

Effect of Aeration on Nitrous Oxide (N₂O) Emission from Nitrogen-Removing Sequencing Batch Reactors

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In this study, nitrous oxide (N₂O) emission was compared between the operations of two different sequencing batch reactors, conventional sequencing batch reactor (CNV-SBR) and simultaneous nitrification and denitrification sequencing batch reactor (SND-SBR), using synthetic wastewater. The CNV-SBR consisted of anoxic (denitrification) and aerobic phases, whereas the SND-SBR consisted of a microaerobic (low dissolved oxygen concentration) phase, which was achieved by intermittent aeration for simultaneous nitrification and denitrification. The CNV-SBR emitted 3.9 mg of N₂O-N in the denitrification phase and 1.6 mg of N₂O-N in the nitrification phase, resulting in a total emission of 5.5 mg from 432 mg of NH₄⁺-N input. In contrast, the SND-SBR emitted 26.2 mg of N₂O-N under the microaerobic condition, which was about 5 times higher than the emission obtained with the CNV-SBR at the same NH₄⁺-N input. From the N₂O yield based on NH₄⁺-N input, the microaerobic condition produced the highest yield (6.1%), followed by the anoxic (0.9%) and aerobic (0.4%) conditions. It is thought that an appropriate dissolved oxygen level is critical for reducing N₂O emission during nitrification and denitrification at wastewater treatment plants.

Key words: Denitrification, dissolved oxygen, nitrification, nitrous oxide (N₂O) emission, sequencing batch reactor (SBR)

Over the past decade, climate change caused by excessive greenhouse gas emission has been a serious problem faced by mankind. Nitrous oxide (N₂O) is a greenhouse gas having an approximately 300-fold stronger global warming effect than carbon dioxide, and its contribution to the total greenhouse effect is about 9% [9]. A significant amount

of N₂O is emitted through the nitrogen compounds transformation processes of waste and wastewater treatments. Nitrogen compounds in wastewater are removed by biological nitrification and denitrification, and it has been known that N₂O is released during the biological nitrogen removal process [23]. The mechanisms involved in N₂O emission from the biological nitrogen transformation process are very diverse. Initially, it was thought that most of the N₂O is produced through the heterotrophic denitrification and incomplete denitrification of nitrite (NO₂⁻), which leads to the accumulation of N₂O in the denitrification pathway. However, it has been found that significant amounts of N₂O are also produced in the nitrification pathway by incomplete oxidation of hydroxylamine (NH₂OH) [4, 8, 14]. Furthermore, ammonia oxidizing bacteria (AOB) are found to have nitrite reductase and nitric oxide (NO) reductase, which denitrify nitrite and nitric oxide (NO) to produce N₂O with ammonia or hydrogen as the electron donor, which is called autotrophic denitrification [2, 23].

Dissolved oxygen is one of the critical factors in denitrification by AOB, and N₂O production is stimulated at low dissolved oxygen concentrations [12, 18, 24]. AOB can use nitrite as an electron acceptor for the oxidation of ammonia to NH₂OH instead of oxygen under oxygen-limited conditions [5, 14]. It has been reported that about 10% of the ammonium (NH₄⁺) load was converted to N₂O when dissolved oxygen was maintained below 1.0 mg/l [5].

Nitrite can also trigger the production of N₂O by autotrophic denitrification [23]. NO and N₂O have been reported to accumulate at high nitrite concentrations when additional nitrite was injected in the nitrification system [18]. A recent survey on N₂O emissions from wastewater treatment has indicated that various nitrification/denitrification pathways and mechanisms are involved in N₂O emission, and these pathways were shown to depend on the environmental and operational conditions of the wastewater treatment

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[13]. The mechanism or the microbial metabolisms involved in the N_2O emission are much more complex than expected, and it is not easy to clearly determine which operational parameter is mostly responsible for N_2O emission from nitrogen removing wastewater treatment plants. However, it is thought that dissolved oxygen is the most important operational parameter in regard to N_2O emission [13, 23]. Therefore, in this study, we used different reactor systems for nitrification and denitrification to compare N_2O emissions under distinct dissolved oxygen conditions.

The sequencing batch reactor (SBR) has been widely used for commercial wastewater treatment and it is suitable for research purposes owing to its diverse and flexible operation scheme [22]. Two types of SBRs were used in this study. The conventional SBR (CNV-SBR) consisted of sequential denitrification and nitrification. During the denitrification phase, wastewater is fed to the reactor to supply electron donors for denitrification. The simultaneous nitrification and denitrification SBR (SND-SBR) operates under a microaerobic (low dissolved oxygen) condition to achieve nitrification and denitrification simultaneously. The advantage of simultaneous nitrification and denitrification is that it does not require separate aerobic and anoxic zones. Therefore, it can utilize existing facilities without further construction. SND has also been estimated to require smaller total tank sizes [11, 17] and maintains a relatively neutral pH in the bioreactor without the addition of external acid/base, since the alkalinity consumed by nitrification is partially recovered by denitrification [6]. In this study, the CNV-SBR was operated under two different conditions, aerobic (nitrification) and anoxic (denitrification), whereas the SND-SBR was operated under the microaerobic condition by intermittent aeration.

