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Spatial and daily variations of nitrous oxide emissions from biological reactors in a full-scale activated sludge anoxic/oxic process

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Nitrous oxide (N_2O) is an important greenhouse gas that can be emitted from wastewater treatment plants (WWTPs). Such emissions are reportedly process specific and related to operational parameters. This study was conducted to clarify spatial and daily variations of N_2O in a full-scale activated sludge anoxic/oxic process that consisted of an anoxic tank and three oxic tanks (oxic-1, oxic-2 and oxic-3), all of which except the final sedimentation tank were fully covered. Higher dissolved N_2O (D-N_2O) loading and gaseous N_2O (G-N_2O) emissions were observed for oxic-3 than for the anoxic, oxic-1, and oxic-2 tanks, implying that there was higher N_2O production potential via nitrification in the latter stage of the oxic tank. Moreover, the sudden decrease in dissolved oxygen concentration after the peak was found to lead to abrupt production of D-N_2O at oxic-3 in the anoxic/oxic process. The increases in AOB *amoA*, AOB *nirK* and the following AOB *nor*B gene transcripts at the end of the oxic-2 tank suggested that nitrifier denitrification occurred to produce N_2O under low dissolved oxygen conditions when the N_2O peak was observed. Additionally, the much lower transcription levels of the two *nosZ* genes suggested lower N_2O consumption. The N_2O emission factors ranged from 0.087% to 0.302%, and lower N_2O emission factors were observed during summer.

[**Key words:** Anoxic/oxic process; Nitrous oxide; Daily variation; Spatial variation; Affecting factors; Production mechanism; Emission factors]

Nitrous oxide (N_2O) is a greenhouse gas with a 100-year global warming potential 265 times more powerful than that of carbon dioxide (CO_2). In addition, N_2O is extremely persistent, with a 121-year lifetime according to the Intergovernmental Panel on Climate Change (1). Nitrous oxide also plays a catalytic role in ozone depletion (2). Since 1750, the concentration of atmospheric N_2O has increased by 20%, and it has been steadily increasing at 0.73 ± 0.03 ppb yr^{-1} over the last three decades. At the same time, N_2O accounted for 6.2% of the total anthropogenic greenhouse gas (GHG) emissions in 2010 (1). Therefore, reduction of N_2O emissions is urgently required.

Wastewater treatment contributed up to 1.4% of the anthropogenic N_2O emissions in the United States in 2016, and this amount has been increasing according to the latest report by the United States Environmental Protection Agency (USEPA) (3). Hence, wastewater treatment plants (WWTPs) have been recognized as important N_2O emission sources. Heterotrophic denitrification under anoxic conditions is widely recognized as the dominant process for N_2O emission from WWTPs, while nitrifier denitrification (4) and NH_2OH oxidation (5) by ammonia-oxidizing bacteria (AOB) also play essential roles in N_2O production in activated sludge systems. Nitrite, dissolved oxygen (DO), and chemical oxygen demand (COD)/N ratio have been identified as the most important factors influencing N_2O emissions from WWTPs (6). High concentrations of nitrite have been shown to affect N_2O emissions in both the nitrification and denitrification stage (6), while very low nitrite concentrations triggered significant N_2O emissions during simultaneous nitrification and denitrification via nitrite in a lab-scale sequencing batch reactor (7). Tallec et al. (8) reported high N_2O emissions at low oxygen concentrations (0.1–2 mg O_2 L^{-1}) because of autotrophic nitrifier denitrification and heterotrophic denitrification, while Itokawa et al. (9) demonstrated that high quantities of N_2O were released by denitrification in the anoxic phase under lower COD/N ratio operations.

Many researchers have made great efforts to identify N_2O sources and emission factors from WWTPs and/or decentralized wastewater treatment facilities based on grab samples and/or off-line analysis (10–12). For example, gaseous N_2O (G-N_2O) was collected from non-fully covered WWTPs using open/closed chamber methods (13). Moreover, Kosonen et al. (14) conducted long-term on-line gaseous N_2O monitoring at a full-scale WWTP and estimated total annual N_2O production based on their observations. They also showed that diurnal N_2O variations were strongly correlated with influent biochemical oxygen demand (BOD) and ammonium nitrogen loading. However, no clear

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relationship was observed between seasonal changes in wastewater temperature and N_2O emission. Wang et al. (15) investigated spatial and daily variations in N_2O emissions by on-line gaseous N_2O monitoring in a full-scale activated sludge anaerobic/anoxic/oxic process and found that N_2O was mainly emitted from the oxic zone and its emission was directly triggered by nitrite accumulation. Song et al. (16) conducted on-line gaseous N_2O monitoring that revealed that an activated sludge system with insufficient nitrification emitted more N_2O than one with complete nitrification in a WWTP. However, the mechanisms and sources of N_2O emissions from full-scale WWTPs are still elusive because of dynamic behaviors of variable flow rates and concentrations of incoming wastewater and the corresponding activities of microorganisms responsible for nitrogen transformation. Recently, on-line dissolved N_2O (D-N_2O) measurements were applied for analysis of full-scale WWTPs, enabling direct clarification of N_2O production in the aqueous phase and revealing their high variabilities within both short and long-term periods. Hence, further intensive and comprehensive studies using on-line D-N_2O measurements are urgently needed, especially those focusing on factors affecting abrupt N_2O emissions in a full-scale WWTP and their correlation with microbial activity.

