Investigation of dissolved N_2O production processes during wastewater treatment system in Ulaanbaatar

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Abstract: Nitrous oxide (N_2O) is an increasing greenhouse gas in the troposphere and a potential destroyer of stratospheric ozone layer. Wastewater treatment plant (WWTP) is one of the anthropogenic N_2O sources because inorganic and organic nitrogen compounds are converted to nitrate (NO_3^-, in the case of standard system) or N_2 (in the case of advanced system) by bacterial nitrification and denitrifcation processes in WWTP. These major processes can be distinguished by isotopocule analysis. In order to reveal production mechanisms of N_2O in a standard wastewater treatment, we made water sampling at the central WWTP in Ulaanbaatar. The water samples collected from seven stations including biological reaction tanks were measured for concentration and isotopocule ratios of dissolved N_2O and other inorganic nitrogen. Dissolved N_2O concentration was extremely higher than that expected under atmospheric equilibrium (about 9 nmol/l) at all stations, indicating that this system is a potential source of N_2O. It showed a gradual increase with the progress of biological reaction and the highest concentration (335.7 nmol/l) was observed at station N5-4 of the aeration tank when the DO was 5.7 mg/l. Nitrification by nitrifying bacteria could actively occur by the concentration of NH_4^+ decreased whereas NO_2^- and NO_3^- showed a temporal and monotonic increase, respectively, under high DO concentration. Although the reported values of site preference (SP) of N_2O, the difference in $^{15}N/^{14}N$ ratio between central (α) and terminal (β) nitrogen, produced via NO_2^- reduction (SP(ND)), including both nitrifier and denitrifier denitrification, and NH_2OH oxidation (SP(HO)) ranged from -10.7‰ to 0‰ and 31.4‰ to 36.3‰, respectively, the observed SP at aeration tank was close to SP(ND) rather than SP(HO). It was ranged from 0.4‰ to 13.3‰ when N_2O concentration was high, implying that the NO_2^- reduction made a greater contribution to N_2O production. Slightly elevated SP (13.3‰) only at station N5-1 was derived from the mixing of N_2O produced via NH_2OH oxidation and the maximal contribution of this pathway was estimated to be about 40%. In other words, the contribution of NO_2^- reduction was more than 60%.

Keywords: *Nitrous oxide; Wastewater; Stable isotope, NO_2^- reduction, NH_2OH oxidation*

INTRODUCTION

Nitrous oxide (N_2O) plays an important role as a greenhouse gas and catalyst in the destruction of ozone in the stratosphere. Concentrations of N_2O in the atmosphere are increasing at the rate of about 0.3% per year [1]. Atmospheric N_2O is derived from natural and anthropogenic sources, but anthropogenic sources are the only ones that are likely to be reduced through improvements in technology. Several anthropogenic sources of N_2O to the atmosphere have been identified, including wastewater treatment plants and agricultural soils [2, 3]. Biological nitrogen removal processes in wastewater treatment system is leading to produce N_2O because inorganic and organic nitrogen compounds are converted to nitrate or N_2 by bacterial nitrification and denitrifcation processes [4]. Nitrification consists of the aerobic oxidation of ammonia (NH_4^+) to nitrate (NO_3^-) via nitrite (NO_2^-), carried out in a two-stepped reaction by ammonia-oxidizing bacteria

that oxidize NH_4^+ to NO_2^-, and by nitrite-oxidizing bacteria that oxidize NO_2^- to NO_3^-. Denitrification is the multi-stepped, anoxic reduction of nitrate (NO_3^-) to dinitrogen gas (N_2) by heterotrophic microorganisms. In Ulaanbaatar, the capital of Mongolia, about one-third of domestic and commercial wastewater is treated by sewer systems with aerobic treatment. Because, most of the population lives in houses, gers using latrine while the 30% of rest inhabitants are living in apartment connected with central sewer system [5]. National estimates of wastewater N_2O emission rates, however, are poorly constrained by fractions of the housing category and wastewater handling method based on population and housing census. For an assessment of the anthropogenic influence on emission rates and climate change projections of wastewater N_2O emissions, a more process-based understanding of the relevance of varying environmental conditions for N_2O production rates is needed. Furthermore, the production processes of N_2O have been the subject of study for many years.

