

## **Nitrous oxide generation in denitrifying phosphorus removal process: Main causes and control measures**

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### **Abstract:**

Despite the many benefits of denitrifying phosphorus removal process, the significant generation of nitrous oxide (N<sub>2</sub>O), a potent greenhouse gas, remains a problem for this innovative and promising process. To better understand and more effectively control N<sub>2</sub>O generation in denitrifying phosphorus removal process, batch experiments were carried out to investigate the main causes of N<sub>2</sub>O generation, based on which the control measures were subsequently proposed. The results showed that N<sub>2</sub>O generation accounted for 0.41% of the total nitrogen removal in denitrifying phosphorus removal process, whereas in contrast, almost no N<sub>2</sub>O was generated in conventional denitrification process. It was further demonstrated that the weak competition of N<sub>2</sub>O reductase for electrons and the higher nitrite accumulation were the two main causes for N<sub>2</sub>O generation, evidenced by N<sub>2</sub>O production and reduction

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rate under different conditions. Accordingly, the reduction of N<sub>2</sub>O generation was successfully achieved via two control measures: (i) the use of continuous nitrate addition reducing N<sub>2</sub>O generation by around 91.4%, and (ii) the use of propionate as the carbon source reducing N<sub>2</sub>O generation by around 69.8%.

*Key words:* Nitrous oxide; Denitrifying phosphorus removal; Causes; Control measures; Continuous nitrate addition; Propionate

## **1. Introduction**

Nitrous oxide (N<sub>2</sub>O) is a potent greenhouse gas. Although the proportion of N<sub>2</sub>O in total greenhouse gas emissions is only 0.03%, N<sub>2</sub>O has a more than 300-fold greater global warming potential (GWP) than carbon dioxide (CO<sub>2</sub>), and it is increasing globally at a surprising rate of 0.31% per year (IPCC, 2007). Moreover, N<sub>2</sub>O can contribute to the destruction of ozone layer through reacting with oxygen (O<sub>2</sub>) to produce nitric oxide (NO) in the stratosphere (Ravishankara, 2009). It has long been known that biological wastewater treatment is an important source of N<sub>2</sub>O emissions (Kampschreur et al., 2009; Li et al., 2010; Wunderlin et al., 2012). Therefore, it is urgent to identify the causes of N<sub>2</sub>O emission and reduce this phenomenon from biological wastewater treatment process.

In the last two decades, many researchers have investigated N<sub>2</sub>O generation from biological wastewater treatment process, and results showed that microbial nitrification and denitrification were the two sources of N<sub>2</sub>O generation, and denitrification was observed to be the dominant process (Meyer et al., 2005; Tallec et

al., 2006; Jia et al., 2012). Denitrification is the reduction of nitrate ( $\text{NO}_3^-$ -N) to molecular nitrogen ( $\text{N}_2$ ) by heterotrophic denitrifiers, with nitrite ( $\text{NO}_2^-$ -N), NO and  $\text{N}_2\text{O}$  as intermediates (Hu et al., 2011a). However, the final product was found to be  $\text{N}_2\text{O}$  rather than  $\text{N}_2$  under certain conditions, such as low COD/N ratio, low pH, high  $\text{NO}_2^-$  concentration and using poly- $\beta$ -hydroxyalkanoates (PHA) as carbon source for denitrification (Schalk-Otte et al., 2001; Itokawa et al., 2001; Zhou et al., 2008a).

Recently, the application of denitrifying phosphorus removal process has attracted intensive attention due to its simultaneously nitrogen (N) and phosphorus (P) removal relying on the ability of denitrifying P accumulating organisms (DPAOs) (Kuba et al., 1996; Zhou et al., 2008b; Wang et al., 2009). DPAOs could take up organic carbon sources in anaerobic phase and store them in the form of PHA with P release. The stored PHA are oxidized in the subsequent anoxic phase, while DPAOs use  $\text{NO}_3^-$ -N/ $\text{NO}_2^-$ -N as the terminal electron acceptor for P removal instead of  $\text{O}_2$  (Oehmen et al., 2007). This process reduces sludge production and the demand for oxygen and carbon sources, which is particularly suitable for treating low COD/N wastewater (Kuba et al., 1996).

However, using PHA for denitrification and high  $\text{NO}_2^-$ -N concentration are the important performance characteristics during denitrifying P removal process, and these characteristics are usually key factors resulting in  $\text{N}_2\text{O}$  generation (Zeng et al., 2003; Zhou et al., 2012). More precisely, it has been reported in previous literature that  $\text{N}_2\text{O}$  generation from denitrifying P removal process was determined to be 2.3%-37.9% of the total N removal (Wang et al., 2011a, b).  $\text{N}_2\text{O}$  generation reduces

the practical application of this process. However, most studies of N<sub>2</sub>O generation from denitrifying P removal process mainly focused on the influence factors such as nitrite, anaerobic reaction time and carbon source (Zhou et al., 2008a; Wang et al., 2011a, b), and the mechanism and control measures for N<sub>2</sub>O generation have not yet been clearly described.

Therefore, the aim of this paper was to identify the causes of N<sub>2</sub>O generation in denitrifying P removal process, and evaluate the control measures of N<sub>2</sub>O generation. For this purpose, the N<sub>2</sub>O generation characteristics between denitrifying P removal process and conventional denitrification process were examined and compared throughout the study. Then, the causes of high N<sub>2</sub>O generation in denitrifying P removal process were investigated by batch experiments. Finally, in order to control N<sub>2</sub>O generation, the effects of nitrate addition strategies and carbon sources on N<sub>2</sub>O generation were investigated.

## **2. Materials and Methods**

### *2.1. Sludge Sources*

Denitrifying P removal sludge from a lab-scale anaerobic-anoxic/nitrifying sequencing batch reactor (A<sub>2</sub>N-SBR) and conventional denitrification sludge from anoxic-aerobic sequencing batch reactor (AO-SBR) were used to carry out the experiments.

