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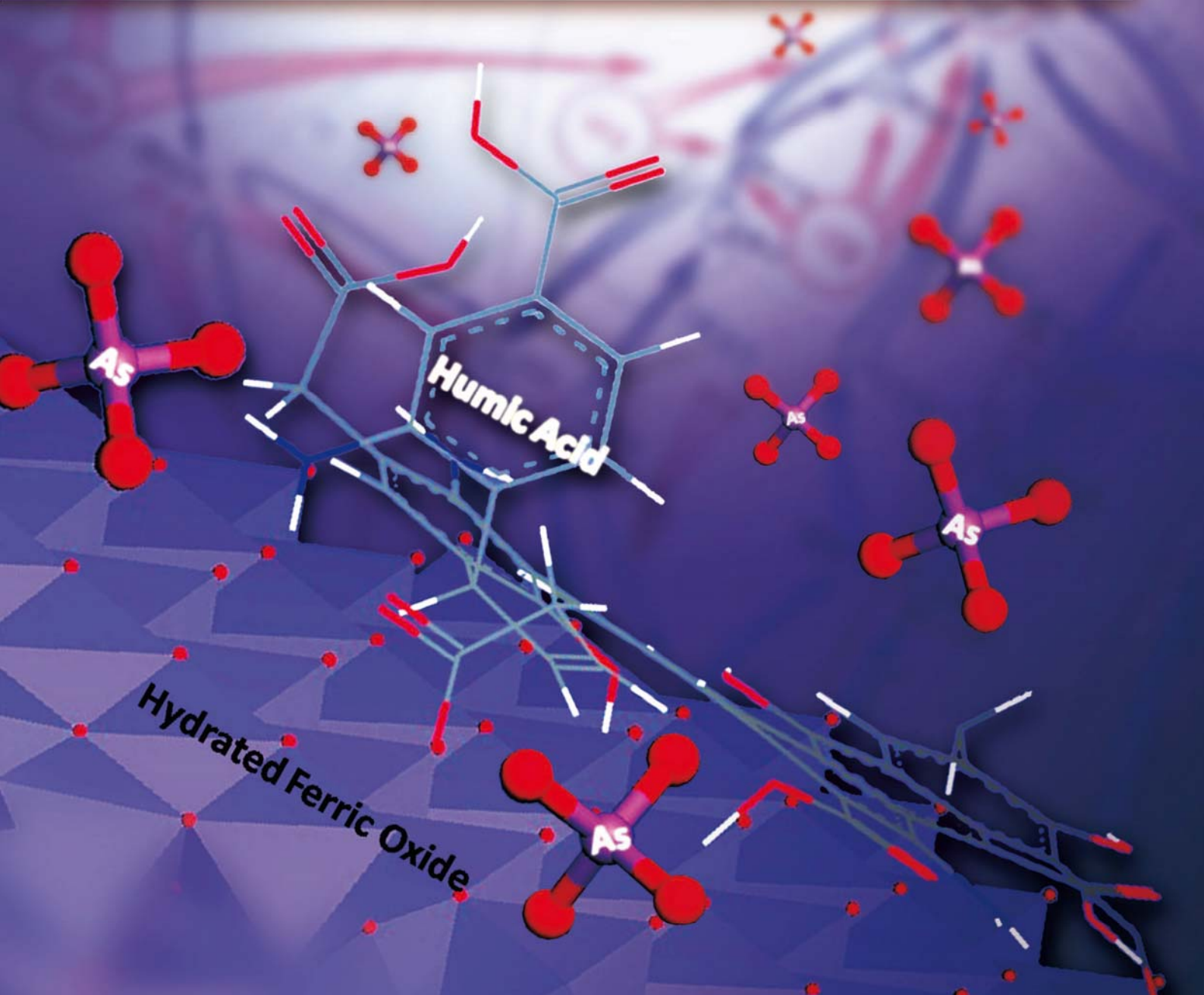

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Effect of temperature on anoxic metabolism of nitrites to nitrous oxide by polyphosphate accumulating organisms

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ABSTRACT

Temperature is an important physical factor, which strongly influences biomass and metabolic activity. In this study, the effects of temperature on the anoxic metabolism of nitrite (NO_2^-) to nitrous oxide (N_2O) by polyphosphate accumulating organisms, and the process of the accumulation of N_2O (during nitrite reduction), which acts as an electron acceptor, were investigated using 91% ± 4% *Candidatus Accumulibacter phosphatis* sludge. The results showed that N_2O is accumulated when *Accumulibacter* first utilize nitrite instead of oxygen as the sole electron acceptor during the denitrifying phosphorus removal process. Properties such as nitrite reduction rate, phosphorus uptake rate, N_2O reduction rate, and polyhydroxyalkanoate degradation rate were all influenced by temperature variation (over the range from 10 to 30°C reaching maximum values at 25°C). The reduction rate of N_2O by N_2O reductase was more sensitive to temperature when N_2O was utilized as the sole electron acceptor instead of NO_2, and the N_2O reduction rates, ranging from 0.48 to 3.53 N_2O-N/(hr·g VSS), increased to 1.45 to 8.60 mg N_2O-N/(hr·g VSS). The kinetics processes for temperature variation of 10 to 30°C were (θ_1 = 1.140–1.216 and θ_2 = 1.139–1.167). In the range of 10°C to 30°C, almost all of the anoxic stoichiometry was sensitive to temperature changes. In addition, a rise in N_2O reduction activity leading to a decrease in N_2O accumulation in long term operations at the optimal temperature (27°C calculated by the Arrhenius model).

Introduction

The enhanced biological phosphorus removal (EBPR) system has been confirmed to be an economical and sustainable process, and plays an increasingly important role in wastewater treatment. A group of bacteria known as polyphosphate accumulating organisms (PAOs) dominate in the EBPR system (Crocetti et al., 2000). The PAOs are able to take up volatile fatty acids and store them as polyhydroxyalkanoates (PHAs), which is attributed to the release of phosphorus in the anaerobic phase. In the subsequent aerobic phase, PHAs are used for growth and phosphate uptake, leading to a net phosphate removal from the wastewater. In most previous studies, one group of PAOs called denitrifying PAOs were shown capable of oxidizing their intracellular PHAs to fulfill their energy requirements and take up phosphorus under anoxic conditions, while utilizing nitrite or nitrate as electron acceptors instead of oxygen (Ahn et al., 2001; Meinhold et al., 1999; Zeng et al., 2003). In this process, the same carbon source can be used to simultaneously remove N and P, requiring 20%–30% less microorganisms, and reducing plant operational costs due to the savings in the consumption of both oxygen and carbon sources (Kishida et al., 2006).

