NITROUS OXIDE EMISSION DEPENDING ON THE TYPE OF ELECTRON ACCEPTOR BY A DENITRIFYING PHOSPHORUS REMOVAL SLUDGE

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ABSTRACT

Denitrifying polyphosphate accumulating organisms (DNPAOs) are very promising for simultaneous nitrogen and phosphorus removal, especially for organic carbon limited wastewater treatment. It possesses characteristics of both denitrification and enhanced biological phosphorus removal. While during denitrification, emission of a greenhouse gas, nitrous oxide (N_2O), may occur under certain conditions, such as high nitrite concentrations, low organic carbon to nitrogen ratios and low dissolved oxygen concentrations. N_2O emission has been reported to be intensified during biological denitrifying phosphorus removal using internally-stored compound as the organic carbon. In this study, DNPAOs were enriched in a lab-scale reactor, and a new phenomenon was examined that N_2O emission did not occur when nitrate was used as the electron acceptor. In addition, during denitrifying nitrate, no nitrite accumulation was observed. However, when nitrite was used as the electron acceptor, a high potential of N_2O emission was observed. Characteristics of the observed phenomena was presented. Within a SBR cycle, PO_4-P was released during the anaerobic phase, and PO_4-P was taken up during both the anoxic and aerobic phases, with the proportion of DNPAOs to PAOs of 87%. During the anoxic phase, with the uptake of PO_4-P, denitrification of nitrate was observed without the accumulation of nitrite. In general, a very low amount of N_2O was produced with nitrate as the electron acceptor, independent of the applied different nitrate concentrations. However, with nitrite as the electron acceptor, a much higher N_2O emission occurred. The N_2O emission ratio to the denitrified NO_2-N was 6.2%, 5.3% and 4.9% at the initial NO_2-N concentration of 10, 20 and 40 mg/L, respectively. In addition, a much higher N_2O emission occurred with the co-existence of NO_3-N and NO_2-N. While the initial organic carbon concentration had no significant effect on N_2O emission with NO_3-N as the electron acceptor. This indicated that at least two types of metabolic modes were presented for DNPAOs. One was denitrifying nitrate with the accumulation of nitrite and the other was without the accumulation of nitrite. When stored organic carbon (PHA) by DNPAOs was used as the electron donor, N_2O emission was depended on the electron acceptor. When nitrate was used, there was not much N_2O emission, while when nitrite was used, a high N_2O emission potential occurred. In addition, when nitrate and nitrite existed simultaneously, N_2O emission was higher than that when only nitrite existed. Therefore, PHA was not the intrinsic reason causing N_2O emission during denitrification for the acclimated DNPAOs and the electron acceptor played an important role in N_2O emission.

Keywords: Nitrous Oxide, Denitrifying Polyphosphate Accumulating Organisms, Nitrite, Intracellular stored organic carbon

1. Introduction

Denitrifying phosphate removal processes are very promising for simultaneous nitrogen and phosphorus removal, especially for treating organic carbon limited wastewater. While during denitrification, emission of a greenhouse gas, nitrous oxide (N_2O), may occur. N_2O has a very high greenhouse effect potential, i.e., 300 times that of carbon dioxide (IPCC, 2001). Therefore, a small amount of N_2O emission may cause a significant greenhouse effect. From the view of

sustainable development, avoidance of secondary pollutants during wastewater treatment should be carried out.

During denitrifying phosphate removal, microbial communities such as denitrifying polyphosphate accumulating organisms (DNPAOs), carry out denitrification using intracellular stored organic carbon (such as polyhydroxybutyrate, PHB) as the electron donor and nitrate or nitrite as the electron acceptor. When the intracellular organic carbon is used for denitrification, whether the intracellular organic carbon itself or the existence of nitrite contributed to N_2O emission has not been clearly clarified (Kampschreur *et al.*, 2009). In addition, during denitrification, a high accumulation of nitrite may also induce N_2O emission, especially when denitrifying activities were inhibited (Li *et al.*, 2013; Zhou *et al.*, 2008; Wang *et al.*, 2011). Wu *et al.* (2013) obtained that when PHB was used as the organic carbon for denitrification, a high N_2O emission occurred when nitrite was coexisted. Therefore, it is necessary to examine effect of different electron donors on N_2O emission during denitrification.

In this study, DNPAOs were enriched in a lab-scale reactor, and N_2O emission characteristics were examined.

