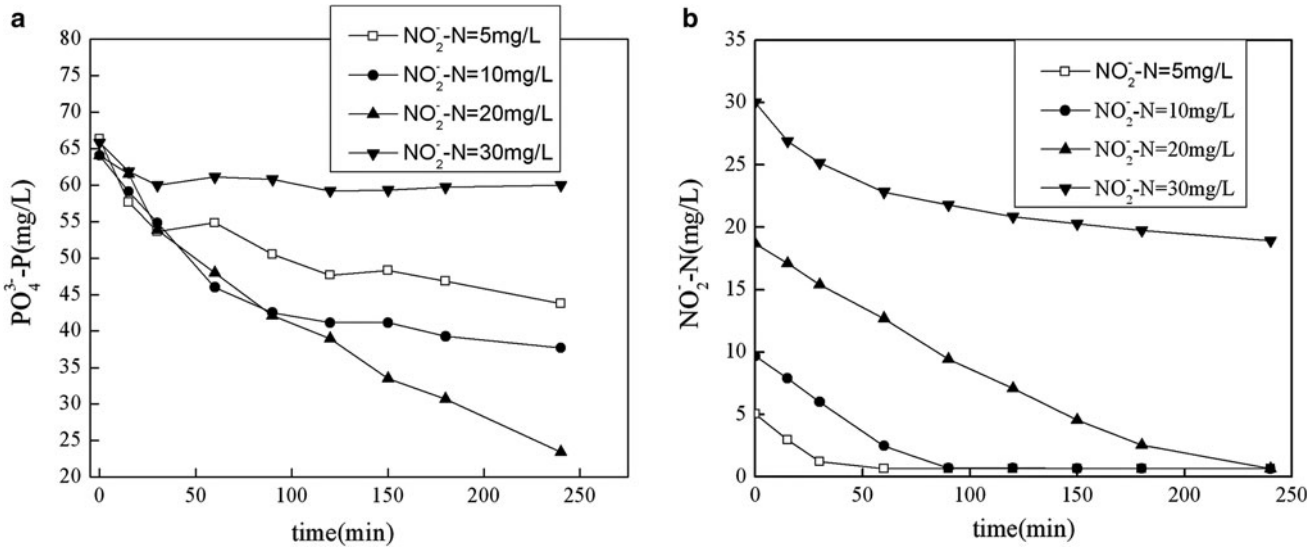

Fig. 3 Variations of PO_4^{3-}-P and NO_2^--N during anoxic P-uptake batch tests of enriched PAOs culture

180 min at the four nitrite levels was 5.45, 4.52, 2.49 and 1.33 C mmol/L, respectively. The outcomes indicated the higher the initial nitrite level, the less consumption of PHA.

Previous studies demonstrated that phosphorus uptake rate (PUR) of PAOs decreased in presence of nitrite, leading to nitrite inhibition on physiological activities of PAOs [8]. The outcomes in this research were in accordance with these studies. Under aerobic conditions, energy generated from oxidation of intercellular stored PHAs was used for biomass increase, glycogen replenishing, P-uptake and poly-P forming by PAOs. As illustrated in Fig. 2b, oxidation of PHA was suppressed when nitrite exposure, leading to decline of energy generated. One possible explanation regarding nitrite inhibition on aerobic P-uptake is based on the fact that denitrification process is a complex biochemical reaction involving a variety of enzymes and intermediates, e.g., nitrite reductase (Nir), nitric oxide reductase (NOR) and nitrous oxide reductase (N_2OR). These enzymes reduce NO_2^- to NO, N_2O, and N_2 in that order, among which, NO_2^- and NO reacts with oxygen respiration reductase, leading to inhibition on aerobic respiration and energy generation of PAOs [8]. Due to the suppression of energy generation, the related metabolism of PAOs such as glycogen replenishing, poly-p synthesis and cell growth would likely have been negatively affected.