Recently, a high-precision analytical technique for determining intramolecular ^{15}N-site preference in and

asymmetric molecules of N_2O was developed [6]. Since N_2O has two N atoms within the molecule (central and outer N), distribution of a stable isotope, ^{15}N, results in the distribution of three isotopomers, such as $^{15}N^{15}NO$, $^{15}N^{14}NO$, and $^{14}N^{15}NO$. By using this newly developed innovative technique, the latter two types of molecules, which exist abundantly in the environment, can be individually measured. The difference in $\delta^{15}N$ between $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$ is the so-called site preference (SP = $\delta^{15}N^\alpha$ - $\delta^{15}N^\beta$, where $^{15}N^\alpha$ and $^{15}N^\beta$ represent the $^{15}N/^{14}N$ ratios at the center and end sites of the nitrogen atoms, respectively). The SP enabled us to identify the source and sinks of N_2O in the environment [6, 7]. Using this technique, Sutka et al. [8-10] found that the SP for N_2O from hydroxylamine oxidation (~33‰) and nitrite reduction (~0‰) differs in a pure culture study and noted that this difference can be used to distinguish the relative contributions of nitrification and denitrification sources to N_2O emissions. There have still been only several reported studies which applied this measurement technique to field N_2O samples [11, 12] or referred to the relative contributions of nitrification and denitrification.

To our knowledge, the present study is the first to (1) apply the stable isotopic analysis to the determination of N_2O sources during the case of WWTS in Mongolia and to (2) understand the actual contributions of nitrification and denitrification to N_2O production.

EXPERIMENTAL

Study site: Municipal wastewater treatment plant (WWTP) located in Ulaanbaatar was studied in this investigation. The plant, which receives wastewater generated by one-third of total inhabitants of 1.3 million, has conventional activated sludge (CAS) treatment system. The system comprises two settlings and a series of biological reaction tanks. Heavy solids are removed from wastewater in the primary clarifier. Then water enters biological reaction tanks to decompose organic matter by activated sludge under aerobic conditions. Subsequently, the microbe-rich liquid flows into the secondary clarifier where activated sludge is separated from treated wastewater by gravity. Some of the settled sludge is continuously recycled back to the aeration tanks to maintain a proper concentration of microorganisms and, the water is moved to disinfection section. Finally, treated effluent is directly discharged into Tuul river, which flow near to the city (Figure 1).

Sample collection: Sampling period was in July 2015 in the CWWTP. For dissolved N_2O (DN_2O) analysis, water samples were collected from seven points in each treatment systems: exit of the primary settling, sections N1-1, N1-4, N5-1, N5-4, secondary settling and return sludge tunnel. The water temperatures during the sampling were ranged in 20.0–23.0°C. Samples for isotopic analyses were transferred into 115 ml glass vials without a headspace, sterilized with 5 ml of saturated $HgCl_2$, and sealed with butyl rubber stoppers and aluminum caps. They were stored at 4°C until analysis. Those for concentration and isotope analysis of NH_4^+ was filtered into 50 ml plastic bottles and kept in a freezer at -35°C until measurement.