In this study, continuous on-line D-N_2O measurement coupled with gas sampling from off-gas pipes was conducted in a fully covered full-scale anoxic/oxic wastewater treatment process. As mentioned above, both heterotrophic denitrification in anoxic tanks and nitrifier denitrification and/or NH_2OH oxidation in oxic tanks play important roles in N_2O production in activated sludge systems. In this study, the authors targeted anoxic/oxic processes to evaluate contributions of these reactions to N_2O production in a WWTP. The specific goals of this study were to quantify N_2O emissions from each biological reactor and clarify the main sources and influencing factors of N_2O production, as well as to investigate both spatial and daily variations in N_2O emissions from biological reactors in a full-scale activated sludge anoxic/oxic process. Additionally, N_2O emission factors and key pathways of N_2O production in the process were estimated based on online N_2O monitoring and transcription of functional genes for nitrogen transformation.

MATERIALS AND METHODS

WWTP process configuration This study was conducted in a full-scale activated sludge anoxic/oxic process (treatment line 6) at Takasu WWTP in Kochi Prefecture, Japan. The process consists of a primary sedimentation tank (PST), an anoxic tank, three oxic tanks (oxic-1, oxic-2, oxic-3) and a final sedimentation tank (FST). All of the tanks except the FST are fully covered. The flow diagram of the investigated anoxic/oxic process is shown in Fig. S1. The off-gas pipes are connected to the surface of the fully-covered tanks and off-gas from the tank is transported to a deodorizer.

The biological reactors (anoxic, oxic-1, oxic-2 and oxic-3) in the process have volumes of 216 m^3 (W = 6.1 m, L = 7.3 m, D = 5.0 m), 323 m^3 (W = 6.1 m, L = 10.9 m, D = 5.0 m), 323 m^3 (W = 6.1 m, L = 10.9 m, D = 5.0 m) and 486 m^3 (W = 6.1 m, L = 16.4 m, D = 5.0 m), respectively, and the cross sectional area of the tanks is 29.6 m^2. Aeration was conducted in the oxic tanks to supply appropriate amounts of oxygen for aerobic treatment.

Over the investigation period (from July 2015 to February 2016), the average daily influent flow rate, hydraulic retention time (HRT), actual retention time, and return sludge ratio were 2615 ± 350.0 m^3/d, 12.5 ± 1.37 h, 7.2 ± 0.57 h, and 72.7 ± 8.53%, respectively. The influent water quality is listed in Table S1.

The lowest influent flow rate was generally observed around 7 AM at Takasu WWTP, and the operators simultaneously increased the aeration rate in accordance with the increase in flow rate in the morning. Hence, DO concentration suddenly increased after the aeration rate was increased, then decreased because of the increase in influent BOD loading rate.

Sampling campaigns The sampling campaigns were mainly conducted in the biological reactors (anoxic, oxic-1, oxic-2 and oxic-3) from July 2015 to February 2016, with 24-h continuous investigations held quarterly on July 23–24, 2015, November 25–26, 2015, and February 23–24, 2016. Operational conditions and treatment performance for each 24-h investigation are summarized in Table S2. A continuous investigation was conducted to observe variations in D-N_2O and DO at a depth of 2.5 m in oxic-3 on November 25–26, 2015, and on December 18–22, 2015, respectively. Sampling sites in the biological reactors are shown in Fig. S2.

Monitoring indexes and analytical methods Biochemical oxygen demand (BOD), dissolved BOD (D-BOD), chemical oxygen demand (COD_{Cr}), dissolved COD_{Cr} (D-COD_{Cr}), total nitrogen (TN), dissolved TN (D-TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), and nitrite nitrogen (NO_2^--N) were analyzed according to Japanese Industrial Standards K 0102 (17). The DO and water temperature were measured using a DO meter (Multi 3410 SET 6, WTW, Weilheim, Germany), and pH was measured with a pH meter (HM-21P, DKK-TOA, Tokyo, Japan). Gas samples were collected from the off-gas pipes and analyzed by gas chromatography using an electron capture detector (6890N, Agilent Technologies, Santa Clara, CA, USA). The gas flow rate in the off-gas pipes was measured using a hot wire anemometer (YK-2004AH, FUSO, Tokyo, Japan) to enumerate G-N_2O fluxes from each tank. Dissolved N_2O (D-N_2O) was detected using a miniaturized Clark-type N_2O Wastewater Sensor (Unisense Environment A/S, Aarhus, Denmark) (18) with a guard cathode. N_2O emission factors were calculated based on G-N_2O emissions from biological reactors (anoxic, oxic-1, oxic-2 and oxic-3) and D-N_2O loading in effluent using the following equations:

$$\text{Emission factor}\left(N_2O\text{-}N/TN_{\text{influent}},\%\right)=\frac{\sum\left(C_{G\text{-}N_2O}\times Q_G+C_{D\text{-}N_2O,\,\text{effluent}}\times Q_{\text{effluent}}\right)}{\sum\left(Q_{\text{influent}}\times C_{TN,\,\text{influent}}\right)}\times 100 \quad (1)$$

$$\text{Emission factor}\left(N_2O\text{-}N/\Delta TN,\%\right)=\frac{\sum\left(C_{G\text{-}N_2O}\times Q_G+C_{D\text{-}N_2O,\,\text{effluent}}\times Q_{\text{effluent}}\right)}{\sum\left\{Q_{\text{influent}}\times\left(C_{TN,\,\text{influent}}-C_{TN,\,\text{effluent}}\right)\right\}}\times 100 \quad (2)$$

where $C_{G\text{-}N_2O}$ is the G-N_2O concentration in an off-gas pipe from each biological reactor (mg N/L); Q_G is the gas flow rate in an off-gas pipe from each biological reactor (m^3/h); $C_{D\text{-}N_2O,\,\text{effluent}}$ is the D-N_2O concentration in the effluent (mg N/L); Q_{effluent} is the effluent flow rate (m^3/h); Q_{influent} is the influent flow rate (m^3/h), which is equal to Q_{effluent}; and $C_{TN,\,\text{influent}}$ and $C_{TN,\,\text{effluent}}$ are the TN concentrations in influent and effluent, respectively (mg N/L). The G-N_2O from a FST could not be observed in this study; therefore, the emission factor may be slightly underestimated.

DNA was extracted using a FastDNA Spin Kit (MP Biomedicals, Carlsbad, CA, USA) according to the manufacturer's protocol. Purities and concentrations of extracted DNA samples were measured using a spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific, Waltham, MA, USA). RNA was extracted using a FastRNA Pro Soil Direct Kit (MP Biomedicals) according to the manufacturer's protocols. Extracted RNA concentrations and purities were measured using a spectrophotometer (NanoDrop 2000c, Thermo Fisher Scientific). Reverse transcription of RNA was conducted with a QuantiTect Reverse Transcription system (Qiagen, Venlo, the Netherlands) as described elsewhere (19). Extracted DNA and complementary DNA were further subjected to real time quantitative PCR (qPCR) for quantification of functional genes encoding AOB ammonia monooxygenase (*amoA*), AOB hydroxylamine oxidoreductase (*hao*), AOB nitric oxide reductase (*norB*), and two different N_2O reductases (*nosZ* clade I and clade II). The detailed primer and qPCR conditions were the same as in a previous study (20).

RESULTS AND DISCUSSION

Spatial and daily variations in N_2O emission Twenty-four-hour investigations were conducted on July 23–24, 2015, November 25–26, 2015, and February 23–24, 2016 to evaluate spatial and daily variations in D-N_2O loading and G-N_2O emissions from the biological reactors in a full-scale anoxic/oxic process (Fig. 1). The highest D-N_2O loading of 31.2 ± 18.7 g N/d (average ± standard deviation) was observed at oxic-3, while D-N_2O loadings from oxic-1 and oxic-2 were as low as 8.21 ± 7.16 g N/d and 9.39 ± 8.70 g N/d, respectively. In the case of the anoxic tank, the D-N_2O loading showed the lowest level of 4.76 ± 7.56 g N/d. Similar spatial variations in G-N_2O emission were also observed. The G-N_2O emitted from oxic-3 was 87.7 ± 58.5 g N/d, which was much higher than the levels emitted from the other tanks, while the lowest G-N_2O emissions were 4.42 ± 2.39 g N/d from the anoxic tank.

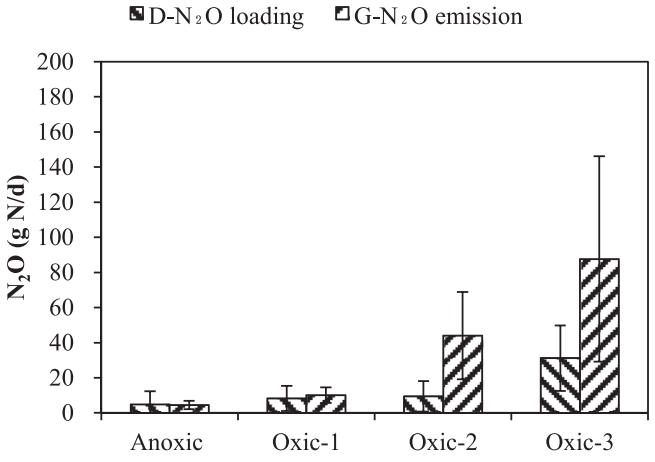

FIG. 1. Spatial variations in D-N_2O loading and G-N_2O emissions from the biological reactors in a full-scale anoxic/oxic process based on three 24-h continuous investigations. Bars are the standard deviations.