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Nitrous oxide (N_2O), a significant greenhouse gas, has 300 times greater warming potential than carbon dioxide (CO_2) (IPCC, 2001). Most studies on nitrification, denitrification, and phosphorus removal pathways have demonstrated that N_2O could be accumulated in wastewater treatment systems. There is an increasing amount of evidence showing that ammonia oxidizing bacteria, heterotrophic denitrification organisms, and denitrifying glycogen accumulation organisms are the major contributors to N_2O emissions from wastewater treatment plants (Tallec et al., 2006; Ahn et al., 2010; Zeng et al., 2003b). Meanwhile, the PAOs, namely *Candidatus Accumulibacter phosphatis*, are often dominant in both lab-scale EBPR reactors (Crocetti et al., 2000; Hesselmann et al., 1999; Lu et al., 2006) and full-scale wastewater treatment plants (Pijuan et al., 2008). PAOs may have contributed to N_2O emission during anoxic metabolism in WWTP operation, which cannot be ignored. The production of N_2O is affected by many parameters such as low dissolved oxygen concentrations, accumulation of nitrite, rapidly changing conditions, types of organic carbon sources, pH, and temperature (Kampschreur et al., 2009; Yoshida, 1988). It has been reported that the accumulation of nitrite leads to an increase in N_2O emission rather than nitrogen (N_2) as the major end-product in denitrifying phosphorus removal processes (Lemaire et al., 2006; Zeng et al., 2003). Indeed, it was reported that free nitrous acid (FNA), rather than nitrite and pH, is likely the real inhibitor of N_2O reduction by denitrifying PAOs (Wang et al., 2011; Zhou et al., 2008). The concentration of free nitrous acid (FNA, HNO_2-N) was calculated by using the following formula:

$$\mathrm{FNA} = S_{(NO_2^-\text{-}N)}/(K_\alpha \times 10^{\mathrm{pH}}) \quad (1)$$

where, $K_\alpha = e^{-2300/(273+T)}$, T (K) is temperature (Anthonisen et al., 1976). Temperature, being an important factor for FNA, should have a major influence on denitrifying phosphorus removal and N_2O metabolism processes. However, most previous studies focused on the aerobic metabolism of PAOs or the influence of temperature on the competition between PAOs and glycogen-accumulating organisms (GAOs). Panswad et al. (2003) investigated the competition between PAOs and GAOs and found that PAOs were dominant at low temperature (e.g., 10°C, and they considered that PAOs were constituted of lower range mesophiles or possibly psychrophiles. Brdjanovic et al. (1997) performed a systematic study concerning the effects of temperature on the aerobic metabolism of PAOs and the dependence of different processes at 5 to 30°C. However, a systematic study on the effects of temperature in N_2O metabolism by a PAO culture under anoxic conditions, with respect to the utilization of nitrite as an electron acceptor, has not been reported yet.

In this study, a series of batch tests were carried out using a highly enriched culture of *Candidatus Accumulibacter phosphatis*. We aimed to find the effects of temperature on PHA oxidation, nitrite reduction, phosphorus uptake, and N_2O metabolism during the denitrifying phosphorus removal process. This study also evaluated the stoichiometry and kinetics of PAOs in combination with N_2O metabolism during this anoxic process.

1 Materials and methods

1.1 Reactor and operation

A laboratory-scale sequencing batch reactor (SBR) with a working volume of 8 L was operated for 240 days under anaerobic and aerobic conditions. The SBR was fed with acetate or propionate, switching at a frequency of one to two sludge ages. The cycle time was 6 hr and consisted of the following: a 150 min anaerobic period, a 180 min aerobic period, 25 min settle/decant period, and a 5 min idle period. In each cycle, 2 L of synthetic wastewater was fed to the reactor in the first 6 min of the anaerobic period, resulting in a hydraulic retention time of 24 hr. At the end of the cycle, 200 mL of sludge was removed to achieve a solids retention time of 10 days and a mixed liquor suspended solid level of 2.5–3.5 g/L. The dissolved oxygen concentration was maintained at 2.0±0.2 mg/L in the aerobic period by using an online on/off controller switch. The pH was controlled during both the anaerobic and aerobic phases at a range of 7.2–8.0 by doses of 0.5 mol/L HCl and 0.5 mol/L NaOH solutions. The temperature was maintained at 20°C.

1.2 Synthetic wastewater

Synthetic wastewater (2 L) described by Lu et al. (2006) was composed of 0.3 L solution A and 1.7 L solution B. The mixed feed of solutions A and B contained 800 COD/L and 40 mg P/L. Solution A contained 3.41 g of acetate and 1.76 mL of propionic acid per liter of solution. In addition, solution A also contained (per liter) 1.02 g NH_4Cl, 0.01 g peptone, 0.01 g yeast extraction, 1.20 g $MgSO_4{\cdot}7H_2O$, 0.19 g $CaCl_2{\cdot}2H_2O$, 7.94 mg allyl-N thiourea (a nitrification inhibitor), and 4.00 mL of a trace elements liquid. Solution B contained 173 mg $K_2HPO_4{\cdot}3H_2O$ and 104 mg KH_2PO_4 per liter of solution. For the propionic acid feed, 10.47 mL of 5 mol/L NaOH was used to adjust the pH to 7.5.

1.3 Batch experiment 1

The sludge sample for testing in batch experiment 1 was taken from the SBR fed with acetate during the normal cycle of operation. At the end of the anaerobic stage, 5 L of mixed liquor was divided into five parts and put into a 1.25 L batch reactor. A nitrite stock solution (sodium nitrite at a concentration of 10 g NO_2-N/L) was added to the batch reactors at the beginning of each experiment, which resulted in initial concentrations of 20 mg NO_2-

N/L. Each batch reactor was controlled within ± 0.5°C of the intended temperature (e.g. 10, 15, 20, 25, and 30°C. The experiments were performed at a controlled pH (7.5 ± 0.5), which was maintained by adding 0.2 mol/L HCl or 0.2 mol/L NaOH solution.

1.4 Batch experiment 2

The sludge sample for testing in batch experiment 2 was also taken from the SBR fed with acetate during the normal cycle of operation. A nitrite stock solution (sodium nitrite at a concentration of 10 g nitrite nitrogen, NO_2-N/L) was added to the batch reactors at the end of the anaerobic phase, which resulted in initial concentrations of 20 mg NO_2-N/L. Two reactors were operated at 27 ± 0.5°C and 20 ± 0.5°C. The experiments were performed at a controlled pH (7.5 ± 0.5), which was maintained by adding 0.2 mol/L HCl or 0.2 mol/L NaOH solution. The cycle time was 5–6.5 hr and consisted of a 150 min anaerobic period, a 90–180 min anoxic period (anoxic time was calculated according to the time taken by most of the nitrite to reduce to nitrogen), 30 min aerobic period, 25 min settle/decant period, and a 5 min idle period.