2.   Materials and methods
2.1. Denitrifying phosphate removal process
A sequencing batch reactor (SBR) was operated at 25^oC and the reactor had a working volume of 6 litres. 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 litres of treated wastewater were exchanged with a new batch of synthetic wastewater. During the anxoci phase, nitrate stock solution was dosed to achieve the initial nitrate nitrogen (NO_3-N) concentration of 30 mg/L. A solids retention time (SRT) of around 15 days was maintained. The influent chemical oxygen demand (COD) was around 400 mg/L, ammonium nitrogen (NH_4-N) of 15 mg/L and orthophoshate (PO_4-P) of 15 mg/L. The reactor was seeded with activated sludge taken from a Wastewater Treatment Plant in Shenzhen, China.

2.2. Batch denitrification experiments
Batch experiments with replications were carried out to examine effects of electron acceptors, different initial nitrite nitrogen (NO_2-N) concentrations and different initial PHB concentrations on N_2O emission. The batch reactors were made from 500 mL capped glass flasks, each with three ports on the cap, one for liquid sampling, one for gas sampling, and the other for gas balance. 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 concentrations (NO_3-N of 15, 30 and 60 mg/L, NO_2-N of 10, 20 and 40 mg/L, and NO_3-N/NO_2-N of 30/5, 30/10, 30/20 mg/L, respectively) of electron acceptors were added for commencing the experiment. For examining the effect of PHB on N_2O emission, the activated sludge was taken from the SBR at the end of the aerobic phase, and then different concetrations of COD (50, 100 and 200 mg/L) was added for accumulating PHB under ananerobic conditions. After that, NO_3-N of 30 mg/L was added to initiate the experiment. In all batch experiments, samples (both liquid and gas samples) were taken at intervals to test NO_2-N and NO_3-N for liquid samples, and N_2O for gas samples.

2.3. Analytical methods
NO_2-N, NO_3-N, suspended solids (SS), volatile suspended solids (VSS) and NH_4-N were determined according to standard methods (APHA, 1995). The PHB concentration was detected by the modified HPLC method (Rodgers and Wu, 2010) with crotonic acid used for calibration. N_2O was detected according to Wu *et al.* (2013).

3.   Results and discussion
The system had been operated for more than 6 months and a very good DNPAOs activities had been obtained. The fraction of DNPAOs to PAOs was around 87% calculated from the aerobic and anoxic PO_4-P uptake experiments (data not shown), showing that DNPAOs were

successfully enriched inside the system. Dynamics of oxidized nitrogen and phosphorus are shown in Figure 1. During the anaerobic phase, a high amount of PO_4-P was released, during the anoxic phase, PO_4-P was taken up, and during the aerobic phase, PO_4-P was further taken up. During the anoxic phase, with the uptake of PO_4-P, denitrification of nitrate was observed without the accumulation of nitrite.
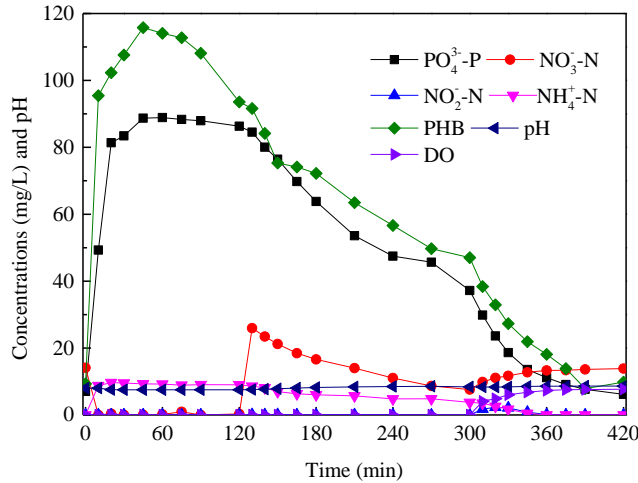

Figure 1: Dynamics of parameters within a typical SBR cycle.

Batch denitrification of nitrate or nitrite for DNPAOs under different oxidized nitrogen (NOx-N) concentrations are shown in Figure 2. In general, a very low amount of N_2O was produced with NO_3-N as the electron acceptor, with the N_2O emission factor to the denitrified NO_3-N of below 0.12%. However, with NO_2-N as the electron acceptor, a much higher N_2O emission occurred. At the initial NO_2-N concentration of 10 mg/L, N_2O emission occurred during the initial 30 min of the batch experiment, and then decreased with the complete consumption of NO_2-N. While under the initial NO_2-N concentration of 20 and 40 mg/L, N_2O emission occurred during the whole batch experiment period. The N_2O emission factor to the denitrified NO_2-N was 6.2%, 5.3% and 4.9% at the initial NO_2-N concentration of 10, 20 and 40 mg/L, respectively. In addition, the NO_2-N reduction rate decreased with increasing NO_2-N concentrations, with values of 11.1, 8.7 and 7.7 mg/g VSS·h at the NO_2-N concentrations of 10, 20 and 40 mg/L, respectively.

