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Effect of ammonium on nitrous oxide emission during denitrification with different electron donors

Guangxue Wu^{1,*}, Xiaofeng Zhai², Chengai Jiang², Yuntao Guan¹

1. Key Laboratory of Microorganism Application and Risk Control of Shenzhen, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, China

2. Department of Environmental Engineering, South China Agricultural University, Guangzhou 510642, China

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Abstract

Nitrous oxide (N₂O) emission during denitrification is receiving intensive attention due to its high potential to cause greenhouse effects. In this study, denitrifiers were acclimated in sequencing batch reactors with methanol or acetate as the electron donor and nitrate as the electron acceptor. The effects of ammonium on N₂O emission were examined in batch experiments with various electron donors. With the addition of ammonium, N₂O emission increased under all the examined conditions compared to experiments without ammonium addition. With different electron donors, the highest ratio of N₂O emission to the removed oxidized nitrogen was 0.70% for methanol, 5.34% for acetate, and 34.79% for polyhydroxybutyrate.

Key words: ammonium; denitrification; greenhouse gas; nitrous oxide; PHB

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Introduction

Nitrous oxide (N₂O) is a type of greenhouse gas, with a global warming potential 310 times that of CO₂ and a life-cycle of 110 years (IPCC, 2001). The annual growth rate of N₂O emission is 0.25%. In addition, during wastewater treatment, the carbon footprint will be increased 30% by 1% increase in N₂O emission (Law et al., 2012). Therefore, it is necessary to control the emission of N₂O during wastewater treatment, specifically during nitrogen removal.

Denitrification has been applied widely in biological nitrogen removal, and N₂O can be released during incomplete denitrification. Many factors affect N₂O emission during denitrification, such as electron donors (organic materials), electron acceptors (nitrate, nitrite, or oxygen), and applied operating conditions (C/N ratios, sludge retention times, etc) (Adouani et al., 2010; Hanaki et al., 1992; Kampschreur et al., 2009). Usually, a high nitrite concentration, a low oxygen concentration, and a low C/N ratio was found to induce high N₂O production (Adouani et al., 2010; Hanaki et al., 1992; Kampschreur et al., 2009). However, few studies have been carried out on the effect of ammonium on N₂O emission during denitrification. As we

know, high N₂O emission was found to occur with high microbial activity during nitrification (Ahn et al., 2010). As the existence of ammonium could enhance the activity of denitrifiers (Nara et al., 2009; Waki et al., 2002), we proposed that the existence of ammonium might enhance N₂O emission during denitrification.

Conventionally, methanol has been often adopted as the external electron donor for denitrification due to its low cost. Nowadays, other electron donors, such as acetate, which can be produced from hydrolysis and acidification of waste activated sludge (Jiang et al., 2007), have been used to replace methanol due to the unsafe nature of methanol. When acetate was used as the electron donor for denitrification, storage of acetate as polyhydroxybutyrate (PHB) during denitrification resulted in a high carbon requirement and an accumulation of nitrite (especially under low C/N conditions) (Ciggin et al., 2009; Itokawa et al., 2001). During denitrification, acetate could induce a high production of N₂O, in the proportion of 74% of the applied influent nitrate (Adouani et al., 2010). In addition, with acetate as the electron donor, it could be used rapidly and further induce endogenous denitrification, while the storage substance and its influence on N₂O emission have not been examined (Itokawa et al., 2001). With PHB as the electron donor, the question of whether PHB itself or the existence of nitrite contributed to N₂O emission

* Corresponding author. E-mail: wu.guangxue@sz.tsinghua.edu.cn

has not been clearly resolved (Kampschreur et al., 2009). Therefore, it is necessary to examine factors influencing N_2O emission during denitrification with different electron donors.

In this study, denitrifiers were acclimated with acetate or methanol as the electron donor. The effect of ammonium addition on the N_2O emission for denitrifiers with various electron donors was also examined.

1 Materials and methods

1.1 Denitrifying activated sludge acclimation

Two sequencing batch reactors (SBR_M with methanol as the electron donor and SBR_A with acetate as the electron donor), each with a working volume of 5.4 L, were operated at 25°C to enrich denitrifiers. The SBRs were operated six cycles per day and each cycle comprised the following phases: fill (20 min), anoxic (150 min), aerobic (10 min), settlement (40 min) and draw/idle (20 min). The reactors were mixed during the fill and anoxic phase by mechanical mixers. The aerobic phase was adopted to remove N_2 produced during the anoxic phase so as to improve the settlement property of the activated sludge. In each cycle, 1.8 L of treated wastewater was exchanged with a new batch of synthetic wastewater, and a hydraulic retention time of 12 hr was used. Sludge removal from the reactor was carried out once a day before the settlement phase to maintain a sludge retention time of 10 days.