As shown in Fig. 2a, c, nitrite of 2 and 5 mg/L was completely reduced at the 120th min and the 150th min, respectively. After that, the PUR was significantly improved. At the nitrite level of 2 mg/L, the PUR of PAOs was 67.83 mg P/(gVSS day) during 0–120 min, and then up to 110.61 mg P/(gVSS day) during 120–180 min after nitrite depletion. At the nitrite level of 5 mg/L, the PUR also rose from 30.89 mg P/(gVSS day) during 0–150 min to 83.48 mg P/(gVSS day) after 150 min. Figure 2b presents that at the nitrite level of 2 mg/L, the PHA oxidizing rate was also increased from 12.89 to 21.45 C mmol/(gVSS day) after nitrite depletion. In this study the triplicate tests at each nitrite level demonstrated that both PHA oxidizing rate and PUR increased after nitrite was exhausted, suggesting the inhibition discontinuing after nitrite depletion.

Impact of nitrite on denitrifying phosphorus removal by PAOs under aerobic conditions

Figure 2c presents that nitrite concentration was gradually decreased under aerobic conditions. Since no nitrate was measured, nitrite was not oxidized to nitrate through nitrification. The decline of nitrite concentration was possibly caused by two reasons: one is nitrite reduction by denitrification of heterotrophs in an anoxic microenvironment; the other one is the presence of denitrifying PAOs using nitrite as electron acceptor. Since DO concentration during the aerobic period was controlled at higher than 5 mg/L and the working volume of batch reactor of only 1 L, the anoxic microenvironment was very difficult to maintain. It has been postulated that type I *Accumulibacter* are able to reduce nitrate, while Type II *Accumulibacter* are unable to reduce nitrate but able to reduce nitrite. Whether type I or type II, both organisms appear capable of nitrite reduction [27–31]. Therefore, presence of PAOs using nitrite as electron acceptor for denitrifying phosphorus removal seems to be the main reason. Identifications of

