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N₂O accumulation from denitrification under different temperatures

Leong Soon Poh^{1,2}

Xie Jiang¹

Zhongbo Zhang³

Yu Liu^{1,2}

Wun Jern Ng^{1,*,2}

Phone (+65)6790-6813

Email WJNg@ntu.edu.sg

Yan Zhou^{1,*,2}

Phone (+65)6790-1103

Email zhouyan@ntu.edu.sg

¹ Advanced Environmental Biotechnology Centre (AEBC), Nanyang Environment and Water Research Institute (NEWRI)

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, Nanyang Technological University, 1 Cleantech Loop, CleanTech One, #06-08, Singapore, 637141 Singapore

² School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore, 639798 Singapore

³ DHI Water & Environment (S) Pte. Ltd., 1 Cleantech Loop, CleanTech One, #03-05, Singapore, 637141 Singapore

Abstract

The effects of temperature on nitrous oxide (N₂O) accumulation during denitrification and denitrification were investigated. Batch experiments were performed to measure N₂O accumulation at 25 and 35 °C. More N₂O accumulation was observed during denitrification at the higher temperature as compared with full denitrification and low temperature tests. The highest

nitrite concentration tested in this study (25 mg/L NO_2^- N and pH 8.0) did not show inhibitory effect on N_2O reduction. It was found that the major cause of more N_2O accumulation during denitrification at higher temperature was due to higher N_2O production rate and lower N_2O solubility. Specific nitrate, nitrite, and N_2O reduction rates increased 62, 61, and 41 %, respectively, when temperature rose from 25 to 35 °C. The decrease of N_2O solubility in mixed liquor at 35 °C (when compared to 25 °C) resulted in faster diffusing rate of N_2O from liquid to gas phase. It was also more difficult for gas phase N_2O to be re-dissolved. The diffused N_2O was then accumulated in the headspace, which was not available for denitrification by denitrifiers. The results of this study suggest higher temperature may worsen N_2O emission from wastewater treatment plants (WWTPs).

Keywords

Nitrous oxide (N_2O)
Denitrification
Temperature
Greenhouse gas
Wastewater treatment

Introduction

In the tropical regions, operating temperatures of wastewater treatment plants (WWTPs) are often 30 °C and above (Metcalf ~~et al~~ & Eddy. 2013). Temperature of wastewater is expected to gradually rise due to global warming.

Denitrification is a part of the nitrogen removal process. In this process, nitrate (NO_3^-) is sequentially reduced to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and nitrogen gas (N_2) by heterotrophic denitrifiers using organic carbon as electron donor (Metcalf ~~et al~~ & Eddy. 2013). As denitrification is a biochemical process, the reaction rate of each step is expected to change with rising temperature. However, the changes may not be proportional for each reduction step. Hence, accumulation of intermediates may occur due to possible “imbalanced” reduction rates.

N_2O accumulation and emission during the nitrogen removal process is a concern because it is a greenhouse gas (GHG) with a global warming potential

which is more than 300 times that of CO_2 (IPCC 2001). Therefore, the emission of N_2O must be minimized from wastewater treatment process. Denitrification has been reported to be a N_2O emission source (Ahn et al. 2010; Kampschreur et al. 2009). Competition for organic carbon (electron donor) and inhibition of N_2O reductase at high nitrite concentrations have been regarded as the causes of N_2O accumulation during denitrification (Ahn et al. 2010; Kampschreur et al. 2009; Pan et al. 2013a; Zhou et al. 2008). It is, however, currently unclear how temperature affects N_2O production, reduction, and accumulation during denitrification.

Most studies on N_2O emission from denitrification had been conducted at indoor room temperature of 21 to 25 °C (Baytshtok et al. 2009; Hu et al. 2013; Li et al. 2013; Lu and Chandran 2010; Pan et al. 2012, 2013a, b). More N_2O emission from an activated sludge process had once been reported at higher temperature (Gejlsbjerg et al. 1998), but it was not clear if the higher emission was due to temperature change or oxygen inhibition of N_2O reductase.

In this study, it is hypothesized that reaction kinetics and N_2O solubility may play significant roles in N_2O accumulation during denitrification at different temperatures. Biological denitrification rates are expected to increase when temperature rises. On the other hand, N_2O solubility in pure water decreases by 23 % when temperature increase from 25 to 35 °C (Weiss and Price 1980). N_2O solubility is an important factor affecting N_2O accumulation and emission because only dissolved N_2O can be degraded by N_2O reductase (Zumft 1997). A lower N_2O solubility at higher temperature will result in more N_2O escaping into the atmosphere before denitrification can be completed. The interactions between these factors are expected to influence N_2O accumulation rates at different temperatures. In this study, the effects of temperature on N_2O accumulation during denitrification were studied using three types of dissolved nitrogen oxides (nitrate, nitrite, and N_2O) as primary electrons acceptors. Specific reduction rates of these nitrogen oxides at two different temperatures (25 and 35 °C) were compared. The possible inhibitory effects of nitrite on N_2O reduction were also investigated. Temperature effects on N_2O distribution between liquid and gas phase in a closed reactor were also studied using computer simulation.

Materials and methods

Sludge source

The sludge used in this study was collected from a local municipal WWTP. Operating temperature of this WWTP varies from 24 to 33 °C, depending on day and night temperature variation, with an average temperature of 30.6 °C. The modified Ludzack–Ettinger (MLE) process is operated for nitrogen removal at this plant (Metcalf ~~Ine et al~~ & Eddy. 2013). Operating conditions of the plant and wastewater characteristics are summarized in Table 1. Prior to sludge collection, the performance of the plant was stable.