The SBRs were fed with synthetic wastewater composed of 510 mg/L sodium acetate or 0.34 mL/L methanol, 607 mg/L $NaNO_3$, 10 mg/L yeast extract, 76 mg/L NH_4Cl , 25 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 following Smolders et al. (1994). The reactors were seeded with activated sludge taken from Nanshan Wastewater Treatment Plant, Shenzhen, China.

1.2 Batch experiments

Batch experiments with replications were carried out to examine the effect of ammonium addition on N_2O emission using various electron donors: methanol, acetate, and

PHB. For each electron donor condition, nitrate or nitrate combined with nitrite was used as the electron acceptor.

Activated sludge mixed liquor was withdrawn from the SBRs at the end of the aerobic phase and used in the batch experiment. 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. After withdrawal from the reactor, the mixed liquor was centrifuged and the remaining sludge was resuspended in a solution of the synthetic wastewater but without the addition of the electron donor, electron acceptor and ammonium. To examine the effect of PHB on N_2O emission, the activated sludge taken from SBR_A was first pretreated under anoxic conditions with the addition of nitrate and acetate to accumulate PHB. After this accumulation step, the activated sludge was centrifuged and resuspended in a solution of the synthetic wastewater but without the addition of the electron donor, electron acceptor and ammonium. The examined conditions are detailed in **Table 1**. In this study, different initial acetate and methanol concentrations were adopted due to their different metabolizing mechanisms. In addition, because it was very difficult to control the initial PHB concentration, the PHB accumulated after adding acetate initially was used during denitrification. In all batch experiments, samples (5 mL for both liquid and gas samples) were taken at intervals to test for methanol or acetate and/or PHB, ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N) and nitrate nitrogen (NO_3-N) for liquid samples, and N_2O for gas samples.

1.3 Analytical methods

NO_2-N and NO_3-N were analyzed with an ICS-1500 ion chromatography system (Dionex, USA). Suspended solids (SS), volatile suspended solids (VSS) and NH_4-N were determined according to standard methods (APHA, 1999).

Sodium acetate was measured with high performance liquid chromatography (HPLC, LC-10A, Shimadzu, Japan) using a UV index detector and an Aminex HPX-87H column (Bio-Rad, USA). Separation during HPLC tests was achieved using a mobile phase of 1/1000 (vol/vol) H_2SO_4 at a flow rate of 0.6 mL/min, a column

Table 1 Examined batch experiment conditions

	Electron donor	Parameters	Nitrate	Nitrate + ammonium	Nitrate + nitrite	Nitrate + nitrite + ammonium
SBR_M	Methanol	Methanol-C (mg/L)	150	150	150	150
		NH_4-N (mg/L)	–	20	–	20
		NO_2-N (mg/L)	–	–	20	20
		NO_3-N (mg/L)	100	100	100	100
SBR_A	Acetate	Acetate-C (mg/L)	300	300	300	300
		NH_4-N (mg/L)	–	20	–	20
		NO_2-N (mg/L)	–	–	20	20
		NO_3-N (mg/L)	100	100	100	100
	PHB	PHB-C (mg/L)	65	65	65	65
		NH_4-N (mg/L)	–	20	–	20
		NO_2-N (mg/L)	–	–	20	20
		NO_3-N (mg/L)	100	100	100	100

temperature of 35°C, and a detector temperature of 40°C. The PHB concentration was detected by the modified HPLC method (Karr et al., 1983; Rodgers and Wu, 2010) and the testing protocol was the same as that for acetate testing, using crotonic acid for calibration.

N₂O was detected 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 specific volume (L) of mixed activated sludge liquor.

2 Results and discussion

The SBRs were acclimated for more than 6 months and denitrifiers were successfully enriched. With the influent

NO₃-N concentration of 100 mg/L, the effluent NO₃-N concentrations were less than 1 mg/L and a low concentration of NO₂-N (below 2 mg/L) was also detected. Under steady state, the biomass (VSS) concentration was 1250 mg/L in SBR_A and 1200 mg/L in SBR_M.

2.1 N₂O emission with methanol as the electron donor

The effects of NH₄-N on N₂O emission with methanol as the electron donor in batch experiments are shown in Fig. 1. The calculated kinetic parameters of denitrification are given in Table 2.

