Nitrite Dosage Strategy On N_2O Production For Denitrifying Phosphorus Accumulating Organisms Acclimated With Different Electron Acceptors

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Abstract

Simultaneous nitrogen and phosphorus removal by denitrifying polyphosphate accumulating organisms (DNPAOs) is a promising process for wastewater treatment. However, high potential of nitrous oxide (N_2O) emission may occur in this process, which would limit its sustainable application. Effect of nitrite dosage strategy on N_2O production for DNPAOs acclimated with different electron acceptors was examined. N_2O emission by the enriched DNPAOs was mainly affected by electron acceptor rather than intracellular organic carbon, and a high emission potential occurred with nitrite as the electron acceptor. By applying pulse dosage of nitrite, N_2O emission was reduced significantly. In addition, a high N_2O emission potential was observed for DNPAOs acclimated with nitrite than nitrate, indicating that the acclimation history had a high effect on system performance. Under all conditions, adequate anoxic duration should be maintained to denitrify the dissolved N_2O, which was another mitigating strategy for N_2O emission.

Keywords: Denitrifying phosphorus removal; DNPAOs; N_2O; Electron acceptor

1. Introduction

Simultaneous nitrogen and phosphorus removal can be achieved in the anaerobic and anoxic denitrifying phosphorus removal process by activities of denitrifying polyphosphate accumulating organisms (DNPAOs). In the anaerobic phase, DNPAOs take up volatile fatty acids (VFAs) from the wastewater with the energy supplied by polyphosphate degradation and store them in the form of poly-β-hydroxyalkanoates (PHAs). In the anoxic phase, PHAs are oxidized to provide energy for phosphate uptake with nitrate and/or nitrite as electron acceptors to achieve denitrifying phosphorus removal (Meinhold *et al.* 1999, Oehmen *et al.* 2007). During denitrifying phosphorus removal, nitrous oxide (N_2O) may be released, preventing its sustainable application. N_2O is a potent greenhouse gas, with the global warming potential of 300 times greater than that of CO_2 and its annually global increasing rate of 0.31%. N_2O not only contributes to the greenhouse effect, but also destructs the ozone layer (Ravishankara *et al.* 2009).

During denitrifying phosphorus removal, as typical denitrification, nitrate nitrogen (NO_3^--N) is reduced sequentially to nitrite nitrogen (NO_2-N), nitric oxide (NO), N_2O and molecular nitrogen (N_2) by DNPAOs. The final product of denitrifying phosphorus removal might be N_2O rather than N_2 under certain conditions (Zeng *et al.* 2003). The utilization of PHAs and high concentration of NO_2-N were considered to, be two main factors inducing high N_2O emission during denitrifying phosphorus removal (Zeng *et al.* 2003). During denitrifying phosphorus removal with PHAs as the organic carbon, the slow degradation of PHAs led to NO_2-N accumulation, consequently inducing a high N_2O accumulation and emission (Lemaire *et al.* 2006). In addition, a high NO_2-N concentration might also inhibit denitrifying of NO_3-N and N_2O, contributing to a high N_2O emission. Furthermore, Goretski and Hollocher (1988) demonstrated that a high NO_2-N concentrations promoted NO generation during denitrification, which was more toxic than that of NO_2-N, and this promoted N_2O accumulation. Zhou *et al.* (2008) and Pijuan *et al.* (2010) found that free nitrous acid (FNA) inhibited aerobic and anoxic phosphorus uptake, and 50% inhibition was observed at the FNA concentration of 0.0007-0.001 mg HNO_2-N/L (approximately 3-4 mg NO_2^--N/L, pH=7), while complete inhibition occurred at the FNA concentration of 0.004 mg HNO_2-N/L. FNA reacts with N_2O reductase (Nos) (copper-containing enzymes) and causes competitive inhibition of N_2O reduction. Further studies are still required to clarify key factors inducing N_2O emission during denitrifying phosphorus removal.

The aims of this study was to examine N_2O emission characteristics by DNPAOs acclimated under different electron acceptors and the NO_2-N dosage strategy for controlling N_2O emission.