Fig.1 shows the schematic diagram of the A<sub>2</sub>N-SBR. The A<sub>2</sub>N-SBR consisted of an anaerobic–anoxic SBR (A<sub>2</sub>-SBR) operated under alternating anaerobic, anoxic, and aerobic conditions, and a separate nitrification SBR (N-SBR) operated under aerobic

condition. Each reactor had a working volume of 5 L and was operated with a cycle time of 8 h. In each cycle, 4 L of synthetic wastewater was fed into the A<sub>2</sub>-SBR leading to a volume exchange ratio of 80%. After a further 90 min anaerobic reaction and 40 min settling, 4 L of supernatant (rich in  $\text{NH}_4^+\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$ ) was flowed into the N-SBR where  $\text{NH}_4^+\text{-N}$  was oxidized to  $\text{NO}_3^-\text{-N}$  during 150 min aerobic reaction. The effluent of N-SBR containing  $\text{NO}_3^-\text{-N}$  and  $\text{PO}_4^{3-}\text{-P}$  was pumped into A<sub>2</sub>-SBR in 2 min before the beginning of anoxic reaction. After 180 min anoxic reaction, 30 min aerobic reaction and 40 min setting, 4 L treated wastewater was discharged from the system.

AO-SBR had a working volume of 5 L and was worked with a cycle time of 6 h consisting of 10 min feeding, 90 min anoxic, 180 min aerobic, 50 min setting and decanting, and 20 min idle periods. In each cycle, 2.5 L of synthetic wastewater was fed into the reactor during the feeding period, resulting a hydraulic retention time of 12 h.

## 2.2. Synthetic wastewater

The synthetic wastewater contained  $\text{CH}_3\text{COONa}$  (77 mg/L),  $\text{CH}_3\text{CH}_2\text{COONa}$  (120 mg/L),  $\text{NH}_4\text{Cl}$  (153 mg/L),  $\text{NaHCO}_3$  (200 mg/L),  $\text{KH}_2\text{PO}_4$  (11 mg/L),  $\text{K}_2\text{HPO}_4\cdot 3\text{H}_2\text{O}$  (18 mg/L),  $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$  (10 mg/L),  $\text{FeSO}_4\cdot 7\text{H}_2\text{O}$  (10 mg/L),  $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$  (10 mg/L) and trace elements solution (1 mL/L). The trace elements solution was:  $\text{H}_3\text{BO}_3$  (150 mg/L),  $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$  (30 mg/L),  $\text{KI}$  (180 mg/L),  $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$  (120 mg/L),  $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$  (60 mg/L),  $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$  (120 mg/L),  $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$  (150 mg/L) and ethylene diamine tetraacetic acid (EDTA, 10 g/L) (Hu et

al., 2011b). The concentrations of COD,  $\text{NH}_4^+\text{-N}$  and TP were approximately 200, 40 and 5 mg/L, respectively.

### *2.3. Batch Experiments*

In order to investigate the causes and control measures of  $\text{N}_2\text{O}$  generation in denitrifying  $\text{P}$  removal process, five series batch experiments were conducted. For batch experiments, two types of reactors were used, one with 0.1 L overhead space had a working volume of 1.4 L, and the other reactor without overhead space had a working volume of 0.25 L. The magnetic stirrers were used to keep the suspension of the sludge, and the temperature was controlled at  $24\pm 1$  °C.

#### *2.3.1. $\text{N}_2\text{O}$ generation characteristics in denitrifying $\text{P}$ removal process and conventional denitrification process*

Batch experiments 1 was carried out to investigate the differences of  $\text{N}_2\text{O}$  generation in denitrifying  $\text{P}$  removal process and conventional denitrification process. The sludge for this experiment was withdrawn from  $\text{A}_2\text{-SBR}$  and  $\text{AO-SBR}$  at the end of aerobic phase, respectively, and then washed three times with distilled water. After being removed the supernatant, the sludge was transferred into two reactors (SBR-1 and SBR-2). Next, 1.12 L of synthetic wastewater was fed into each reactor. Then, distilled water was added to make the final volume of each reactor to be 1.4 L, resulting in an initial COD,  $\text{NH}_4^+\text{-N}$ ,  $\text{PO}_4^{3-}\text{-P}$  concentrations of 160, 10 and 5 mg/L, respectively. SBR-1 was operated with 90 min anaerobic reaction and 180 min anoxic reaction, and  $\text{KNO}_3$  was pulse added at the end of anaerobic phase, giving an initial concentration of  $\text{NO}_3^-\text{-N}$  of approximately 25 mg/L. SBR-2 was operated with 180

min anoxic reaction, and  $\text{KNO}_3$  was pulse added at the beginning of anoxic phase, and the initial concentration of  $\text{NO}_3^-$ -N was also 25 mg/L.

### *2.3.2 Identifying causes of $\text{N}_2\text{O}$ generation in denitrifying P removal process*

Batch experiments 2 and 3 were carried out to analyze the causes of  $\text{N}_2\text{O}$  generation in denitrifying P removal process, by investigating the  $\text{N}_2\text{O}$  production and reduction rates under different conditions. Batch experiments 2, 4 L mixed liquor was withdraw from  $\text{A}_2$ -SBR at the end of anaerobic phase, and divided evenly into four batch reactors (R1, R2, R3 and R4). R1 and R2 were then amended with  $\text{KNO}_3$  solution to give an initial  $\text{NO}_3^-$ -N concentration of 25 mg/L, while R3 and R4 received  $\text{NaNO}_2$  solution to provide an initial  $\text{NO}_2^-$ -N concentration of 20 mg/L. At the same time, a certain amount of acetate was added into R2 and R4 to give an initial COD concentration of 80 mg/L. The anoxic reaction lasted for 180 min, and  $\text{N}_2\text{O}$  microsensor (Unisense, Denmark) was used to measure the dissolved  $\text{N}_2\text{O}$  concentration.

The sludge mixture (1L) for batch experiments 3 was taken from  $\text{A}_2$ -SBR at the end of anaerobic phase, and divided equally into four 250 mL reactors (r1, r2, r3 and r4). A certain amount of  $\text{N}_2\text{O}$  saturated solution was added to the reactors, resulting in an initial dissolved  $\text{N}_2\text{O}$ -N concentration of about 4 mg/L. [N<sub>2</sub>O saturated solution was prepared according to the method of Pan et al. \(2012\).](#) Meanwhile 80 mg/L COD were prepared in r2 and r4 by adding acetate, and 25 mg/L  $\text{NO}_3^-$ -N were prepared in r3 and r4. The anoxic reaction lasted for 30 min, and dissolved  $\text{N}_2\text{O}$  concentration was detected by  $\text{N}_2\text{O}$  microsensor.