With nitrate as the electron acceptor, there was not much difference in the utilization of methanol or reduction of nitrate under all the applied conditions, while a relatively small amount of nitrite was produced, indicating a slightly higher nitrate reduction rate than the nitrite reduction rate. With both nitrite and nitrate as electron acceptors, similar results were obtained as that with nitrate as the electron acceptor, while reduction of nitrite was observed, indicating that a high nitrite reduction rate occurred.

Under all the conditions, a higher N₂O emission occurred with the addition of ammonium. With the electron

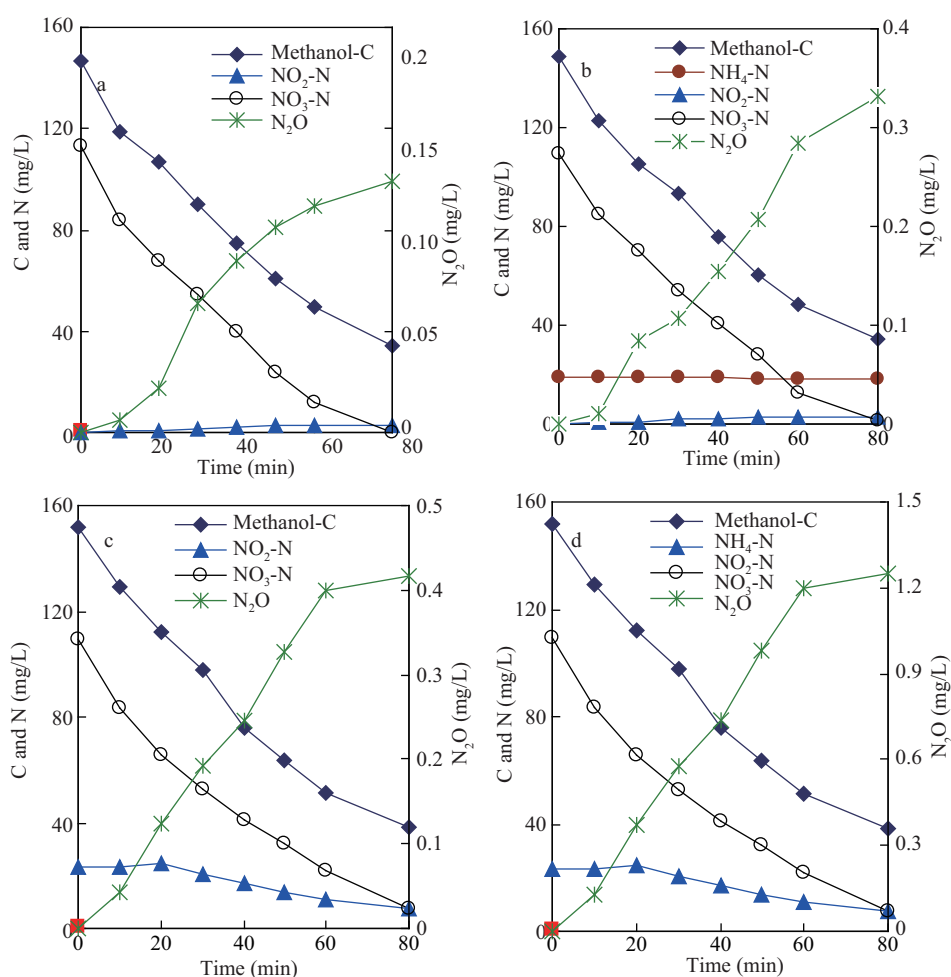


Fig. 1 Effects of ammonium addition on N₂O emission with methanol as the electron donor. (a) nitrate; (b) nitrate + ammonium; (c) nitrate + nitrite; (d) nitrate + nitrite + ammonium.

Table 2 Denitrification kinetics with methanol as the electron donor.

	Nitrate	Nitrate + ammonium	Nitrate + nitrite	Nitrate + nitrite + ammonium
Methanol-C (mg/(g·hr))	-84.6	-87.5	-90.2	-91.0
NH ₄ -N (mg/(g·hr))	-	-1.0	-	-1.1
NO ₂ -N (mg/(g·hr))	2.85	2.77	-12.4	-14.4
NO ₃ -N (mg/(g·hr))	-87.0	-83.0	-74.8	-72.0
N ₂ O (mg/(g·hr))	0.113	0.253	0.361	0.947
N ₂ O-N emission ratio	0.08%	0.20%	0.26%	0.70%

acceptors of nitrate and nitrate + nitrite, ratios of the released N₂O to the removed oxidized nitrogen were 0.08% and 0.26% without the addition of ammonium, and 0.20% and 0.70% with the addition of ammonium. Therefore, by adding ammonium, the increase in N₂O was 2.50 and 2.69 times higher than that for the experiments without the addition of ammonium for the electron acceptors of nitrate and nitrate + nitrite, respectively. With the addition of nitrite, the produced N₂O was significantly increased compared with experiments with only nitrate. The results

indicated that nitrite also had a large effect on N₂O emission when methanol was the electron donor.