2. Materials and methods

2.1. SBRs

Two 6-L sequencing batch reactors (SBRs) were operated at 27°C to acclimate DNPAOs with NO_2-N ($DNPAO_{N2}$) or NO_3-N ($DNPAO_{N3}$) as the electron acceptor, respectively. Both SBRs were operated by alternating anaerobic/anoxic/aerobic conditions with a reaction cycle of 8 h, including 10 min filling period, 110 min anaerobic

period, 180 min anoxic period, 120 min aerobic period and 60 min sludge setting and decanting period. 150 mL of $NaNO_2$ ($DNPAO_{N2}$) or $NaNO_3$ ($DNPAO_{N3}$) was added into both SBRs at the beginning of the anoxic phase with the dosed final concentration of both 30 mg/L to induce denitrifying phosphorus removal, respectively. In each cycle, 3 L treated wastewater was pumped out and 3 L synthetic wastewater was fed into the reactors, resulting in a hydraulic retention time (HRT) of 16 h. 300 mL activated sludge was removed at the end of the aerobic phase with an applied solids retention time (SRT) of approximately 20 d.The synthetic wastewater contained CH_3COONa of 513 mg/L (COD around 400 mg/L), $MgSO_4$ of 90 mg/L, $CaCl_2$ of 14 mg/L, Na_2HPO_4 of 74 mg/L (16 mg/L PO_4-P), NH_4Cl of 76 mg/L (NH_4-N 20 mg/L), yeast of 10 mg/L, and trace elements of 0.4 mL/L.

2.2. Batch experiments

For determination of DNPAOs fraction in PAOs, activated sludge (900 mL) was withdrawn from both SBRs at the end of the anaerobic phase and divided into three batch reactors. One was dosed with $NaNO_2$ at an initial NO_2-N concentration of 20 mg/L, the other was dosed with $NaNO_3$ at an initial NO_3-N concentration of 30 mg/L, and the last one was only aerated without dosing of $NaNO_2$ or $NaNO_3$. The batch reaction lasted for 90 min. For the effect of types of electron acceptors on N_2O emission during denitrifying phosphorus removal, mixed liquor of 600 mL was withdrawn from both SBRs at the end of the anaerobic phase and divided into two batch reactors. $NaNO_2$ or $NaNO_3$ solution was added to both reactors, achieving an initial NO_2-N and NO_3-N concentration of 20 and 30 mg/L, respectively. Both reactors were sealed and the denitrification reaction lasted for 90 min. For the effect of NO_2-N dosage strategy on N_2O emission during denitrifying phosphorus removal, mixed liquor of 1200 mL was withdrawn from both SBRs at the end of the anaerobic phase and divided into two batch reactors. $NaNO_2$ was dosed by two or three pulses, respectively. The initial NO_2-N concentration of each pulse addition for two and three pulses was approximately 10 and 6.7 mg/L, respectively.

During all batch experiments, samples were taken from each batch reactor at intervals and N_2 was introduced to balance the atmospheric pressure. Gas samples were collected using syringes and analyzed for N_2O. Solid and liquid samples were analyzed for NH_4-N, NO_2-N, NO_3-N, PO_4-P, PHB, SS and VSS.

2.3. Analytical methods

The liquid samples were immediately filtered for analysis. NH_4-N, NO_2-N, NO_3-N, PO_4-P, PHB, SS and VSS were measured according to Standard Methods. PHB was measured in accordance with Rodgers and Wu (2010). The concentration of N_2O in gas samples were analyzed by gas chromatograph (GC) (Agilent Technologies 6280, USA) equipped with an electron capture detector (ECD). The dissolved N_2O was measured by Microsensor Multimeter (Unisense, Denmark). FNA was calculated according to the method described by Anthonisen *et al*. (1976). The N_2O emission factor was calculated as the ratio of the produced N_2O-N to the removed oxidized nitrogen. The reaction rates for denitrification were calculated by linear regression of the related parameters.