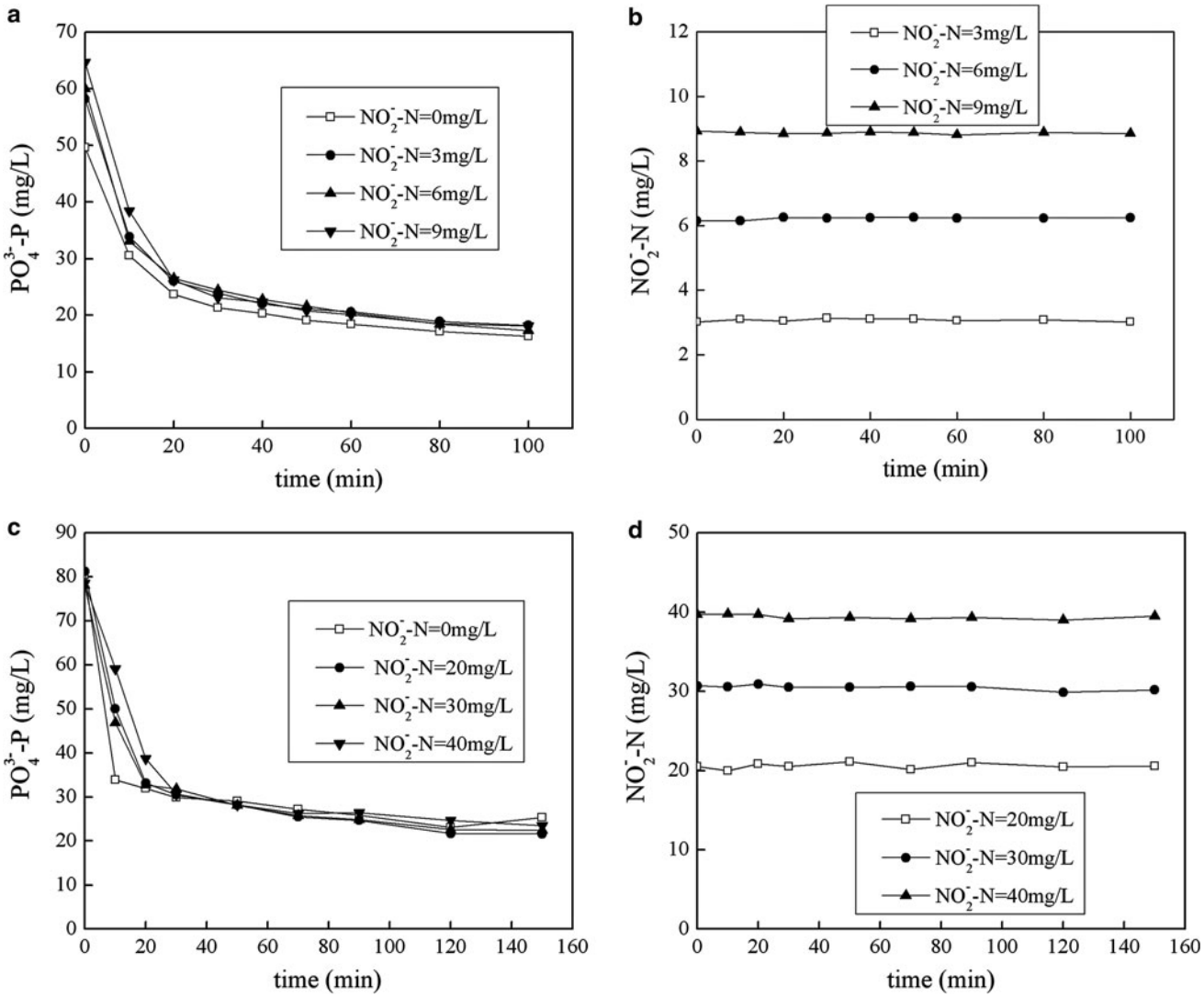

Fig. 4 Variations of PO_4^{3-}-P and NO_2^--N concentration in the batch tests of activated sludge 2

distinct types and multiple sub-clades of *Accumulibacter* should be further investigated.

To investigate nitrite impact and denitrifying capabilities of the enriched PAOs culture (activated sludge 1), batch tests of anoxic P-uptake were carried out. Tested sludge 1 was taken from SBR1 at the end of anaerobic phase and was averagely put into four batch reactors of 1 L. The initial NO_2^--N and FNA concentration is shown in Table 3. Figure 3 depicts the variations of PO_4^{3-}-P and NO_2^--N concentration during anoxic P-uptake process by PAOs. As shown in Fig. 3, at the nitrite concentration of 5, 10 and 20 mg/L, i.e., the calculated FNA concentration below 1.5×10^{-3} mg HNO_2-N/L, anoxic P-uptake using nitrite as an electron acceptor was not inhibited. Whereas when the FNA concentration reached 2.25×10^{-3} mg HNO_2-N/L, P-uptake and denitrification was both suppressed. These outcomes demonstrated that under aerobic conditions, FNA of high level not only inhibited aerobic P-uptake, but also suppressed anoxic P-uptake using nitrite as electron acceptor.

Impact of nitrite on aerobic P-uptake in nutrient removal system through nitrite-pathway nitritation

The activated sludge 2 taken from SBR2 treating real domestic wastewater with nitrite-pathway nitritation was used to investigate the impact of nitrite on aerobic P-uptake. PAOs in activated sludge 2 represented 7.6 % of the total bacterial population by FISH analysis (Fig. 1b).

In the aerobic P-uptake batch tests of activated sludge 2, nitrite concentration was controlled at a range of 0–40 mg/L and the highest calculated FNA concentration was 3×10^{-3} mg HNO_2-N/L (Table 3). Figure 4 presents the variations of PO_4^{3-}-P and NO_2^--N concentration in the