2.2 N₂O emission with acetate as the electron donor

The effects of NH₄-N on N₂O emission with acetate as the electron donor in batch experiments are shown in **Fig. 2**. The calculated kinetic parameters of denitrification are given in **Table 3**.

Under all conditions, acetate was taken up and partially stored as PHB. Because a high initial acetate concentration

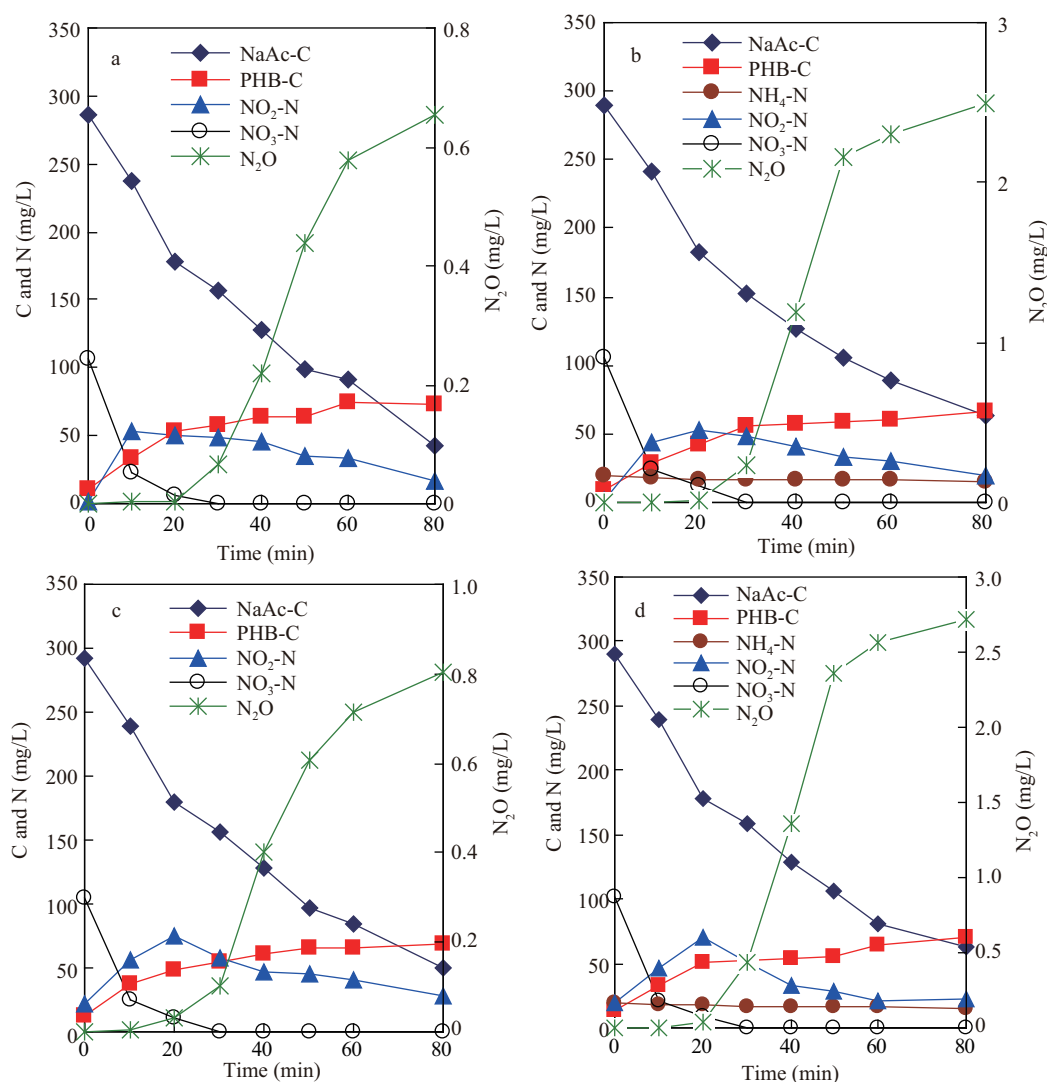


Fig. 2 Effects of ammonium on N₂O emission with acetate as the electron donor. (a) nitrate; (b) nitrate + ammonium; (c) nitrate + nitrite; (d) nitrate + nitrite + ammonium.