Analysis of dissolved gases and inorganic N species: Water temperature, DO, pH were measured using a pH-temperature electrode with gel electrolyte (SenTix 21; 0-14 pH; 0-80°C; ±0.2°C) and DO sensor (FDO 925; 0-20 mg/l ± 0.5%; 0-50 ± 0.2°C), connected to a portable two channel multi meter (3430 IDS; WTW GmbH). DN_2O concentrations were measured using a

Number	Unit procss	Sample point(s)	Number	Unit procss	Sample point(s)
1	Primary settling	effluent PS	5	Nitrification (5-4)	effluent of the tank
2	Nitrification (1-1)	Influent of the tank	6	Secondary settling	effluent water
3	Nitrification (1-4)	mid of the tank	7	Return sludge	Excess sludge
4	Nitrification (5-1)	mid of the tank			

Fig. 1. Aerial view of the Central Wastewater Treatment Plant (CWWTP) in Ulaanbaatar with the different unit processes and sampling points for dissolved N_2O

gas chromatograph equipped with an electron capture detector (ECD). Using a gastight syringe, gas samples were injected to a precolumn (1/8 in. o.d., 1-m long stainless steel tube packed with Porapak Q) maintained at 70°C. The concentrations of dissolved NH_4^+, NO_3^- and NO_2^- were measured using a portable spectrophotometer (DR 2800™; Hach company, Colorado, US).

Analysis of isotopocule ratios: For analysis of isotopocule ratios of DN_2O, samples were prepared by injecting 60 ml of ultrapure helium (He) and subsequent equilibrating of liquid and gas phase at constant temperature (20°C). Then, gas was taken from the headspace into 115 ml glass bottles, which had been flushed with N_2. Analyses were performed using a Delta XP isotope ratio mass spectrophotometer (IRMS, MAT 251, Thermo–Finnigan, Bremen, Germany) allowing simultaneous detection of m/z 30, 31, 44, 45 and 46. The notation of the isotopocule ratios is the following:

$$\delta^{15}N^i = (^{15}R^i_{sample} / ^{15}R^i_{std} - 1) \times 1000 \ (‰)$$

$$\delta^{18}O = (^{18}R_{sample} / ^{18}R_{std} - 1) \times 1000 \ (‰)$$

$$(i = \alpha, \beta \text{ or bulk}) \quad (4)$$

where $^{15}R^\alpha$ and $^{15}R^\beta$ represent the $^{15}N/^{14}N$ ratios of α and β N atoms, respectively. $^{15}R^{bulk}$ and ^{18}R denote average isotope ratios for $^{15}N/^{14}N$ and $^{18}O/^{16}O$, respectively. Subscripts "sample" and "std", respectively, signify isotope ratios for the sample and the standard, atmospheric N_2 for N and Vienna Standard Mean Ocean Water (VSMOW) for O. The ^{15}N site preference (hereinafter, SP) was also defined as an illustrative parameter of the intramolecular distribution of ^{15}N [6, 7]:

$$^{15}N - \text{site preference (SP)} = \delta^{15}N^\alpha - \delta^{15}N^\beta \quad (5)$$

Site-specific N isotope analysis in NO was conducted using ion detectors modified for mass analysis of the N_2O fragment ions (NO^+), which contained N atoms in the a position of the N_2O molecules, whereas bulk (average) N and O isotope ratios were determined from molecular ions (N_2O^+) [5]. Assuming that the N_2O reduction process is to be negligible, the contributions of NO_2^- reduction (x) and NH_2OH

oxidation $(1 - x)$ to N_2O production can be estimated using the SP value as presented here:

$$SP_{sample} = x \, SP_{NO2\text{-}reduction} + (1-x) \, SP_{NH2OH\,oxidation} \quad (6)$$

There in, $SP_{NO2\text{-}reduction}$ and $SP_{NH2OH\,oxidation}$, respectively, signify the SP values when N_2O is produced only by NO_2^- reduction and when N_2O is produced only by NH_2OH oxidation.

The $\delta^{15}N$ value of NH_4^+ was measured using the diffusion method [13] and analyzed by an EA1110 elemental analyzer (Thermo Fisher Scientific K.K.) coupled with the IRMS.