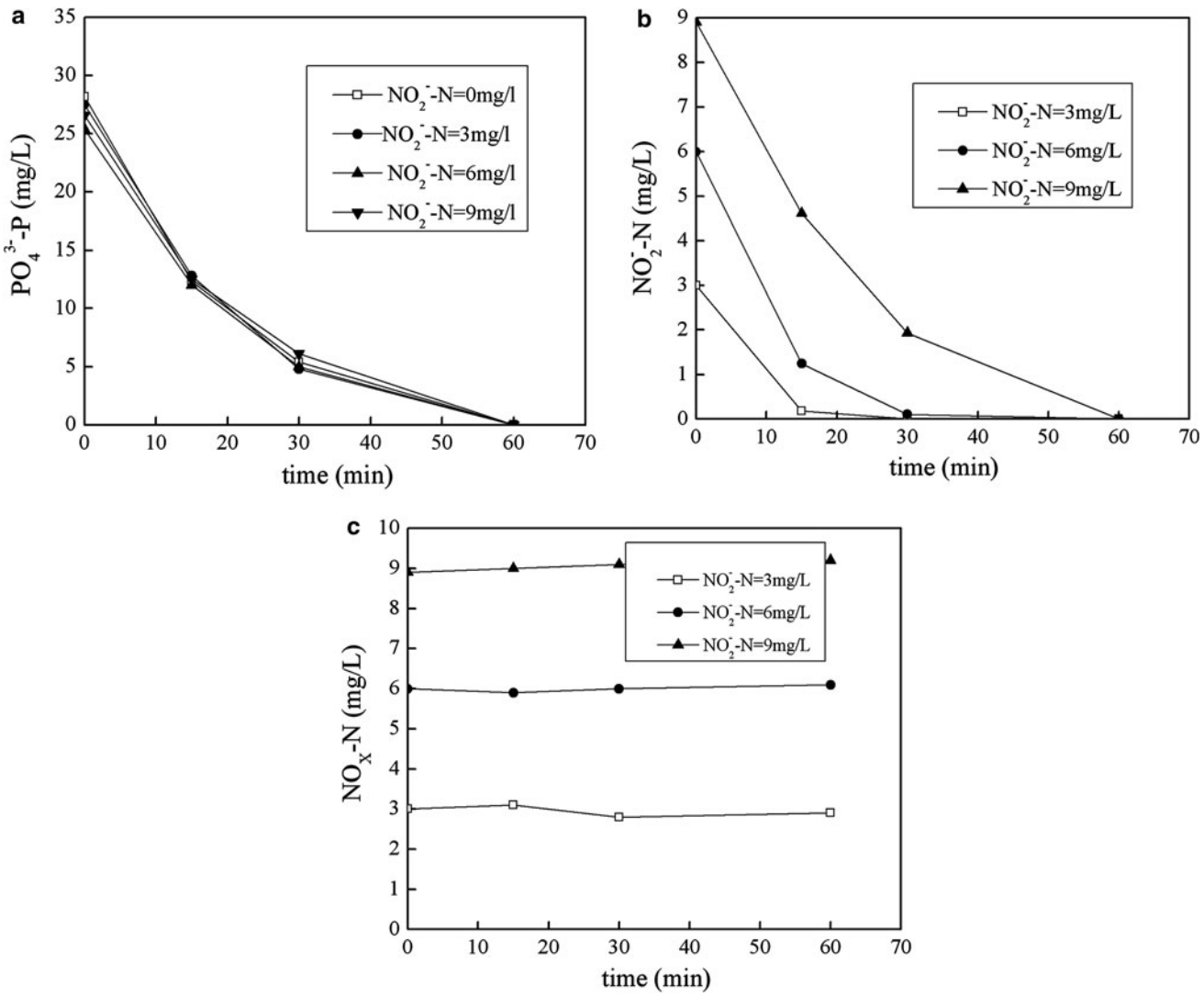

Fig. 5 Variations of PO_4^{3-}-P, NO_2^--N and NO_x^--N concentration in the batch tests of activated sludge 3

batch tests of activated sludge 2. As shown in Fig. 4a, c, aerobic P-uptake of PAOs was not inhibited at the tested NO_2^--N and FNA concentrations. Furthermore, Fig. 4b, d suggested that the nitrite was not oxidized to nitrate by aerobic nitrification even though DO concentration was above 5 mg/L. Because activated sludge 2 was long-term operated at nitrite-pathway nitritation with the nitrite accumulation ratio of above 95 %, nitrite oxidizing bacteria (NOB) were washed out of the reactor or suppressed. Thus, the nitrite concentration throughout the aerobic period was stabilized at the initial level.