Table 1

Characteristics of MLE process at the WWTP

	Anoxic tank	Aerobic tank
Temperature (°C)	30.6	30.8
pH	7.5	6.8
DO (mg/L)	0.02	0.5
	Influent	Effluent
COD (mg/L)	520	25
NH_4^+ (mg/L $\text{NH}_4^+ - \text{N}$)	43	0.5
NO_3^- (mg/L $\text{NO}_3^- - \text{N}$)	Not detected	16

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The sludge was washed with a mineral solution twice to remove dissolved organics and nitrogen compounds. The mineral solution was formulated to be similar to domestic wastewater with trace organic matter and nitrogen compounds. It was made by adding to tap water the following compounds (mg/L): $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (47), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (27), KH_2PO_4 (16), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.6), H_3BO_3 (0.06), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.012), KI (0.072), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.048), $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.048), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.06), EDTA- $\text{Na} \cdot 2\text{H}_2\text{O}$ (5.1), bacto-peptone (3.5), and yeast extract (3.5). The biomass was then diluted to approximately 2500 mg/L volatile suspended solids (VSS) with the mineral solution and prepared for the batch experiments. Washed and diluted sludge was acclimatized to required temperature in a water bath for at least 2 h before the start of each experiment to minimize the effects of temperature shock.

Batch experiments

Batch experiments were carried out to determine the specific reduction rates

of three types of dissolved nitrogen oxides (NO_3^- , NO_2^- , and N_2O) at two temperatures (25 and 35 °C). The temperatures at the two extreme ends of the temperature range of the WWTP were selected to better illustrate the effects of temperature on N_2O accumulation. The effect of nitrite on N_2O accumulation at different temperatures was studied with two nitrite concentrations (15 and 25 mg/L NO_2^- -N). Batch experiments were repeated with and without headspace to investigate the effect of N_2O solubility on N_2O emission at different temperatures. In total, 14 sets of batch experiments were performed in this study (Table 2).

Table 2

Summary of all batch experiments

Set	Temperature (°C)	Primary NO_x concentration (mg/L - N)	Headspace volume (L)	Liquid volume (L)	Remarks
1	25	NO_3^- , 25	0.75	1.5	NO_3^- with headspace
2	35				
3	25	NO_2^- , 25	0.75	1.5	NO_2^- with headspace
4	35				
5	25	NO_2^- , 25	0	2.25	NO_2^- without headspace
6	35				
7	25	NO_2^- , 15	0.75	1.5	Low NO_2^- with headspace
8	35				
9	25	NO_2^- , 15	0	2.25	Low NO_2^- without headspace
10	35				
11	25	N_2O , 25	0	2.25	N_2O without headspace
12	35				
13	25	N_2O , 25	0.75	1.5	N_2O with headspace

Set	Temperature (°C)	Primary NO _x , concentration (mg/L - N)	Headspace volume (L)	Liquid volume (L)	Remarks
14	35				

A 2.25-L gas tight, water-jacketed reactor was used for all experiments. Temperature of the mixed liquor was controlled at 25.0 or 35.0 ± 0.5 °C by continuously circulating chilled or warm water through the water jacket from a water bath (PolyScience, IL, USA). The mixed liquor was continuously stirred with a magnetic stirrer at 300 RPM. For experiments with headspace, the liquid volume was 1.5 L and headspace volume was 0.75 L. The liquid volume was 2.25 L for experiments without headspace.

Before each experiment, the biomass was sparged with N₂ gas for 5 min to remove residual oxygen. The reactor was sealed immediately after sparging. Sodium acetate was used as the dissolved organic carbon source for denitrification in all sets. Organic carbon concentration, in terms of chemical oxygen demand (COD), was controlled at a COD/N ratio of 10 (250 or 150 mg/L) at the start of each set. The high COD/N ratio was selected to avoid electron donor limitation for denitrification. pH of the mixed liquor was maintained at 8.0 ± 0.1 by manual dosing of 0.25 M HCl or NaOH. Every batch experiment was carried out in duplicate. The details of all batch experiments are summarized in Table 2.

Nitrate as primary electron acceptor (set 1–2)

Sodium nitrate (25 mg/L NO₃⁻-N) was used in experiment sets 1 and 2 with headspace. Each test lasted for 90 min; gas and liquid samples were taken at 15-min intervals. The specific reduction rates of nitrate, intermediate nitrite, and N₂O at 25 and 35 °C were determined. N₂O accumulation in dissolved and gas phase were also monitored.

Nitrite as primary electron acceptor (set 3–10)

Sodium nitrite was used in sets 3 to 10. In these eight sets of experiments, the specific reduction rates of nitrite and N₂O at different temperatures were determined. The effects of temperature, nitrite, and N₂O solubility on gaseous and dissolved N₂O accumulation during denitrification (nitrite reduction) were also analyzed. The initial concentration of sodium nitrite was 25 mg/L NO₂⁻-N for sets 3 to 6, while that was 15 mg/L NO₂⁻-N in sets 7 to 10. The

experiments lasted for 90 min and gas and liquid samples were taken at 15-min intervals for sets 3 to 6. In sets 7 to 10 where lower nitrite was applied, the experiments lasted for 60 min with samples taken at 10-min intervals. Sets 3, 4, 7, and 8 were operated with headspace and the rest were without headspace.

N₂O as primary electron acceptor (set 11–14)

Saturated N₂O solution was used as the source of dissolved N₂O for sets 11 to 14. In these sets of experiment, the specific N₂O reduction rates under different temperatures were determined. As a part of the study of the effects of N₂O solubility on specific N₂O reduction rates at different temperatures, headspace and dissolved N₂O concentration data from sets 11 to 14 were used in computer simulation to evaluate N₂O distribution in a closed reactor system. Saturated N₂O solution was prepared by sparging deionized water with pure N₂O gas for 5 min (Zhou et al. 2008). When the saturated N₂O solution was ready, a measured volume was injected into the mixed liquor for a final dissolved concentration of 25 mg/L N₂O-N. Sets 11 and 12 were performed without headspace, while sets 13 and 14 had headspace. The 25 °C experiments (sets 11 and 13) lasted for 90 min with gas sampling (set 13) at 15-min intervals. The 35 °C sets (12 and 14) were operated for 60 min with gas sampling (set 14) at 10-min intervals.