1.5 Analytical methods

The liquid samples were immediately filtered through Millipore filter units (0.45 μm pore size) for analysis of NO_2^--N, and PO_4^{3-}-P. PO_4^3-P and NO_2-N were analysed using a Lachat Quikchem 8500 flow injection analyser (FIA USA). PHA analysis was performed using the Oehmen method to determine poly-β-hydroxybutyrate (PHB), poly-β-hydroxyvalerate (PHV), and poly-β-hydroxy-2-methylvalerate (PH2MV) (Oehmen et al., 2005). Freeze-dried biomass (20–30 mg), 2 mL chloroform, and 2 mL methanol acidified with 3% H_2SO_4 solution were added into a glass tube, the contents were mixed and then heated at 100°C for 20 hr after being mixed. After cooling to room temperature, 1 mL of Milli-Q water was put into the tubes and the contents were mixed. After centrifugation, 1.4 mL of the bottom organic phase was separated, and added into a gas chromatography vial for analysis. The temperature of injector and flame ionization detector were maintained at 200 and 250°C, respectively. The temperature program was set as follows: held at 80°C for 2 min; increased to 140°C at the rate of 10°C min, and then maintained for 1 min. Polysaccharides such as glycogen were detected using the method introduced by Zeng et al. (2003c). A 0.6 mol/L HCl (5 mL) solution was added to weighed freeze-dried biomass in screw-top glass tubes, and then heated at 105°C for 6 hr. After cooling the solution to room temperature, it was centrifuged and then 1 mL of the supernatant solution was transferred to a high performance liquid chromatography vial for glucose analysis. N_2O was analyzed using a N_2O sensor by Microsensor Multimeter (BD Diagnostics, USA). The approximate rate of reduction of N_2O-N was determined by linear regression of the measured total nitrogen (TN = NO_2-N + N_2O-N) profiles. The transformation rates of NO_2-N, PO_4^3-P, and N_2O-N were determined by linear regression of the measured profiles.

Fluorescence *in situ* hybridization (FISH) was performed with cy5-labelled EUBMIX probes (for most bacteria) (Daims et al., 1999), cy3-labelled GAOMIX probes (for Competibacter, comprising equal amounts of probes GAO431 and GAO 989)[21], cy3-labelled PAOMIX (for *Candidatus Accumulibacter phosphatis* or *Accumulibacter*, comprising equal amounts of probes PAO462, PAO651, and PAO846) (Crocetti et al., 2000).

2 Results and discussion

2.1 Reactor performance and microbial community

The SBR was operated for 240 days under anaerobic/aerobic conditions, and more than 140 days under steady-state conditions. The P removal performance is shown in **Fig. 1a**. During the start-up stage (0–45 day), the P removal efficiency was not stable, and the P concentration in the effluent was higher than that in influent. It reached a steady-state condition after the first 95 days of operation, and the P concentration of the effluent was stably maintained at less than 0.8 mg/L throughout the subsequent operations. **Figure 1a** shows that the P concentration at the end of the anaerobic phase was 130 mg/L on average when fed with acetate, which is higher than when fed with propionate as the carbon source (80 mg/L). **Figure 1b** and **c** illustrate a quantification of the

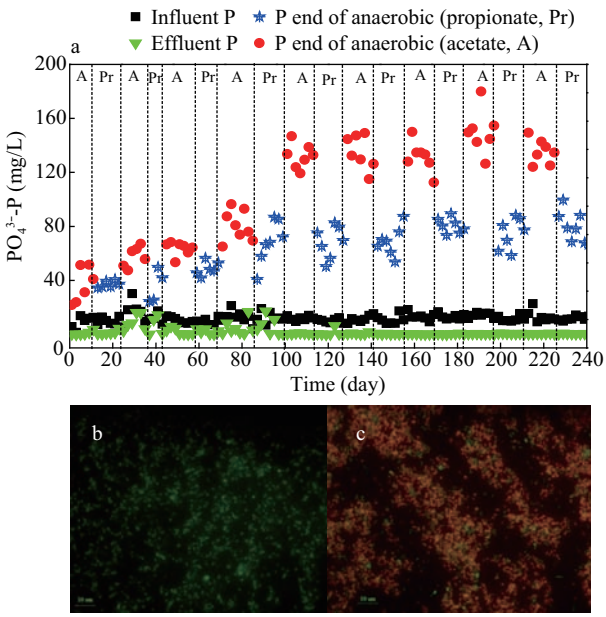

Fig. 1 Performance of the reactor and FISH results: (a) Phosphorus removal performance; (b) FISH image on 1st day; (c) FISH image on 241st day.

biomass population distribution obtained by FISH analysis indicating that *Accumulibacter* rose from 3% to 91% (± 4%) of total bacteria from the 1st day to the 240th day, whereas no GAOs were detected.

2.2 Effect of temperature on denitrifying phosphorus removal and N_2O accumulation

2.2.1 Comparison of phosphorus and nitrite in batch experiment 1

Figure 2 illustrates the effect of temperature on the anaerobic denitrifying phosphorus process. Alinear fits were obtained for the nitrite and phosphorus concentrations before nitrite was completely exhausted. The results showed that the linear regression coefficients of nitrite reduction were > 0.944. At the initial addition of 20 mg NO_2-N/L, the nitrite concentration decreased rapidly at 20, 25, and 30°C with a corresponding nitrite reduction rate of 4.86, 5.89, 5.28 and mg NO_2-N/(hr·g VSS), respectively. The nitrite reduction rate decreased as the temperature was lowered, and reached a value of 0.74 and 1.84 mg NO_2-N/(hr·g VSS) at 10 and 15°C, respectively. Additionally,