In the aerobic P-uptake batch experiments of activated sludge 1, FNA concentration gradually decreased along with the consumption of nitrite. As shown in Fig. 2, FNA inhibited aerobic P-uptake of PAOs at the initial low level of 0.47×10^{-3}, and 2.37×10^{-3} mg HNO_2-N/L caused a severe inhibition of aerobic P-uptake. Contrastively, in the batch tests of activated sludge 2, aerobic P-uptake of PAOs was not inhibited even when the FNA concentration was constantly maintained at the high level of 3×10^{-3} mg HNO_2-N/L (Fig. 4). This difference possibly resulted from that activated sludge 2 had undergone a long period of nitrite accumulation and the PAOs in it had a strong tolerance to nitrite exposure.

Impact of nitrite on aerobic P-uptake in nutrient removal system through nitrate-pathway nitrification

The activated sludge 3 taken from SBR3 treating real domestic wastewater with nitrate-pathway nitrification was used to investigate the impact of nitrite on aerobic P-uptake. PAOs in activated sludge 3 represented 6.5 % of the total bacterial population by FISH analysis (Fig. 1c).

Table 4 A comparison of this study and previous studies in terms of aerobic and anoxic P-uptake rate (PUR)

	Aerobic P-uptake		Anoxic P-uptake	
	Concentration of NO_2^--N (mg N/L) or FNA (mg HNO_2-N/L)	Aerobic PUR	Concentration of NO_2^--N (mg N/L) or FNA (mg HNO_2-N/L)	Anoxic PUR
Saito et al. [6]	0 mg N/L (pH 7)	576 mg P/(gVSS day)	3-10 mg N/L (pH 7)	240 mg P/(gVSS day)
	2 mg N/L (pH 7)	57.6 mg P/(gVSS day) (severe inhibition)	12 mg N/L (pH 7)	168 mg P/(gVSS day)
	>6 mg N/L (pH 7)	<14 mg P/(gVSS day) (complete inhibition)		
Yoshida et al. [8]	0 mg N/L (pH 7)	216 mg P/(gVSS day)		
	3 mg N/L (pH 7)	144 mg P/(gVSS day)		
	5 mg N/L (pH 7)	96 mg P/(gVSS day) (severe inhibition)		
Sin et al. [10]	0 mg N/L (pH 7.5)	8.1 mg P/(L h)	>25 mg N/L (pH 7.5)	74.4 mg P/(gSS day) (NO_2^--N as electron acceptor) and 48 mg P/(gSS day) (NO_3^--N as electron acceptor)
	5 mg N/L (pH 7.5)	5.5 mg P/(L h)		
	10 mg N/L (pH 7.5)	2.7 mg P/(L h)		
Zhou et al. [11]			0.001 mg HNO_2-N/L	40.8 mg P/(gVSS day)
			0.005 mg HNO_2-N/L	19.2 mg P/(gVSS day)
			0.02 mg HNO_2-N/L	Completely inhibited
Zhou et al. [13]			0.0007-0.01 mg HNO_2-N/L	Sharply decreased
			0.01 mg HNO_2-N/L	28.8 mg P/(g biomass day) (inhibited by 50 %)
			0.037 mg HNO_2-N/L	Completely inhibited
Zhou et al. [15]	0 mg HNO_2-N/L	518.4 mg P/(g biomass day) (maximum aerobic P-uptake rate)	0 mg HNO_2-N/L	259.2 mg P/(g biomass day) (maximum anoxic P-uptake rate)
	0.01 HNO_2-N/L	100 % inhibition	5×10^{-3} mg HNO_2-N/L	100 % inhibition
This study (enriched PAOs)	0 mg HNO_2-N/L	145 mg P/(gVSS day)	0.75×10^{-3} mg HNO_2-N/L (10 mg N/L at pH 7.5)	70.4 mg P/(gVSS day)
	0.47×10^{-3} mg HNO_2-N/L (2 mg N/L at pH 7)	82 mg P/(gVSS day)	1.5×10^{-3} mg HNO_2-N/L (20 mg N/L at pH 7.5)	107 mg P/(gVSS day)
	1.18×10^{-3} mg HNO_2-N/L (5 mg N/L at pH 7)	40 mg P/(gVSS day)	2.25×10^{-3} mg HNO_2-N/L (30 mg N/L at pH 7.5)	13 mg P/(gVSS day) (complete inhibition)
This study (nitritation sludge 2)	3×10^{-3} mg HNO_2-N/L (40 mg N/L at pH 7.5)	230 mg P/(gVSS day) (no inhibition)		
This study (nitrification sludge 3)	2.13×10^{-3} mg HNO_2-N/L (9 mg N/L at pH 7)	261 mg P/(gVSS day) (no inhibition)		