Sampling and analytical methods

Liquid samples (8 mL) were collected at regular intervals as described above (except for sets 11–14). Collected mixed liquor samples were filtered through 0.45-µm disposable syringe filters and stored at 4 °C before measurement. In order to balance the headspace gas pressure, equal volume of washed biomass was replaced into the reactor immediately after each liquid sample was taken. NO₃[−] and NO₂[−] concentrations were measured according to methods 4500-NO₃[−] B and 4500-NO₂[−] described in standard methods (APHA 2012). Measurements of nitrogen oxide compounds are expressed as mass-nitrogen (NO_x-N) for easier comparison. Soluble COD concentration was measured using low range COD measurement kit (HACH, Loveland, CO, USA). Soluble COD concentrations decreased proportionally according to denitrification rates in all experiments of this study (data not shown).

Dissolved N₂O concentration in the mixed liquor was continuously read and logged with an online N₂O sensor at 1 s intervals (N2O-R, Unisense A/S,

Aarhus, Denmark). The online N₂O sensor was calibrated strictly according to the manufacturer's instructions. Gas phase samples (0.2 mL) were collected from the headspace before each liquid sampling for N₂O measurements. Gaseous N₂O concentration was measured using GC-μECD (7890A GC, Agilent, Santa Clara, CA, USA) equipped with a capillary column (GS-Gaspro, Agilent J&W, Santa Clara, CA, USA).

Gas pressure in the headspace was measured with a handheld manometer before each gas sampling. Gas pressure and volume were used for calculation of gaseous N₂O quantity. The amount of N₂O in the headspace was calculated with the ideal gas law equation.

$$PV = nRT$$

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where P is the measured absolute gas pressure in the headspace (Pa), V is measured volume of N₂O in the headspace (m³), n is amount of N₂O (moles), R is the universal gas constant (8.314 J/mol. K), and T is the absolute temperature (298 or 308 K). The amount of N₂O in moles was then multiplied by 0.028 to convert to mass in terms of nitrogen (mg N).

Mixed liquor samples were collected in triplicates before and at the end of all batch experiment for mixed liquor volatile suspended solids (MLVSS) measurements according to Standard Methods 2540E (APHA 2012).

Calculations

Specific reduction rates of nitrogen oxides

The specific nitrogen oxide reduction rate of primary nitrogen oxides (nitrate in sets 1 and 2, nitrite in sets 3 to 10, N₂O in sets 11 to 14) was calculated by linear regression of the measured amount of nitrate, nitrite, and N₂O and then dividing the gradient from linear regression by the measured amount of biomass.

The apparent reduction rates of intermediate nitrogen oxides (NO₂⁻ and N₂O) were calculated by regression of profiles of the intermediate nitrogen oxides. The real reduction rates of intermediate nitrogen oxides were then calculated by adding the apparent reduction rates to the reduction rates of nitrogen oxide immediately upstream (Pan et al. 2013a).

$$r_{\text{NO}_3^-} = r_{\text{NO}_{3,a}^-}$$

$$r_{\text{NO}_2^-} = r_{\text{NO}_{2,a}^-} + r_{\text{NO}_3^-}$$

$$r_{\text{N}_2\text{O}} = r_{\text{N}_2\text{O}_a} + r_{\text{NO}_2^-}$$

where $r_{\text{NO}_{x,a}}$ are apparent reduction rates and r_{NO_x} are real reduction rates.

NO was assumed to be quickly broken down after formation and of negligible quantity (Richardson et al. 2009; Schreiber et al. 2009; Zeng et al. 2003).

Simulation of N_2O distribution between gas and liquid phase

The model was developed to simulate the N_2O degradation and transfer between gas–liquid phases under anoxic condition based on ASM_N model (Hiatt and Grady 2008). Carbon removal and four-step denitrification involving N_2O generation and degradation were incorporated in the model. Biomass decay and hydrolysis of organic nitrogen in biomass were also considered in this model. Aerobic growth and nitrification were not included. In order to simulate the transfer of N_2O between gas–liquid phases, the following formula was employed:

$$F_{\text{N}_2\text{O}} = V_{\text{N}_2\text{O}} \left(\frac{P_{\text{N}_2\text{O}} * 28}{K'_H} - C_{\text{N}_2\text{O}} \right)$$

where $F_{\text{N}_2\text{O}}$ is the N_2O transfer rate between mixed liquor and headspace in the reactor (mg N/L·day), $V_{\text{N}_2\text{O}}$ is the specific mass transfer coefficient of N_2O in the system (day^{-1}), $P_{\text{N}_2\text{O}}$ is the partial pressure of N_2O in the headspace (atm), K'_H is the Henry's Law constant (atm·l/mmol) and $C_{\text{N}_2\text{O}}$ is the concentration of dissolved N_2O in the mixed liquor (mg N/L).

The mass transfer coefficient and Henry's Law constant of N_2O in the system were calibrated with the measured concentrations of N_2O in headspace and mixed liquor (sets 13 and 14) using the Monte Carlo method in Matlab®. The flux of N_2O between liquid and gas phase was then calculated with the following equation:

$$\text{N}_2\text{O}_{\text{flux}} = F_{\text{N}_2\text{O}} * V$$

where $\text{N}_2\text{O}_{\text{flux}}$ is the flux of N_2O between gas–liquid phases (mg N/d), V is the

volume of liquid phase (l). A positive flux indicates N_2O transfer from liquid to gas phase and conversely, dissolution of N_2O from the headspace into the liquid phase is indicated by a negative flux. When the flux is 0, it indicates that N_2O in the gas and liquid phases are in equilibrium.