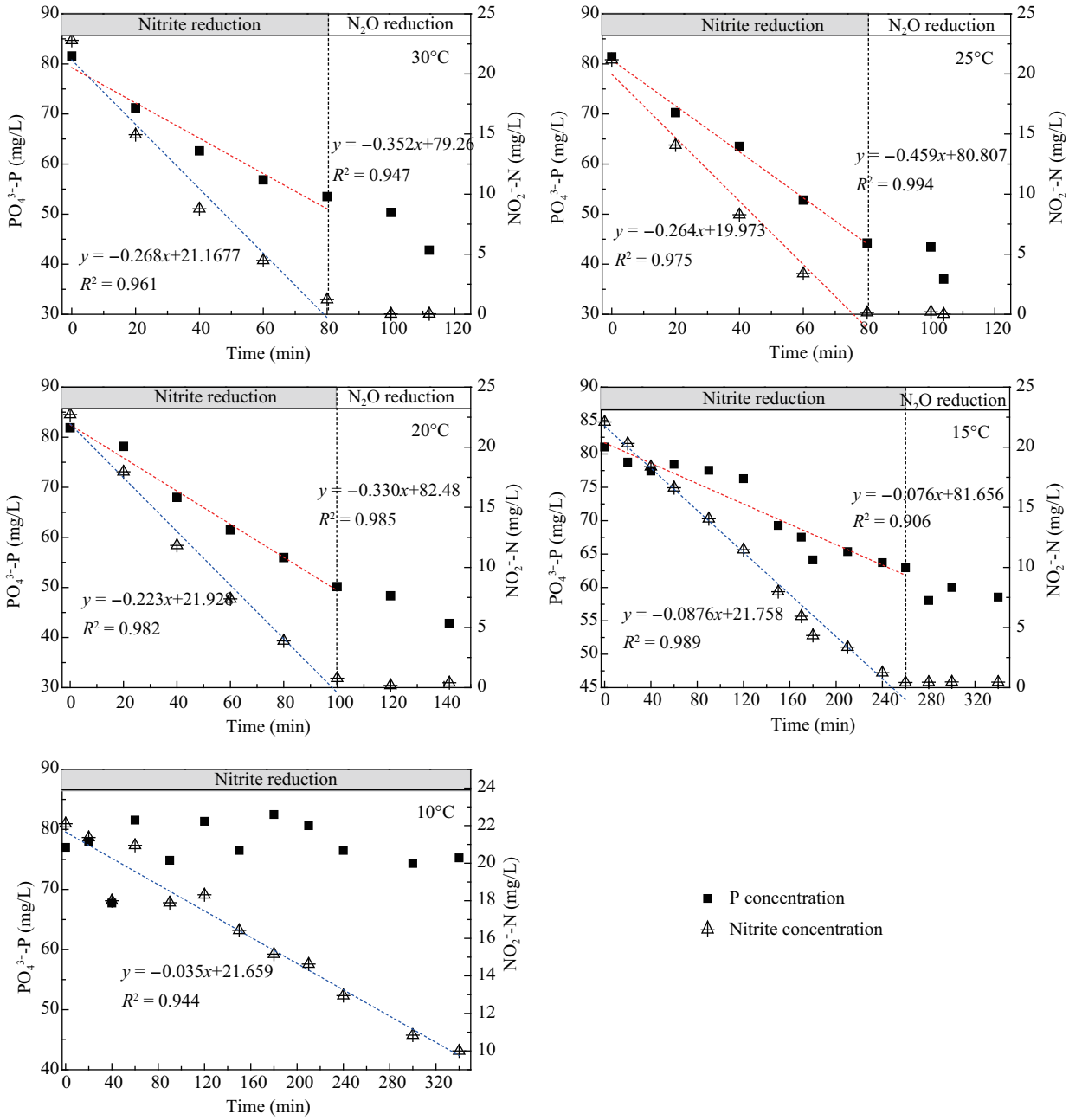

Fig. 2 Effect of temperature on anoxic phosphorus uptake.

Fig. 2 shows that some group of PAOs could take up phosphorus by using nitrite as the electron acceptor in spite of the fact that the sludge was only operated under anaerobic/aerobic conditions. Further, similar to the nitrite reduction rate, the phosphorus uptake occurred rapidly at 20, 25, and 30°C with the rates of 7.03, 10.41, and 6.87 mg P/(hr·g VSS), respectively. The phosphorus uptake rate also decreased below 15°C and almost stopped at 10°C as shown in **Table 1**. This suggests that temperature had a significant effect on the anoxic metabolism of PAOs and the denitrifying phosphorus removal process using nitrite as the electron acceptor, which could be completely inhibited at low temperatures (e.g., below 10°C). In several studies, the denitrifying phosphorus removal process was found to be restrained at lower nitrite concentrations of 5–10 mg N/L (Kuba et al., 1993). Bassin et al. (2012) utilized a SBR reactor run at 30°C to study the conditions when PAO and GAO coexisted in the system, and the results showed that the highest nitrite reduction rate and phosphorus uptake rate were 2.5 mg NO_2-N/(hr·g VSS) and 3.8 mg P/(hr·g VSS), respectively, which were lower than the values calculated from these experiments. This means that *Accumulibacter* was cultivated in anaerobic/aerobic conditions, and not only utilized nitrite instead of oxygen as the electron acceptor but also demonstrated very high denitrifying phosphorus removal efficiency. In this study, the initial addition of 20 mg NO_2-N/L was not the main inhibition factor since the substrate was sufficient for the anoxic metabolism. Therefore, the nitrite reduction and phosphorus uptake rates varied with changing temperature. Both these rates were sensitive to the temperature from 10 to 30°C. As shown in **Table 1**, it is also clear that the nitrite reduction rate and phosphorus uptake rate increase with increasing temperature, and both these rates reached a maximum value at 25°C.

2.2.2 Comparison of nitrous oxide in batch experiment 1

As shown in **Fig. 3**, N_2O is produced in the denitrifying phosphorus removal process by PAOs under different temperatures. Compared to previous studies on the denitrifying phosphorus removal process, a lot more N_2O was accumulated by PAOs in this study (Wang et al., 2011). The proportion of highest N_2O accumulation to TN at 10, 15, 20, 25, and 30°C was 20.8%, 31.6%, 33.6%, 43.2%, and 39.3%, respectively. Further, the process of N_2O accumulation during nitrite reduction could be utilized by PAOs by delaying the anoxic operation time, and this benefited in reducing N_2O emission. In this study, the accumulated N_2O was completely exhausted in 32, 24, 42, and 80 min at 30, 25, 20, and 15°C, respectively. This suggested that the anoxic metabolism process using N_2O as an electron acceptor was sensitive to temperature variation. In the denitrifying phosphorus removal process using N_2O as the electron acceptor, PAOs needed less time to complete the reaction when temperature was increased from 15 to 25°C. However, this trend was reversed at 25 to 30°C.

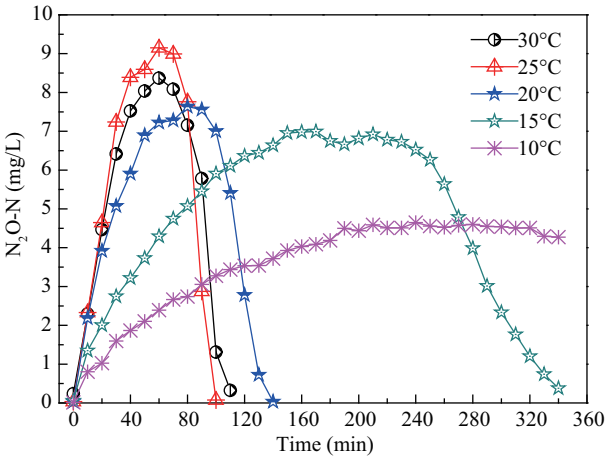

Fig. 3 Effect of temperature on N_2O production.