The objectives of this study were to compare the characteristics of nitrogen removal and N_2O emission of the CNV-SBR and the SND-SBR, and to quantitatively analyze the effects of dissolved oxygen during nitrogen removal operations (nitrification, denitrification, simultaneous nitrification and denitrification) on N_2O emission. Cumulated N_2O emission and N_2O yields were analyzed for this purpose.

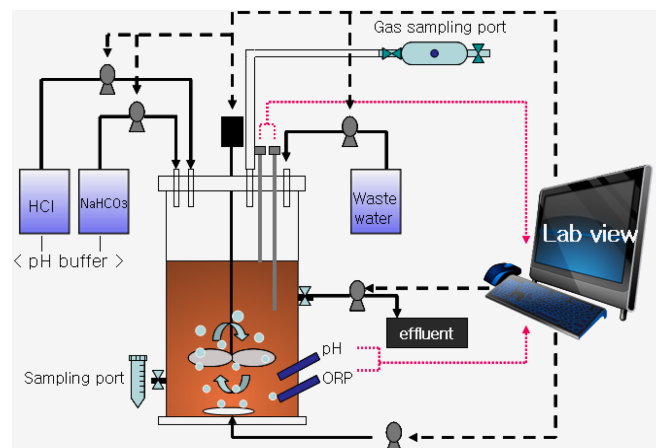


Fig. 1. Experimental set-up of the laboratory scale SBR for nitrogen removal from the synthetic wastewater.

MATERIALS AND METHODS

Experimental Set-Up of the CNV-SBR and SND-SBR

The SBRs (CNV-SBR and SND-SBR) were made of an acrylic column that had a height of 40 cm and a diameter of 20 cm (working volume: 9 L). A schematic diagram of the SBR is shown in Fig. 1. All the operations of the SBRs, wastewater pumping, aeration, agitation, settling, decanting and pH control, were controlled using a personal computer (LabView). A mechanical agitator was used to mix the reactor. The pH was maintained between 7.0 and 8.0 by the addition of $NaHCO_3$ and HCl solutions. One cycle of the SBRs took 8 h, and the operation procedures of the two SBRs are shown in Table 1. The CNV-SBR had a separate denitrification and nitrification procedures, whereas nitrification and denitrification were performed simultaneously when the SND-SBR was used. The CNV-SBR cycle consisted of 180 min of wastewater feeding without aeration (anoxic) for denitrification and 265 min of aeration for nitrification. The SND-SBR cycle consisted of 445 min of simultaneous nitrification and denitrification by consecutive on/off of the aeration. During the aeration-off period, the same flow rate (0.8 L/min) of pure nitrogen gas was purged into the SBRs. After allowing 25 min for sludge settling, 20% (1.8 L) of the total wastewater was decanted from the SBRs over 10 min. The activated sludge from a municipal wastewater treatment plant of Chuncheon City was used for inoculation. N_2O

Table 1. Operation cycles and conditions of the CNV-SBR and SND-SBR applied in this study.

	Operation cycle (min)			Operation condition	
	CNV-SBR	SND-SBR		CNV-SBR	SND-SBR
Anoxic (Feeding)	180 (180)	445 (440) microaerobic (aeration:10 min on/10 min off)	DO	0~6.5 mg/l	0~1.0 mg/l
			MLSS	1,600 mg/l	1,850 mg/l
Aeration	265		HRT ^a	40 h	
Settling	25	25	SRT ^b	25 days	
Draw	10	10	Aeration	0.8 L/min	
Total	480	480	Temp.	27~30°C	

^a HRT: hydraulic retention time.

^b SRT: sludge retention time.

Table 2. Composition of the synthetic wastewater used in the SBR experiments.

Contents	Concentration (mg/l)
N((NH ₄) ₂ SO ₄)	240
Acetate	800
NaHCO ₃	1,700
MgSO ₄ ·H ₂ O	32
KCl	45
NaHPO ₄ ·12H ₂ O	186
CaCl ₂ ·2H ₂ O	45
KH ₂ PO ₄	70
FeCl ₃ ·6H ₂ O	6

emissions and nitrogen components in the wastewater were periodically monitored and compared to analyze the characteristics of the two SBRs when they reached steady state 30 days after the start-up. The synthetic wastewater composition used in this study is shown in Table 2. Acetate was used as the electron donor for denitrification, and NaHCO₃ was used to buffer the wastewater pH. The wastewater had a COD/N ratio of 3.56, which was higher than the theoretical COD (COD/N = 2.68) needed for complete denitrification.