Higher N_2O production potential via nitrification in the latter part of an oxic stage than in the anoxic stage was demonstrated in a multiple anoxic and aerobic process. Therefore, our subsequent studies focus on the oxic tanks in the process.

Daily variations in D-N_2O loading and G-N_2O emissions during the anoxic/oxic process are shown in Figs. 2 and 3, which provide very interesting variation trends. As shown in Fig. 2A, the highest D-N_2O loading of 3.98 g N/h at oxic-3 was attained at 13:00 on July 23, while it decreased to a lower level at midnight and early morning, regardless of other factors. The highest G-N_2O emission of 2.60 g N/h was observed at oxic-3 at 15:00 on July 23 as shown in Fig. 3A. Additionally, the highest level of total G-N_2O emissions from the biological reactors was 5.00 g N/h at 15:00 on July 23.

The D-N_2O loading and G-N_2O emission for the investigations in November 2015 and February 2016 are shown in Fig. 2B, C, and Fig. 3B, C, respectively. The peak D-N_2O loading and G-N_2O emissions appeared at 15:00 for the oxic-3 tank. The highest D-N_2O loading and G-N_2O emission values were 4.37 g N/h and 17.7 g N/h, respectively, on November 25, while they were 5.09 g N/h and 13.8 g N/h on February 23. D-N_2O loadings in biological reactors were only observed in oxic-3 from November 25 to 26, 2015. The highest level of total G-N_2O emissions from the biological reactors was 24.8 g N/h at around 13:00 on November 25, while it was 23.8 g N/h at 15:00 on February 23.

Similarly, D-N_2O loading in the effluent showed interesting fluctuations. The highest D-N_2O loading of 1.45 g N/h was observed at 13:00 on July 23, while it remained 0.00 g N/h from November 25 to 26, 2015. The highest loading of 0.73 g N/L was observed at 11:00 on February 23, 2016. As mentioned above, the oxic-3 tank had higher N_2O emissions than the other tanks. There were also obvious fluctuations in N_2O throughout the day, with an upward trend occurring from 11:00 to 15:00, a gradual decline from 15:00 to midnight and early morning, and a further increase from 9:00.

Effect of DO on D-N_2O in the oxic tank The 4-day D-N_2O continuous online measurement was conducted at the oxic-3 tank from December 18 to 22, 2015. During this period, the water temperature remained stable in the range of 21.1°C–21.9°C. Large daily fluctuations in both D-N_2O and DO concentrations were observed at oxic-3 under aerobic conditions (Fig. 4A). The DO concentration greatly increased from 6:42 to 9:47 on December 19 because of aeration management by the operator as mentioned above, while the D-N_2O concentration was maintained at almost stable levels during this time. Afterwards, the D-N_2O concentration sharply increased when the DO concentration began to decrease,