### 2.3.3 Controlling $N_2O$ generation in denitrifying $P$ removal process

In order to decrease  $N_2O$  generation in denitrifying  $P$  removal system, batch experiments 4 and 5 were used to study the effects of nitrate addition strategies and carbon sources on  $N_2O$  generation, respectively. Batch experiments 4, sludge mixture (2.8 L) were withdrawn from  $A_2$ -SBR at the end of anaerobic phase, and directly transferred to two batch reactors (Run 1 and Run 2). For Run 1, 35 mL  $KNO_3$  solution (1 g/L) was pulse added. However, 35 mL  $KNO_3$  solution was continuously fed into Run 2 in 2 hours by a peristaltic pump (BQ50-1J, Longer). The anoxic reaction lasted for 180 min.

Batch experiments 5, 4.2 L sludge mixture were taken from  $A_2$ -SBR at the end of aerobic phase. The washed sludge was divided equally into three reactors (run 1, run 2 and run 3). Like batch experiment 1, synthetic wastewater and distilled water were supplied to each reactor. However, the carbon sources in three reactors were different, and run 1, run 2 and run 3 were fed with acetate, acetate/propionate and propionate, respectively. The three reactors were operated as SBR-1.

### 2.4 Analytical methods

The analyses of COD,  $NH_4^+$ -N,  $NO_3^-$ -N,  $NO_2^-$ -N,  $PO_4^{3-}$ -P, MLSS and MLVSS were conducted in accordance with Standard Methods (Chinese SEPA, 2002). PHA including Poly-b-hydroxybutyrate (PHB), poly-hydroxyvalerate (PHV) and poly-3-hydroxy-2-methylvalerate (PH2MV) were measured according to the method described by Oehmen et al. (2005).  $NO_3^-$ -N and  $NO_2^-$ -N reduction rates were determined according to the method described by Wang et al. (2011b). The off-gas



$\text{N}_2\text{O}$  collection and calculation were based on the methods of Yang et al. (2009).  $\text{N}_2\text{O}$  concentration in gas phase and liquid phase were measured with Gas chromatography (GC) (SP-3410, China) and  $\text{N}_2\text{O}$  microsensor (Unisense, Denmark), respectively.

### 3. Results and discussion

#### 3.1. $\text{N}_2\text{O}$ generation characteristics

After over four months of operation period, stable N and P removal were achieved in  $\text{A}_2$ -SBR and AO-SBR. Then batch experiments 1 were carried out to investigate the differences of  $\text{N}_2\text{O}$  generation between denitrifying P removal process and conventional denitrification process.

Figures 2 and 3 show the variations of COD, N, P, and PHA during N removal via denitrifying P removal (in SBR-1) and conventional denitrification (in SBR-2) in a typical cycle. During the anaerobic phase of SBR-1, COD was rapidly decreased to the lowest level within 30 min, and this was accompanied by the synthesis of PHA, release of  $\text{PO}_4^{3-}\text{-P}$ . In the subsequent anoxic phase, simultaneous denitrification and  $\text{PO}_4^{3-}\text{-P}$  uptake was occurred, and PHA was consumed (Fig. 2). SBR-1 showed a typical DPAOs phenotype. Unlike the SBR-1, there was only anoxic phase in SBR-2. During the anoxic phase, COD consumption,  $\text{NO}_3^- \text{-N}$  reduction and  $\text{PO}_4^{3-}\text{-P}$  release were simultaneously occurred, and only a small amount of PHA was formed (Fig. 3). SBR-2 showed a conventional denitrifying bacteria phenotype.

In SBR-1, it was found that  $\text{N}_2\text{O}$  was mainly produced in anoxic phase, and nearly no  $\text{N}_2\text{O}$  was detected in anaerobic phase; once  $\text{KNO}_3$  solution was added to the reactor, dissolved  $\text{N}_2\text{O}\text{-N}$  concentration increased rapidly to 0.87 mg/L in the first 15

min and decreased drastically to around zero (Fig. 2). Like SBR-1, a transient accumulation of N<sub>2</sub>O was also observed at the beginning of anoxic period in SBR-2 (Fig. 3). However, the highest dissolved N<sub>2</sub>O concentration in SBR-2 was only 0.08 mg/L, which was much lower than that in SBR-1. Furthermore, the N<sub>2</sub>O emission was also measured and the total N<sub>2</sub>O generation amount (in gas and liquid phases) was quantified in Table 1. It can be seen that although N<sub>2</sub>O generation amount in SBR-1 (0.41±0.034% of the TN removal) was much higher than that (0.013±0.0056%) in SBR-2, it was much lower than those (2.34%~21.6%) reported in previous denitrifying P removal systems (Wang et al., 2011ab). This may be attributed to the use of a mixing carbon source containing both of propionate and acetate rather than the use of acetate alone in previous studies. Compared with acetate, the use of propionate as carbon source significantly decreased N<sub>2</sub>O generation in wastewater treatment process (Zhu and Chen, 2011).

### *3.2. Identifying the causes of N<sub>2</sub>O generation in denitrifying P removal process*

SBR-1 used PHA as a carbon source for denitrification, while SBR-2 used acetate and propionate as carbon source for denitrification, and the NO<sub>2</sub><sup>-</sup>-N accumulation concentration in SBR-1 was much higher than that in SBR-2. Therefore, we speculated that using PHA for denitrification and higher NO<sub>2</sub><sup>-</sup>-N accumulation caused the difference of N<sub>2</sub>O generation in the two reactors.

#### *3.2.1. Using PHA for denitrification caused N<sub>2</sub>O generation*

The cause was first tested by adding acetate in anoxic batch experiments 2 using denitrifying P removal sludge (Fig. 4). The obtained results showed that when acetate

was not added to the reactor, DPAOs used PHA which was synthesized in anaerobic phase as carbon source for denitrification, and the maximum N<sub>2</sub>O-N accumulation reached to 0.85 mg/L. However, when acetate was added, DPAOs could use acetate as a carbon source for denitrification, and the maximum N<sub>2</sub>O-N accumulation was only 0.21 mg/L. These results indicated that using PHA for denitrification would increase the N<sub>2</sub>O generation. Compared with acetate, the rate of PHA degradation was slow, which cannot provide enough electrons for denitrification, and this would lead to competition for electrons between denitrifying enzymes (Kampschreur et al., 2009). N<sub>2</sub>O reductase (Nos) could not have a competitive advantage at capturing electrons due to the reduction of N<sub>2</sub>O is the last step of denitrification, and the N<sub>2</sub>O reduction would be inhibited (Kampschreur et al., 2009; Wang et al., 2011b).

