EFFECT OF HETEROOTROPHIC ACTIVITIES ON NITROUS OXIDE EMISSION DURING NITRIFICATION UNDER DIFFERENT AERATION RATES

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EXTENDED ABSTRACT

Nowadays, biological nutrient removal (BNR) processes have been applied widely for wastewater treatment. However, one type of greenhouse gases, nitrous oxide (N\textsubscript{2}O), can be released from both nitrification and denitrification during BNR. In our previous study, we found that N\textsubscript{2}O emission during nitrification could be affected by heterotrophic activities significantly in a BNR system. Therefore, it is necessary to examine N\textsubscript{2}O emission during nitrification from the viewpoint of "ecology of activated sludge", which has not been received much attention in the research area of N\textsubscript{2}O emission during nitrification. In this study, effect of heterotrophic activities on N\textsubscript{2}O emission under different aeration rates was investigated in batch experiments for activated sludge taken from a BNR system. The BNR system was acclimated to remove nitrogen and phosphorus from synthetic wastewater. N\textsubscript{2}O emission under three aerobic conditions were examined, one without heterotrophic effect, one with heterotrophic activities by using internal stored organic carbon (polyhydroxybutyrate, PHB), and the other with heterotrophic activities by using external organic carbon (acetate). Under each condition, the applied aeration rates were 100 ml/min, 250 ml/min and 500 ml/min, respectively. Under the condition with PHB as the organic carbon, the released N\textsubscript{2}O-N to the produced oxidized nitrogen (NO\textsubscript{x}-N) was 10.0% at 100 ml/min, 3.6% at 250 ml/min, and 0.6% at 500 ml/min. Under the condition with acetate as the organic carbon, the released N\textsubscript{2}O-N to the produced NO\textsubscript{x}-N was 14.5% at 100 ml/min, 4.1% at 250 ml/min, and 0.7% at 500 ml/min. Under the condition without organic carbon, the released N\textsubscript{2}O-N to the produced NO\textsubscript{x}-N was 14.5% at 100 ml/min, 4.1% at 250 ml/min, and 0.7% at 500 ml/min. These results showed that (i) heterotrophic activities affected N\textsubscript{2}O emission during nitrification significantly; (ii) there was no significant difference in N\textsubscript{2}O emission during nitrification affected by heterotrophic activities by using internal or external organic carbon; (iii) a high aeration rate with a high dissolved oxygen (DO) concentration reduced N\textsubscript{2}O emission significantly under aerobic conditions. For controlling N\textsubscript{2}O emission during nitrification concurrent with heterotrophic activities, DO concentrations should be carefully controlled.

Keywords: nitrous Oxide, nitrification, heterotrophic activity; aeration rate

1. INTRODUCTION

Nowadays, biological nutrient removal (BNR) processes have been applied widely for wastewater treatment. However, one type of greenhouse gases, nitrous oxide (N\textsubscript{2}O), can be released from both nitrification and denitrification during BNR. The global warming potential of N\textsubscript{2}O is around 300 times that of CO\textsubscript{2} and its life-cycle is around 129 years (IPCC, 2001). The atmospheric N\textsubscript{2}O concentration is about 310 ppbv and its annual increasing rate is about 0.25% (IPCC, 2001). In spite of the contribution of N\textsubscript{2}O emission from sewage treatment is only 3.2% to the total N\textsubscript{2}O emission, a small amount of N\textsubscript{2}O emission can cause significant influence on the global greenhouse gas effect. In addition, by increasing only 1% of N\textsubscript{2}O emission during wastewater treatment, the carbon footprint of wastewater treatment plants will be increased by 30% (Law et al., 2012). Therefore, it is necessary to investigate and further control the N\textsubscript{2}O emission during wastewater treatment.
N₂O emission during nitrogen removal can be from either nitrification or denitrification or both (Rassamee et al., 2011). Some studies showed that nitrification under aerobic condition was the main source for N₂O emission during wastewater treatment (Geijlsbjerg et al., 1998; Liu et al., 2008), while others showed that denitrification was the main source (Meyer et al., 2005). Therefore, further investigations are still required to clarify which process is the main contributor to N₂O emission in wastewater treatment processes.

