

Effect of nitrite on nitrous oxide generation and emission during denitrifying phosphorus removal

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Abstract

Nitrous oxide (N₂O) generation and emission may hamper the wide application of denitrifying phosphorus removal technology. In this study, batch experiments were carried out to investigate the effect of nitrite on N₂O generation and emission during denitrifying phosphorus removal. Compared to NO₃-N, N₂O emission was much higher when NO₂-N was used as the electron acceptor. N₂O emission had no obvious difference with an increase in the NO₂-N concentration (5, 10, 20 and 40 mg/L), with the emission factor of 2.29%-3.25%. However, N₂O generation increased with increasing NO₂-N concentrations, with concentrations of 3.35, 5.55, 6.16, 8.18 mg N₂O/L, respectively. With the initial anaerobic dosage acetate concentration of 100, 200 and 400 mg/L, the N₂O emission factor was 3.95%, 3.16% and 3.08%, respectively, and the generated dissolved N₂O concentration was 7.13, 13.48 and 12.31 mg N₂O/L.

Keywords

N₂O generation and emission; denitrifying phosphorus removal; electron acceptor; carbon source

INTRODUCTION

Denitrifying phosphorus removal can be applied to remove nitrogen and phosphorus simultaneously from wastewater. This technology is achieved by denitrifying polyphosphate-accumulating organisms (DNPAOs) that capable of utilizing nitrate or nitrite instead of oxygen as the electron acceptor for phosphorus uptake under anoxic conditions. In comparison with conventional biological phosphorus removal, denitrifying phosphorus removal can save aeration energy and the amount of carbon sources needed for denitrification, as well as lowering the sludge production (Kuba et al., 1996). However, a large amount of nitrous oxide (N₂O) production (ratio of anoxic N₂O production to TN removal was 7.77%) was confirmed for denitrifying phosphorus removal system, especially with nitrite nitrogen (NO₂-N) as the electron acceptor (Wang et al., 2011b).

In Denitrifying phosphorus removal system, N₂O generation and emission affected by many factors, including type of electron acceptors (Schulthes et al., 1995; Tang et al., 2016), free nitrous acid (FNA) or NO₂-N (Zhou et al., 2008; Li et al., 2013; Zhou et al., 2011), and the amount of anaerobic Poly-β-hydroxyalkanoates (PHAs) produced (Li et al., 2013; Wang et al., 2011a; Zhou et al., 2012). Previous studies showed that the ability of Nos (nitrous oxide reductase) in competition of electron was weak. Hence, deficient in PHAs was the main reason stimulating N₂O emission (Li et al., 2013). The toxicity of NO₂-N to the activity of Nos was considered as the main cause in the study of Alinsafi et al. (2008). In addition, Lemire et al. (2006) demonstrated that N₂O generation and emission was mainly attributed to Glycogen-accumulating organisms (GAOs). Therefore, the exactly determining factor regulating N₂O production and emission in denitrifying phosphorus removal system is still unclear.

This study aimed to examine the effect of nitrite and the concentration of PHAs on the emission of N₂O during denitrifying phosphorus removal. Batch experiments was conducted by adding different electron acceptors, different concentrations of NO₂-N and different concentrations of initial acetate. The generation and emission of N₂O during denitrifying phosphorus removal was investigated

simultaneously.

MATERIALS AND METHODS

Denitrifying phosphate removal process

A sequencing batch reactor (SBR) with a working volume of 6 liters was operated at 25°C. The SBR had three cycles per day and each cycle comprised the following phases: fill (10 min), anaerobic (110 min), anoxic (180 min), aerobic (120 min), settle (40 min) and draw/idle (20 min). In each cycle, 3 liters of treated wastewater were exchanged with a new batch of synthetic wastewater. The reactor was constantly stirred with a mixer during the fill, anaerobic and aerobic phases. During the aerobic phase, air was supplied with an air diffuser located at the bottom of the reactor. During the anoxic phase, nitrate stock solution was dosed to achieve the initial nitrate nitrogen ($\text{NO}_3\text{-N}$) concentration of 30 mg/L. Once a day, 400 mL of mixed liquor was withdrawn from the reactor at the end of the aerobic phase, resulting in a solids retention time (SRT) of around 15 days if no solids loss occurred during the settling phase.