In order to prove the above hypothesis, batch experiments 3 were conducted and the results were shown in Fig 5. Fig 5 showed that acetate addition significantly increased N<sub>2</sub>O reduction rate, because acetate degradation rate is 6-20 times faster than PHA, and Nos could obtain enough electrons for N<sub>2</sub>O reduction (Third et al., 2003). When nitrate was added to the batch reactors, N<sub>2</sub>O reduction was inhibited, because nitrate reductase (Nar) had a competitive advantage for electrons over Nos, and the electrons provided by PHA oxidation were first used for nitrate reduction (Kampschreur et al., 2009). Whereas when acetate and nitrate were both supplied, the N<sub>2</sub>O reduction was not inhibited, because the electrons provided by acetate were sufficient for both nitrate and N<sub>2</sub>O reduction. These results indicated that when using PHA for denitrification, the limited electrons provided by PHA could not satisfy the

requirement of denitrifying enzymes, and Nos had a weak competition for electrons when compared to Nar.

### 3.2.2. High $\text{NO}_2^-$ -N concentration caused $\text{N}_2\text{O}$ generation

From Figs. 2 and 3, it was found that  $\text{NO}_2^-$ -N accumulated in SBR-1 up to 8.51 mg/L, which was much higher than that in SBR-2 (4.91 mg/L). The possible reason is that SBR-1 used the internal carbon source (PHA) as carbon source for denitrification, while SBR-2 used acetic acid and propionic acid for denitrification. As the lower PHA degradation rate caused denitrifying enzymes competing for electrons, the nitrate reduction rate was much higher than nitrite reduction rate (Kampschreur et al., 2009; Wang et al., 2011b). The batch experiment 2 was then conducted to confirm  $\text{NO}_2^-$ -N accumulation caused  $\text{N}_2\text{O}$  generation.

From Fig. 4, it was also found that nitrite addition to the DPAOs sludge significantly stimulated the  $\text{N}_2\text{O}$  generation, and the maximum  $\text{N}_2\text{O}$ -N accumulation reached to 6.19 mg/L which was much higher than that in the nitrate addition reactor. This finding is consistent with the results reported by Lemaire et al. (2006), indicating that the  $\text{N}_2\text{O}$  production rate with nitrite addition was five times higher than that with nitrate addition. Likewise, Zhou et al. (2008a) demonstrated that the high level of  $\text{NO}_2^-$ -N caused the accumulation of  $\text{N}_2\text{O}$ . They later revealed that the activity of Nos was inhibited by high  $\text{NO}_2^-$ -N accumulation (Zhou et al., 2011).

However, it was observed an interesting phenomenon from batch tests 2 (Fig. 4). When nitrite and acetate was both added to the DPAOs sludge, the  $\text{N}_2\text{O}$  generation was much lower than that in the only nitrite addition reactor. This result also

explained why nearly no  $N_2O$  was generated in conventional denitrification process, although  $NO_2^-$ -N accumulation reached to 4.91 mg/L. The external carbon source could decrease the nitrite inhibition on Nos activity.

Therefore, the weak competition of Nos for electrons and higher  $NO_2^-$ -N accumulation were the two main reasons for  $N_2O$  generation in denitrifying phosphorus removal system.

### *3.3. Controlling $N_2O$ generation during denitrifying phosphorus removal process*

As N and P could be simultaneously removed using the same carbon source, denitrifying P removal offered a great potential to save the requirement for carbon sources and oxygen. However, lots of  $N_2O$  would be generated, which severely impedes the application of this process. Based on the above analysis, two measures could possibly reduce  $N_2O$  generation during denitrifying P removal: (a) using continuous nitrate addition to decrease the competition for electrons between denitrifying enzymes; (b) using propionate as carbon source to decrease nitrite accumulation.

#### *3.3.1. The effects of nitrate addition strategies on $N_2O$ generation*

Nitrate was pulse added to the reactors, and the  $NO_3^-$ -N concentration was always at relatively high levels (25 mg/L) at the beginning of anoxic phase. The electrons which were provided by PHA oxidation could not be satisfied the requirement for reducing  $NO_3^-$  to  $N_2$ , and the limited electrons were firstly used to reduce  $NO_3^-$ -N to  $NO_2^-$ -N and  $N_2O$ . If applying the continuous nitrate addition strategy, the  $NO_3^-$ -N concentration could be managed at the relatively low levels. So,

the requirement of electrons for complete conversion of  $\text{NO}_3^-$ -N to  $\text{N}_2$  would be enough, and the  $\text{N}_2\text{O}$  accumulation would be decreased.  $\text{N}_2\text{O}$  and  $\text{NO}_2^-$ -N accumulation under pulse nitrate addition condition was compared with that under continuous addition condition by batch experiments 4. Fig. 6 showed that  $\text{N}_2\text{O}$  generation varied between the two different kinds of nitrate addition strategies. The continuous addition significantly decreased the  $\text{N}_2\text{O}$  generation and  $\text{NO}_2^-$ -N accumulation. Zhou et al. (2008b) also found that the  $\text{N}_2\text{O}$  generation was decreased in a novel 2-sludge 3-stage system by feeding the nitrite-containing stream continuously to the anoxic stage.

### 3.3.2. The effects of carbon sources on $\text{N}_2\text{O}$ generation

$\text{NO}_2^-$ -N accumulation was the main reason for  $\text{N}_2\text{O}$  generation in denitrifying P removal process.  $\text{N}_2\text{O}$  generation would be decreased if the  $\text{NO}_2^-$ -N accumulation was reduced. Propionate was good carbon source for DPAOs, and it could decrease the  $\text{NO}_2^-$ -N accumulation in denitrifying P removal process (Carvalho et al., 2007). Therefore, the  $\text{N}_2\text{O}$  generation would be reduced if using propionate as carbon source for DPAOs. The superiority of propionate for  $\text{N}_2\text{O}$  reduction was clearly evidenced by the results of batch tests 5 (Fig. 7, Table 1). It showed that compared with acetate and the mixture of acetate and propionate, using propionate as the sole carbon source significantly decreased  $\text{NO}_2^-$ -N accumulation and  $\text{N}_2\text{O}$  generation.  $\text{NO}_2^-$ -N is an intermediate of denitrification, and its accumulation is affected by the activities of Nar and nitrite reductase (Nir) (Wang et al., 2011a). The carbon sources can exert different effects on Nar and Nir activities, leading to a different ratio of nitrate reduction rate

(N1) to nitrite reduction rate (N2). The high ratio of N1/N2 could cause high  $\text{NO}_2^-$ -N accumulation (Wang et al., 2011a; Zhu and Chen, 2011). As can be seen in Table 2, the use of propionate as carbon source decreased the ratio of N1/N2, which was consistent with the lower  $\text{NO}_2^-$ -N accumulation.