The N_2O specific mass transfer coefficient is influenced by factors such as the gas–liquid interfacial area, liquid depth, agitation speed, and temperature, of which gas–liquid interfacial area, liquid depth, and agitation speed were kept identical in all batch experiments. Henry’s Law constant is dependent on temperature and concentration of dissolved solids in the liquid phase.

Contributions from these factors were consolidated into these two terms ($V_{\text{N}_2\text{O}}$ and K'_H) for simplicity.

Results

Nitrate as primary electron acceptor

Denitrification started immediately after the addition of sodium nitrate and sodium acetate at both temperatures (25 and 35 °C). Nitrate decreased in linear trends (Fig. 1). The specific denitrification rates for nitrate were 5.8 and 9.4 mg N/g VSS·h at 25 and 35 °C respectively, which was a 62 % increase in specific nitrate reduction rates from 25 to 35 °C.

Fig. 1

Denitrification of 25 mg/L NO_3^- -N; **a** set 1, 25 °C; **b** dissolved and gas phase N_2O accumulation of set 1; **c** set 2, 35 °C; **d** dissolved and gas phase N_2O accumulation of set 2

Nitrite was accumulated under both temperature conditions. At 25 °C, nitrite was accumulated in a gradual and linear trend across the 90-min test period. At 35 °C, nitrite was accumulated in the first 60 min before decreasing as nitrate was depleted in the final 30 min. The specific reduction rates for intermediate nitrite were 3.8 and 6.8 mg N/g VSS·h for 25 and 35 °C, respectively, increasing by 79 % with the increase in temperature.

N_2O accumulation displayed different trends at the two temperatures. At 25 °C, dissolved N_2O was below 0.10 mg N_2O -N throughout the experiment. N_2O in the gas phase was below detection limits in the first 60 min and increased to 0.10 and 0.06 mg N_2O -N at the 75th and 90th minute, respectively. At 35 °C, most N_2O accumulation occurred in the gas phase. The highest amount of total N_2O accumulation (gaseous and dissolved) was recorded at the 90th minute at 2.7 mg N_2O -N, of which 1.9 mg N_2O -N was in the gas phase. The specific reduction rates for intermediate N_2O were 3.8 and 6.3 mg N/g VSS·h at 25 and 35 °C, respectively, which increased by 66 %.

Nitrite as primary electron acceptor

In denitrification experiments, sets 3 to 10, nitrite decreased in linear patterns at both temperatures. Examples of the linear nitrite reduction patterns (sets 3 and 4) are illustrated in Fig. 2. Specific nitrite reduction rate increased 61 % between 25 and 35 °C when initial nitrite concentration was 25 mg/L NO_2^- N.

The specific nitrite and N_2O reduction rates of sets 3 to 10 are presented in Table 3. At 35 °C, specific nitrite reduction rates were 55 to 72 % faster than those at 25 °C. In sets with higher initial nitrite concentrations (sets 3 to 6), specific nitrite and N_2O reduction rates were higher, ranging from 4.3 to 7.9 $\text{mg NO}_2^- \text{N/g VSS}\cdot\text{h}$ and 4.3 to 7.2 $\text{mg N}_2\text{O-N/g VSS}\cdot\text{h}$ as compared to the specific nitrite and N_2O reductions rates in sets 7 to 10 with lower initial nitrite concentrations at 4.0–7.0 $\text{mg NO}_2^- \text{N/g VSS}\cdot\text{h}$ and 3.7–6.3 $\text{mg N}_2\text{O-N/g VSS}\cdot\text{h}$, respectively. Specific nitrite reduction rate is dependent on the initial nitrite concentration.

Fig. 2

Denitrification of 25 $\text{mg/L NO}_2^- \text{N}$; **a** set 3, 25 °C; **b** set 4, 35 °C

Table 3

Specific nitrite reduction rates (rNO_2^-), specific N_2O reduction rates (rN_2O), and total N to 10

Set	Temperature (°C)	Initial nitrite concentration (mg/L $\text{NO}_2^- \text{N}$)	Headspace	rNO_2^- (mg N/g VSS·h)	Increase (%)	rN_2O (mg N/g VSS·h)
3	25	25	Yes	4.9	61	4.7
4	35			7.9		6.4
5	25	25	No	4.3	72	4.3
6	35			7.4		7.2
7	25	15	Yes	4.0	75	3.7
8	35			7.0		4.6

Set	Temperature (°C)	Initial nitrite concentration (mg/L NO_2^- -N)	Headspace	rNO_2^- (mg N/g VSS·h)	Increase (%)	rN_2O (mg N/g VSS·h)
9	25	15	No	4.2	55	4.2
10	35			6.5		6.3

Specific N_2O reduction rates between 25 and 35 °C increased by a smaller magnitude when compared to specific nitrite reduction rates. These different increments of specific nitrite and N_2O reduction rates were observed in both denitrification experiments with and without headspace. In sets with headspace (sets 3, 4, 7, and 8), increment of specific nitrite reduction rate was two to three times of the increment of specific N_2O reduction rate when temperature increased from 25 to 35 °C. However, in experiments without headspace (sets 5, 6, 9, and 10), increment of specific nitrite and N_2O reduction rate differed less significantly when temperature was raised (Table 3). Nitrite and N_2O reduction occurred at identical rates in experiments without headspace at 25 °C (4.3 mg N/g VSS·h for both specific nitrite and N_2O reduction rates in set 5 and 4.2 mg N/g VSS·h for both specific nitrite and N_2O reduction rates in set 9). In sets 6 and 10 (35 °C without headspace), N_2O reduction occurred at slightly slower rates (7.2 and 6.3 mg N_2O -N/g VSS·h respectively) compared to nitrite reduction (7.4 and 6.5 mg NO_2^- -N/g VSS·h respectively), leading to accumulations of dissolved N_2O (Fig. 3 d, h).

