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Effect of heterotrophic activities on nitrous oxide emission during nitrification under different aeration rates

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ABSTRACT

Nitrous oxide (N_2O), a greenhouse gas, can be released from both nitrification and denitrification during wastewater treatment. In this study, N_2O emission under three aerobic conditions (aeration rates of 100, 250, and 500 ml/min, respectively) were examined, one with only endogenous heterotrophic activities, one with heterotrophic activities using internal stored organic carbon (polyhydroxybutyrate, PHB), and the other with heterotrophic activities using external organic carbon (acetate). Under the condition with PHB as the organic carbon, the released N_2O-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_2O-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_2O-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. These results showed that (i) heterotrophic activities affected N_2O emission during nitrification significantly; (ii) there was no significant difference in N_2O emission during nitrification affected by heterotrophic activities using internal or external organic carbon; and (iii) a high aeration rate with a high dissolved oxygen concentration reduced N_2O emission significantly under aerobic conditions.

Keywords: Nitrous oxide; Nitrification; Denitrification; Heterotrophic activity; Aeration rate

1. Introduction

Nowadays, biological nutrient removal (BNR) processes have been applied widely for wastewater treatment. However, one of the greenhouse gases, nitrous oxide (N_2O), can be released from both nitrification and denitrification during BNR. The global warming potential of N_2O is around 300 times that of CO_2 and its life cycle is around 129 years [1]. The atmospheric N_2O concentration is about 310 ppbv and its annual increasing rate is about 0.25% [1]. In spite of the contribution of N_2O emission from sewage treatment is only 3.2% to the total N_2O emission, a small amount of N_2O emission can cause a significant

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Presented at the 13th International Conference on Environmental Science and Technology (CEST 2013), 5–7 September 2013, Athens, Greece influence on the global greenhouse gas effect. In addition, by increasing only 1% of N_2O emission during wastewater treatment, the carbon footprint of wastewater treatment plants will be increased by 30% [2]. Therefore, it is necessary to investigate and further control the N_2O emission during wastewater treatment.

N_2O emission during nitrogen removal can be from either nitrification or denitrification or both [3]. Some studies showed that nitrification under aerobic condition was the main source for N_2O emission during wastewater treatment [4,5], while others showed that denitrification was the main source [6]. Therefore, further investigations are still required to clarify which process is the main contributor to N_2O emission in wastewater treatment processes.

N_2O emission during nitrification is mainly through two processes: (i) nitrifier denitrification and (ii) biological or chemical hydroxylamine oxidation [7]. Lots of factors affect the emission of N_2O during nitrification, including substrate concentrations such as ammonium and oxygen, product concentrations such as nitrite, environmental conditions such as sludge retention time (SRT) and pH, and dynamic conditions, etc. [3,8–12]. Among these studies, very few have examined N_2O emission from the viewpoint of microbial ecology. While, in our previous study, we found that N_2O emission during nitrification could be affected by heterotrophic activities significantly in a BNR system. The reason might be due to that during nitrification, competition between nitrifiers and heterotrophs for oxygen might affect N_2O emission due to less competition of nitrifiers for oxygen, which might induce oxygen-limited condition for nitrifiers. Therefore, it is necessary to examine N_2O emission during nitrification from the viewpoint of "ecology of activated sludge," which has not received much attention in the research area of N_2O emission during nitrification.

In this study, the effect of heterotrophic activities on N_2O 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_2O emission under three aerobic conditions were examined, one with only endogenous heterotrophic effect, one with heterotrophic activities using internal stored organic carbon (polyhydroxybutyrate, PHB), and the other with heterotrophic activities using external organic carbon (acetate). Under each condition, the applied aeration rates were 100, 250, and 500 ml/min, respectively.

2. Materials and methods

2.1. BNR system

A six-liter lab-scale sequencing batch reactor (SBR) was operated at 25℃ for BNR. 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 phases, while it 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 h and the SRT was around 10 d.