Table 3 Denitrification kinetics with acetate as the electron donor

Duration (min)	Nitrate		Nitrate + ammonium		Nitrate + nitrite		Nitrate + nitrite + ammonium	
	0–20	20–80	0–20	20–80	0–20	20–80	0–20	20–80
Acetate-C (mg/(g·hr))	-276.3	-114.1	-269.9	-100.9	-288.2	-111.8	-286.9	-102.5
PHB-C (mg/(g·hr))	108.3	18.4	84.6	16.6	90.8	16.7	97.1	16.6
NH ₄ -N (mg/(g·hr))	–	–	-5.08	-1.51	–	–	-4.91	-1.49
NO ₂ -N (mg/(g·hr))	127.1	-29.0	131.2	-28.4	136.9	-35.9	127.2	-39.4
NO ₃ -N (mg/(g·hr))	-256.2	–	-237.9	–	-237.5	–	-233.1	–
N ₂ O (mg/(g·hr))	0.010	0.620	0.015	2.380	0.081	0.722	0.097	2.512
N ₂ O-N emission ratio	0.005%	1.36%	0.009%	5.34%	0.052%	1.28%	0.058%	4.06%

was used, there was still some acetate remaining at the end of the experiment. With NO₃-N as the electron acceptor, the reduction rate of acetate was 270 mg/(g·hr), while it decreased to 100 mg/(g·hr) with nitrite as the electron acceptor. The production ratio of PHB from acetate was 31%–39% with nitrate as the electron acceptor and it was 14.9%–16.5% with nitrite as the electron acceptor.

Under the condition with nitrate as the electron acceptor, nitrate was denitrified rapidly during the initial 20 min and nitrite accumulation occurred, indicating a lower nitrite reduction rate compared to the nitrate reduction rate. Previous studies have shown that nitrite accumulation occurred under acetate limitation conditions (Oh and Silverstein, 1999; Van Rijn et al., 1996). The reason could be due to that (1) a higher nitrate reduction rate compared to the nitrite reduction rate, (2) priority utilization of nitrate rather than nitrite as the electron acceptor, and (3) priority storage of acetate resulting in acetate limitation conditions. With the complete consumption of nitrate, denitrification with nitrite as the electron acceptor proceeded. Compared with reduction of nitrate, a much lower nitrite reduction rate existed, with the denitrifying rate of 269.9–288.2 mg/(g·hr) for nitrate and 100.9–114.1 mg/(g·hr) for nitrite.

Under conditions with nitrate as the electron acceptor, there was a lag time for N₂O emission. During the initial 20 min, less than 0.1% of reduced nitrate was converted to N₂O. N₂O was mainly produced during denitrification of nitrite after the completion of the denitrification of nitrate (from minutes 20–80). These results showed that N₂O was mainly produced during denitrification due to the denitrifying of nitrite rather than during the denitrification of nitrate. This was due to the fact that N₂O was produced from denitrifying NO₂-N rather than from denitrifying NO₃-N to NO₂-N (Thomson et al., 2012). With the addition of ammonium, N₂O production increased under conditions with nitrate or nitrate + nitrite as the electron

acceptor, and the emission ratio of N₂O to the reduced oxidized nitrogen increased from 1.36% to 5.34% with nitrate and from 1.28% to 4.06% with nitrate + nitrite as the electron acceptor. Because nitrite was produced during denitrification of nitrate and a high concentration of nitrite existed during most of the reaction, the coexistence of nitrite and nitrate did not increase the N₂O emission significantly compared with experiments using only nitrate as the electron acceptor.

2.3 N₂O emission with PHB as the electron donor

The effects of NH₄-N on N₂O emission with PHB as the electron donor in batch experiments are shown in **Fig. 3**. The calculated kinetic parameters of denitrification are given in **Table 4**.

Under all the conditions, PHB and nitrate was denitrified slowly. With the denitrification of nitrate, nitrite accumulation occurred, indicating a lower nitrite reduction rate compared to the nitrate reduction rate. As mentioned above, previous studies have shown that nitrite accumulation occurs under electron donor limiting conditions (Oh and Silverstein, 1999; Van Rijn et al., 1996). The reason for nitrite accumulation when PHB was the electron donor could be due to the fact that the degradation of PHB was a rate-limiting step during biological degradation (Beun et al., 2000).