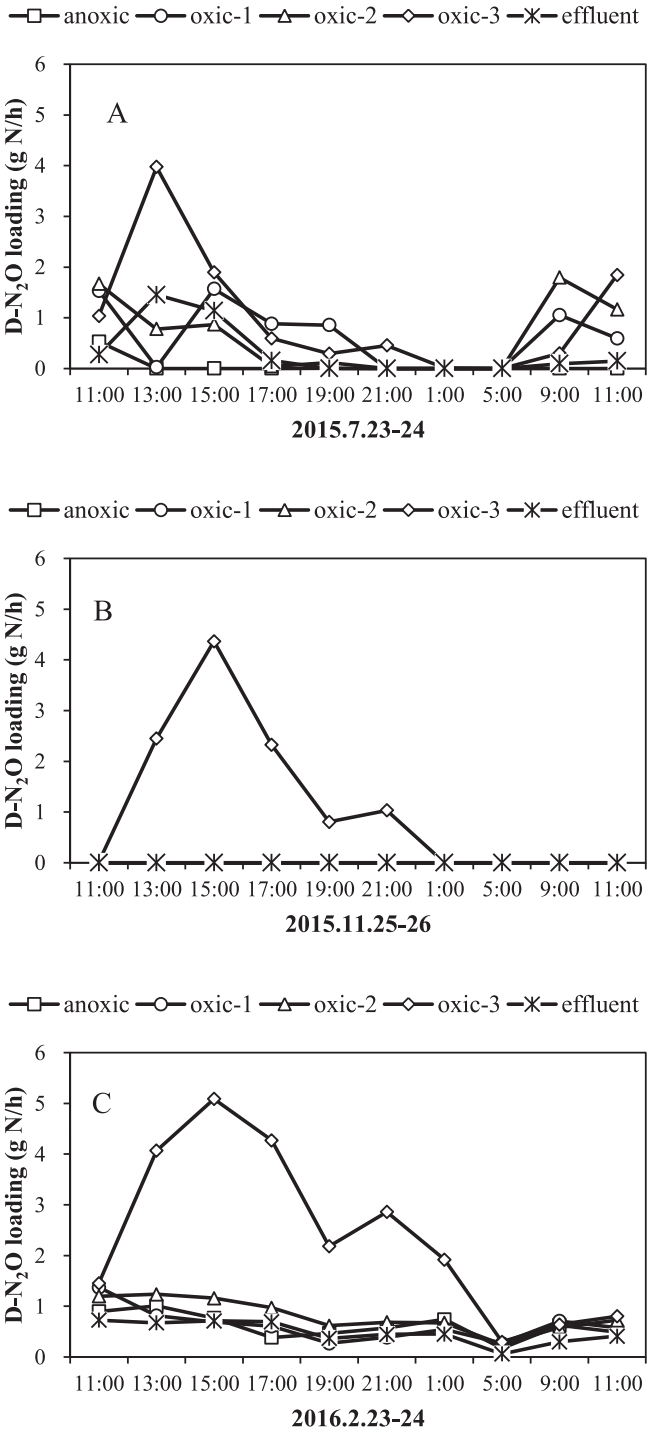

FIG. 2. Daily variations in D-N_2O loading in the anoxic/oxic process on (A) July 23–24, 2015; (B) November 25–26, 2015; and (C) February 23–24, 2016.

reaching a peak of 35.9 μg N/L when the DO decreased to a lower value of around 0.24 mg/L at 15:07. On December 20, the DO levels fluctuated obviously from 7:32 and the peak of 4.2 mg/L appeared at around 8:42. Moreover, the D-N_2O gradually increased from 8:40 to 15:30, reaching the highest value of 22.2 μg N/L when the DO was around 0.3 mg/L. On December 21, the DO concentration increased between 7:17 and 9:47, showing a peak of 2.4 mg/L at 9:47. The D-N_2O increased after the DO decreased from 2.4 mg/L to 0.2 mg/L, reaching a peak of 8.83 μg N/L at a DO concentration of around 0.4 mg/L at 14:08. On the last day, obvious variations in DO

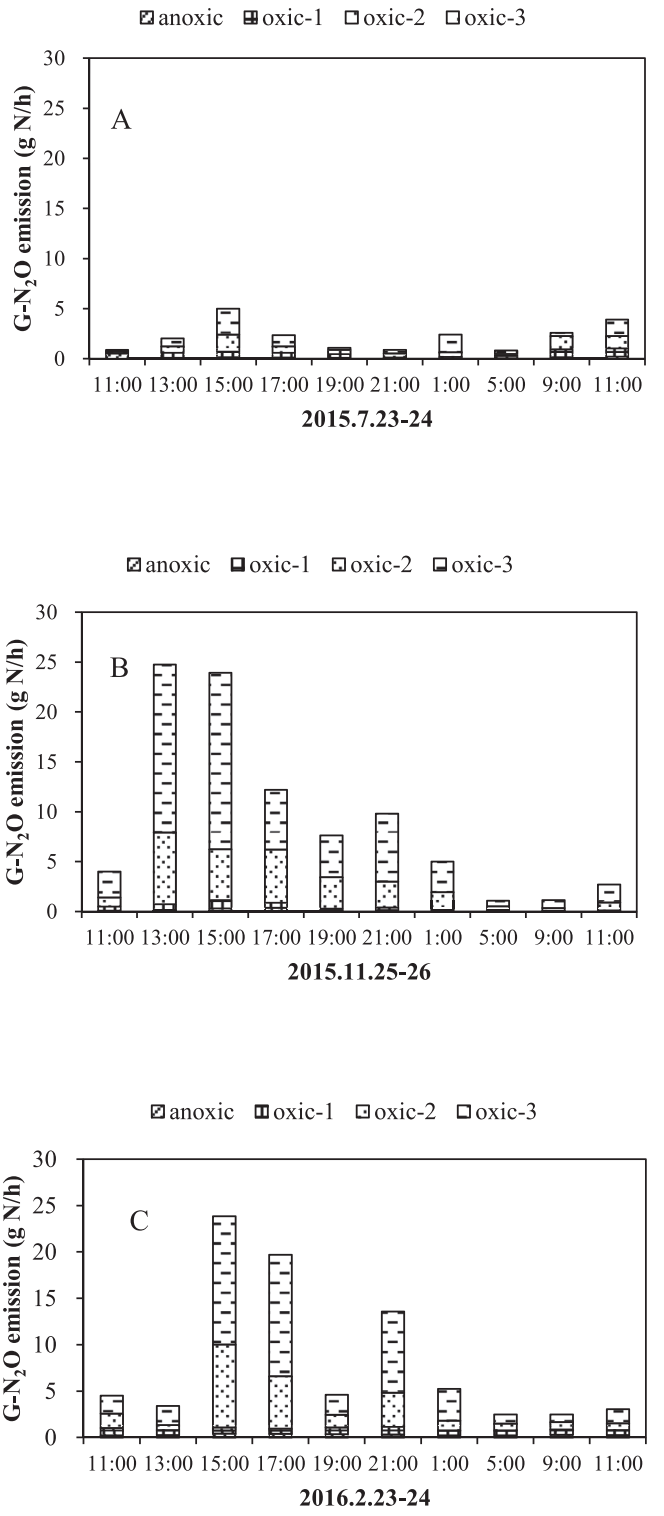

FIG. 3. Daily variations in G-N_2O emissions during the anoxic/oxic process on (A) July 23–24, 2015; (B) November 25–26, 2015; and (C) February 23–24, 2016.