Synthetic wastewater was fed into the SBR with the following components: 510 mg/l sodium acetate, 10 mg/l yeast extract, 153 mg/l NH_4Cl, 46 mg/l Na_2HPO_4, 90 mg/l $MgSO_4{\cdot}7H_2O$, 14 mg/l $CaCl_2{\cdot}2H_2O$, and 1 ml of trace elements. Trace elements were added according to Smolders et al. [13]. 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℃ to examine the effect of heterotrophic activities on N_2O emission during nitrification under three conditions. One condition (Batch-I) was with only endogenous 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, 250, 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 placed static for 2 h, and then aerated under different aeration rates. For Batch-II experiment, the suspended mixed liquor was initially placed under anaerobic condition for 2 h 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 h 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 ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), and orthophosphate (PO_4-P) in the liquid, and N_2O in the gas phase. DO was measured directly inside the batch reactor.

2.3. Analytical methods

NO_2-N and NO_3-N were analyzed by an ICS-1500 ion chromatography (Dionex, USA). PO_4-P, mixed liquor suspended solids (SS), and mixed liquor volatile suspended solids (VSS) were determined according to standard methods [14]. DO was measured by the WTW DO probe (WTW 3010oxi, Germany).

N_2O 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℃ for the injection port, 50℃ for the oven, and 300℃ for the detector. Nitrogen gas was used as the carrier gas at the flow rate of 15 ml/min. Pure N_2O gas was used as the standard for calibration. For convenient comparison, the produced N_2O in the gas phase was expressed as mg/l, representing mg N_2O (gas) produced from the unit volume (liter) of mixed activated sludge liquor. The ratio of N_2O emission to the produced oxidized nitrogen (NOx-N) was obtained by dividing the N_2O emission rate by the NO_3-N production rate (N_2O-N/NOx-N).

3. Results

After long-term acclimation, the SBR had achieved efficient biological nitrogen and phosphorus removal. Under steady state, the SS concentrations were 2,367 ± 173 mg/l and the VSS concentrations were 1,865 ± 112 mg/l. For the effluent, the NH_4-N concentration was around 0.28 ± 0.10 mg/l, the NOx-N (dominated by NO_3-N) concentration was 14.3 ± 1.7 mg/l, and the PO_4-P concentration was below 0.5 mg/l.