The components of the synthetic wastewater contained sodium acetate, yeast extract, NH_4Cl , Na_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$ and trace elements. The influent chemical oxygen demand (COD) was around 400 mg/L, ammonium nitrogen ($\text{NH}_4\text{-N}$) of 15 mg/L and ortho-phosphate ($\text{PO}_4\text{-P}$) of 15 mg/L. The reactor was seeded with activated sludge taken from a Wastewater Treatment Plant in Shenzhen, China.

Batch experiments

Batch experiments with replications were carried out to examine effects of electron acceptors, initial $\text{NO}_2\text{-N}$ concentrations and initial PHAs concentrations on N_2O generation and emission. Average results from replications were presented. The batch reactors were made from 800 mL capped glass flasks.

For the effect of electron acceptors, activated sludge mixed liquor was withdrawn from the SBR at the end of the anaerobic phase, and then different electron acceptors (30 mg/L of $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$) were added for commencing the experiment.

For the effect of different concentrations of $\text{NO}_2\text{-N}$, activated sludge mixed liquor was withdrawn from the SBR at the end of the anaerobic phase, and then different concentration of electron acceptors (5, 10, 20 and 40 mg/L of $\text{NO}_2\text{-N}$) were added for commencing the experiment.

For examining the effect of PHAs on N_2O generation and emission, the activated sludge was taken from the SBR at the end of the aerobic phase, and then different concentrations of acetate (100, 200 and 400 mg/L) was added for accumulating PHAs under anaerobic conditions for 120 min. Then, 40 mg/L of $\text{NO}_2\text{-N}$ was added to initiate the experiment.

In all batch experiments, the batch reactor was carefully sealed and heated in water bath, and samples were taken at intervals to test $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, PHB and PHV for liquid samples, and N_2O for gas samples.

Analytical methods

$\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, $\text{PO}_4\text{-P}$, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) concentrations were determined according to standard methods (APHA, 1995).

N_2O was determined using an Agilent GC (GC 9720, Zhejiang, China) equipped with ECD detector and HP-PLOT/Q capillary column (WAX-HT, $30m \times 0.25mm \times 0.25\mu m$, USA). Dissolved N_2O was on-line monitoring by N_2O microelectrodes (Unisense, Denmark).

The Poly- β -hydroxybutyrate (PHB) and Polyhydroxyvalerate (PHV) were extracted using the method of Comeau et al. (1998) with some modification. Briefly, 5 mL of sludge mixed liquor was dewatered by centrifuging at 10000 rpm for 10 min. The sludge was freeze-dried for more than 20 h. The sludge was heated at $100^\circ C$ for 20 h after the biomass was suspended in 2 mL n-propanol (benzoic acid internal standard) and 2 mL dichloromethane. After cooling, 4 mL water was added and the sample was mixed. When the phases separated, approximately 1 mL of the bottom organic layer was transferred for analysis via gas chromatography (GC-2014, Shimadzu).

RESULTS

N_2O emission with different electron acceptors

Figure 1 shows dynamics of N, P, PHB and PHV during denitrifying phosphorus removal with NO_2^- -N (30 mg/L) and NO_3^- -N (30 mg/L) as the electron acceptor. The denitrification and phosphorus-uptake rates with NO_2^- -N as the electron acceptor (5.79 mg N/g VSS/h and 11.95 mg N/g VSS/h, respectively) were a little higher than NO_3^- -N (3.11 mg N/g VSS/h and 7.28 mg N/g VSS/h, respectively), indicating that DNPAOs which long term acclimated with NO_3^- -N could utilize NO_2^- -N as electron donor. In addition, the emitted concentration of N_2O were 0.14 mg/L and 0.006 mg/L with NO_2^- -N and NO_3^- -N as the electron acceptor, respectively. This result suggested that N_2O emission was stimulated when NO_2^- -N was used as the electron acceptor.