Batch denitrification of DNPAOs under different initial PHB concentrations with NO_3-N as the electron acceptor are shown in Figure 3. Similar to the effect of NO_3-N alone, there was not much N_2O emission, with the N_2O emission factor to the denitrified NO_3-N also below 0.12% under all conditions. This result indicated that the carbon to nitrogen ratio had no significant effect on N_2O emission for the enriched DNPAOs when NO_3-N was used as the electron acceptor.
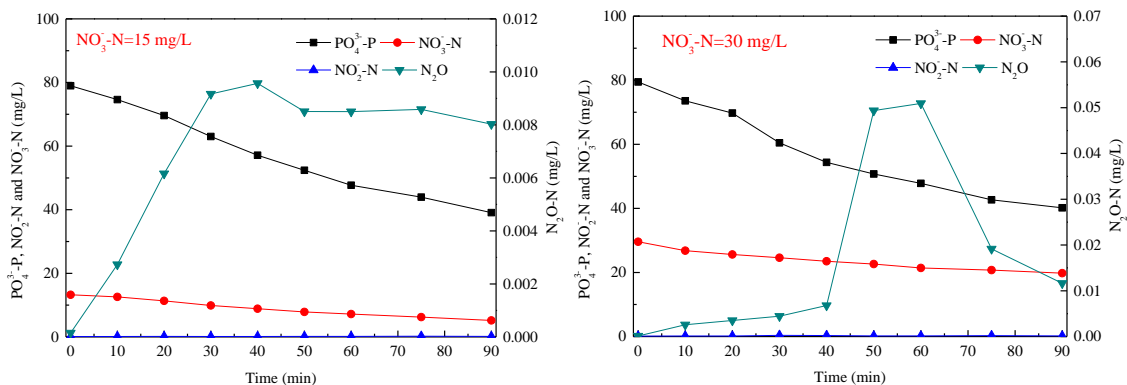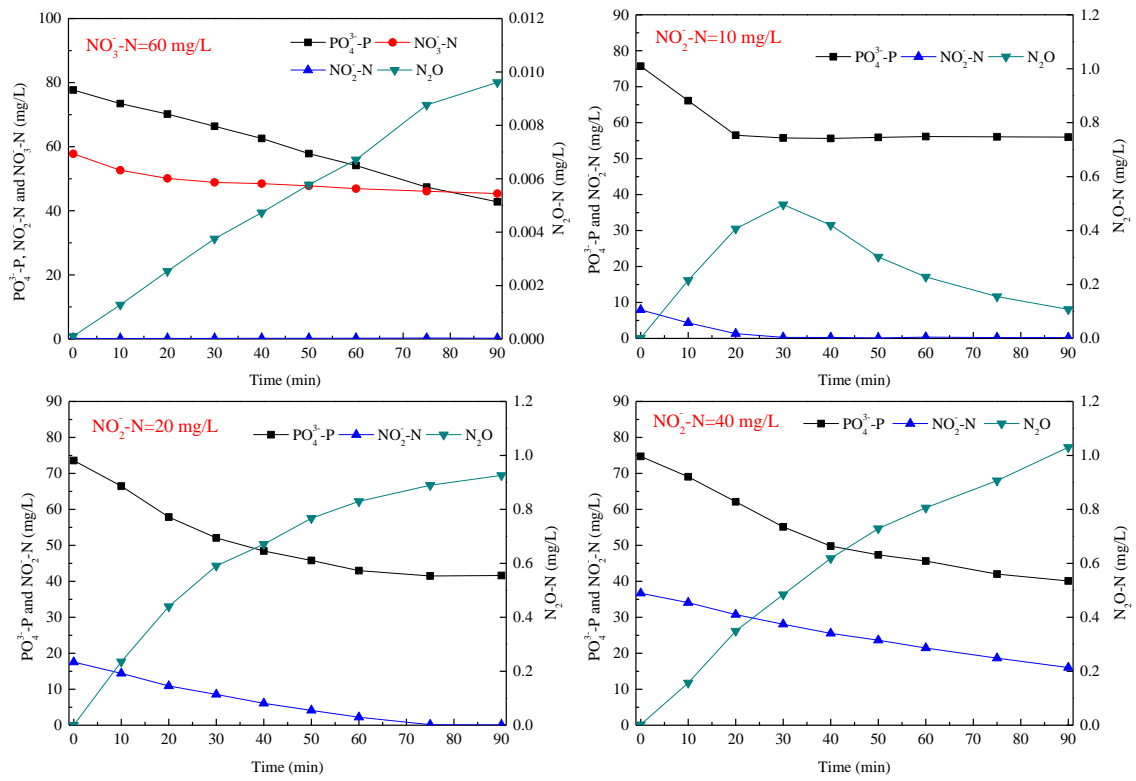
Figure 2: N$_2$O emission under different electron acceptor concentrations.
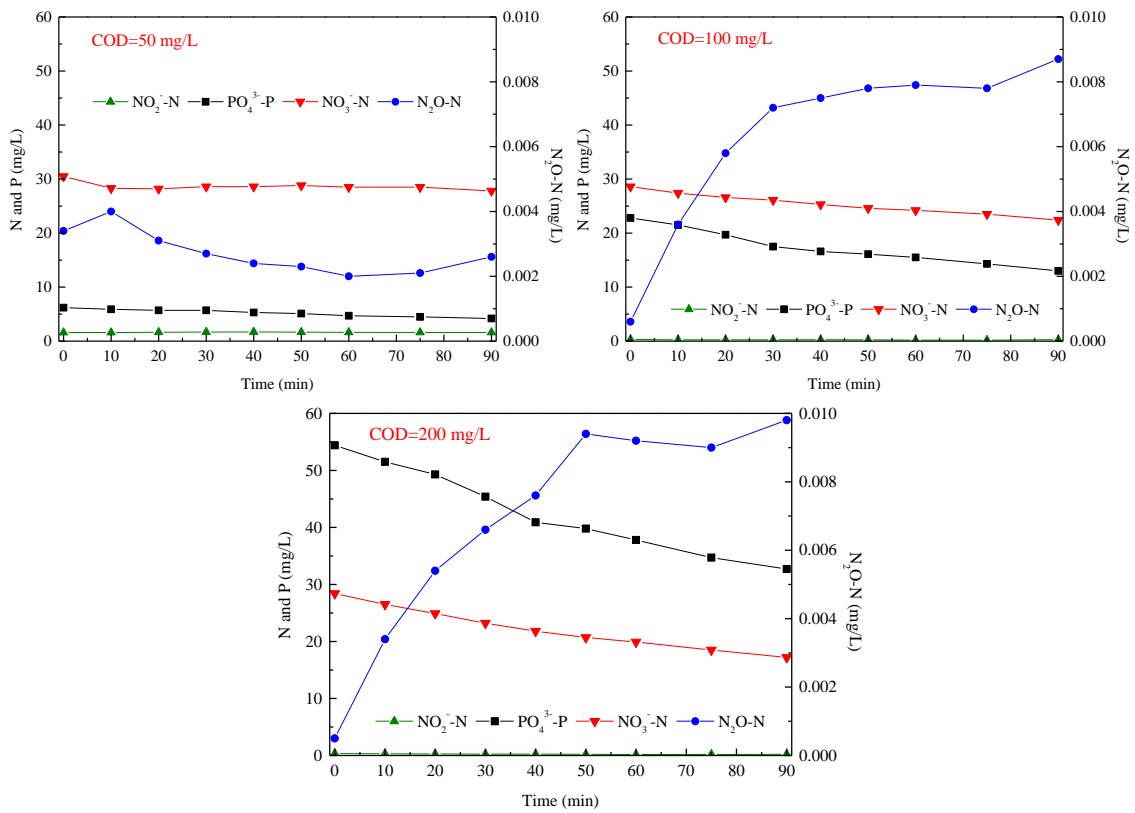

Figure 3: N$_2$O emission under different initial organic carbon concentrations.

4. Conclusions

When stored organic carbon of DNPAOs was used as the electron donor, N_2O emission was depended on the electron acceptor. When nitrate was used, there was not much N_2O emission, while when nitrite was used, a high N_2O emission potential occurred. In addition, when nitrate and nitrite existed simultaneously, N_2O emission was higher than that when only nitrite existed. Therefore, PHA was not the intrinsic reason causing N_2O emission during denitrification for the acclimated DNPAOs and the electron acceptor played an important role in N_2O emission.

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