Fig. 3

N_2O accumulation during denitrification sets 3 to 10; **a** 25 mg/L NO_2^- -N, 25 °C, with headspace; **b** 25 mg/L NO_2^- -N, 35 °C, with headspace; **c** 25 mg/L NO_2^- -N, 25 °C, no headspace; **d** 25 mg/L NO_2^- -N, 35 °C, no headspace; **e** 15 mg/L NO_2^- -N, 25 °C, with headspace; **f** 15 mg/L NO_2^- -N, 35 °C, with headspace; **g** 15 mg/L NO_2^- -N, 25 °C, no headspace; **h** 15 mg/L NO_2^- -N, 35 °C, no headspace

N_2O accumulation was observed in all denitrification experiments and more N_2O accumulation occurred at 35 °C compared to 25 °C. In sets with headspace, gas phase N_2O made up most of the accumulation, consisting of

33–98 % of total N_2O accumulated (Fig. 3). The accumulation of N_2O in headspace is more severe when denitritation was performed at 35 °C compared to 25 °C (Fig. 3 a, b, e, f).

Figure 4 presents the net flux of N_2O between liquid and gas phases (sets 3 and 4) from the simulation study. At 35 °C, N_2O flux peaked mildly at around 0.2 mg N/min while the maximum N_2O flux at 25 °C was close to 0.02 mg N/min. The flux then decreased as dissolved N_2O was removed by biological reduction in the liquid phase (Fig. 4). The decrement indicated less N_2O was diffusing to gas phase until the flux reached the equilibrium point (flux = 0 mg N/min). The time taken for N_2O flux reached the equilibrium is around 44 and 54 min when temperature was at 25 and 35 °C, respectively. After the equilibrium point, flux remained in the negative zone indicating that N_2O in the headspace was re-dissolving into the mixed liquor. Removal of N_2O by biological denitrification and diffusion from liquid phase were calculated based on mass balance. Upon reaching the equilibrium point, 15.6 and 28.0 mg of N_2O -N were derived from upstream NO_2^- reduction at 25 and 35 °C. From the N_2O produced, 93 % (15.0 mg N_2O -N) was removed from liquid phase through biological reduction at 25 °C. At higher temperature (35 °C), only 73 % (20.5 mg N_2O -N) of the N_2O produced was biologically denitrified.

Fig. 4

Net N_2O transfer rate in sets 3 (25 °C, with headspace) and 4 (35 °C, with headspace) with NO_2^- as electron acceptor

Nitrous oxide as primary electron acceptor

Four sets (11 to 14) of experiments were conducted using dissolved N_2O as

the primary electron acceptor. In sets 11 (25 °C) and 12 (35 °C) without headspace, dissolved N_2O concentration expressed a linearly decreasing pattern (Fig. 5 a, b). Specific N_2O reduction rate increased 41 % from 8.0 to 11.3 mg N/g VSS·h from 25 to 35 °C. In sets 13 (25 °C) and 14 (35 °C) with headspace, dissolved N_2O concentrations decreased in a non-linear pattern (Fig. 5 c, d). Specific N_2O reduction rates for total N_2O (gaseous and dissolved) were 7.9 and 9.4 mg N/g VSS·h for 25 and 35 °C, respectively, increasing by 19 %.

Fig. 5

N_2O reduction sets 11 to 14; **a** 25 °C, no headspace; **b** 35 °C, no headspace; **c** 25 °C, with headspace; **d** 35 °C, with headspace

In the initial period of sets 13 and 14, dissolved N_2O depleted quickly through both biological reduction and conversion into the gaseous form, the amount of dissolved N_2O decreased with a steeper gradient. Gaseous N_2O built up rapidly in the headspace after saturated N_2O solution was injected into the mixed liquor. In later period of the experiments when dissolved N_2O concentrations were lower, gaseous N_2O re-dissolved into the mixed liquor as biological reduction continued, resulting in a more gradual dissolved N_2O

concentration gradient towards the end of the experiment. The interference of N_2O phase exchanges resulted in specific N_2O reduction rates falling by 1.3 % at 25 °C and 20 % at 35 °C when compared with sets without headspace (Fig. 5).

Henry's Law constant (K'_H) and specific mass transfer coefficient ($V_{\text{N}_2\text{O}}$) of N_2O at 25 and 35 °C were calibrated using specific N_2O reduction rates from sets 11 and 12 and N_2O concentration data from sets 13 and 14. The calibration results were summarized in Table 4.

Table 4

Calibrated N_2O Henry's Law constant and specific mass transfer coefficient

Temperature (°C)	Henry's Law constant, K'_H (atm·l/mmol)	Specific mass transfer coefficient, $V_{\text{N}_2\text{O}}$ (day ⁻¹)
25	0.061	0.051
35	0.087	0.420

Higher specific mass transfer coefficient in the system indicated a more active gas–liquid exchange at higher temperature. The specific mass transfer coefficient was eight times at 35 °C of the value at 25 °C (0.42 vs. 0.051 day⁻¹).