The transformation of nitrite to nitrogen is shown in Eq. (2), and the metabolism of NO_2 to N_2 is usually divided into two steps. In the first step, NO_2 is reduced to N_2O (via NO) by nitrite reductase. Then, in the second step, N_2O is reduced to N_2 by N_2O reductase.

$$NO_2^- \longrightarrow NO \longrightarrow N_2O \longrightarrow N_2 \quad (2)$$

Previous studies showed that both nitrite reductase and N_2O reductase play important roles in denitrifying

Table 1 Various rates of denitrifying phosphorus removal process by PAOs

		10°C	15°C	20°C	25°C	30°C
Nitrite reduction rate (mg NO_2-N/(hr·g VSS))		0.74	1.84	4.86	5.89	5.28
Phosphorus uptake rate (mg P/ (hr·g VSS))	Nitrite existence	–	1.36	7.03	10.41	6.87
	Nitrite exhaustion	–	1.76	4.08	5.37	6.98
N_2O reduction rate (mg NO_2-N/(hr·g VSS))	Nitrite existence	0.48	1.36	3.31	3.71	3.53
	Nitrite exhaustion	–	1.45	3.87	8.60	4.46
PHA degradation rate (mmol C(hr·g VSS))	Nitrite existence	0.165	0.263	0.596	0.733	0.568
	Nitrite exhaustion	–	0.098	0.273	0.247	0.277
N_2O-N/TN ratio (%)		20.8	31.6	33.6	43.2	39.3

–: no data.

metabolism processes (Poth and Focht, 1985; Rasmussen et al., 2000). N_2O reductase contains two metal centers, a binuclear copper center: Cu_A that serves to receive electrons from soluble donors; and a tetranuclear copper-sulfide center: Cu_Z present at the active site (Rasmussen et al., 2005). At similar substrate concentrations, reaction rates are positively correlated with the enzyme activities. In this study, the various reaction rates are illustrated in **Table 1**, which indicates that the denitrifying phosphorus removal process is divided into two phases: nitrite existence and nitrite exhaustion (N_2O as the electron acceptor). The N_2O reduction rates under nitrite existence are 3.53, 3.71, 3.31, 1.36, and 0.48 mg N_2O-N/(hr·g VSS), corresponding to 30, 25, 20, 15, and 10°C, respectively.

A previous study reported that N_2O reduction was affected by the FNA concentration. Zhou et al. (2007) demonstrated that denitrification by PAOs was found to be inhibited by HNO_2 and the denitrification rate decreased by approximately 40% when the FNA concentration was increased from 0.002 to 0.02 mg HNO_2-N/L. In this study, FNA concentrations were 1.43×10^3, 1.51×10^3, 1.85×10^3, 2.01×10^3, and 2.37×10^3 mg HNO_2-N/L at 30, 25, 20, 15, and 10°C, respectively, which could inhibit the metabolism of the denitrifying phosphorus removal process. However, the N_2O reduction rate increased from 3.53 (at 30°C) to 3.71 (at 25°C) mg N_2O-N/(hr·g VSS) even though the FNA concentration increased from 0.00143 to 0.00153 mg HNO_2-N/L. These results suggest that the N_2O reduction rate was not strictly linearly correlated with the FNA concentration, but rather was affected by the combined action of temperature and FNA concentration. Zhou et al. (2008) reported that FNA could bind to the active sites of N_2O reductase, thereby causing competitive inhibition of N_2O reduction. The results also showed that temperature could affect the active sites of N_2O reductase, thereby causing a higher N_2O reduction rate at 25°C. Moreover, the N_2O reduction rates have similar values at 20, 25, and 30°C in the presence of nitrite, and they changed intensively when N_2O was utilized as the sole electron acceptor instead of nitrite at the same temperature (**Table 1**). These results also suggested that compared to inhibition factors (such as FNA concentration), temperature had less effect on N_2O reductase activity in the temperature range of –20 to 30°C. Once the inhibitions completely disappeared under the nitrite exhaustion condition, the N_2O reduction rate was raised and became sensitive changes in temperature. The N_2O reduction rates were raised to 4.46, 8.60, 3.87, and 1.45 mg N_2O-N/(hr·g VSS) at 30, 25, 20, and 15°C, respectively, and reached a maximum value at 25°C.

2.2.3 Comparison of PHA in the batch experiment 1

Unlike the external carbon source, PHA contributed to N_2O production during anoxic metabolism when PAOs utilized nitrite as the electron acceptor. Wang et al. (2011) demonstrated that PHA played an important role in N_2O production during the denitrifying phosphorus removal process. In this study, the types of PHA used were PHB and PHV, and the surplus levels of PHA at the end of the anoxic phase were all above 63.2%. These results indicated that the shortage of carbon source was not the main factor influencing the outcome in this experiment. As shown in **Table 1**, the PHA degradation rate under nitrite existence conditions was 0.165, 0.263, 0.596, 0.733 and 0.568 mmol C/(hr·g VSS) at 10, 15, 20, 25, and 30°C, respectively. It is clearly shown in **Fig. 4** that the PHA degradation rates depended strongly on temperature at 10–25°C when nitrite was present in the system. However, between 25 and 30°C the PHA metabolism performed well at a lower temperature in the denitrifying phosphorus removal process. Moreover, lower PHA degradation rates were observed at 15, 20, 25, and 30°C when nitrite was exhausted from the system, which resulted in lower PHA degradation rates due to the change in electron acceptor from nitrite to N_2O. During the nitrite exhaustion stage, the PHA degradation rates were similar at 20, 25, and 30°C with values of 0.273, 0.247, and 0.277 mmol C/(hr·g VSS), respectively. These results suggested that the anoxic maintenance requirements decrease, and therefore lower amounts of PHAs should be consumed in the denitrifying phosphorus removal process when PAOs utilize N_2O as the electron acceptor instead of nitrite. Moreover, a temperature change from 20 to 30°C did not have any negative impact on the PHA metabolism when N_2O was utilized as the sole electron acceptor.