Analytical Methods

Liquid samples taken from the SBRs were filtered through a 0.2 µm syringe filter before ionic compound analysis. NH₄⁺-N was analyzed using an ion chromatograph (ICS-1500, Dionex) equipped with a cationic column (CS15, Dionex), and 10 mM H₂SO₄ + 9% acetonitrile was used as the eluent. NO₂⁻-N, NO₃⁻-N, and acetate were analyzed using an ion chromatograph (DX-500, Dionex) equipped with an anionic column (AS14, Dionex), and 0.5 M Na₂CO₃ + 0.5 M NaHCO₃ was used as the eluent. MLSS (mixed liquor suspended solids) and MLVSS (mixed liquor volatile suspended solids) in the SBRs were measured after drying the solids in an oven at 100–110°C for 2 h and heating at 550°C for 15 min. Dissolved oxygen was measured using a DO meter (235D; Istek, Korea) and the pH was measured with a pH meter (720P; Istek, Korea).

The gas sampling port, which consisted of a 250 ml glass cylinder with valves at the inlet and outlet, was used to measure N₂O in the off-gas of the SBRs. The valves were normally open, but were closed during gas sampling, and the gas samples were acquired using a 5 ml gas-tight syringe for analysis. N₂O in the off-gas was measured using a gas chromatograph (6890, Agilent) with a HP-FFTP column and an electron capture detector at an oven temperature of 50°C, injector temperature of 100°C, and detector temperature of 250°C. High-purity N₂ gas was used as the carrier gas. Nitrogen mass balance was carried out based on the measured values of nitrogen compounds (NH₄⁺-N, NO₂⁻-N, NO₃⁻-N, N₂O-N) in the SBRs and the estimated nitrogen mass of the nitrifying bacteria.

Dissolved N₂O in the wastewater was measured as follows [15]: 5 ml of wastewater was mixed vigorously with 0.1 ml of 5% HgCl₂ in a serum vial (15 ml) for 1 min to stop the microbial activity. The vial was purged with argon gas for 30 min to keep the pressure slightly higher than the atmospheric pressure. The vial was incubated in a shaking incubator for 1 day at room temperature to achieve gas–liquid equilibrium. Gas in the head space of the vial was sampled using a gas-tight syringe to measure N₂O. Dissolved N₂O (C_{N₂O}) was calculated using the following equation (1):

$$C_{N_2O} = \frac{C_{gas} \times (V_{gas} + \alpha \times V_{liq})}{V_{liq}} \quad (1)$$

C_{gas}: N₂O concentration in the gas phase

V_{gas}: volume of the gas phase

V_{liq}: volume of the liquid sample

α: N₂O solubility in water at 25°C (0.544 ml N₂O/ml water)

RESULTS

Nitrogen Removal and N₂O Emission in CNV-SBR

Nitrogen removal in the CNV-SBR occurs by cyclic denitrification and nitrification. Denitrification of nitrified wastewater was carried out under an anoxic condition by supplying 1.8 L of wastewater containing 240 mg/l of NH₄⁺-N and 800 mg/l of acetate for 180 min at a wastewater flow rate of 10 ml/min. After denitrification, nitrification was carried out for 265 min under the aerobic condition without wastewater feeding. Fig. 2A shows a typical nitrogen compound profile of a cycle of the CNV-SBR in steady state. Nitrogen compounds were expressed in mass units to show quantitative nitrogen transformation in the SBR as the wastewater volume varied during the SBR operation cycle.

After 180 min of wastewater feeding in the anoxic phase, a total of 360 mg of NH₄⁺-N was obtained and the concentration was 40 mg/l in the CNV-SBR. Based on the nitrogen mass balance, the CNV-SBR should have 432 mg of NH₄⁺-N after feeding if there was no reaction or stripping of NH₄⁺-N. It was thought that some of the missing NH₄⁺-N had assimilated into bacteria and was transformed or stripped to gaseous compounds. However, most of the NO₃⁻-N (about 280 mg) was denitrified by the consumption of acetate in the wastewater. After the anoxic phase, the system was placed under the aerobic condition for 265 min through aeration and the remaining NH₄⁺-N was nitrified, which resulted in the production of about 310 mg of NO₃⁻-N and very little accumulation of NO₂⁻-N.

About 3.9 mg of N₂O-N was emitted during the denitrification phase, while 1.6 mg was emitted during the nitrification phase, corresponding to a total N₂O-N emission of 5.5 mg, which was 1.27% of the total influent NH₄⁺-N. With regard to nitrogen mass balance of the CNV-SBR, the wastewater nitrification efficiency was close to 100% and the total nitrogen removal efficiency reached 86% at an influent NH₄⁺-N concentration of 240 mg/l and effluent NO₂⁻-N and NO₃⁻-N concentrations of 1.1 and 32.8 mg/l, respectively.