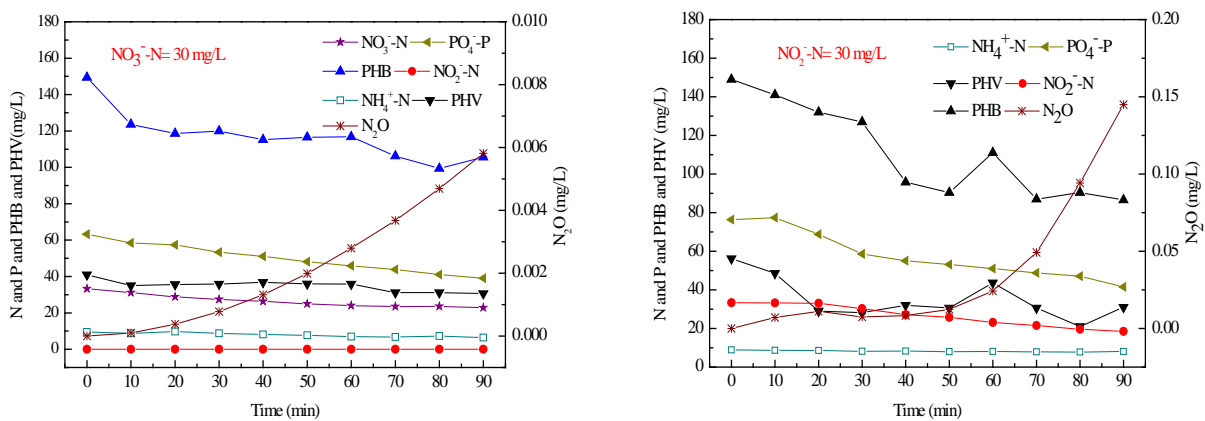


Figure 1. Dynamics of different types of nitrogen and phosphorus during batch experiments with different electron acceptors.

N_2O emission under different nitrite concentrations

Batch experiments were carried out by adding different concentrations of nitrite (5, 10, 20 and 40 mg/L) after 2 h anaerobic reaction. From Table 1 and Figure 2, denitrification rate and phosphorus-uptake rate were 7.42 mg N/g VSS, 13.15 mg P/g VSS; 7.20 mg N/g VSS and 15.15 mg P/g VSS; 7.67 mg N/g VSS and 12.48 mg P/g VSS; 6.76 mg N/g VSS and 12.26 mg P/g VSS when NO_2^- -N was 5, 10, 20 and 40 mg/L. N and P removal rates were similar under all applied conditions, indicating that the activity of DNPAOs was less affected by nitrite or FNA within the range of 5–40 mg/L and the corresponding HNO_2^- -N of 0.004–0.027 mg/L. In addition, the N_2O emission factor was 2.29%, 2.50%, 2.60% and 3.25% when the concentration of NO_2^- -N was 5, 10, 20 and 40 mg/L, respectively.

Table 1. The parameters in batch experiments with different concentration of nitrite.

NO ₂ -N (mg/L)	FNA (mg HNO ₂ - N/L)	V _{NO2-N} (mg N/g VSS/h)	V _{PO4-P} (mg P/g VSS/h)	N ₂ O _{peak} (mg/L)	N ₂ O emission rate (mg N/g VSS/h)	N ₂ O emission factor (%)
5	0.004	7.42	13.15	0.15 ^a /3.35 ^b	0.17	2.29
10	0.007	7.20	15.15	0.17/5.55	0.20	2.50
20	0.014	7.67	12.48	0.17/6.16	0.20	2.60
40	0.027	6.76	12.26	0.20/8.18	0.22	3.25

a: N₂O (mg/L); b: dissolved N₂O (mg N₂O/L)