Similar to experiments using the external electron donors methanol and acetate, N₂O emission was also significantly increased by adding ammonium with PHB as the electron donor under all the applied conditions. The emission ratio of N₂O to the reduced oxidized nitrogen increased from 0.032% to 34.79% with nitrate and from 8.09% to 27.79% with nitrate + nitrite as the electron acceptor. Here, for the simplification, linear regression was used to calculate the N₂O production ratio, while the production of N₂O with the use of nitrate or nitrate + nitrite

Table 4 Denitrification kinetics for various electron acceptors with PHB as the electron donor

	Nitrate	Nitrate + ammonium	Nitrate + nitrite	Nitrate + nitrite + ammonium
PHB-C (mg/(g·hr))	-7.6	-8.2	-8.4	-9.9
NH ₄ -N (mg/(g·hr))	–	-1.92	–	-1.42
NO ₂ -N (mg/(g·hr))	0.97	7.2	2.5	10.4
NO ₃ -N (mg/(g·hr))	-3.6	-7.56	-5.1	-12.4
N ₂ O (mg/(g·hr))	0.0014	0.197	0.322	0.859
N ₂ O-N emission ratio	0.032%	34.79%	8.09%	27.79%

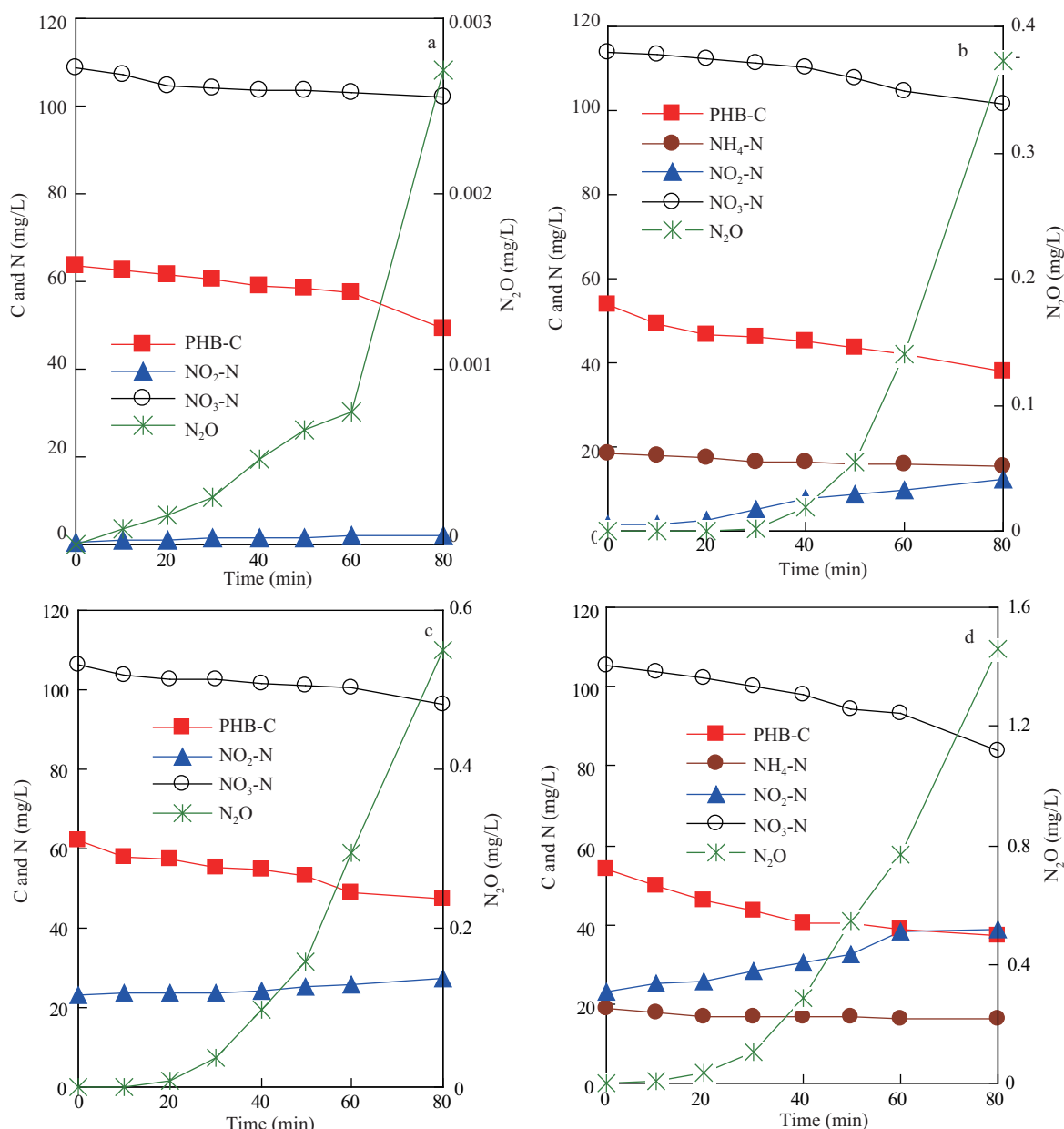


Fig. 3 Effects of ammonium on N₂O emission with PHB as the electron donor. (a) nitrate; (b) nitrate + ammonium; (c) nitrate + nitrite; (d) nitrate + nitrite + ammonium.

seems to be better fitted by other equations rather than the linear equation.

2.4 Effect of ammonium and electron donor on N₂O emission

In this study, it was shown that with the addition of ammonium, N₂O emission increased with methanol, acetate or PHB as the electron donor. Previous studies have shown that with the addition of ammonium, the denitrification rate could be increased (Nara et al., 2009; Waki et al., 2002). In our study, with methanol or acetate as the electron donor, the denitrifying rate was similar under conditions with and without the addition of ammonium; with PHB as the electron donor, the denitrifying rate increased significantly

with the addition of ammonium. The possible reason for increased N₂O emission could be that the addition of ammonium enhanced biomass production and induced some precursors (such as synthesis of more enzymes) responsible for N₂O emission, as denitrifiers would prefer ammonium over nitrate or nitrite as the nitrogen source for growth. During nitrification, N₂O emission has been shown to increase with increasing nitrification activities (Ahn et al., 2010; Yu et al., 2010). Li et al. (2008) found that N₂O emission and the denitrification efficiency both increased with increasing C/N ratios, which showed that high N₂O emission occurred with high microbial activities.