The flux of N_2O from liquid phase to headspace in sets 13 and 14 were simulated using the calibrated values and concentration data and illustrated in Fig. 6a. N_2O flux from liquid to headspace peaked rapidly upon the introduction of N_2O into the mixed liquor. The net N_2O transfer rate decreased at a slower pace at 35 °C (Fig. 6a). At 25 °C (set 13), the net N_2O flux reached equilibrium after 20 min while it reached equilibrium at the 26th minute when temperature was 35 °C (set 14). The cumulative amount of N_2O diffused from liquid to gas phase is presented in Fig. 6b. In the first 20 min, when N_2O mainly diffused from liquid to gas phase, 27 % more of N_2O diffused to headspace at 35 °C compared with the amount diffused at 25 °C. More N_2O was thus emitted into headspace at higher temperature (35 °C). Upon reaching the equilibrium point at 25 °C, the amount of N_2O degraded by denitrifiers was 8.3 mg N_2O -N whereas 9.3 mg N_2O -N diffused to headspace. The amount of N_2O diffused to gas phase was 12.1 mg N_2O -N and 12 mg N_2O -N was removed through denitrification at 35 °C.

Fig. 6

a Flux of N_2O from liquid phase to headspace in set 13, 25 °C, and set 14, 35 °C. **b** Cumulative N_2O transfer from liquid to gas phase in set 13, 25 °C, and set 14, 35 °C

AQ4

Discussion

The results indicated denitrification rates, nitrite concentrations, headspace, and N_2O solubility at different temperatures had varied effects on N_2O accumulation during denitrification. The contribution of each mechanism on N_2O accumulation is discussed in this section.

Imbalanced denitrification rates increment and electron competition

The specific nitrate, nitrite, and N_2O reduction rates did not increase uniformly when temperature increased from 25 to 35 °C (Table 5). Data

collected in this study is in agreement with previous reports that denitrification rates increase with rising temperature (Dawson and Murphy 1972; Lewandoswki 1982). However, the extents of increase observed in this study are lower than that of earlier studies.

Table 5

Specific reduction rates of sets 1, 2, 3, 4, 11, and 12 (initial dissolved $\text{NO}_x = 25 \text{ mg/L NO}_x\text{-N}$).

Specific reduction rates (mg N/g VSS·h) AQ5					
Set	Temperature	Nitrate	Nitrite	N_2O	Remarks
1	25 °C	5.8	3.8	3.8	25 mg/L $\text{NO}_3^- \text{N}$ with headspace
2	35 °C	9.4	6.8	6.3	
% increase		62	79	66	
3	25 °C		4.9	4.7	25 mg/L $\text{NO}_2^- \text{N}$ with headspace
4	35 °C		7.9	6.4	
% increase			61	36	
11	25 °C			8.0	25 mg/L $\text{N}_2\text{O-N}$ without headspace
12	35 °C			11.3	
% increase				41	

The differences between upstream and downstream specific reduction rates between nitrate, nitrite, and N_2O lead to accumulation of intermediate nitrite and N_2O during denitrification and denitrification (sets 1 to 10). Accumulation of intermediate NO_x was higher when there were larger differences in specific reduction rates at the higher temperature (35 °C).

Intracellular competition for electrons is a possible cause for the lower specific N_2O reduction rate during denitrification. It was reported that nitrate reduction receives electrons from the ubiquinone/ubiquinol (UQ/UQH_2) pool, while nitrite, nitric oxide, and nitrous oxide reductions receive electrons from

the cytochrome c550 pool which in turn obtains electrons from the UQ/UQH₂ pool (Richardson et al. 2009). From this configuration of intracellular electrons distribution, it can be seen that nitrate reductase receives its electrons nearer to the electron source (UQ/UQH₂ pool), without direct competition from other nitrogen oxide reductases. While nitrite, NO, and N₂O reductases receive their electrons from a shared source after an additional step (cytochrome c550 pool), the competition for electrons between nitrite, NO, and N₂O reductases is therefore higher than that for nitrate reductase. It was reported that electrons competition occurred despite carbon abundant conditions in a methanol fed denitrification process at 23 °C (Pan et al. 2013a). Ribera-Guardia et al. (2014) also demonstrated that a preference in electron flow, which gives priority to nitrate/nitrite reduction in their work. The authors suggested that N₂O reduction rate could be affected more significantly compared to nitrate and nitrite reduction when electron competition occurred. The preference of electron flow would also be the reason, which led to the difference of denitrification rate increment. Higher specific reduction rates at warmer temperature (35 °C), shown in this study, might induce a tighter competition for electrons, leading to higher accumulation of N₂O. Further investigation is required to understand the true cause of both higher nitrite and N₂O accumulation at high temperature.

AQ6

Nitrite inhibition

N₂O accumulation was previously linked to high nitrite concentration during biological wastewater treatment. It was later revealed that free nitrous acid (FNA) was the true inhibitor of N₂O reductase (Zhou et al. 2011). In this study, the highest nitrite concentration was 25 mg/L NO₂⁻-N (sets 3 and 4) and FNA concentrations at pH 8.0 were 0.56 and 0.44 µg/L N₂O-N at 25 and 35 °C, respectively. According to the equilibrium equation, a higher temperature will result in lower FNA concentration (Anthonisen et al. 1976). These FNA concentrations were much lower than 10 µg/L N, which was the threshold found to cause inhibition of anoxic metabolism (Zhou et al. 2010).

Comparing the specific N₂O reduction rates in sets 3 (25 mg/L NO₂⁻-N, 25 °C) and 7 (15 mg/L NO₂⁻-N, 25 °C) (Table 2 and Fig. 3), it can be observed that N₂O reduction occurred at rather similar rates. Higher initial nitrite concentration did not cause lower specific N₂O reduction rate. Same observations were also found in experiments at 35 °C (Set 4 and 8). Based on the similarities in specific N₂O reduction rates, it can be concluded that nitrite

up to 25 mg/L NO_2^- -N at pH 8.0 did not significantly affect N_2O reduction during denitrification between 25 and 35 °C. This suggested that inhibitory effect of FNA was not the major cause of N_2O accumulation in this study.