N₂O emission during nitrification is mainly through two processes: (1) nitrifier denitrification, and (2) biological or chemical hydroxylamine oxidation (Wunderlin et al., 2012). Lots of factors affect the emission of N₂O during nitrification, including substrate concentrations such as ammonium and oxygen, products concentrations such as nitrite, environmental conditions such as sludge retention time (SRT) and pH, and dynamic conditions etc (Colliver and Stephenson, 2000; Tallec et al., 2006; Kampschreur et al., 2008; Law et al., 2011; Yu et al., 2010; Rassamee et al., 2011). Among these studies, very few have examined N₂O emission from the viewpoint of microbial ecology. While, in our previous study, we found that N₂O emission during nitrification could be affected by heterotrophic activities significantly in a BNR system. The reason could be that during nitrification, competition between nitrifiers and heterotrophs for oxygen may affect N₂O emission due to less competition of nitrifiers for oxygen, which may induce oxygen limited condition for nitrifiers. Therefore, it is necessary to examine N₂O emission during nitrification from the viewpoint of “ecology of activated sludge”, which has not been received much attention in the research area of N₂O emission during nitrification.

In this study, effect of heterotrophic activities on N₂O emission under different aeration rates was investigated in batch experiments for activated sludge taken from a BNR system. The BNR system was acclimated to remove nitrogen and phosphorus from synthetic wastewater. N₂O emission under three aerobic conditions were examined, one without heterotrophic effect, one with heterotrophic activities by using internal stored organic carbon (polyhydroxybutyrate, PHB), and the other with heterotrophic activities by using external organic carbon (acetate). Under each condition, the applied aeration rates were 100 ml/min, 250 ml/min and 500 ml/min, respectively.

2. MATERIALS AND METHODS

2.1. Biological nutrient removal system
A 6-litre lab-scale sequencing batch reactor (SBR) was operated at 25°C for biological nutrient removal. The operational mode of SBR included fill/anoxic (10 min), anoxic/anaerobic (110 min), aerobic (180 min), settlement (40 min) and draw/idle (20 min). The reactor was well mixed during the fill and anoxic phase, while was also mixed by aeration during the aerobic phase. The dissolved oxygen (DO) concentration during the aerobic phase was above 2 mg/l. The applied hydraulic retention time was 12 hours and the SRT was around 10 days.

Synthetic wastewater was fed into the SBR with the following components: 510 mg/l sodium acetate, 10 mg/l yeast extract, 153 mg/l NH₄Cl, 46 mg/l Na₂HPO₄, 90 mg/l MgSO₄·7H₂O, 14 mg/l CaCl₂·2H₂O, and 1 ml of trace elements. Trace elements were added according to Smolders et al. (2004). The SBR was inoculated with activated sludge taken from Nanshan Wastewater Treatment Plant, Shenzhen, China.

2.2. Batch experiments
Batch experiments were carried out at 25°C to examine the effect of heterotrophic activities on N₂O emission during nitrification under three conditions. One condition (Batch-I) was without heterotrophic effect (without the addition of organic carbon), another condition (Batch-II) was with heterotrophic activities by using internal stored
organic carbon (PHB), and the other condition (Batch-III) was with heterotrophic activities by using external organic carbon (acetate). Under each condition, three aeration rates of 100 ml/min, 250 ml/min and 500 ml/min were applied to achieve different DO concentrations inside the batch reactor. All the batch experiments were carried out in 500 ml capped glass flasks, each with several ports on the caps, one for liquid sampling, one for gas sampling and flow rate measurement, and the other for aeration. All experiments were carried out with duplicates and the average results were presented in this study.