However, these results disagreed with the conclusions of Wang et al. (2011a), who reported that  $\text{N}_2\text{O}$  generation was significantly increased when using acetate/propionate or propionate as carbon source in short-term experiments. The differences between the two studies were that DPAOs in this study was acclimatized by acetate/propionate, whereas DPAOs in their study was only acclimatized by acetate. The species of DPAOs in the two studies might be different. More research efforts are needed to further investigate the relationship between  $\text{N}_2\text{O}$  generation and microbial community in denitrifying P removal process acclimatized with different carbon sources.

Therefore, using continuous nitrate addition or propionate as a carbon source could decrease the  $\text{N}_2\text{O}$  generation in denitrifying P removal process. Furthermore, several measures have been reported to decrease  $\text{N}_2\text{O}$  generation in biological nitrogen and phosphorous removal process, such as reducing anaerobic time, using sludge alkaline fermentation liquid, and adding copper ion ( $\text{Cu}^{2+}$ ) (Wang et al. 2011b; Zhu and Chen, 2011; Zhu et al. 2012). The applicability of these measures in denitrifying P removal process is therefore worth further research efforts.

#### **4. Conclusion**

Compared with the conventional denitrification process, the denitrifying P removal process significantly increased N<sub>2</sub>O generation. Two main reasons were responsible for N<sub>2</sub>O generation. One is using PHA for denitrification could lead to competition for electrons between denitrifying enzymes, and N<sub>2</sub>O reductase could not have a competitive advantage at capturing electrons; the other is too much nitrite would be accumulated, and the activity of N<sub>2</sub>O reductase would be inhibited. Therefore, N<sub>2</sub>O generation could be decreased via two pathways: (a) using continuous nitrate addition to decrease the competition for electrons between denitrifying enzymes; (b) using propionate as a carbon source to decrease the nitrite accumulation.

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**Table 1**N<sub>2</sub>O generation amount and denitrification efficiency in batch experiments<sup>a</sup>

Reactor	N <sub>2</sub> O generation amount (mg N/L )	Ratio of N <sub>2</sub> O generation to denitrified nitrogen (%)	Denitrification efficiency (%)
SBR-1	0.083±0.0076	0.41±0.034	90.66±3.47
SBR-2	0.0027±0.00092	0.013±0.0056	100
pulse feed	0.089±0.0074	0.40±0.036	88.51±3.60
continuous feed	0.0071±0.0033	0.035±0.014	81.58±4.73
acetate	0.21±0.028	0.83±0.011	100
acetate + propionate	0.096±0.010	0.43±0.0054	90.01±3.17
propionate	0.025±0.0035	0.13±0.0076	76.93±6.40

<sup>a</sup>The data are the averages and their standard deviations in triplicate tests.

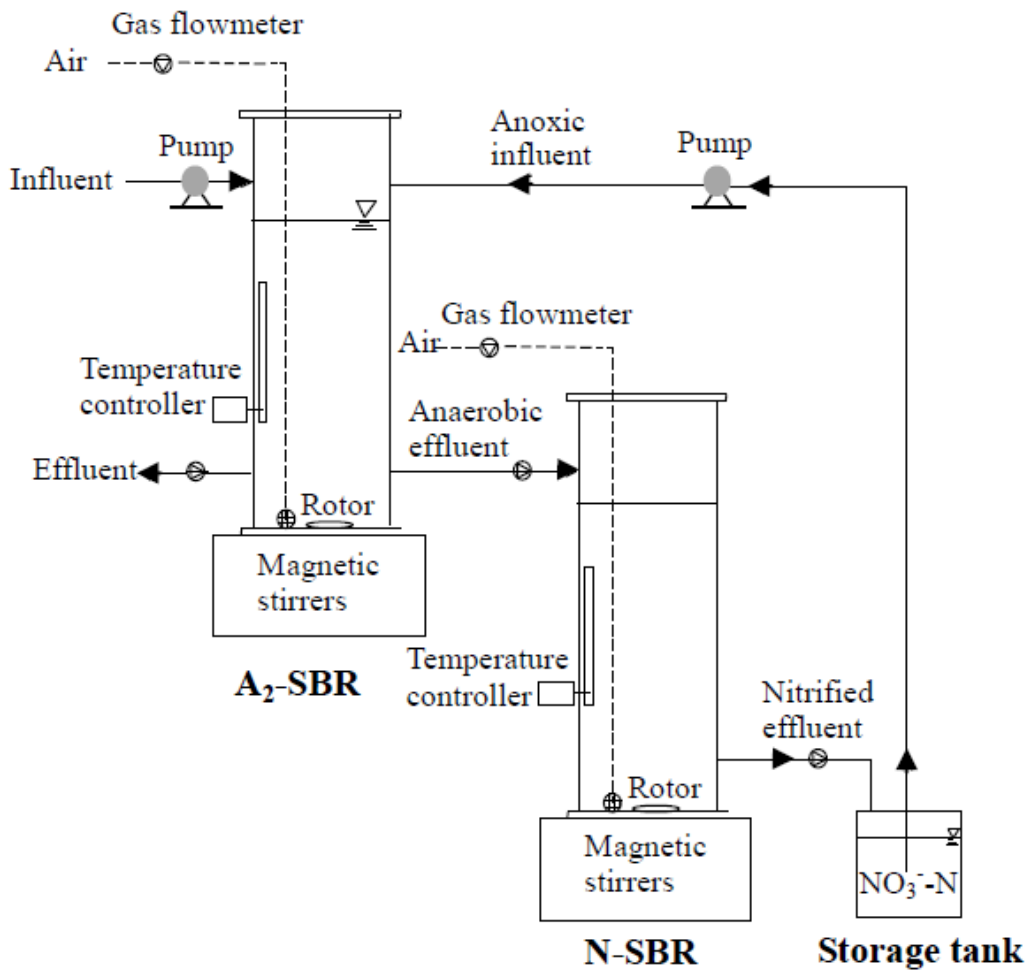
**Table 2**

Comparison of the reduction rates of  $\text{NO}_3^-$ -N and  $\text{NO}_2^-$ -N with different carbon sources<sup>a</sup>