Headspace and effects of N_2O solubility

N_2O accumulation was recorded in all denitrification experiments (sets 3 to 10, Fig. 3). The highest N_2O accumulation occurred in sets with headspace and warm temperature (sets 4 and 8, Fig 3b, f). In these sets, most of the accumulated N_2O occurred in the headspace. At 35 °C, the solubility of N_2O in mixed liquor is 30 % lower than 25 °C (Table 54). The presence of headspaces in sets 4 and 8 allowed N_2O to diffuse and accumulate in the gas phase due to the lower solubility at warmer temperature. The accumulation of more N_2O in gas phase resulted in less-dissolved N_2O available for denitrification. In sets without headspace (sets 5, 6, 9, and 10), N_2O produced from nitrite reduction were unable to diffuse into the gas phase. Dissolved N_2O was quickly reduced by denitrifiers resulting in higher specific N_2O reduction rates that were closer to its production rates and hence, less N_2O accumulations.

The calibrated Henry's constants of N_2O developed in this study (Table 4) were in similar range to the Henry's constant of N_2O in pure water (0.04 and 0.053 atm·l/mmol at 25 and 35 °C, respectively) (Haynes 2013). The higher Henry's constant at 35 °C reflected a lower N_2O solubility at higher temperature. N_2O solubility had been reported to decrease by 20.4 to 28.2 % when temperature increased from 25 to 35 °C (Clarke 1964; Haimour and Sandall 1984; Versteeg and Van Swaalj 1988; Yaghi and Houache 2008). The effects of solubility on N_2O accumulation could be more obvious when N_2O was used as primary electron acceptor with headspace in sets 13 and 14. The simulation results demonstrated that the lower solubility of N_2O could lead to higher mass transfer coefficient at 35 °C. The mass transfer coefficient represented how fast N_2O was transferred between liquid and gas phases. N_2O exchange between headspace and mixed liquor is expected to occur faster and be more responsive to changes in gaseous and dissolved N_2O concentration at higher temperature. As a result, cumulative N_2O diffused from liquid to headspace was higher when temperature rose. In addition, lower solubility hindered N_2O re-dissolve back to liquid phase after soluble N_2O was reduced by microorganisms. It took longer time for net N_2O transfer rate to achieve equilibrium at 35 °C. More N_2O diffused to the headspace and slower re-dissolution of N_2O caused higher accumulation of N_2O in gas phase. The

gaseous form of N_2O was not available to be removed by biological denitrification even though the potential specific N_2O reduction is higher at 35 °C (11.3 mg N/g VSS·h) compared with the rate at 25 °C (8.0 mg N/g VSS·h). The lower solubility at higher temperature thus resulted in more N_2O emission.

Implications to denitrification process in wastewater treatment plant operation

This study described higher operating temperature may increase denitrification kinetics, which can potentially reduce capital costs of treatment systems. However, imbalanced enhancement of different reactions may also cause accumulation of intermediates. Care should be taken to ensure the proper management of these accumulated intermediate nitrogen oxides to avoid release into the environment.

More gaseous N_2O accumulation occurred at higher temperature due to higher N_2O production rate and lower N_2O solubility. Thus, a significant amount of N_2O may be emitted from the WWTP under high-temperature condition. Operators running anoxic denitrification processes at warm temperature may need to consider covering their reactors so as to encourage the re-dissolution of gaseous N_2O and minimize unintentional release of N_2O into the atmosphere. It is also suggested that denitrification off-gas be re-circulated to prolong the retention time of N_2O in the system to enhance N_2O reduction and reduce emissions. This study would also suggest warmer temperatures may worsen GHG emission from WWTPs. The case could be more severe in tropical countries. It would be interesting to investigate if the reaction kinetics can adapt to temperature changes and establish a new equilibrium following long term operation.

In summary, the impact of temperature on nitrate, nitrite, and N_2O reduction was investigated in this study. It was found that denitrification rates of different nitrogen oxide compounds do not respond in similar magnitudes to temperature changes. When temperature increased from 25 to 35 °C, specific nitrate, nitrite, and N_2O reduction rates were increased by 62, 61, and 41 %, respectively. The imbalanced changes in specific reduction rates resulted in more intermediate nitrogen oxides accumulations. Higher N_2O production rates and lower N_2O solubility were found to cause more N_2O accumulation at higher temperature.

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Conflict of interest The authors declare that they have no competing interests.

References

- Ahn JH, Kim S, Park H, Rahm B, Pagilla K, Chandran K (2010) N₂O emissions from activated sludge processes, 2008-2009: results of a national monitoring survey in the United States. *Environ Sci Technol* 44(12):4505–4511. doi: 10.1021/es903845y
- Anthonisen AC, Loehr RC, Prakasam TBS, Srinath EG (1976) Inhibition of nitrification by ammonia and nitrous acid. *J Water Pollut Control Fed* 48(5):835–852
- APHA (2012) Standard methods for examination of water and wastewater. American Public Health Association, Washington DC
- Baytshtok V, Lu HJ, Park H, Kim S, Yu R, Chandran K (2009) Impact of varying electron donors on the molecular microbial ecology and biokinetics of methylotrophic denitrifying bacteria. *Biotechnol Bioeng* 102(6):1527–1536. doi: 10.1002/bit.22213
- Clarke JKA (1964) Kinetics of absorption of carbon dioxide in monoethanolamine solutions at short contact times. *Ind Eng Chem Fundam* 3(3):239–245. doi: 10.1021/i160011a012
- Dawson RN, Murphy KL (1972) The temperature dependency of biological denitrification. *Water Res* 6(1):71–83. doi: 10.1016/0043-1354(72)90174-1
- Gejlsbjerg B, Frette L, Westermann P (1998) Dynamics of N₂O production from activated sludge. *Water Res* 32(7):2113–2121. doi: 10.1016/s0043-1354(97)00456-9
- Haimour N, Sandall OC (1984) Absorption of carbon dioxide into aqueous

methyldiethanolamine. *Chem Eng Sci* 39(12):1791–1796. doi: 10.1016/0009-2509(84)80115-3