For methanol, acetate or PHB, a high N₂O emission potential was obtained with PHB or acetate as the electron

donor, while less N₂O emission was obtained for methanol as the electron donor. The highest N₂O emission ratio was 0.70% for methanol, 5.34% for acetate, and 34.79% for PHB. These results are similar to those obtained by Adouani et al. (2010), where a high N₂O emission potential was obtained with acetate rather than ethanol as the electron donor. However, Spector (1998) found that the N₂O emission ratio was in the range from 50%–80% with methanol as the electron donor, and these high ratios could be due to stripping applied under the anoxic condition. Wang et al. (2011) found that the ratio of anoxic-produced N₂O to the removed total nitrogen was around 7.77% with acetate as the electron donor, and Hanaki et al. (1992) found that the influent NO₃-N converted to N₂O could be up to 8% with acetate as the electron donor, and these ratios were similar to that obtained in the present study. In addition, Li et al. (2008) showed that acetate was a better electron donor for denitrification due to its suppression of N₂O emission, when the acetate supply was adequate. To date, few studies have investigated the contribution of PHB to N₂O emission. Here, the emission of N₂O with PHB as the electron donor could reach a very high level, which could be due to its low denitrifying rate, as the consumption of PHB was shown to be the rate-limiting step (Beun et al., 2002). Kampschreur et al. (2009) proposed that N₂O emission with PHB as the electron donor could be due to the existence of nitrite. Our results showed that the emission of N₂O with PHB as the electron donor could not be due to the existence of nitrite, as it was not produced during the initial phase even with both nitrate and nitrite coexisting (Fig. 3c).

3 Conclusions

With methanol, acetate or PHB as the electron donor, N₂O emission during denitrification increased with the addition of ammonium. By adding ammonium, with electron acceptors of nitrate or nitrate + nitrite, the N₂O emission ratio was 2.50 and 2.69 times higher for methanol, 3.93 and 3.17 times higher for acetate, and 1087 and 3.44 times higher for PHB compared to the ratios without the addition of ammonium. The highest ratio of the N₂O emission to the removed oxidized nitrogen was 0.70% for methanol, 5.34% for acetate, and 34.79% for PHB.