As presented in Table 3, FNA concentration in aerobic P-uptake batch tests of activated sludge 3 was controlled at 0, 0.71×10^{-3}, 1.42×10^{-3} and 2.13×10^{-3} mg HNO_2-N/L, respectively. Figure 5 shows the variations of PO_4^{3-}-P, NO_2^--N and NO_x^--N (the sum of NO_2^--N and NO_3^--N) concentration in the batch tests of activated sludge 3. As shown in Fig. 5a, at different concentrations of FNA, the amount of aerobic P-uptake was almost the same, i.e., 2.13×10^{-3} mg HNO_2-N/L did not inhibit aerobic P-uptake of activated sludge 3. Figure 5b, c also indicated that nitrite was gradually oxidized to nitrate, and NO_x^--N concentration maintained at a constant level during aerobic period.

The nitrite concentration gradually decreased during aerobic P-uptake of activated sludge 3, which was similar to the nitrite variations in the batch tests of activated sludge 1. At the same FNA levels, PAOs in activated sludge 3 were not suppressed; whereas PAOs in activated sludge 1 was inhibited. A possible explanation for this difference was that activated sludge 3 ("real" sludge) was taken from real wastewater treatment system and activated sludge 1 was from enriched PAOs culture fed with synthetic wastewater. In general, synthetic wastewater treatment system exhibits a lower microbial diversity than real wastewater treatment system. In comparison with enriched PAOs culture, community structure in activated sludge 3 was more complicated and diverse due to complex influent composition in real municipal wastewater. Thus, the diversity may provide the functional redundancy and a higher system resiliency in activated sludge 3 where the nitrite disturbance occurred [32].

A comparison of this study and previous studies in terms of aerobic and anoxic P-uptake rate obtained under the different FNA or nitrite concentrations is given in Table 4. Although a certain concentration of FNA or nitrite inhibited aerobic P-uptake of PAOs, the inhibition levels varied in a large range due to the differences of seed sludge, operation modes and wastewater composition. In this study, tolerance degree of the three different sludges to FNA was activated sludge 2 with nitritation > activated sludge 3 with nitrification > enriched PAOs culture.

Conclusions

PAOs taken from different EBPR systems had different tolerance to nitrite. To the enriched PAOs culture, FNA of 0.47×10^{-3} mg HNO_2-N/L inhibited the aerobic P-uptake and the PHA oxidation, and FNA of 2.25×10^{-3} mg HNO_2-N/L suppressed denitrifying phosphorus removal using nitrite as electron acceptor. PAOs from real wastewater treatment system with nitrite-pathway nitritation had undergone a long period of nitrite accumulation, and thus a high level FNA of 3×10^{-3} mg HNO_2-N/L did not cause inhibition. To the PAOs in real wastewater treatment system with nitrate-pathway nitrification, nitrite inhibition did not occur even when FNA reached 2.13×10^{-3} mg HNO_2-N/L. Although a certain concentration of nitrite inhibited the aerobic P-uptake of PAOs, the inhibition levels varied in a large range due to the differences of seed sludge, operation modes and wastewater composition. The microbial diversity in real wastewater treatment system may provide the functional redundancy and performance resiliency in case of the nitrite exposure. Thus, real wastewater treatment system, especially with nitrite-pathway nitritation, exhibited to be more tolerant to nitrite than synthetic wastewater treatment system.

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