Activated sludge mixed liquor was taken from the parent SBR before the end of the aerobic phase. The mixed liquor was centrifuged and the supernatant was discharged. The solid sludge was suspended in a solution whose composition was the same as that of the synthetic wastewater but without the addition of organic carbon. For Batch-I experiment, the suspended mixed liquor was place static for 2 hours, and then aerated under different aeration rates. For Batch-II experiment, the suspended mixed liquor was initially placed under anaerobic condition for 2 hours with the addition of acetate to accumulate PHB, and then aerated under different aeration rates. For Batch-III experiment, the suspended mixed liquor was initially placed under anaerobic condition for 2 hours without the addition of acetate, and then aerated under different aeration rates after the addition of acetate (the initial acetate concentration of 500 mg/l).

For all batch experiments, both liquid and gas samples were taken at intervals of 10-15 min to test NH₄-N, NO₂-N, nitrate nitrogen (NO₃-N) and orthophosphate (PO₄-P) in the liquid, and N₂O in the gas phase. DO was measured directly inside the batch reactor.

2.3. Analytical methods
NO₂-N and NO₃-N were analysed by an ICS-1500 ion chromatography (Dionex, USA). PO₄-P, mixed liquor suspended solids (SS) and mixed liquor volatile suspended solids (VSS) were determined according to standard methods (APHA, 1995). DO was measured by the WTW DO probe (WTW 3010oxi, Germany).

N₂O was measured by a gas chromatography (GC, Agilent 6820, Agilent Technologies, USA) with an electron capture detector (ECD) and a HP-PLOT/Q column (J&W GC Columns, Agilent Technologies, USA). Temperatures during testing were 50°C for the injection port, 50°C for the oven, and 300°C for the detector. Nitrogen gas was used as the carrier gas at the flow rate of 15 ml/min. Pure N₂O gas was used as the standard for calibration. For convenient comparison, the produced N₂O in the gas phase was expressed as mg/l, representing mg N₂O (gas) produced from the unit volume (litre) of mixed activated sludge liquor. The ratio of N₂O emission to the produced NOx-N was obtained from dividing the N₂O emission rate by the NO₃-N produced rate (N₂O-N/NOx-N).

3. RESULTS AND DISCUSSION
After long-term acclimation, the SBR had achieved efficient biological nitrogen and phosphorus removal. Under steady state, the SS concentrations were 2367 ± 173 mg/l and the VSS concentrations were 1865 ± 112 mg/l. For the effluent, the NH₄-N concentration was around 0.28 ± 0.10 mg/l, the oxidized nitrogen (NOx-N, dominated by NO₃-N) concentration was 14.3 ± 1.7 mg/l, and the PO₄-P concentration was below 0.5 mg/l.

3.1. N₂O emission during nitrification without organic carbon utilisation
Under conditions without the existence of organic carbon, the DO concentration was in the range from 3.4 to 4.4 mg/l at the aeration rate of 100 ml/min, from 4.3 to 5.2 mg/l at the aeration rate of 250 ml/min, and 4.7 to 5.9 mg/l at the aeration rate of 500 ml/min.
Ammonium reduction and NOx-N production could be regressed by linear relationship at all the conditions. The NOx-N production rate was 4.3 mg N/g VSS·h at the aeration rate of 100 ml/min, 5.1 mg N/g VSS·h at 250 ml/min, and 5.6 mg N/g VSS·h at 500 ml/min. During nitrification, NO2-N production was observed, and the highest concentration was 1.8 mg/l at the aeration rate of 100 ml/min, 2.3 mg/l at the aeration rate of 250 ml/min, and 2.7 mg/l at the aeration rate of 500 ml/min. With high DO concentrations under all the conditions, N2O emission was relatively small. The released N2O-N to the produced NOx-N was 0.18% at 100 ml/min, 0.20% at 250 ml/min, and 0.41% at 500 ml/min. The N2O emission ratio was slightly high at the aeration rate of 500 ml/min.