Haynes WM (2013) *CRC handbook of chemistry and physics*, 94th edn. Taylor & Francis, Florida

Hiatt WC, Grady CPL (2008) Application of the activated sludge model for nitrogen to elevated nitrogen conditions. *Water Environ Res* 80(11):2134–2144. doi: 10.2175/106143008x304767

Hu Z, Zhang J, Li S, Xie H (2013) Impact of carbon source on nitrous oxide emission from anoxic/oxic biological nitrogen removal process and identification of its emission sources. *Environ Sci Pollut Res* 20(2):1059–1069. doi: 10.1007/s11356-012-1018-6

IPCC (2001) *Climate change 2001: the scientific basis*. Cambridge Univ. Press, Cambridge

Kampschreur MJ, Temmink H, Kleerebezem R, Jetten MSM, van Loosdrecht MCM (2009) Nitrous oxide emission during wastewater treatment. *Water Res* 43(17):4093–4103. doi: 10.1016/j.watres.2009.03.001

Lewandowski Z (1982) Temperature dependency of biological denitrification with organic materials addition. *Water Res* 16(1):19–22. doi: 10.1016/0043-1354(82)90048-3

Li C, Zhang J, Liang S, Ngo H, Guo W, Zhang Y, Zou Y (2013) Nitrous oxide generation in denitrifying phosphorus removal process: main causes and control measures. *Environ Sci Pollut Res* 20(8):5353–5360. doi: 10.1007/s11356-013-1530-3

Lu HJ, Chandran K (2010) Factors promoting emissions of nitrous oxide and nitric oxide from denitrifying sequencing batch reactors operated with methanol and ethanol as electron donors. *Biotechnol Bioeng* 106(3):390–398. doi: 10.1002/bit.22704

Metcalf, ~~Ine, E~~ & Eddy, Tchobanoglous G, Burton FL, Stensel HD (2013) *Wastewater engineering: treatment and resource recovery*. McGraw-Hill Education, New York

- Pan Y, Ye L, Ni B-J, Yuan Z (2012) Effect of pH on N₂O reduction and accumulation during denitrification by methanol utilizing denitrifiers. *Water Res* 46(15):4832–4840. doi: 10.1016/j.watres.2012.06.003
- Pan Y, Ni B-J, Bond PL, Ye L, Yuan Z (2013a) Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. *Water Res* 47(10):3273–3281. doi: 10.1016/j.watres.2013.02.054
- Pan Y, Ye L, Yuan Z (2013b) Effect of H₂S on N₂O reduction and accumulation during denitrification by methanol utilizing denitrifiers. *Environ Sci Technol* 47(15):8408–8415. doi: 10.1021/es401632r
- Ribera-Guardia A, Kassotaki E, Gutierrez O, Pijuan M (2014) Effect of carbon source and competition for electrons on nitrous oxide reduction in a mixed denitrifying microbial community. *Process Biochem* 49(12):2228–2234. doi: 10.1016/j.procbio.2014.09.020
- Richardson D, Felgate H, Watmough N, Thomson A, Baggs E (2009) Mitigating release of the potent greenhouse gas N₂O from the nitrogen cycle—could enzymic regulation hold the key? *Trends Biotechnol* 27(7):388–397. doi: 10.1016/j.tibtech.2009.03.009
- Schreiber F, Loeffler B, Polerecky L, Kuypers MMM, de Beer D (2009) Mechanisms of transient nitric oxide and nitrous oxide production in a complex biofilm. *ISME J* 3(11):1301–1313. doi: 10.1038/ismej.2009.55
- Versteeg GF, Van Swaalj W (1988) Solubility and diffusivity of acid gases (carbon dioxide, nitrous oxide) in aqueous alkanolamine solutions. *J Chem Eng Data* 33(1):29–34. doi: 10.1021/je00051a011
- Weiss RF, Price BA (1980) Nitrous oxide solubility in water and seawater. *Mar Chem* 8(4):347–359. doi: 10.1016/0304-4203(80)90024-9
- Yaghi B, Houache O (2008) Solubility of nitrous oxide in amine aqueous solutions. *J Eng Comput Arch* 2(1):1-14
- Zeng RJ, Yuan ZG, Keller J (2003) Enrichment of denitrifying glycogen-accumulating organisms in anaerobic/anoxic activated sludge system. *Biotechnol Bioeng* 81(4):397–404. doi: 10.1002/bit.10484

Zhou Y, Pijuan M, Zeng RJ, Yuan Z (2008) Free nitrous acid inhibition on nitrous oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge. *Environ Sci Technol* 42(22):8260–8265. doi: 10.1021/es800650j

Zhou Y, Ganda L, Lim M, Yuan ZG, Kjelleberg S, Ng WJ (2010) Free nitrous acid (FNA) inhibition on denitrifying poly-phosphate accumulating organisms (DPAOs). *Appl Microbiol Biotechnol* 88(1):359–369. doi: 10.1007/s00253-010-2780-3

Zhou Y, Oehmen A, Lim M, Vadivelu V, Ng WJ (2011) The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants. *Water Res* 45(15):4672–4682. doi: 10.1016/j.watres.2011.06.025

Zumft WG (1997) Cell biology and molecular basis of denitrification. *Microbiol Mol Biol Rev* 61(4):533–616

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