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References

- Adouani N, Lendormi T, Limousy L, Sire O, 2010. Effect of the carbon source on N₂O emissions during biological denitrification. *Resources, Conservation and Recycling*, 54(5): 299–302.
- Ahn J H, Kim S, Park H, Rahm B, Paglia K, Chandran K, 2010. N₂O emission from activated sludge processes, 2008–2009: results from a national monitoring survey in the united states. *Environmental Science and Technology*, 44(12): 4505–4511.
- APHA (American Public Health Association), AWWA (American Water Works Association), WEF (Water Environment Federation), 1999. *Standard Methods for the Examination of Water and Wastewater* (20th ed.). Washington DC, USA.
- Beun J J, Dircks K, Van Loosdrecht M C M, Heijnen J J, 2002. Poly-β-hydroxybutyrate metabolism in dynamically fed mixed microbial cultures. *Water Research*, 36(5): 1167–1180.
- Ciggin A S, Karahan O, Orhon D, 2009. Effect of high nitrate concentration on PHB storage in sequencing batch reactor under anoxic conditions. *Bioresource Technology*, 100(3): 1376–1382.
- Hanaki K, Hong Z, Matsuo T, 1992. Production of nitrous oxide gas during denitrification of wastewater. *Water Science and Technology*, 26(5-6): 1027–1036.
- IPCC, 2001. *Climate Change 2001: the Scientific Basis*. Cambridge University Press, Cambridge, UK.
- Itokawa H, Hanaki K, Matsuo T, 2001. Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. *Water Research*, 35(3): 657–664.
- Jiang S, Chen Y G, Zhou Q, Gu G W, 2007. Biological short-chain fatty acids (SCFAs) production from waste-activated sludge affected by surfactant. *Water Research*, 41(14): 3112–3120.
- Kampschreur M J, Temmink H, Kleerebezem R, Jetten M S M, Van Loosdrecht M C M, 2009. Nitrous oxide emission during wastewater treatment. *Water Research*, 43(17): 4093–4103.
- Karr D B, Waters J K, Emerich D W, 1983. Analysis of poly-β-hydroxybutyrate in *Rhizobium japonicum* bacteroids by ion-exclusion high-pressure liquid chromatography and UV detection. *Applied and Environmental Microbiology*, 46(6): 1339–1344.
- Law Y, Ye L, Pan Y, Yuan Z, 2012. Nitrous oxide emissions from wastewater treatment processes. *Philosophical Transactions of the Royal Society B*, 367(1593): 1265–1277.
- Li Q H, Li P, Zhu P P, Wu J H, Liang S Z, 2008. Effects of exogenous electron donor substrates on nitrous oxide emissions during the denitrification process of sequence batch reactors. *Environmental Engineering Science*, 25(8): 1221–1228.
- Nara K, Iwata K, Matsui T, Shigeno T, Omori T, 2009. Functional analysis of the thermophilic denitrifying bacterium *Geobacillus* sp. Strain TDN01 in continuous culture. *Journal of General and Applied Microbiology*, 55(2): 87–92.
- Oh J, Silverstein J, 1999. Acetate limitation and nitrite accumulation during denitrification. *Journal of Environmental Engineering*, 125(3): 234–242.
- Rodgers M, Wu G X, 2010. Production of polyhydroxybutyrate by activated sludge performing enhanced biological phosphorus removal. *Bioresource Technology*, 101(3): 1049–1053.

- Smolders G J F, Van der Meij J, Van Loosdrecht M C M, Heijnen J J, 1994. Model of the anaerobic metabolism of the biological phosphorus removal process: Stoichiometry and pH influence. *Biotechnology and Bioengineering*, 43(6): 461–470.
- Spector M, 1998. Production and decomposition of nitrous oxide during biological denitrification. *Water Environment Research*, 70(5): 1096–1098.
- Thomson A J, Giannopoulos G, Pretty J, Baggs E M, Richardson D J, 2012. Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. *Philosophical Transactions of the Royal Society B*, 367(1593): 1157–1168.
- Van Rijn J, Tal Y, Barak Y, 1996. Influence of volatile fatty acids on nitrite accumulation by a *Pseudomonas stutzeri* strain isolated from a denitrifying fluidized bed reactor. *Applied and Environmental Microbiology*, 62(7): 2615–2620.
- Waki M, Tanaka Y, Osada T, Suzuki K, 2002. Effects of nitrite and ammonium on methane-dependent denitrification. *Applied Microbiology and Biotechnology*, 59(2-3): 338–343.
- Wang Y Y, Geng J J, Guo G, Wang C, Liu S H, 2011. N₂O production in anaerobic/anoxic denitrifying phosphorus removal process: the effect of carbon sources shock. *Chemical Engineering Journal*, 172(2-3): 999–1007.
- Yu R, Kampschreur M J, Van Loosdrecht M C M, Chandran K, 2010. Mechanisms and specific directionality of autotrophic nitrous oxide and nitric oxide generation during transient anoxia. *Environmental Science and Technology*, 44(4): 1313–1319.

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