3.2. N2O emission during nitrification concurrent with PHB utilisation
Under conditions with the utilisation of intracellular PHB, the DO concentration was in the range from 0.6 to 4.9 mg/l at the aeration rate of 100 ml/min, from 2.0 to 5.1 mg/l at the aeration rate of 250 ml/min, and 3.5 to 5.4 mg/l at the aeration rate of 500 ml/min. The gradient DO concentration during ammonium nitrification was about 1.0 mg/l at 100 ml/min, 2.0 mg/l at 250 ml/min, and 4.0 mg/l at 500 ml/min. During the aerobic condition, PO4-P uptaken was observed, indicating the activities of polyphosphate accumulating organisms (PAOs). The high activity of PAOs caused the
DO gradients obtained above. Ammonium reduction and NOx-N production were carried out concurrent with PO₄-P uptake. During nitrification, NO₂-N accumulation during the initial stage was observed, and the highest concentration was 3.6 mg/l at the aeration rate of 100 ml/min, 4.4 mg/l at the aeration rate of 250 ml/min, and 4.8 mg/l at the aeration rate of 500 ml/min. The NOx-N production rate was 4.8 mg N/g VSS-h at the aeration rate of 100 ml/min, 8.8 mg N/g VSS-h at 250 ml/min, and 9.1 mg N/g VSS-h at 500 ml/min. N₂O emission decreased with increasing aeration rates. The released N₂O-N to the produced NOx-N was 10.0% at 100 ml/min, 3.6% at 250 ml/min, and 0.6% at 500 ml/min.

Figure 2. Dynamics of nitrogen, phosphorus and DO under different aeration rates with the effect of heterotrophs by utilising of PHB.

3.3. N₂O emission during nitrification concurrent with acetate utilisation
Under conditions with the utilisation of acetate, the DO concentration was in the range from 1.1 to 4.7 mg/l at the aeration rate of 100 ml/min, from 1.7 to 5.2 mg/l at the aeration rate of 250 ml/min, and 2.3 to 5.5 mg/l at the aeration rate of 500 ml/min. The gradient DO concentration during ammonium nitrification was about 1.1 mg/l at 100 ml/min, 2.5 mg/l at 250 ml/min, and 3.6 mg/l at 500 ml/min.

During the aerobic condition, with the addition of acetate, PO₄-P was initially released and then taken up by PAOs. The high activity of PAOs caused the DO gradients obtained
above. Ammonium reduction and NOx-N production were carried out concurrent with PO4-P release or uptake. During nitrification, NO2-N accumulation during the initial stage was observed, and the highest concentration was 7.3 mg/l at the aeration rate of 100 ml/min, 7.1 mg/l at the aeration rate of 250 ml/min, and 6.6 mg/l at the aeration rate of 500 ml/min. The NOx-N production rate was 3.8 mg N/g VSS-h at the aeration rate of 100 ml/min, 5.9 mg N/g VSS-h at 250 ml/min, and 6.8 mg N/g VSS-h at 500 ml/min. N2O emission decreased with increasing aeration rates. The released N2O-N to the produced NOx-N was 14.5% at 100 ml/min, 4.1% at 250 ml/min, and 0.7% at 500 ml/min.

![Figure 3](image_url)

**Figure 3.** Dynamics of nitrogen, phosphorus and DO under different aeration rates with the effect of heterotrophs by utilising of acetate.

4. **DISCUSSION**

Compared with the results from conditions without or with the activities of heterotrophs, it showed that N2O emission during nitrification was affected by heterotrophic activities significantly. At the aeration rate of 100 ml/min, the N2O emission ratio was increased from 0.18% without heterotrophic activities to 10% or 14.5% with heterotrophic activities. Usually, a low N2O emission ratio has been obtained during nitrification by activities of nitrifiers without the effect of heterotrophs. Such as, Law et al. (2011) obtained that the proportion was of 1% for AOB of halophilic and halotolerant members of *Nitrosomonas* sp., and Ahn et al. (2011) reported the N2O emission ratio was in the range of 0.13-1.9%
due to activities of *N. europaea* and *N. eutropha*. From our study, it showed that a much high N$_2$O emission ratio was obtained with the effect of heterotrophs. Therefore, N$_2$O emission during nitrification should be controlled carefully by considering the effect of heterotrophs from the view of microbial competition.