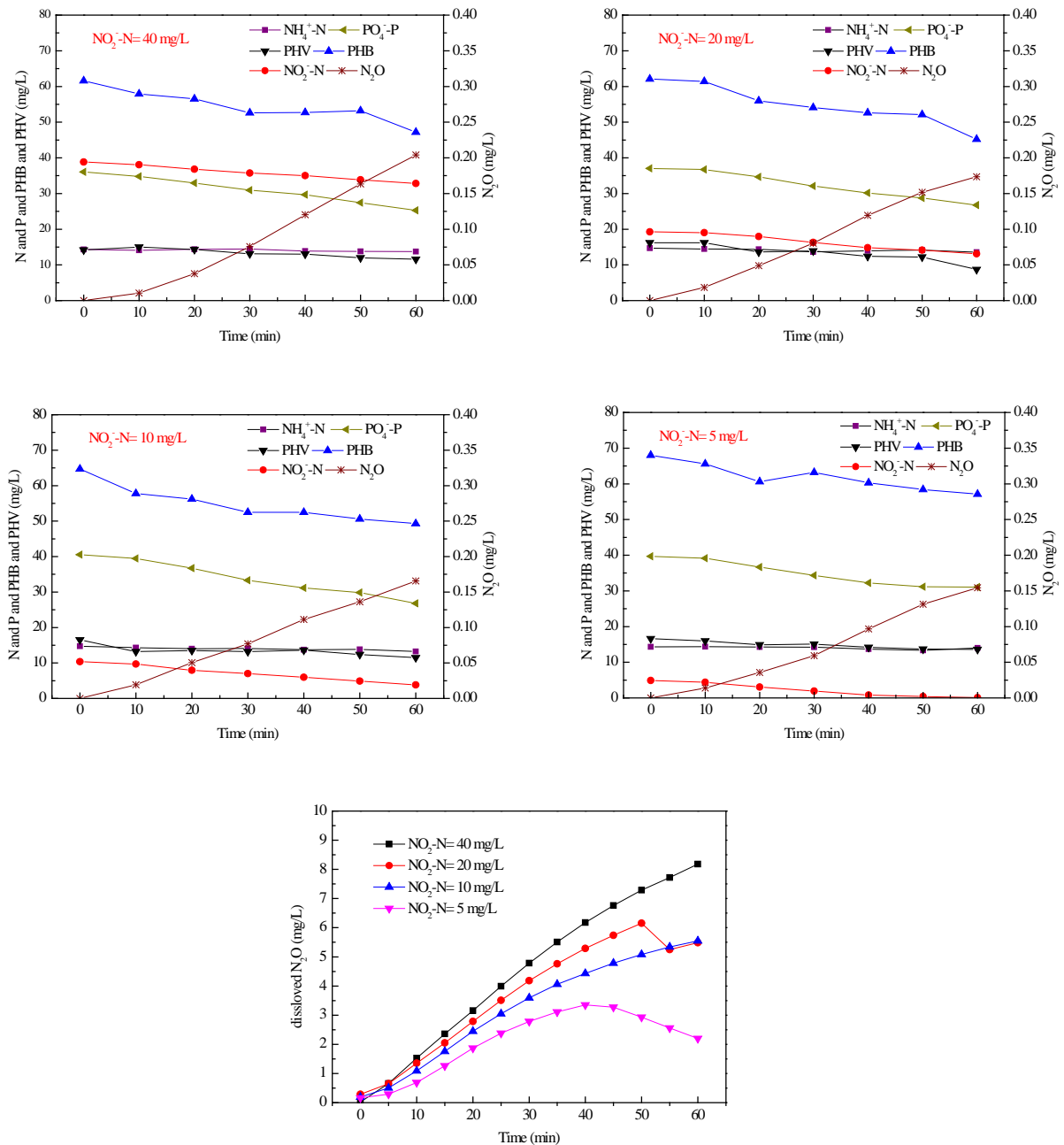


Figure 2. Dynamics of different types of nitrogen and phosphorus during batch experiments with different concentration of nitrites.

N₂O emission characteristics with different level of PHAs

The effect of concentrations of PHAs on N₂O emission was tested by adding different concentration of acetate (100, 200 and 400 mg/L) during the anaerobic phase. After 2 h anaerobic reaction, 40 mg/L NO₂-N were added for simultaneous removals of nitrogen and phosphorus. As shown in Figure 3, with different PHAs concentrations (display with PHB and PHV), denitrification rate and phosphorus-uptake rate were 5.85 mg N/g VSS/h and 9.89 mg P/g VSS/h, 10.96 mg N/g VSS/h and 21.19 mg P/g VSS/h, and 11.94 mg N/g VSS/h and 19.93 mg P/g VSS/h, respectively. These findings indicated that sufficient PHAs facilitated the nitrogen and phosphorus removal, and there was little difference when the concentration of initial acetate was 400 mg/L comparing with 200 mg/L.