occurred from 6:37, with the highest concentration of 3.5 mg/L occurring at around 9:00. The D-N_2O began to increase around 12:00, then reached 7.40 μg N/L when the DO decreased to 0.25 mg/L at 14:59. A lower DO concentration was detected without obvious variation between 17:00 and 6:00 the following early morning, while the D-N_2O concentration remained at a relatively steady state at midnight and in the morning throughout the investigation period. The effects of DO on N_2O emissions have been studied in many previous investigations. Pijuan et al. showed that N_2O emissions decreased from 6% to 2.2% when DO increased from 1.0 to 4.5 mg/L in a pilot-scale continuous granular airlift nitrification reactor (21). Ahn et al. (22) reported higher N_2O emissions during partial nitrification under low DO (1.1 ± 0.38 mg/L) conditions. Frison et al. (23) reported that DO levels at or above 1.5 mg/L can limit N_2O emissions during the nitrification process. However, in this study, no specific concentration or increases in DO concentration were found to be the source of variations in N_2O. In contrast, a sudden decrease in DO concentration after the peak led to abrupt production of D-N_2O in the latter part of the oxic stage in the anoxic/oxic process.

As shown in Fig. 4B, the 24-h continuous online measurement of D-N_2O and DO was conducted at site D2 in the oxic-3 tank on November 25–26, 2015. Water samples were also collected from site E (see Fig. S2) immediately before flowing into the oxic-3 tank for NH_4^+-N, NO_3^--N, and NO_2^--N measurement (Fig. 5). The water temperature ranged from 22.7°C to 24.0°C in the oxic-3 tank, and the average ammonium removal efficiency was 99.5% in the anoxic/oxic process. As shown in Fig. 4B, obvious fluctuations in D-N_2O concentration started from 12:00, immediately after which DO decreased to 0.19 mg/L, then increased to a peak of 32.4 μg N/L at 15:13 on November 25. During the peak hours of D-N_2O from 12:10 to 20:40, DO was in the low range of 0.08–0.33 mg/L. As mentioned above, higher D-N_2O emissions were not directly related to lower DO concentrations because the D-N_2O concentration was maintained at almost 0.00 μg N/L at midnight and early morning, when the DO ranged from 0.03 to 0.52 mg/L. Furthermore, D-N_2O remained stable when the DO increased from 0.15 mg/L to 2.6 mg/L after 8:00 on November 26. Therefore, the sudden drop in DO after the peak might have been a primary factor influencing the D-N_2O increase in the oxic-3 tank.

As shown in Fig. 5, the ammonia concentration immediately before oxic-3 peaked at 8.8 mg N/L at 13:00, then decreased to 6.5 mg N/L at 15:00 on November 25, while nitrate and nitrite nitrogen increased from 13:00 and peaked at 15:00 immediately before the D-N_2O peak appeared at 15:13. Increases in nitrate and nitrite concentrations of 83% and 44%, respectively, were observed between 13:00 and 15:00. Nitrate and nitrite concentrations gradually decreased after 19:00 until 5:00 on November 26, after which they started to increase. These results suggest that nitrification was a mechanism for production of D-N_2O.

Mechanism of N_2O production As shown in Fig. 6, on November 25, a relatively higher AOB *amoA* gene transcript was attained from 10:00 to 18:00 than from 18:00 to 6:00. The level of gene transcripts then increased again at 9:00 on November 26, which was concordant with an increase in DO concentration (Fig. 4B). The gene transcript of *haoA* was much lower than that of *amoA* and no noticeable trend was observed. When compared with the trends of *amoA* and *haoA*, the AOB *nirK* and *norB* gene transcripts were relatively constant during the sampling period. Increases in AOB *amoA* and AOB *nirK* gene transcripts in the activated sludge at 14:00 coincided with the peak D-N_2O concentration at 15:13. The average flow rate of activated sludge between 14:00 and 16:00 in the downstream direction was 173 m^3/h, and the average flow velocity was calculated to be 5.84 m/h by assuming plug flow in the reactor. Given that the samples for the gene transcripts (Fig. 6) were taken at E (upstream) and D-N_2O concentrations (Fig. 4B) were monitored at D2 (downstream) with a distance of 6.2 m from E, it is reasonable to assume that the increases in AOB *amo*A and AOB *nir*K gene transcripts at E at 14:00 were linked with the peak in the D-N_2O concentration at D2 at 15:13. An increase in the AOB *norB* gene transcript observed from 15:00 followed a similar increase in the