Effect of heterotrophs on N$_2$O emission during nitrification could be through two aspects: (1) lowering DO concentrations and (ii) increasing NO$_2$-N concentrations (Gejlsbherg et al., 1998; Tallec et al., 2006; Kampschreur et al., 2007; Wunderlin et al., 2012). With the activities of heterotrophs, DO concentration was lowered at the same aeration rate. For example, the DO was around 1 mg/l at the flow rate of 100 ml/min with the activity of heterotrophs, while it was above 4 mg/l without the effect of heterotrophs. This is due to that heterotrophs possesses a high DO requirement and therefore reduce the DO concentrations. In addition, ammonia oxidizing bacteria (AOB) have less competition ability for DO compared with heterotrophs. Besides the DO effect, the highest NO$_2$-N concentration was also increased from 1.8-2.7 mg/l without heterotrophic activities to 3.6-4.8 mg/l or 6.6-7.3 mg/l with heterotrophic activities. This is due to nitrite oxidizing bacteria (NOB) have the least competition ability compared with both AOB and heterotrophs and their activities were affected significantly.

Under conditions with the effect of heterotrophs, N$_2$O emission ratio decreased with increasing the aeration rate (also increasing the DO concentrations). For example, the ratio of N$_2$O emission decreasing from above 10% at the aeration rate of 100 ml/min (around 1 mg/l) to around 0.5% at 500 ml/min (above 3.5 mg/l). These results are consistent with previous studies that a low DO concentration induced a high N$_2$O emission (Gejlsbherg et al., 1998; Tallec et al., 2006). Tallec et al. (2006) obtained the highest N$_2$O emission at the DO concentration of 1 mg/l. A high aeration rate with a high dissolved oxygen (DO) concentration reduced N$_2$O emission significantly under aerobic conditions. For controlling N$_2$O emission during nitrification concurrent with heterotrophic activities, DO concentrations should be carefully controlled.

There was no significant difference in N$_2$O emission during nitrification affected by heterotrophic activities by using internal or external organic carbon. However, using acetate as the organic carbon, a relatively small high NO$_2$-N and N$_2$O emission were observed at the aeration rate of 100 ml/min and 250 ml/min than those with PHB as the organic carbon. The reason could be that PHB degradation is the limiting step for heterotrophs and heterotrophs can use acetate faster then using PHB. Therefore, when acetate was used as the organic carbon, a relatively high heterotrophic activity might occur and induced a high N$_2$O emission. In addition, it is surprising to see that the NOx-N production rate was increased with the effect of heterotrophs compared with without their effects. The possible reason underlying is not so clear at the moment.

5. CONCLUSIONS

Under the condition with PHB as the organic carbon, the released N$_2$O-N to the produced oxidized nitrogen (NOx-N) was 10.0% at 100 ml/min, 3.6% at 250 ml/min, and 0.6% at 500 ml/min. Under the condition with acetate as the organic carbon, the released N$_2$O-N to the produced NOx-N was 14.5% at 100 ml/min, 4.1% at 250 ml/min, and 0.7% at 500 ml/min. Under the condition without organic carbon, the released N$_2$O-N to the produced NOx-N was 0.18% at 100 ml/min, 0.2% at 250 ml/min, and 0.4% at 500 ml/min.

These results showed that (i) heterotrophic activities affected N$_2$O emission during nitrification significantly; (ii) there was no significant difference in N$_2$O emission during nitrification affected by heterotrophic activities by using internal or external organic carbon; (iii) a high aeration rate with a high DO concentration reduced N$_2$O emission
significantly under aerobic conditions. For controlling N₂O emission during nitrification concurrent with heterotrophic activities, DO concentrations should be carefully controlled.

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