2.2.4 Temperature effects on the anoxic stoichiometry and kinetics

A series of anoxic tests at 10 to 30°C were executed to address the effects of temperature on the anoxic stoichiometry and kinetics of PAOs. Tests for short-term effects were conducted to rule out the influence of changes in bacterial population in the system. The average of different

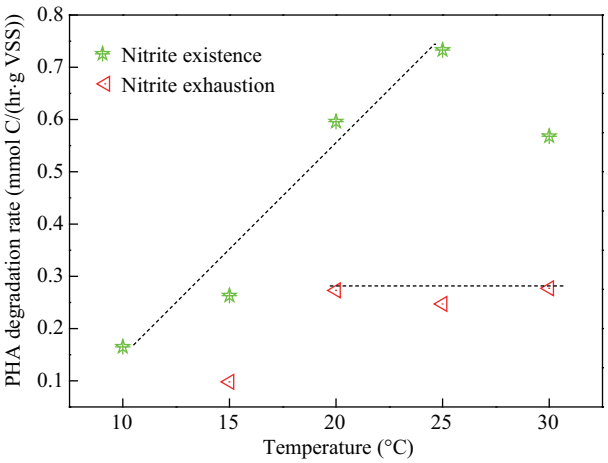

Fig. 4 Correlation between the PHA degradation and temperature.

metabolism rates observed at 10 to 30°C is illustrated in **Table 1**. The simplified Arrhenius expression was used to describe the effect of temperature on the conversion rates of biomass as follows:

$$r_T = r_{20}\theta^{(T-20)} \quad (3)$$

where, r_T is the reaction at the temperature T (°C) and θ is the temperature coefficient. In order to describe the decline in bacterial activity above the optimal thermal point, an extended Arrhenius equation was used as follows:

$$r_T = r_{20}\theta_1^{(T-20)}[1 - \theta_2^{(T-T_{MAX})}] \quad (4)$$

where, θ_1 is the temperature coefficient θ calculated from Eq. (3), T_{MAX} is the temperature at which the microbial activity ceases, and θ_2 is a second temperature coefficient used to describe the decline in biomass activity at temperatures higher than the optimal temperature (Lopez-Vazquez et al., 2009). **Figure 5** shows the short-term temperature dependency of the phosphorus uptake rate, nitrite reduction rate, N_2O reduction rate, and PHA degradation rate observed in Section 2.2. The kinetic processes involved in the anoxic metabolism of PAOs in the temperature range from 10 to 30°C were θ_1 = 1.140–1.216 and θ_2 = 1.139–1.167. Compared to the temperature coefficient θ value reported by Brdjanovic et al. (1997), an average θ of anaerobic and aerobic on temperature from 5 to 30°C (1.078 and 1.057) were lower than this study. This is probably due to the fact that PAOs first utilized nitrite instead of oxygen as the electron acceptor, and have a higher temperature dependency. According to the Activated Sludge Model No. 2 (Henze et al., 1999), temperature has a moderate influence on the anoxic metabolism by PAOs, which is comparable to that for ordinary heterotrophic organisms, fermentation, and nitrification processes and GAOs.

The anoxic stoichiometry observed in the temperature range 10 to 30°C was in the range of different ratios calculated from the two conditions: nitrite existence and nitrite exhaustion. The results are shown in **Table 2**, which revealed a remarkable difference between the runs of nitrite existence and runs of nitrite exhaustion in terms of variation with temperature. As shown in **Table 2**, the PHB degradation/TN consumption ratio and PHV degradation/TN consumption ratio were similar under nitrite existence conditions observed in the temperature range 15 to 25°C. These results suggest that the same amount of PHA was exhausted equivalent to TN removal; even

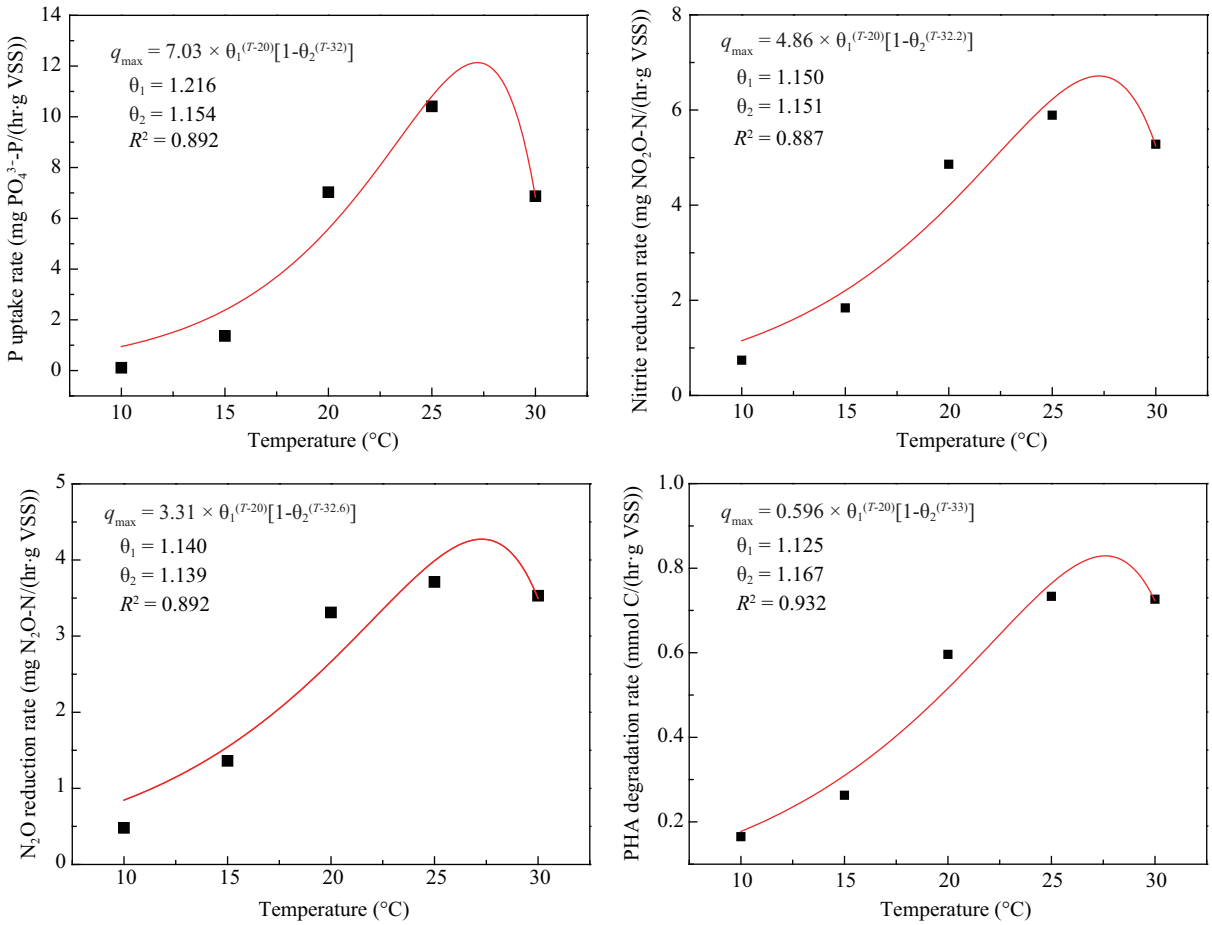

Fig. 5 Temperature effects on the anoxic metabolism kinetics.

though it had different N_2O reduction rates in the temperature range 15 to 25°C. Regardless of this ratio, the anoxic stoichiometry seemed to be sensitive to temperature fluctuations in both nitrite existence and exhaustion conditions. However, some stoichiometry values had shown a decrease in the nitrite existence conditions at 30°C compared to 25°C. This is probably due to the fact that higher metabolism rates required more energy during the anoxic metabolism process at 25°C. Indeed, the combined trend of kinetics and metabolism rate indicated that temperature has a significant effect on the anoxic stoichiometry of PAOs as it increases above 25°C.