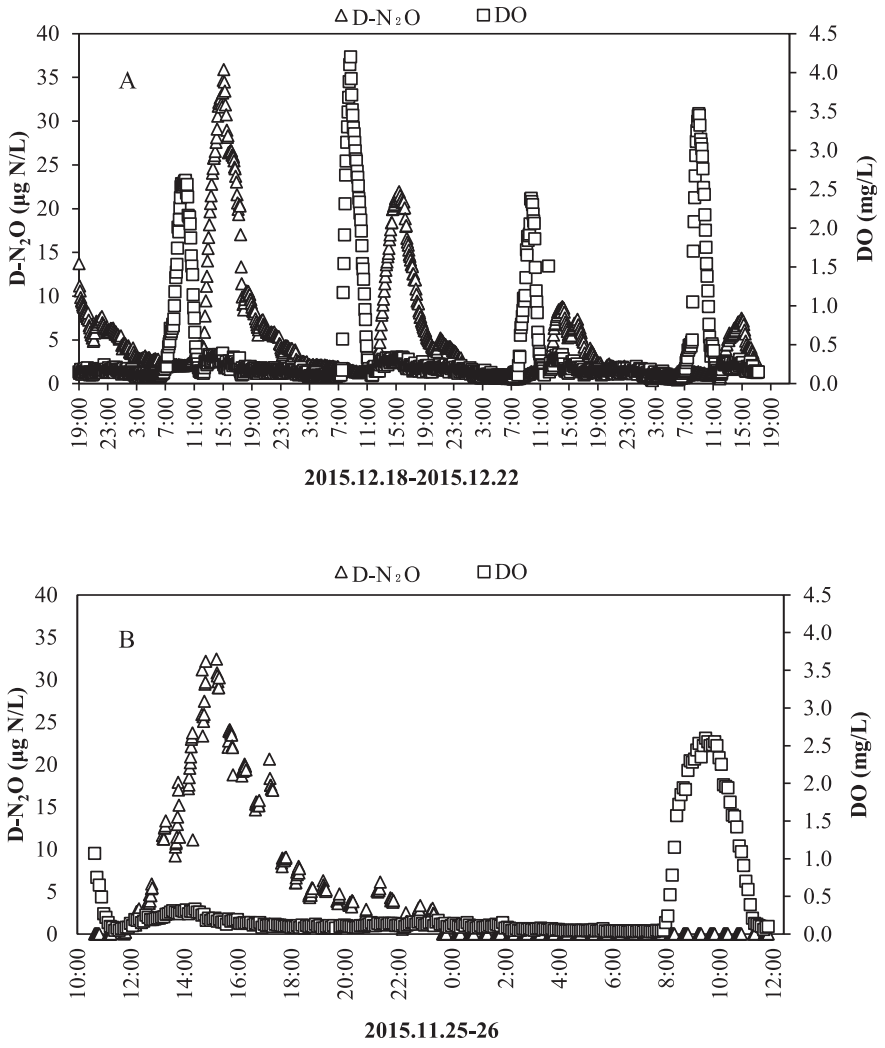

FIG. 4. Continuous online measurement of D-N_2O and DO, (A) 4-day measurement at site D2 in oxic-3 on December 18–22, 2015 (the detection interval was 5 min); (B) 24-h measurement at site D2 in oxic-3 on November 25–26, 2015 (the detection interval was 1 min).

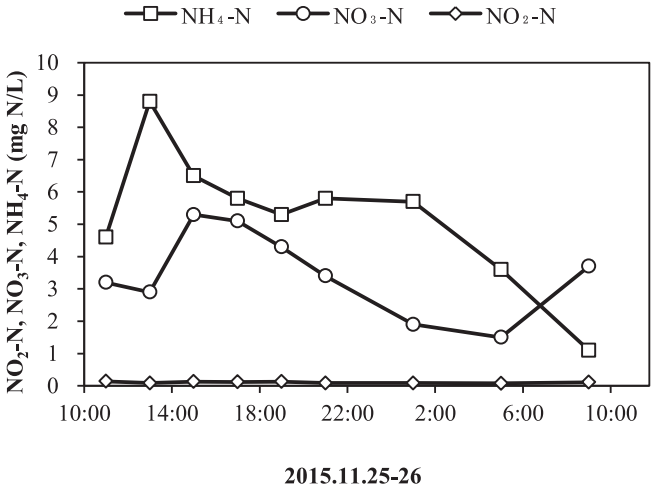

FIG. 5. Daily variations in nitrite, nitrate and ammonia concentrations at site E on November 25–26, 2015.

AOB *nirK* gene. The increases in AOB *amoA*, AOB *nirK* and the following AOB *norB* gene transcripts suggested occurrence of nitrifier denitrification to produce N_2O, which is a major N_2O production pathway that has been previously reported (24,25). Although nitrite concentrations were as low as the detection limit (Fig. 5), the presence of ammonium induced AOB *nirK* expression during the sampling period. The trend was likely to occur according to a previous report (4) in which induction of the AOB *nirK* gene was also observed in activated sludge during nitrification when there was a low level of nitrite (<2 mgN/L). The expression of AOB *norB* may indicate the occurrence of nitric oxide reduction to N_2O. Conversely, the far lower level of *haoA* expression suggests a marginal effect of N_2O emission via NH_2OH oxidation (26,27), which disagrees with the results of a previous study in which NH_2OH oxidation was found to induce production of N_2O at a higher ammonium and lower nitrite level (28). This is likely because of a difference in DO concentration [<0.33 mg/L in our study (12:00–8:00 in Fig. 4B) vs. 2.0 mg/L in the previous study (28)]. Taking the results of AOB gene transcripts into consideration, nitrifier denitrification was likely responsible for the increased N_2O emissions under the low DO condition of 0.08–0.33 mg/L when the N_2O levels peaked.