RESULTS AND DISCUSSION

Concentrations of dissolved inorganic nitrogen (DIN) compounds and DN_2O: The distribution of NH_4^+, NO_2^- and NO_3^-, DN_2O and dissolved oxygen (DO) in the water from different sampling points at CWWTP are presented, respectively, in Figure 2. The concentration of NH_4^+ at station PS was gradually decreased from 3464.3 to 2314.3 µmol/l at the end of aeration tank (N5-4) which implies NH_4^+ oxidation was occurred in this system by the effect of ammonia-oxidizing bacteria. However, it is clear that the process of NH_4^+ oxidation into NO_3^- was not completely conducted as seen from the fraction of NH_4^+ conversion rate. This problem can be caused by either treatment capacity of the plant or old facilities applying for operation. The NO_3^- concentration was highly fluctuated throughout treatment, and the highest concentration (139.3 µmol/l) was at N1-4. We found that total DIN compounds were removed about 30.1% and those removed N fraction probably converted into other gaseous forms based on mass balance estimation (Figure 2a). The DO concentration at primary settling (PS) and beginning of aerobic tank (N1-1) was initially low, however, it increased gradually from station N1-4, indicating that more oxygen was consumed for the oxidation of NH_4^+ and organics (Figure 2b). The highest DN_2O concentration (335.7 nmol/l) was observed at station N5-1 following great decline until secondary settling (SS) in biological reaction basin. This high value can be linked to relatively lower DO concentration compared to DO at other stations. Generally, the DN_2O concentration at all stations was always higher than the value expected under
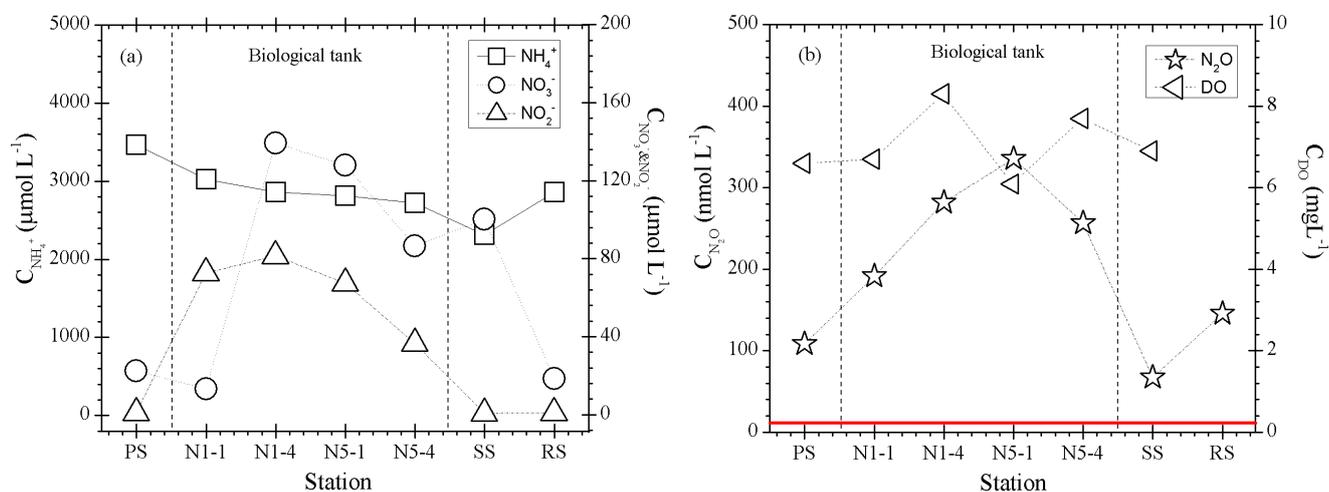