2.3 Long-term temperature effect on N_2O reduction

Reactor operation conditions were introduced in the batch experiment 2. According to Arrhenius model of Eq. (2), the maximum N_2O reduction rate was reached at approximately 27°C (**Fig. 5**). In order to know the long-term temperature effects on N_2O reduction, two reactors were operated at 27°C and room temperature (20 ± 2°C) for 7 days. **Figure 6** shows the reduction rate of N_2O-N, which was determined by linear regression of the measured NO_2^--N + N_2O-N (TN) profiles at 1, 3, 5, and 7 days (the details of data are not shown). Both the N_2O reduction rate and the maximum N_2O accumulation changed after 7 days, and the N_2O reduction rate was increased from 3.79 to 5.92 mg N_2O-N/(hr·g VSS) at 27°C. Consequently, the maximum N_2O accumulation was decreased while the N_2O reduction ability was strengthened, and the maximum N_2O/TN ratio was decreased from 36% to 13% over the same period of time. Compared to 27°C, the N_2O reduction rate was increased slowly from 3.35 to 4.32 mg N_2O-N/(hr·g VSS). Changes in the biomass population could not be observed in these long-term experiments. These observations suggest that potential utilization of nitrite (as an electron acceptor) by PAOs in long-term operation could be enhanced by N_2O reduction. Moreover, the increase of N_2O reduction activity and decrease of N_2O accumulation at the optimal temperature of the Arrhenius model benefited the denitrifying phosphorus removal process.

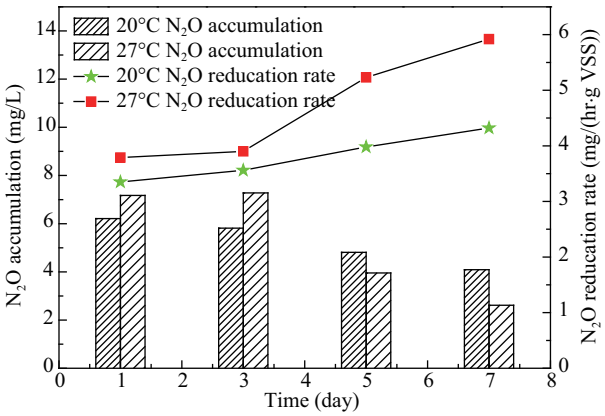

Fig. 6 Long term temperature effects on N_2O reduction by PAOs.

3 Conclusions

In the present study, the effects of temperature on an enriched PAO culture were evaluated using nitrite as the electron acceptor. The results showed that high N_2O accumulation occurred (the maximum N_2O/TN value reached 43.2%) when PAOs first utilized nitrite instead of oxygen as the electron acceptor. The N_2O accumulation in turn was also utilized by PAOs through extending the anoxic operation time. N_2O reduction rate (by N_2O reductase) was more sensitive to temperature when N_2O was utilized as the sole electron acceptor instead of nitrite, and the N_2O reduction rates increased from a range of 0.48–3.53 to a range of 1.45–8.60 mg N_2O-N/(hr·g VSS)).

Table 2 Summary of stoichiometry for anoxic batch experiment at different temperatures

		10°C	15°C	20°C	25°C	30°C
P uptake/NO_2-N reduction ratio (mmol P/mmol N)		–	0.34	0.65	0.80	0.59
PHB degradation/NO_2-N reduction ratio (mmol C/mmol N)		1.81	1.17	1.42	1.31	1.13
PHV degradation/NO_2-N reduction ratio (mmol C/mol N)		0.50	0.39	0.29	0.44	0.38
P uptake/TN consumption ratio (mmol P/mmol N)	Nitrite existence	–	0.45	0.96	1.27	0.88
	Nitrite exhausion	–	0.55	0.48	0.42	0.71
PHB degradation/P uptake ratio (mmol C/mmol P)	Nitrite existence	–	4.52	2.18	1.64	1.91
	Nitrite exhausion	–	1.31	1.69	1.68	0.79
PHV degradation/P uptake ratio (mmol C/mmol P)	Nitrite existence	–	1.45	0.45	0.55	0.65
	Nitrite exhausion	–	0.42	0.39	0.24	0.17
PHB degradation/TN consumption ratio (mmol C/mmol N)	Nitrite existence	3.44	2.04	2.08	2.07	1.68
	Nitrite exhausion	–	0.68	0.80	0.46	0.44
PHV degradation/TN consumption ratio (mmol C/ mmol N)	Nitrite existence	1.38	0.66	0.63	0.69	0.57
	Nitrite exhausion	–	0.22	0.18	0.17	0.30

–: no data.

Compared to the inhibition factors (such as nitrite or FNA concentration), temperature in the range 20–30°C had less effect on N_2O metabolism until these inhibition factors completely disappeared from the system. The kinetics processes involved in the anoxic metabolism by PAOs in the temperature range 10–30°C were θ_1 = 1.140–1.216 and θ_2 = 1.139–1.167. Between 10 and 30°C, the anoxic stoichiometry of PAOs was found to be sensitive to temperature changes. Furthermore, high N_2O reduction activity and low N_2O accumulation at the optimal temperature of the Arrhenius model in long term operations could benefit the denitrifying phosphorus removal process.

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REFERENCES

Ahn, J. H., Kim, S., Park, H., Rahm, B., Pagilla, K., Chandran, K., 2010. N_2O emissions from activated sludge processes, 2008–2009: Results of a national monitoring survey in the United States. Environ. Sci. Technol. 44, 4505–4511.

Ahn, J., Daidou, T., Tsuneda, S., Hirata, A., 2001. Metabolic behavior of denitrifying phosphate-accumulating organisms under nitrate and nitrite electron acceptor conditions. J. Biosci. Bioeng. 92, 442–446.