Much lower transcription levels of the two *nosZ* genes than those of the other genes were obtained, suggesting lower activity of N_2O consumption in the oxic-2 tank. This is reasonable because *nosZ* genes encode N_2O reductase, which shows impaired activity in the presence of oxygen (29). The transcription levels of the two

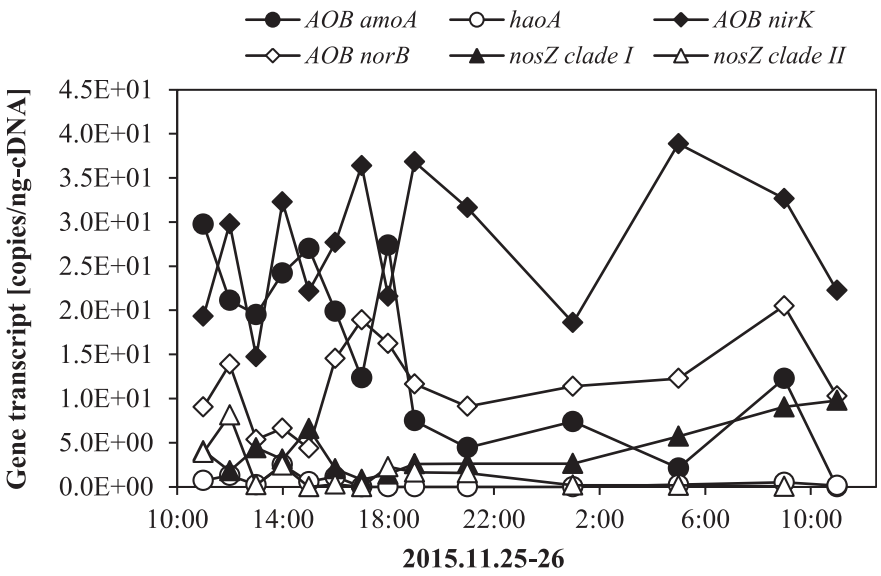

FIG. 6. Daily variations in functional gene transcripts at sampling site E in the oxic-2 tank.

nosZ genes were higher for clade I than clade II during the sampling period. The higher gene transcription of clade I is not congruent with recent observations in which clade II type N_2O reducers were shown to be highly effective at N_2O consumption (29,30). However, these studies were based on biokinetic analysis of isolated bacteria harboring either clade I or clade II type. It has been reported that N_2O production is governed by metabolic activity rather than gene expression (31). More comprehensive investigation regarding the link of gene expression with metabolic activity should be investigated in future studies.

N_2O emission factors N_2O Emission factors were calculated based on 24 h of measurements conducted on July 23–24, 2015, November 25–26, 2015 and February 23–24, 2016. The average water temperatures on these dates were 25.5°C, 23.6°C and 19.9°C. Table S3 summarizes the emission factors in comparison with previous data that were obtained in anoxic/oxic (AO), modified Ludzack–Ettinger (MLE), and anaerobic/anoxic/oxic (A^2O) processes. Emission factors in November and February were calculated to be 0.322 and 0.302 (N_2O-N/ΔTN, %) using Eq. 2, which were similar to the value of 0.587 (N_2O-N/ΔTN, %) reported by Masuda et al. (32), but much lower than those reported by Foley et al. (10) and Sun et al. (33). Although the authors investigated fully covered anoxic/oxic process, various differences may exist among operation parameters, sampling sites and detection methodologies. Moreover, because of the difficulty in sampling at other tanks in this study, evaluation of N_2O emission factors was based on the G-N_2O emissions from biological reactors and effluent D-N_2O loading, which may lead to underestimation.

There were obvious seasonal differences in the N_2O emission factors. As shown in Table S2, the BOD-SS loading rate in February 2016 (0.296 kg/(kg·d)) was 36% and 53% higher than those in July and November 2015 (0.217 and 0.194 kg/(kg·d)), whereas the average TN removals were almost the same (65.9% in July 2015, 71.2% in November 2015, and 71.9% in February 2016). The maximum emission factor (0.322%) was observed in November, which was 4.0 times higher than the minimum value of 0.087% observed in July, indicating that temperature had a negative effect on N_2O emissions. Conversely, Wang et al. (34) reported a gradual increase in N_2O production rates with increasing temperature from lab-scale denitrifying activated sludge, which was attributed to the maximum NO_3^- reduction rate being more sensitive to temperature change than the corresponding N_2O reduction rates. Based on these findings, the effects of temperature in a full scale anoxic/oxic process should be investigated in further detail in future studies.

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jbiosc.2018.08.003.

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