3. Results and discussion

3.1. System long-term operation and performance

After a long-term acclimation, both reactors operated under steady state. In $DNPAO_{N2}$, SS and VSS concentrations were 2470±80 and 1990±30 mg/L, with the VSS/SS ratio of 0.81. The concentrations of PO_4-P at the end of the anaerobic, anoxic and aerobic phases were 58.9, 39.9 and 8.0 mg/L, respectively. The NO_2-N concentration at the end of the anoxic phase was 1.2 mg/L. The Effluent NO_3-N concentration was 2.6 mg/L, and the effluent NO_2-N was not detected. In $DNPAO_{N3}$, SS and VSS concentrations were 1980±60 and 1740±30 mg/L, with the VSS/SS ratio of 0.87. The concentration of PO_4-P at the end of the anaerobic, anoxic and aerobic phases were 83.3, 43.6 and 9.0 mg/L, respectively. The concentration of NO_3-N at the end of the anoxic phase and in the effluent was 3.2 and 11.4 mg/L, and the effluent NO_2-N was not detected. The influent PO_4-P concentration was similar in both reactors, PO_4-P release in the anaerobic phase was less in $DNPAO_{N2}$ than in $DNPAO_{N3}$, indicating a high PO_4-P release capacity in $DNPAO_{N3}$. While the capacity of PO_4-P removal in both reactors were similar, owing to the similar effluent PO_4-P concentration. Dynamics of N, P, PHB, DO and pH in typical cycles of $DNPAO_{N2}$ and $DNPAO_{N3}$ are shown in Figure 1. In $DNPAO_{N2}$, during the anoxic phase, the concentration of NO_2-N and PO_4-P decreased from 31.97 to 0.06 mg/L and 60.34 to 35.81 mg/L, and the denitrification rate and the PO_4-P uptake rate were 6.36 and 4.30 mg/g VSS·h, respectively. In $DNPAO_{N3}$, during the anoxic phase, the concentration of NO_3-N and PO_4-P declined from 29.72 to 12.43 mg/L and 88.75 to 46.91 mg/L, and the denitrification rate and the PO_4-P uptake rate were 4.19 and 8.64 mg/g VSS·h, respectively. The rate of PO_4-P uptake during the anoxic phase in $DNPAO_{N3}$ was higher than in $DNPAO_{N2}$, and the denitrification rate (6.36 mg/g VSS·h) in $DNPAO_{N2}$ was higher than that in $DNPAO_{N3}$. The ratios of PO_4-P uptake/NO_x-N reduction were 1.72 and 0.74 g P/g NO_x-N in $DNPAO_{N3}$ and $DNPAO_{N2}$, respectively. For $DNPAO_{N2}$, PO_4-P uptake rates were 8.2, 4.2 and 17.1 mg P/g VSS·h with NO_2-N, NO_3-N and O_2 as electron acceptors, and the fractions of DNPAOs with nitrite or nitrate as the electron acceptor to the total PAOs were 60.0% and 30.8%. For $DNPAO_{N3}$, the PO_4-P uptake rates were 12.0, 4.2 and 21.2 mg P/g VSS·h with NO_2-N, NO_3-N and O_2 as electron acceptors, and the fractions of DNPAOs with nitrite or nitrate as the electron acceptor to the total PAOs were 60.1% and 64.4%. These indicated that DNPAOs enriched in both reactors were able to use NO_2-N. The rates of PO_4-P uptake and denitrification in $DNPAO_{N3}$ were higher than those in $DNPAO_{N2}$ with the same type of electron acceptor, indicating higher PAOs activities in $DNPAO_{N3}$ than in $DNPAO_{N2}$. For $DNPAO_{N2}$ and $DNPAO_{N3}$, the rate of PO_4-P uptake was higher with NO_2-N as the electron acceptor than that with NO_3-N, confirming that no NO_2-N accumulated in typical cycles.