Fig. 2. Concentration profiles of DIN (a), DN_2O and DO (b) in conventional activated sludge system at CWWTP. Red line in right figure indicates water-atmospheric equilibrium for dissolved N_2O

water–atmosphere equilibrium (about 9 nmol/kg at 20°C, showed by red line in Figure 2b [14]) which means that this treatment process is potential source of N_2O emitted to the atmosphere. An operating parameter believed to play a critical role in influencing emissions is DO [15]. An insufficient supply of oxygen in a nitrifying process can lead to incomplete nitrification, whereby autotrophic ammonia-oxidising bacteria (AOB) reduce NO_2^- to N_2O, instead of oxidation to NO_3^-. While the presence of oxygen can inhibit denitrification enzymes, particularly N_2O reductase, which converts N_2O to dinitrogen gas (N_2) in denitrification process. Therefore, DO may be key in determining the metabolic mechanisms that trigger N_2O production, from either nitrifying or denitrifying microorganisms, depending on whether conditions are aerobic and/or anoxic [16]. High N_2O observed at biological reaction tanks under sufficient DO, therefore, probably due to not favourable environment for denitrifying enzymes locally existed in activated sludge. We found that water in biological tanks was supersaturated with DN_2O between 2144 and 3760% which suggests CAS system of this plant is a potential source of N_2O emitted to the atmosphere.

Source identifying of N_2O deduced from stable isotope ratios: N_2O has a strong greenhouse effect, and its emissions must be mitigated. To devise a strategy for mitigation, it is necessary to understand its sources in detail. Stable isotopic analysis is promising tool to differentiate the main N_2O production processes such as NH_2OH oxidation and bacterial NO_2^- reduction in several environments. In this study, isotope ratios of N_2O ($\delta^{15}N$, $\delta^{18}O$, and SP values) showed slight variations during the treatment process (Figure 3).
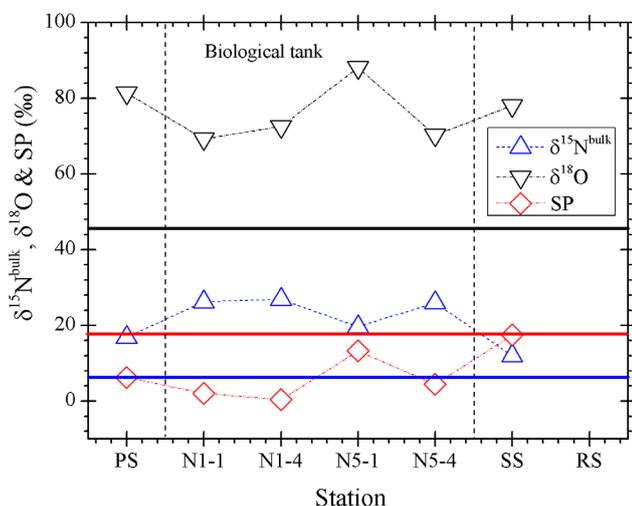

Fig. 3. Isotope ratios of DN_2O in water sampled from CWWTP

In general, the $\delta^{15}N$ value of N_2O was slightly increased from the PS (+16.8‰) to station N5-4 (+26.9‰) except for sudden decline at station N5-1. At the return sludge (RS), it was the lowest around 11.9‰, however, all the values at all stations were higher than that of atmospheric $\delta^{15}N$ value. The $\delta^{18}O$ values were within +70 to +80‰ that is nearly two-fold greater than atmospheric value. Basically, N_2O reduction is accompanied by a simultaneous increase in $\delta^{15}N$ and $\delta^{18}O$ values [17] and a diffusive loss of N_2O from the water to the atmosphere occurs with a marginal

isotope effect [18]. Therefore, the observed decrease in the $\delta^{15}N$ and $\delta^{18}O$ values of N_2O at station N5-1 is interpreted as an isotope effect in microbial N_2O production during the treatment process.