The N₂O emission factor were 3.95%, 3.16% and 3.08% when the initial acetate concentration was 100, 200 and 400 mg/L, respectively. In addition, the peak dissolved N₂O concentration was 7.13, 13.48 and 12.29 mg N₂O/L. Insufficient carbon source in wastewater triggered the N₂O emission during denitrifying phosphorus removal via NO₂-N as the electron acceptor, whereas the N₂O generation was lower in 100 mg/L (acetate) than that in 200 and 400 mg/L. This was possibly due to the electron competition between Nir(nitric oxide reductase) and Nos, and the inhibitory effect of NO₂-N (Li et al., 2013; Kampschreur et al., 2009; Wang et al., 2015).

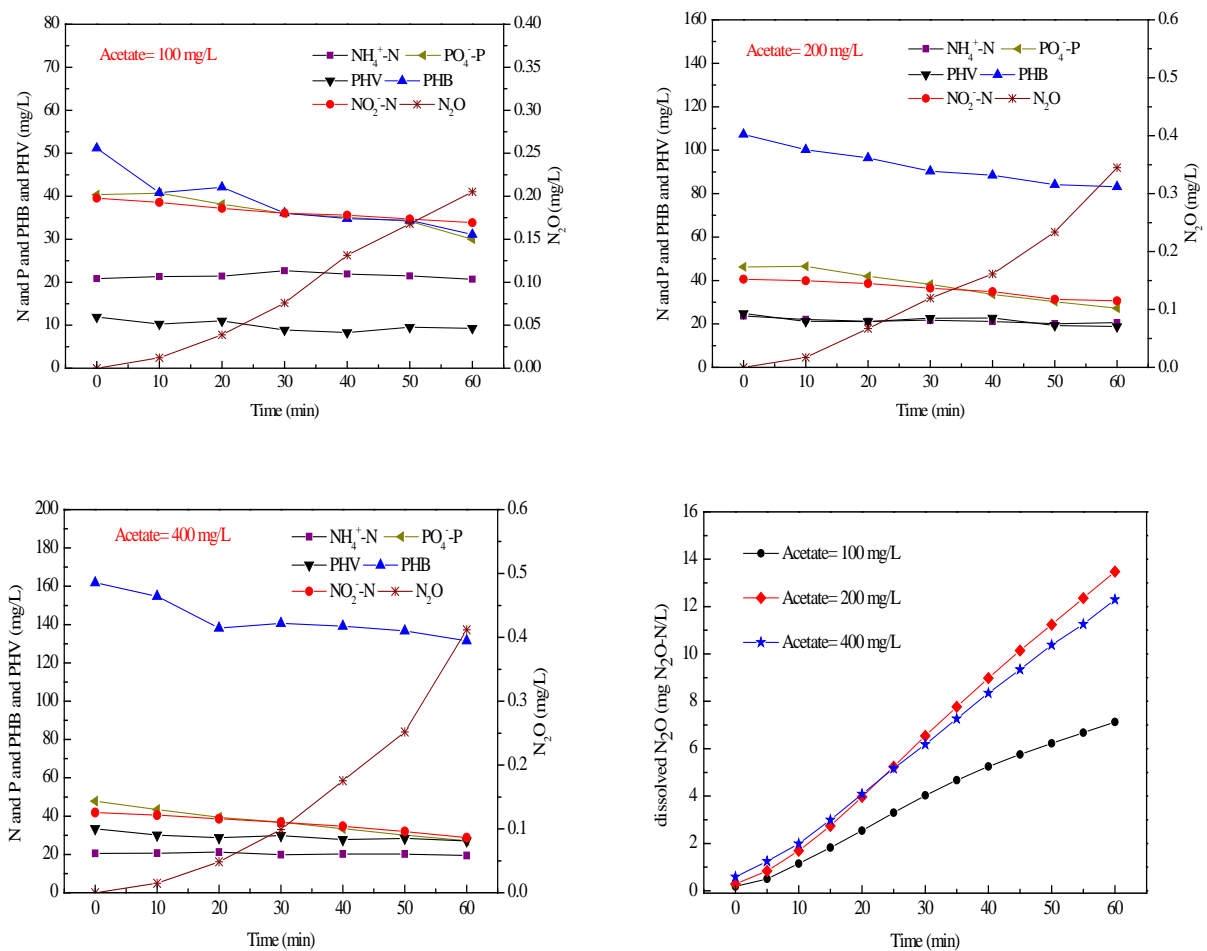


Figure 3. Dynamics of different parameters during the batch experiments with different initial concentrations of acetate.