Carbon source	$\text{NO}_3^-$ -N reduction rate (N1) <sup>b</sup>	$\text{NO}_2^-$ -N reduction rate (N2) <sup>b</sup>	N1/N2
acetate	0.43±0.036	0.18±0.031	2.39
acetate + propionate	0.33±0.029	0.17±0.025	1.94
propionate	0.19±0.022	0.17±0.028	1.12

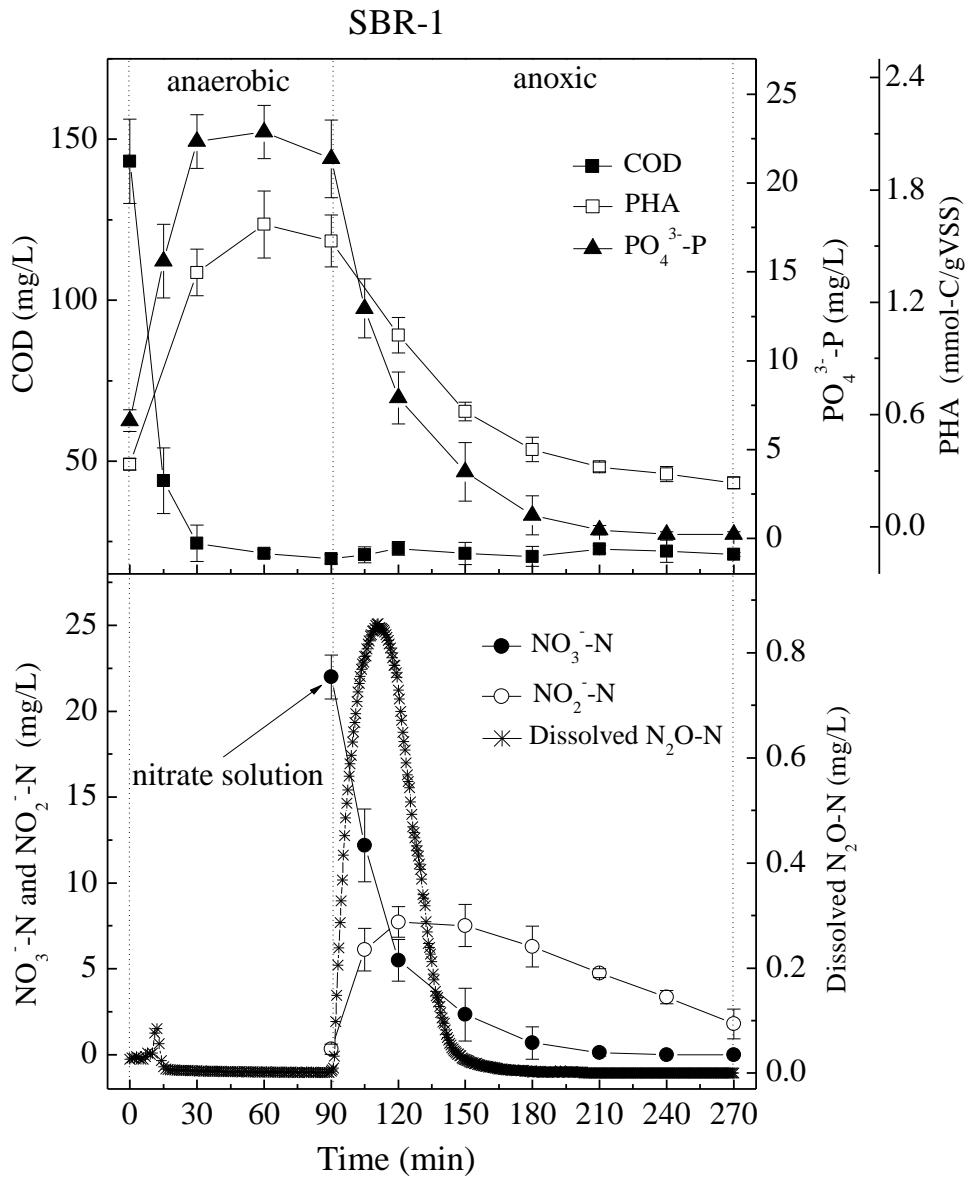
<sup>a</sup> The data are the averages and their standard deviations in triplicate tests.

<sup>b</sup> The unit of reduction rate is mg N/g VSS/min.