The SP value, which is unique tool for differentiating the microbial processes, was ranged from 0.4‰ at N1-1 to 13.3‰ at N5-1 with high DN_2O concentration. Exceptions were observed at station N5-1 and SS that can be partly produced by NH_2OH oxidation. Production and consumption of N_2O can be revealed using $\delta^{15}N^{bulk}$-SP mapping approach, in which the $\delta^{15}N$ of substrate (NH_4^+) is used to interpret the relation between SP and $\delta^{15}N^{bulk}$ (Figure 4, Eq.7).

$$\delta^{15}N_{N2O} = \delta^{15}N_{NH4^+ \to (NO2^-)} + \varepsilon(^{15}N)_{substrate \to N2O} \qquad (7)$$

The N_2O produced by NO_2^- reduction (nitrifier-denitrification) is assumed to have SP of -13.6‰ to +5.0‰ [8-10, 19] and the $\delta^{15}N^{bulk}$ of +10.7‰ to +38.9‰ according to the measured $\delta^{15}N_{NH4+}$ and the expected isotopic fractionation during NO_2^- reduction to N_2O (-76 to -11‰ [20]). In contrast, N_2O produced by NH_2OH oxidation (nitrification) is supposed to be SP of +27.2‰– +35.6‰ and the expected isotopic fractionation during NO_2^- reduction to N_2O (-76 to -11‰ [20]). Note that the $\delta^{15}N$ value of substrate was identical as NO_2^- reduction process.
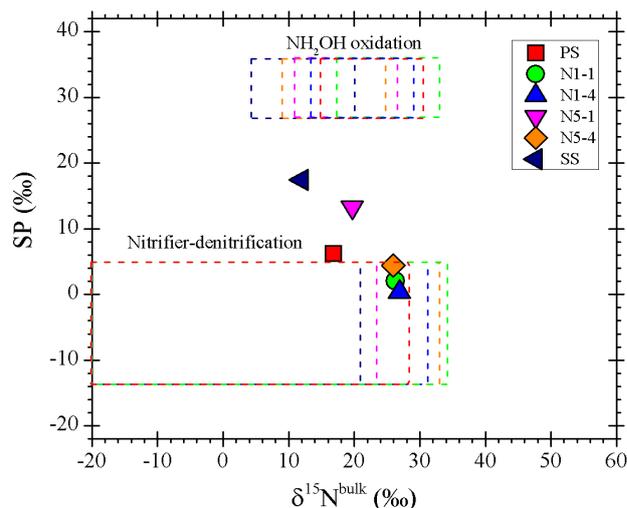

Fig. 4. Schematic diagram portraying N_2O production processes in CWWTP. The range of $\delta^{15}N_{NH4+}$ is shown as horizontal side of colored-dashed box at up and bottom in the graph

Based on this mapping approach, most of data was fallen near to NO_2^- reduction suggesting that the nitrifier-denitrification was dominant pathway to N_2O production. This is agreed with previous results that found nitrifier-denitrification was a key process for N_2O production in aerobic tank of CAS system applied in Japanese WWTP [11,12]. The contribution of this pathway is estimated as 87-99% at stations N1-1, N1-4 and N5-4, respectively. At station N5-1 and SS, data were observed at middle of two boxes demonstrated that N_2O produced by two microbial processes. It means that NH_2OH oxidation and NO_2^- reduction were partly produced to N_2O. NH_2OH oxidation was contributed to N_2O production about 40 and 53%.

CONCLUSION

Results of this study particularly underscore the usefulness of isotope and isotopocule measurements of N_2O to investigate source of N_2O produced during WWT system in Mongolia. The main findings showed as following:

1. Dissolved N_2O concentration at all stations was higher than water-atmospheric equilibrium indicating that WWTP is a potential source of N_2O

2. Water in the biological reaction tank was supersaturated with N_2O as about 3760%, and those N_2O were partly emitted to the atmosphere.

3. Total nitrogen removal efficiency in the process was about 30%.

4. SP value of N_2O implied that the NH_2OH oxidation (nitrification) was partly produced to N_2O with 47-60% contributions at station N5-1 and SS, whereas the NO_2^- reduction (nitrifier-denitrification) was major pathway to N_2O production at other stations including PS, N1-1, N1-4 and N5-4.

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