DISCUSSION

Noted that N_2O emission in denitrifying P removal process with NO_2-N as the electron acceptor was much higher than NO_3-N , with total emitted N_2O concentration of 0.14 mg/L and 0.006 mg/L, respectively. Similar results were found by Wang et al. (2011a), who revealed that N_2O generation was 4.7 times when NO_2-N used as the electron donor than that of NO_3-N , possibly due to Nos activity inhibited by high NO_2-N or FNA. The high nitrite concentration resulted in the high N_2O concentration was also observed in the study of (Gong et al., 2012), with the emitted N_2O of 1.20 mg/L and 0.23 mg/L when NO_3-N and NO_2-N supplemented as the sole electron acceptor during denitrification, respectively.

Unlike findings from previous studies, the activity of DNPAOs in this study was less affected by nitrite or FNA. Zhou et al. (2007) found that the activity of DNPAOs was significant affected by FNA. The denitrification rate decreased by 40% when elevated FNA from 0.002 to 0.02 mg HNO_2/L , but this decrease was not observed when FNA continue to increase. Besides, denitrification rate and phosphorus-uptake rate were conspicuously suppressed due to that a high FNA (0.01 mg HNO_2/L) inhibited the electron transfer activity of DNPAOs (Zhou et al., 2010). It should be noted that acetic acid and propionic acid were used as mixed carbon source in the study of Zhou et al. (2010). This maybe one of the key reasons responding for the difference. In addition, different concentration of NO_2-N (10, 20, 30 and 40 mg/L) had inhibitory effect on DNPAOs (display with denitrification rate and phosphorus-uptake rate), with long term acclimation with mixed electron acceptors (NO_2-N and NO_3-N) (Tang et al., 2016). The degree of inhibition increased with increase concentrations of NO_2-N . Hence, various microbe communities were obtained after long term enrichment with different types of carbon source and electron acceptor. The DNPAOs acclimated with NO_3-N had strong ability to endure the fluctuation of NO_2-N in our study. Therefore, the denitrification rate and phosphorus-uptake rate were less affected by NO_2-N .

As shown in Table 1 and Figure 2, the N_2O emission factor increased with increasing nitrite concentrations, as did the dissolved N_2O . NO_2-N accumulation stimulated the N_2O generation and emission in denitrifying phosphorus removal at the same initial concentration of PHAs. Firstly, numerous studies have reported that nitrite accumulation (10-50 mg/L) inhibited the growth of denitrifying bacteria. An inhibition of N_2O reductases by the toxicity of NO_2-N was observed (Alinsafi et al., 2008). According to Li et al. (2013), the weak competition of Nos for electrons and high NO_2-N accumulation (20 mg/L) were the two main reasons for N_2O generation in denitrifying phosphorus removal system. Secondly, the results obtained by Zhou et al. (2008a, b, 2011) suggested that FNA, rather NO_2-N , was the true inhibitor. Since FNA could react with the enzymes involved in the N_2O reduction. 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 , at the active site. Both Cu_A and Cu_Z were affected by changes in pH and FNA. HNO_2 could bind to the active sites of copper-contained enzymes, leading to competitive inhibition of N_2O reduction. Besides, fifty percent inhibition was observed at an FNA concentration of 0.0007-0.001 mg HNO_2-N/L , while complete inhibition occurred when the FNA concentration was higher than 0.004 mg HNO_2-N/L . In our study, the FNA concentration ranged from 0.004-0.027 mg HNO_2-N/L . Correspondingly, maximal dissolved N_2O concentration ranged from 3.35-8.18 mg N_2O/L , indicating that N_2O reduction was inhibited. Finally but not the last, the difference in microbial community in denitrifying phosphorus removal systems could not be ruled out. Zeng et al. (2003a) demonstrated that N_2O emission was observed even with low levels of NO_2-N (less than 2 mg/L) as the electron acceptor. Similarly, in the study of Lemaire et al. (2006), 77% of nitrite and 26% of nitrate was convert to N_2O in the absence of NO_2-N in denitrifying phosphorus removal process. In addition, an overall (liquid + gas) N_2O accumulation of about 0.20 mg N/L occurred in spite of the