3.2. Effect of types of electron acceptors on N_2O emission

Dynamics of N and P with NO_2-N or NO_3-N as the electron acceptor during denitrifying phosphorus removal in $DNPAO_{N2}$ and $DNPAO_{N3}$ are shown in Figure 2. With NO_3-N as the electron acceptor, no NO_2-N accumulation occurred and N_2O emission was relatively low during
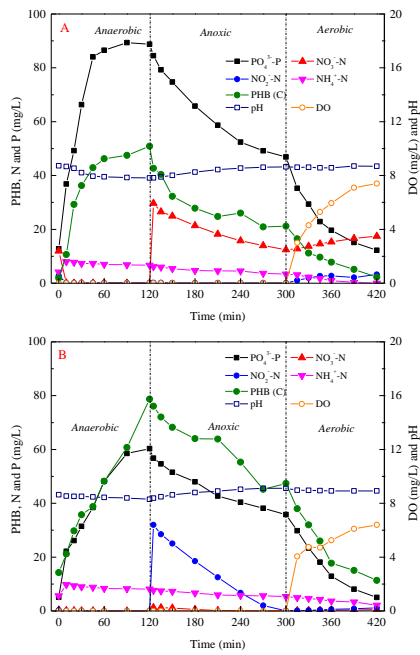
Figure 1. Dynamics of N, P, PHB, DO and pH in typical cycles of DNPAO$_{N3}$ (A) and DNPAO$_{N2}$ (B).

With NO$_2$-N as the electron acceptor, N$_2$O emission was relatively high during denitrifying phosphorus removal and it was high in DNPAO$_{N2}$ compared to that in DNPAO$_{N3}$, with the N$_2$O emission factor of 7.5% in DNPAO$_{N2}$ and 0.1% in DNPAO$_{N3}$. During the anoxic uptake of PO$_4$-P, denitrification of NO$_3$-N was observed without the accumulation of NO$_2$-N. In the study of Oehmen *et al*. (2010), similar phenomenon was also reported but was not emphasized. However, the phenomenon was different from other previous studies, where under anoxic phase, denitrification of NO$_3$-N was accompanied with the NO$_2$-N accumulation in DNPAO systems, such as Wang *et al*. (2011). N$_2$O generation was apparently higher with NO$_2$-N as the electron acceptor than that with NO$_3$-N, showing that NO$_2$-N was an important inducer for N$_2$O generation. The production of N$_2$O was comparatively low with NO$_3$-N as the electron acceptor and PHB as the carbon source, indicating that electron acceptor rather than PHB was the key factor causing N$_2$O generation during denitrifying phosphorus removal. denitrifying phosphorus removal, while the dissolved N$_2$O concentration rose initially and then decreased thereafter. The rates of PO$_4$-P uptake and denitrification in DNPAO$_{N3}$ were higher than those in DNPAO$_{N2}$. The emission factor of N$_2$O was 0.06% in DNPAO$_{N2}$ and 0.05% in DNPAO$_{N3}$.

3.3. Effect of two pulses of NO$_2$-N dosage on N$_2$O emission

Effect of two pulses of NO$_2$-N dosage on N$_2$O emission during denitrifying phosphorus removal is shown in Figure 3. For microbial activities, the rates of PO$_4$-P uptake and denitrification after the second NO$_2$-N dosage were lower than those of the first dosage, indicating that denitrifying phosphorus removal activities were inhibited. Meinhold *et al*. (1999) showed that the PO$_4$-P uptake was inhibited completely with the addition of 10 mg/L NO$_2$-N during denitrifying phosphorus removal; after nitrite was completely consumed, NO$_3$-N (5 mg/L) was further added, but this did not lead to PO$_4$-P uptake and NO$_3$-N was consumed at a relatively low rate. This demonstrated that