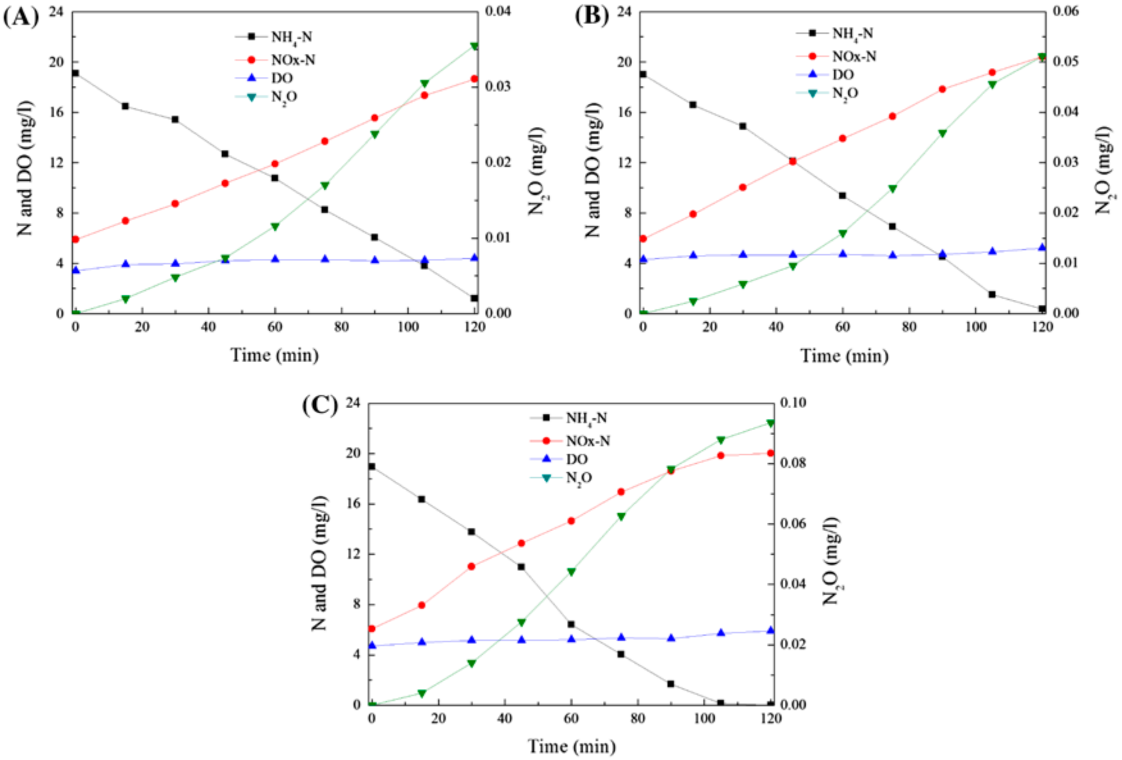

Fig. 1. Dynamics of nitrogen, phosphorus, and DO under different aeration rates with only endogenous heterotrophic effect. (A) The aeration rate of 100 ml/min; (B) the aeration rate of 250 ml/min; and (C) the aeration rate of 500 ml/min.

3.1. N_2O emission during nitrification without organic carbon utilization

Dynamics of different types of nitrogen and DO under various aeration rates with only endogenous heterotrophic effect is shown in Fig. 1.

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, NO_2-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, N_2O emission was relatively small. The released N_2O-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 N_2O emission ratio was slightly high at the aeration rate of 500 ml/min.

3.2. N_2O emission during nitrification concurrent with PHB utilization

Dynamics of different types of nitrogen and DO under various aeration rates with the effect of heterotrophic activities using PHB is shown in Fig. 2.

Under conditions with the utilization 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 phase, PO_4-P uptake 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 PO_4-P uptake. During