concentration of FNA lower than 0.06×10^{-3} mg HNO₂-N/L (Wei et al., 2014). The reactors were highly enriched in PAOs and GAOs in these studies. GAOs have previously reported to be a major contributor to N₂O production in denitrifying phosphorus removal systems, since the N₂O was the major product of denitrification in the DGAOs system (Zhu and Chen, 2011; Zeng et al., 2003b). Hence, the microbial community might play an important role in triggering N₂O emission even under the condition of low NO₂-N or FNA.

Previous study showed that high N₂O emission at low COD/N ratios, due to endogenous denitrification and the presence of NO₂-N at the same time (Itokawa et al., 2001). In addition, Li et al. (2013) and Zhou et al. (2012) showed that the degradation rate of PHAs was much slower than that of the external carbon sources, leading to the competition between Nir and Nos, resulting in the accumulation of N₂O. From the result of Wang et al. (2011a), the amount of anaerobic PHA produced was 1.8 times increased when decreasing the anaerobic time from 90 min to 60 min, while N₂O production was reduced. This phenomenon suggested that the increase of PHAs production in anaerobic phase accelerated the reduction of NO₃-N, NO₂-N and N₂O in denitrifying phosphorus removal process, thus N₂O production could be alleviated. To further explore whether PHAs exert a decisive effect on this process. The denitrifying phosphorus removal process via fixed concentration of NO₂-N (20-25 mg/L, a constant and low FNA level) was carried out. Results showed that N₂O production stably maintained at approximately 40% of the amount of nitrite reduced with the decrease in the PHAs degradation rate. In other words, N₂O production was not affected by the PHAs degradation rate (Wei et al., 2014). Recently, Wang et al. (2015) emphasized that the slow rate of PHAs degradation was the main reason triggering N₂O production when DNPAOs using NO₃-N as the electron acceptor. Herein, the N₂O production was affected by PHAs through enhancement of NO₂-N accumulation. Although the amount of NO₃-N was only 30 mg/L, the anoxic N₂O production was up to 3.56 mg/L (8.78 mg/L of NO₂-N accumulation). Tang et al. (2016) suggested that PHAs was not the intrinsic reason causing N₂O emission during denitrification for the acclimated DNPAOs, and the electron acceptor played an important role in N₂O emission. Moreover, the different composition of PHAs was also an important factor influencing N₂O production. Based on Wang et al. (2011b), the more PHV composition in PHAs led to more N₂O production. Therefore, PHAs significantly influenced the N₂O generation and emission in denitrifying phosphorus removal processes. In our study, the batch experiment with different concentration of acetate was conducted by adding 100, 200 and 400 mg/L acetate and 40 mg/L NO₂-N (FNA=0.027). This concentration of FNA was sufficient to inhibit the activity of Nos. However, the activity of DNPAOs was less affected by FNA or NO₂-N. The generation of dissolved N₂O markedly increased when the concentration of acetate elevated from 100 mg/L to 200 mg/L and 400 mg/L.

Therefore, the process of denitrifying phosphorus removal by DNPAOs needs carefully balance in case of accumulation of NO₂-N. Continuous and step NO₂-N or NO₃-N dosing were proved to be effective approaches to reduce the inhibitory effect of NO₂-N on N₂O reduction, thus lower the N₂O generation and emission (Wei et al., 2014; Li et al., 2013; Zhou et al., 2008a; Yang et al., 2009). In addition, using carbon source that decreasing or avoiding the nitrite accumulation (such as propionate) can be another way to reduce N₂O.

CONCLUSIONS

High concentration of NO₂-N as well as insufficient PHAs triggered N₂O generation in denitrifying phosphorus removal process. An increase in NO₂-N from 5 mg/L to 40 mg/L resulted in an increase in N₂O generation. In addition, High N₂O emission factor (3.95%) was observed at lower PHAs concentrations. NO₂-N rather than PHAs played the decisive role in N₂O generation and emission

in the process of denitrifying phosphorus removal.

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