NO$_2$-N inhibition could last for a period of time even after NO$_2$-N was completely utilized.
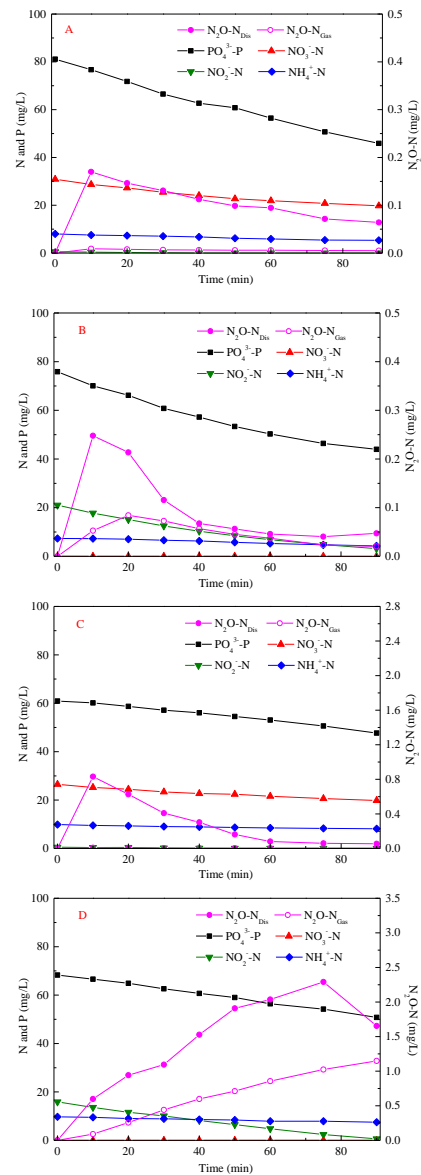

Figure 2. Dynamics of N and P with NO$_3$-N (A, C) or NO$_2$-N (B, D) as the electron acceptor for DNPAOs from DNPAO$_{N3}$ (A, B) and DNPAO$_{N2}$ (C, D), respectively.

In both DNPAO$_{N2}$ and DNPAO$_{N3}$, the dissolved N$_2$O concentration increased after each dosage of NO$_2$-N and then decreased after reaching a peak; the off-gas N$_2$O concentration gradually rose during denitrifying phosphorus removal. The concentration of N$_2$O generated in DNPAO$_{N2}$ was high and the N$_2$O emission factor during the first and second pulse was 3.3% and 5.0%, respectively, while those were 0.2% and 0.1% in DNPAO$_{N3}$. The maximum dissolved N$_2$O concentration during the first and second pulse was 0.07 and 0.08 mg/L in DNPAO$_{N3}$, respectively, which were lower than those of 0.98 and 2.14 mg/L in DNPAO$_{N2}$. The maximum accumulated N$_2$O concentration in the secondary pulse was higher than that in the first pulse in both reactors. The possible reasons were as follows. Firstly, the detrimental effect of NO$_2$-N still remained after the first dosage, resulting in inhibition of denitrifying activities. Secondly, the degradation of PHA were slow during denitrifying

phosphorus removal, resulting in the competition between denitrifying enzymes. In addition, Nos enzyme was weak at competing for the electrons and N_2O reduction was the last step in denitrification, which inhibited the reduction of N_2O. Finally, considering that PHAs was consumed during the first pulse dosage, less would be available during the second pulse dosage, intensifying the competition for electrons and stimulating more N_2O accumulation.
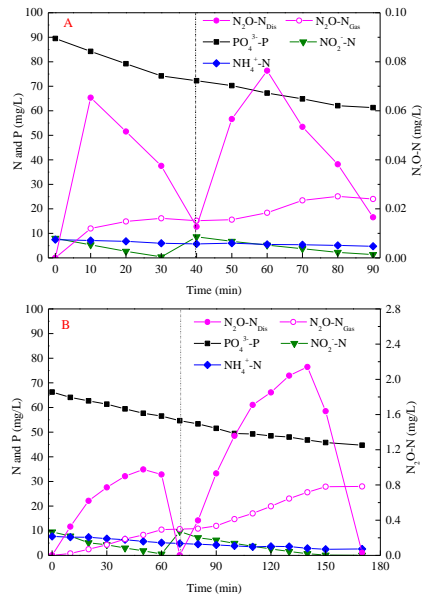
analysis, the inhibition of N_2O reductase might be the key factor inducing N_2O accumulation.

Figure. 4. Dynamics of N and P under conditions with three dosages of NO_2-N for DNPAOs from $DNPAO_{N3}$ (A) and $DNPAO_{N2}$ (B), respectively.