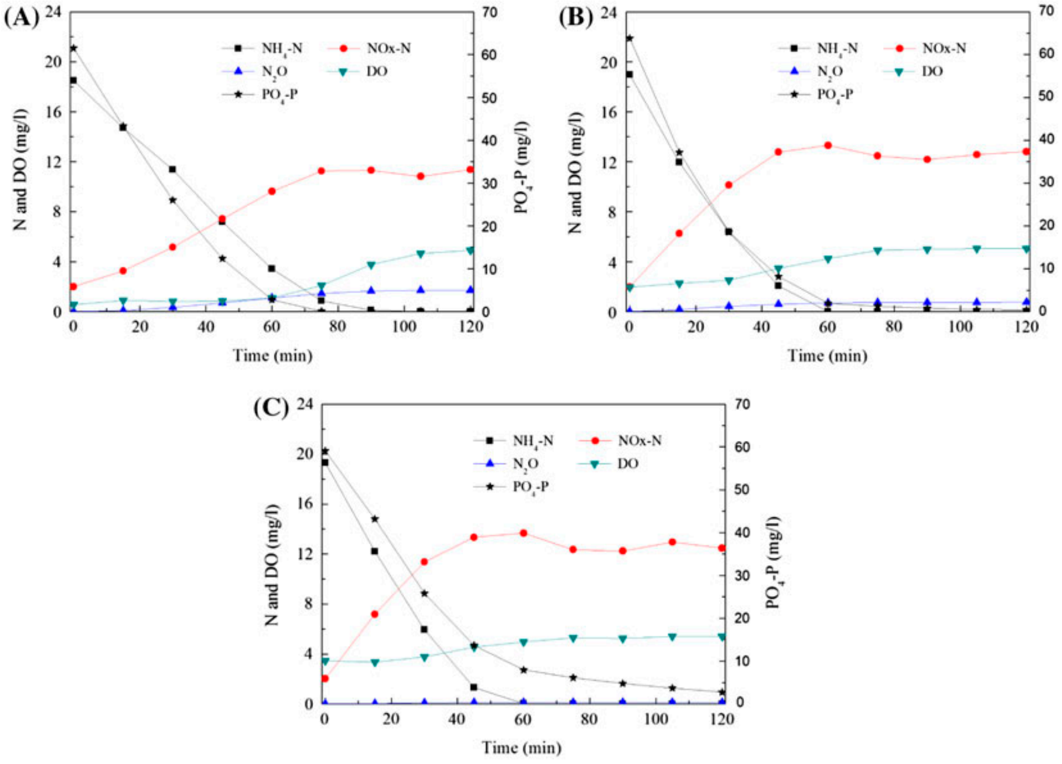

Fig. 2. Dynamics of nitrogen, phosphorus, and DO under different aeration rates with the effect of heterotrophs by utilizing PHB. (A) The aeration rate of 100 ml/min; (B) the aeration rate of 250 ml/min; and (C) the aeration rate of 500 ml/min.

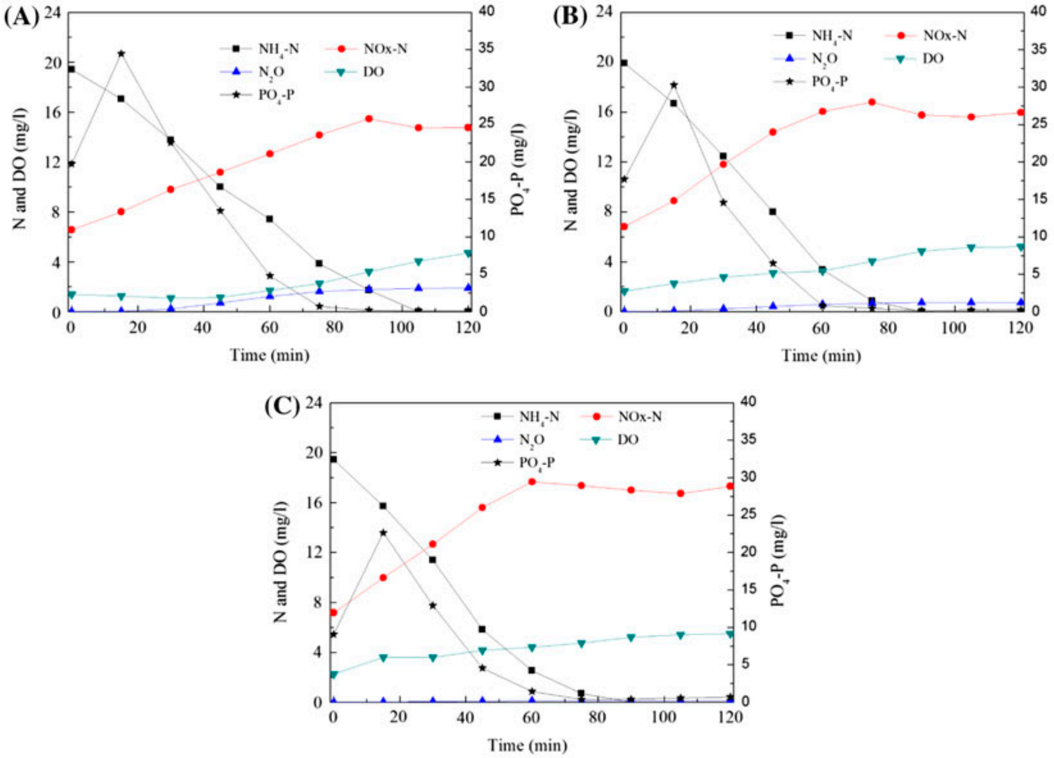

Fig. 3. Dynamics of nitrogen, phosphorus, and DO under different aeration rates with the effect of heterotrophs by utilizing acetate. (A) The aeration rate of 100 ml/min; (B) the aeration rate of 250 ml/min; and (C) the aeration rate of 500 ml/min.

nitrification, NO_2-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. N_2O emission decreased with increasing aeration rates. The released N_2O-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.

3.3. N_2O *emission during nitrification concurrent with acetate utilization*

Dynamics of different types of nitrogen and DO under various aeration rates with the effect of heterotrophic activities using acetate is shown in Fig. 3.