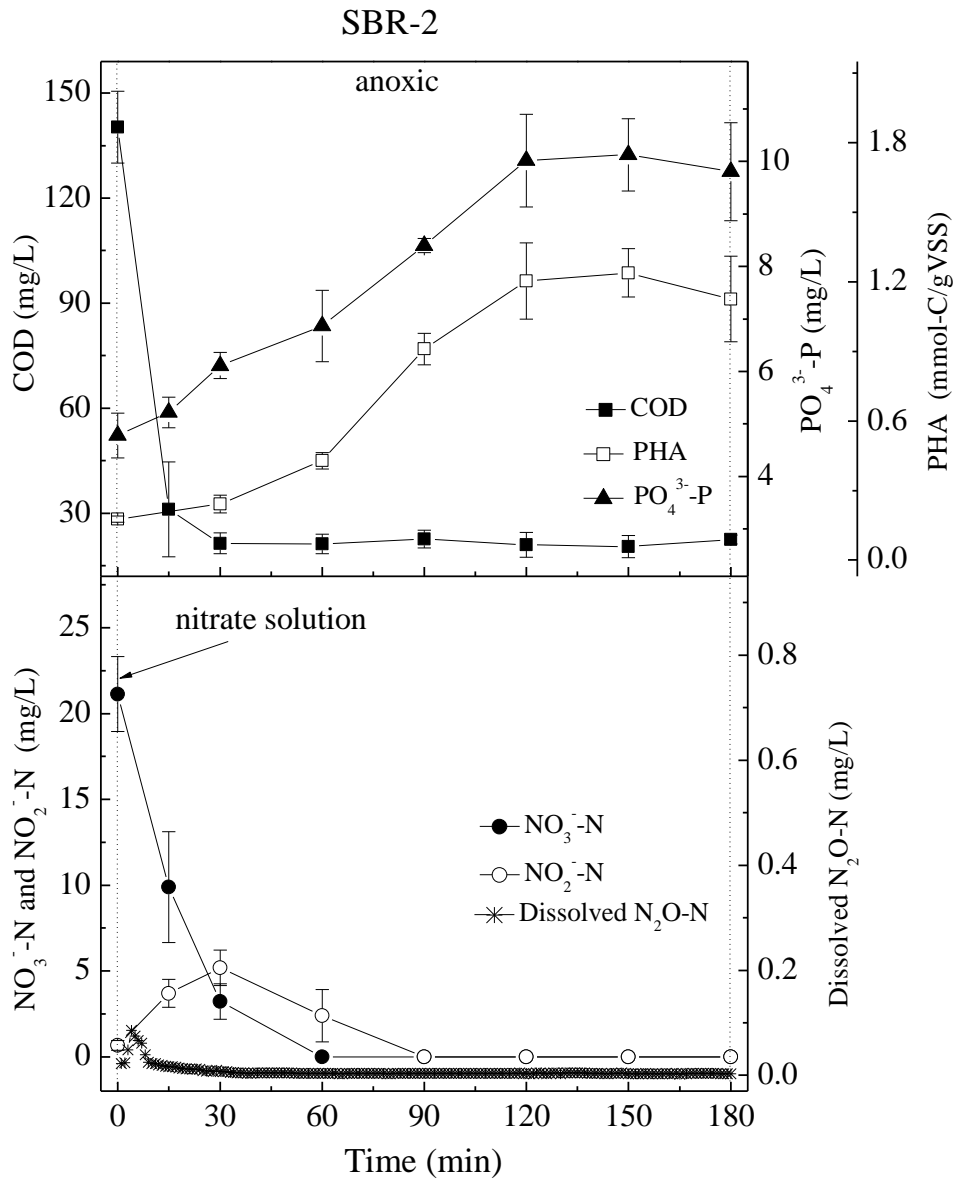


**Fig. 1.** The schematic diagram of the A<sub>2</sub>N-SBR.

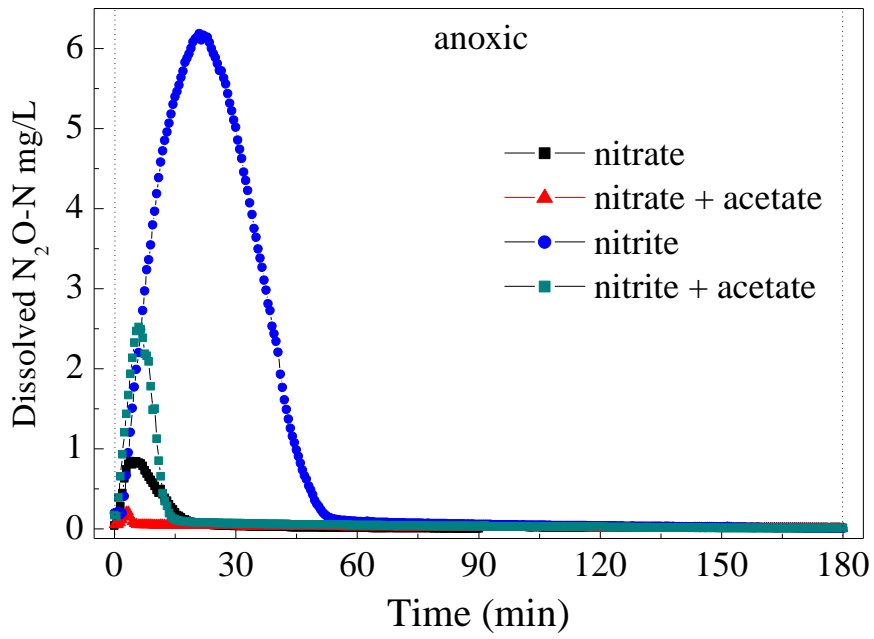




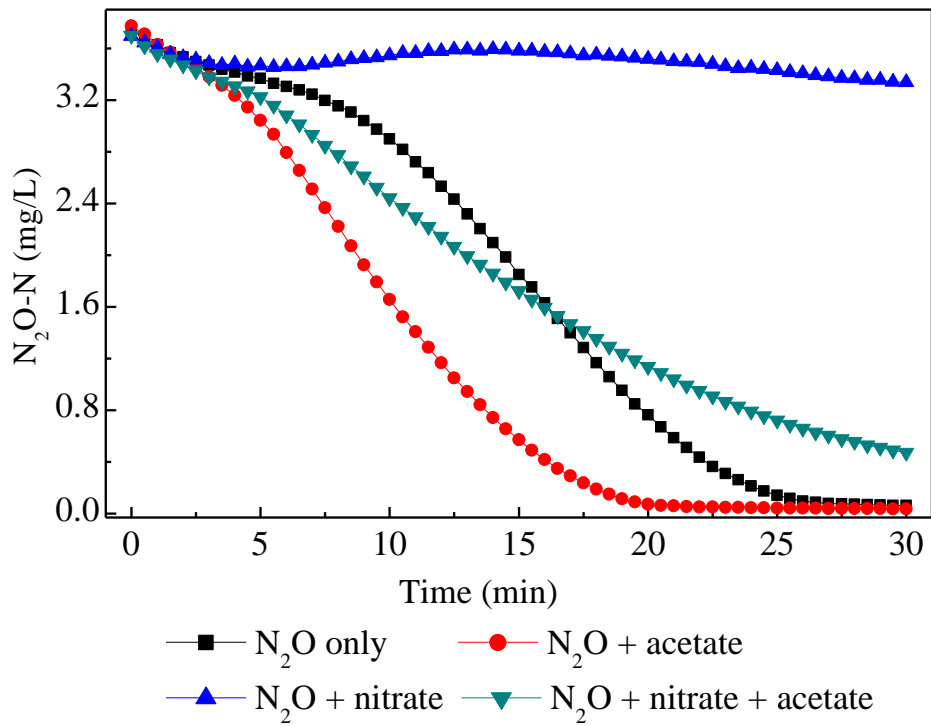
**Fig. 2.** Variations of COD, PHA, PO<sub>4</sub><sup>3-</sup>-P, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and dissolved N<sub>2</sub>O concentrations during nitrogen removal via denitrifying phosphorus removal in SBR-1. Error bars represent standard deviations of triplicate tests.