3.5. Effect of NO_2-N dosage strategy on N_2O emission and its control

Several times pulse dosage of NO_2-N could reduce N_2O emission, especially for the NO_2-N acclimated DNPAOs. Therefore, pulse addition of NO_2-N could reduce the N_2O emission. In addition, the above results showed that the acclimation history had a high effect on N_2O emission during denitrifying phosphorus removal, with a high N_2O emission potential for DNPAOs acclimated with NO_2-N. Under all conditions, the dissolved N_2O was initially produced and then consumed thereafter. For example, in $DNPAO_{N2}$, the maximum dissolved N_2O concentration under three dosage strategy conditions was 0.83, 2.14 and 2.17 mg/L, respectively. The produced dissolved N_2O increased as increasing the dosage times, owing to the inhibitory effect of NO_2-N. Therefore, the effect of long period presence of NO_2-N on N_2O emission during denitrifying phosphorus removal should be further investigated so as to provide effective N_2O emission control strategy. However, special attention should be paid to the dissolved N_2O that adequate reaction time could minimize the N_2O production and consequently decrease N_2O emission. Therefore, for controlling N_2O emission under anoxic conditions, adequate anoxic reaction duration should be maintained.
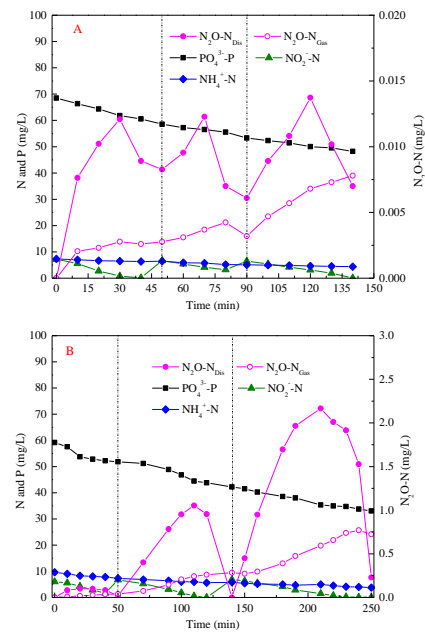

Figure 3. Dynamics of N and P under conditions with two dosages of NO_2-N for DNPAOs from $DNPAO_{N3}$ (A) and $DNPAO_{N2}$ (B), respectively.

3.4. Effect of three pulses of NO_2-N dosage on N_2O emission

Effect of three pulses of NO_2-N dosage on N_2O emission during denitrifying phosphorus removal is shown in Figure 4. In $DNPAO_{N3}$, the dissolved N_2O concentration increased after each pulse dosage of NO_2-N, and then decreased after reaching the peak, while the off-gas N_2O concentration constantly increased during denitrifying phosphorus removal with the emission factor below 0.07%. In $DNPAO_{N2}$, the characteristics of dissolved N_2O production was similar to that of $DNPAO_{N3}$, but the dissolved N_2O accumulation and reduction rates were high; the off-gas N_2O kept increasing during the whole process. The N_2O emission factor was 0.8% during the first pulse, and then it increased rapidly in the following two pulses with values of 3.3% and 6.6%, respectively. The potential of N_2O production by $DNPAO_{N2}$ was high, especially during the second and third pulses of NO_2-N addition. The presence of NO_2-N promoted the N_2O generation since it was the substrate for denitrification. Inhibitory effect of initial NO_2-N concentration was not the reason for N_2O accumulation considering that the NO_2-N concentration after each addition was the same, and it might be the long period of NO_2-N presence inhibited the activity of Nos rather than NO_2-N reductase (Nir) (Meinhold *et al*. 1999), resulting in a high N_2O generation in the following two pulses. The dissolved N_2O concentration in $DNPAO_{N2}$ was also relatively high, but it could be reduced after the depletion of NO_2-N by denitrification. Based on the above

4.   Conclusions

The main conclusions were obtained as follows: (1) N_2O emission was mainly affected by the electron acceptor rather than the intracellular organic carbon during denitrifying phosphorus removal, (2) N_2O emission potential was high for DNPAOs acclimated with NO_2-N rather than NO_3-N as the electron acceptor, and (3)

adequate anoxic duration should be maintained as a controlling strategy for mitigating N$_2$O emission.

Acknowledgements

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