Under conditions with the utilization 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 phase, with the addition of acetate, PO_4-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 PO_4-P release or uptake. During nitrification, NO_2-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. N_2O emission decreased with increasing aeration rates. The released N_2O-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.

4. Discussion

Compared to the results from conditions with only endogenous heterotrophic activities or with heterotrophic activities, it showed that N_2O emission during nitrification was affected by heterotrophic activities significantly. At the aeration rate of 100 ml/min, the N_2O emission ratio was increased from 0.18% with only endogenous heterotrophic activities to 10.0% or 14.5% with heterotrophic activities. Usually, a low N_2O emission ratio has been obtained during nitrification by activities of nitrifiers without the effect of heterotrophs. Such as, Law et al. [11] obtained that the proportion was of 1% for ammonia-oxidizing bacteria (AOB) of halophilic and halotolerant members of *Nitrosomonas* sp., and Ahn et al. [15] reported the N_2O 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 higher N_2O emission ratio was obtained with the effect of heterotrophs. Therefore, N_2O emission during nitrification should be controlled carefully by considering the effect of heterotrophs from the view of microbial competition.

Effect of heterotrophs on N_2O emission during nitrification could be through two aspects: (i) lowering DO concentrations and (ii) increasing NO_2-N concentrations [4,7,9,16]. With the activities of heterotrophs, DO concentration was lowered at the same aeration rate compared with only endogenous heterotrophic activities. For example, the DO was around 1 mg/l at the aeration rate of 100 ml/min with the activity of heterotrophs, while it was above 4 mg/l with only endogenous heterotrophic activities. This is due to that heterotrophs possess a high DO requirement and therefore reduce the DO concentrations. In addition, 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 with only endogenous heterotrophic activities to 3.6–4.8 or 6.6–7.3 mg/l with heterotrophic activities. Existence of NO_2-N can enhance N_2O emission during nitrification [7]. This was because the nitrite-oxidizing bacteria had the least competition ability compared with both AOB and heterotrophs, and their activities were affected significantly. In spite of the aerobic condition, simultaneous heterotrophic denitrification might also occur under low DO concentrations due to the diffusion limitation of DO within the activated sludge flocs, which might contribute to the high N_2O emission under the low aeration rates, as also shown by Wunderlin et al. [7]. Further studies are required to examine this effect.

Under conditions with the effect of heterotrophs, N_2O emission ratio decreased with increasing aeration rates (also increasing DO concentrations). For example, the ratio of N_2O emission decreased 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 were consistent with previous studies that a low DO concentration induced a high N_2O emission [4,9]. Tallec et al. [9] obtained the highest N_2O emission at the DO concentration of 1 mg/l. A high aeration rate with a high DO concentration reduced N_2O emission significantly under aerobic conditions. For controlling N_2O emission during nitrification concurrent with heterotrophic activities, the DO concentrations should be carefully controlled.

There was no significant difference in N_2O emission during nitrification affected by heterotrophic activities using internal or external organic carbon. However, using acetate as the organic carbon, a slightly higher NO_2-N and N_2O emission was observed at the aeration rate of 100 and 250 ml/min than those with PHB as the organic carbon. The reason could be that PHB degradation was the limiting step for heterotrophs and it could use acetate faster than using PHB. Therefore, when acetate was used as the organic carbon, a relatively high heterotrophic activity might occur and induce a high N_2O emission. In addition, it was surprising to see that the NOx-N production rate was increased with the effect of heterotrophs compared with that with only the endogenous heterotrophic effect. 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_2O-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. Under the condition with acetate as the organic carbon, the released N_2O-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_2O-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_2O emission during nitrification significantly; (ii) there was no significant difference in N_2O emission during nitrification affected by heterotrophic activities using internal or external organic carbon; and (iii) a high aeration rate with a high DO concentration reduced N_2O emission significantly under aerobic conditions. For controlling N_2O emission during nitrification concurrent with heterotrophic activities, the DO concentrations should be carefully controlled.

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