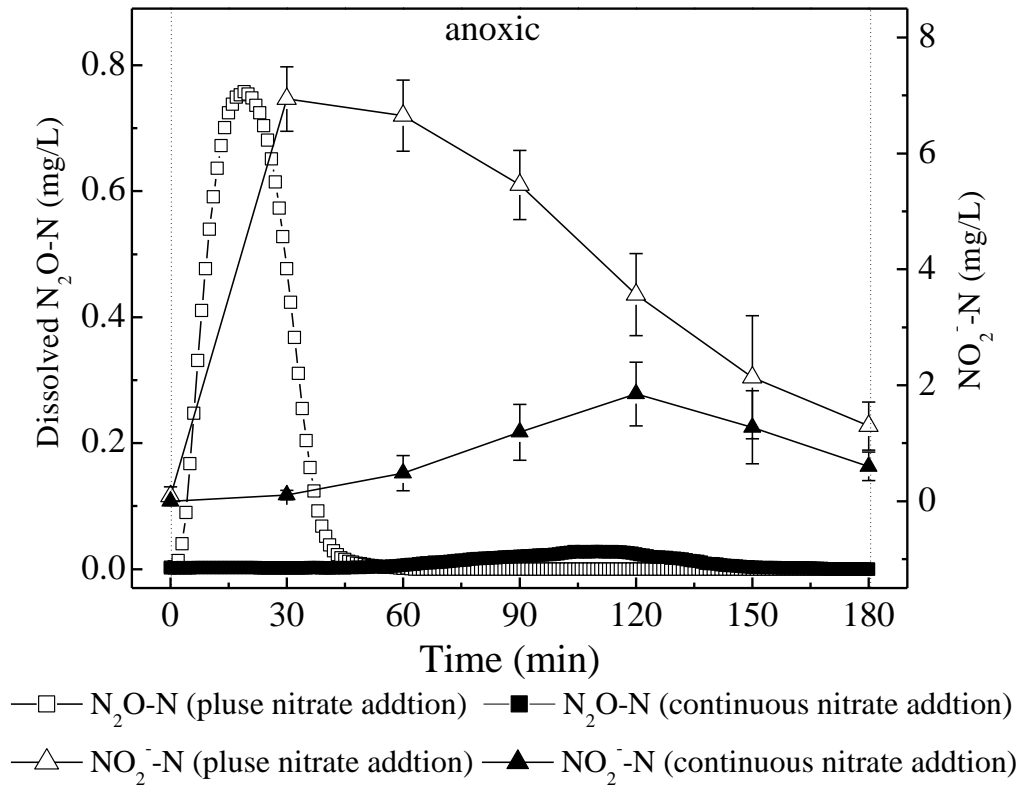
**Fig. 3.** Variations of COD, PHA, PO<sub>4</sub><sup>3-</sup>-P, NO<sub>3</sub><sup>-</sup>-N, NO<sub>2</sub><sup>-</sup>-N and dissolved N<sub>2</sub>O concentrations during nitrogen removal via conventional denitrification in SBR-2. Error bars represent standard deviations of triplicate tests.



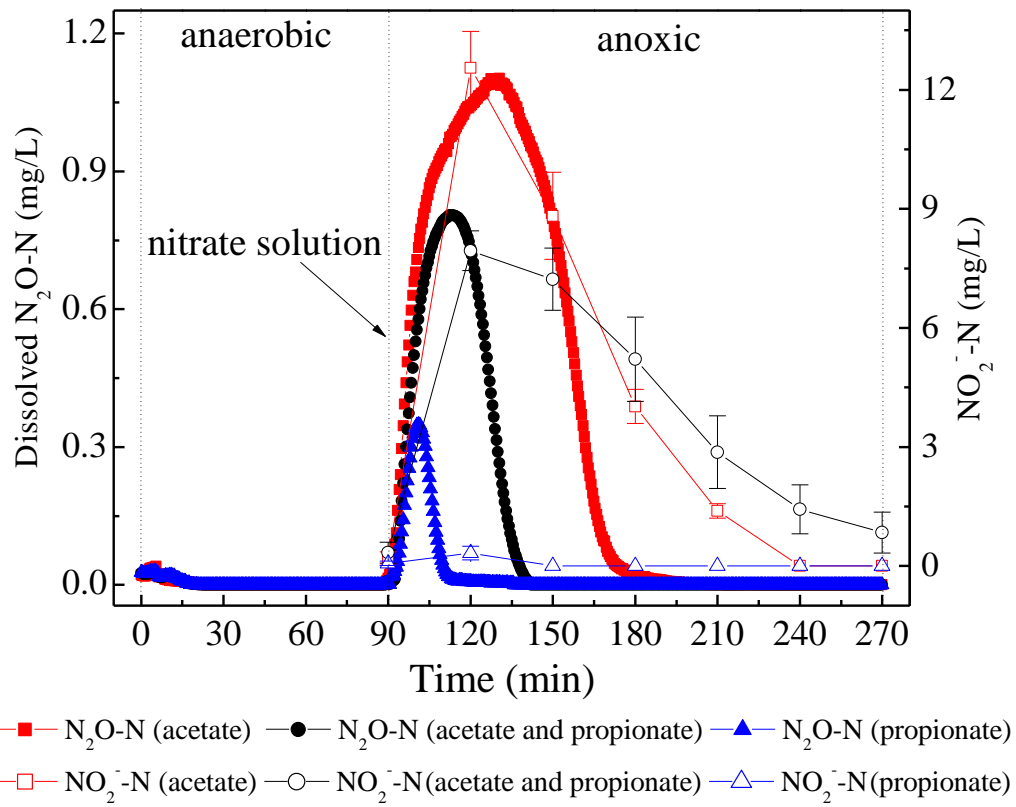
**Fig. 4.**  $N_2O$  production profiles during four batch experiments with the addition of nitrate, nitrate+acetate, nitrite, and nitrite+acetate using denitrifying phosphorus removal sludge.



**Fig. 5.** N<sub>2</sub>O reduction profiles during four batch experiments with the addition of acetate, nitrate, and nitrate+acetate using denitrifying phosphorus removal sludge.



**Fig. 6.**  $\text{N}_2\text{O}$  generation and  $\text{NO}_2^- \text{-N}$  accumulation profiles during two batch experiments with the pulse and continuous feed strategies. Error bars represent standard deviations of triplicate tests.



**Fig. 7.** N<sub>2</sub>O generation and NO<sub>2</sub><sup>-</sup>-N accumulation profiles during three batch experiments with acetate alone, the mixture of acetate and propionate or propionate alone as carbon source. Error bars represent standard deviations of triplicate tests.



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