Anthonisen, A. C., Loehr, R. C., Prakasam, T., Srinath, E. G., 1976. Inhibition of nitrification by ammonia and nitrous-acid. J. Water Pollut. Control Federat. 48, 835–852.

Bassin, J. P., Kleerebezem, R., Dezotti, M., van Loosdrecht, M. C. M., 2012. Simultaneous nitrogen and phosphate removal in aerobic granular sludge reactors operated at different temperatures. Water Res. 46, 3805–3816.

Brdjanovic, D., Van Loosdrecht, M. C. M., Hooijmans, C. M., Alaerts, G. J., Heijnen, J. J., 1997. Temperature effects on physiology of biological phosphorus removal. J. Environ. Eng.-Asce, 123, 144–153.

Crocetti, G. R., Hugenholtz, P., Bond, P. L., Schuler, A., Keller, J., Jenkins, D., et al., 2000. Identification of polyphosphate-accumulating organisms and design of 16S Rrna-directed probes for their detection and quantitation. Appl. Environ. Microbiol. 66, 1175–1182.

Daims, H., Bruhl, A., Amann, R., Schleifer, K. H., Wagner, M., 1999. The domain-specific probe Eub338 is insufficient for the detection of all bacteria: Development and evaluation of a more comprehensive probe set. System. Appl. Microbiol. 22, 434–444.

Henze, M., Gujer, W., Mino, T., Matsuo, T., Wentzel, M. C., Marais, G. V. R., van Loosdrecht, M. C. M., 1999. Activated sludge model No.2D, Asm2D. Water Sci. Technol.39, 165–182.

Hesselmann, R., Werlen, C., Hahn, D., van der Meer, J. R., Zehnder, A., 1999. Enrichment, phylogenetic analysis and detection of a bacterium that performs enhanced biological phosphate removal in activated sludge system. Appl. Microbiol. 22, 454–465.

Kampschreur, M. J., Temmink, H., Kleerebezem, R., Jetten, M., van Loosdrecht, M., 2009. Nitrous oxide emission during wastewater treatment. Water Res. 43, 4093–4103.

Kishida, N., Kim, J., Tsuneda, S., Sudo, R., 2006. Anaerobic/oxic/anoxic granular sludge process as an effective nutrient removal process utilizing denitrifying polyphosphate-accumulating organisms. Water Res. 40, 2303–2310.

Kuba, T., Smolders, G., Vanloosdrecht, M., Heijnen, J. J., 1993. Biological phosphorus removal from wastewater by anaerobic-anoxic sequencing batch reactor. Water Sci. Technol. 27, 241–252.

Lemaire, R., Meyer, R., Taske, A., Crocetti, G. R., Keller, J., Yuan, Z. G., 2006. Identifying causes for N_2O accumulation in a lab-scale sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal. J. Biotechnol. 122, 62–72.

Lopez-Vazquez, C. M., Hooijmans, C. M., Brdjanovic, D., Gijzen, H. J., van Loosdrecht, M. C. M., 2009. Temperature effects on glycogen accumulating organisms. Water Res. 43, 2852–2864.

Lu, H. B., Oehmen, A., Virdis, B., Keller, J., Yuan, Z. G., 2006. Obtaining highly enriched cultures of candidatus accumulibacter phosphates through alternating carbon sources. Water Res. 40, 3838–3848.

Meinhold, J., Arnold, E., Isaacs, S., 1999. Effect of nitrite on anoxic phosphate uptake in biological phosphorus removal activated sludge. Water Res. 33, 1871–1883.

Oehmen, A., Zeng, R. J., Yuan, Z. G., Keller, J., 2005. Anaerobic metabolism of propionate by polyphosphate-accumulating organisms in enhanced biological phosphorus removal systems. Biotechnol. Bioeng. 91, 43–53.

Panswad, T., Doungchai, A., Anotai, J., 2003. Temperature effect on microbial community of enhanced biological phosphorus removal system. Water Res. 37, 409–415.

Pijuan, M., Oehmen, A., Baeza, J. A., Casas, C., Yuan, Z., 2008. Characterizing the biochemical activity of full-scale enhanced biological phosphorus removal systems: A comparison with metabolic models. Biotechnol. Bioeng. 99, 170–179.

Poth, M., Focht, D. D., 1985. N-15 Kinetic-analysis of N_2O production by nirosomonas-europaea-an examination of nitrifier denitrification. Appl. Environ. Microbiol. 49, 1134–1141.

Rasmussen, T., Berks, B. C., Sanders-Loehr, J., Dooley, D. M., Zumft, W. G., Thomson, A. J., 2000. The catalytic center in nitrous oxide reductase, CuZ, is a copper-sulfide cluster. Biochemistry 39, 12753–12756.

Rasmussen, T., Brittain, T., Berks, B. C., Watmough, N. J., Thomson, A. J., 2005. Formation of a cytochrome C-nitrous oxide reductase complex is obligatory for N_2O reduction by paracoccus pantotrophus. Dalton Transact. 3501–3506.

Tallec, G., Garnier, J., Billen, G., Gousailles, M., 2006. Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: Effect of oxygenation level. Water Res. 40, 2972–2980.

Wang, Y. Y., Geng, J. J., Guo, G., Wang, C., Liu, S. H., 2011. N_2O production in anaerobic/anoxic denitrifying phosphorus removal process: The effects of carbon sources shock. Chem. Eng. J. 172, 999–1007.

Yoshida, N., 1988. 15N-Depleted N_2O as a product of nitrification. Nature 335, 528–529.

Zeng, R. J., Lemaire, R., Yuan, Z., Keller, J., 2003a. Simultaneous nitrification, denitrification, and phosphorus removal in a lab-scale sequencing batch reactor. Biotechnol. Bioeng. 84, 170–178.

Zeng, R. J., Saunders, A. M., Yuan, Z. G., Blackall, L. L., Keller, J., 2003b. Identification and comparison of aerobic and denitrifying

polyphosphate-accumulating organisms. Biotechnol. Bioeng. 83, 140–148.

Zeng, R. J., van Loosdrecht, M., Yuan, Z. G., Keller, J., 2003c. Metabolic model for glycogen-accumulating organisms in anaerobic/aerobic activated sludge systems. Biotechnol. Bioeng. 81, 92–105.

Zhou, Y., Pijuan, M., Yuan, Z. G., 2007. Free nitrous acid inhibition on anoxic phosphorus uptake and denitrification by poly-phosphate accumulating organisms. Biotechnol. Bioeng. 98, 903–912.

Zhou, Y., Pijuan, M., Zeng, R. J., Yuan, Z., 2008. Free nitrous acid inhibition on nitrous oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge. Environ. Sci. Technol. 42, 8260–8265.

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