Fig. 2B shows the profiles of pH and dissolved oxygen of the CNV-SBR at the same cycle. The pH increased from 7.3 to 8.0 over the first 180 min in the anoxic phase as denitrification increased the alkalinity (HCO₃⁻). The pH decreased to 7.0 in the aerobic phase as nitrification continued. Dissolved oxygen was maintained at zero level during the denitrification, and it increased up to 6.0 mg/l in

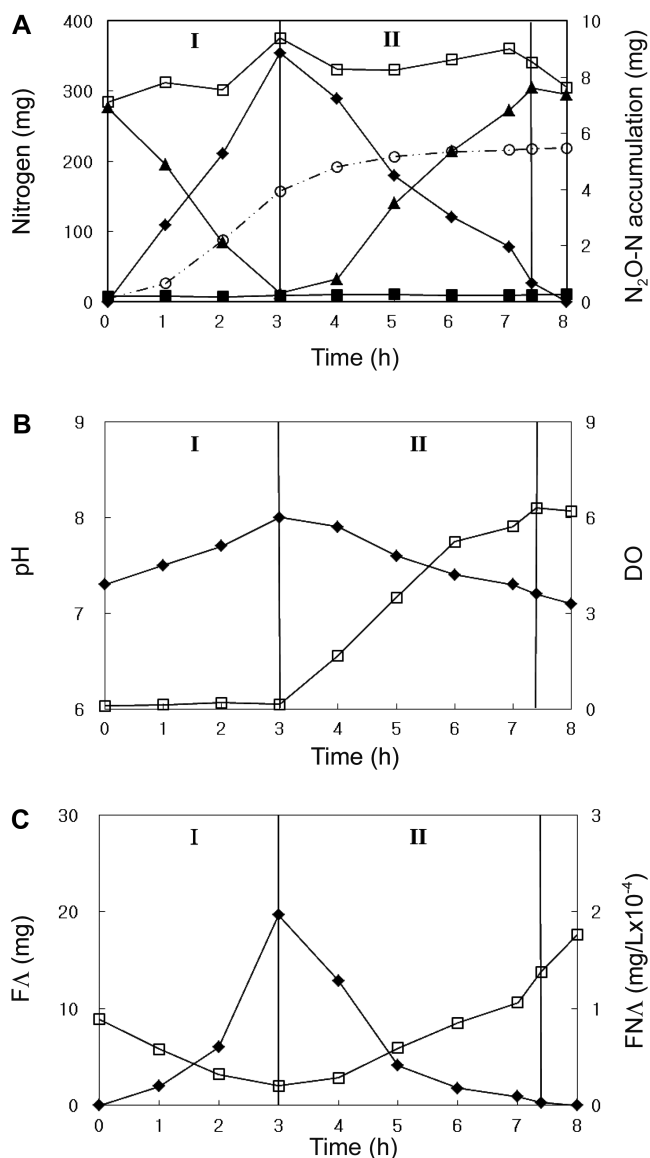


Fig. 2. N₂O emission from the CNV-SBR during nitrification and denitrification.

(A) Nitrogen concentration profiles in the CNV-SBR (◆: NH₄⁺-N; ■: NO₂⁻-N; ▲: NO₃⁻-N; □: Total N; ○: N₂O-N; I: anoxic phase; II: aerobic phase). (B) Profiles of the pH and DO in the CNV-SBR (◆: pH; □: DO). (C) Profiles of free ammonia (FA) and free nitrous acid (FNA) in the CNV-SBR (◆: free ammonia; □: free nitrous acid).

the aerobic phase for nitrification. The pH and dissolved oxygen profiles of the CNV-SBR are in agreement with the sequential denitrification and nitrification of the CNV-SBR.

Fig. 2C shows the profiles of free ammonia (NH₃, FA) and free nitrous acid (HNO₂, FNA) at the same cycle of the CNV-SBR. FA and FNA are in equilibrium with NH₄⁺-N and NO₂⁻-N, respectively, depending on the pH and temperature [1]. When the pH was higher, the equilibrium shifted to more FA and less FNA, and *vice versa*. FA

increased to 2.1 mg/l during the anoxic phase as the NH₄⁺-N concentration increased owing to increased wastewater feeding, and it decreased to zero in the aerobic phase with the oxidation of NH₄⁺-N. The concentration profile of FNA was the opposite of the FA concentration profile. FNA decreased to zero in the anoxic phase owing to the denitrification of NO₂⁻-N, and increased up to 2.0×10^{-4} mg/l in the aerobic phase with nitrification. Both FA and FNA are inhibitors of nitrification, and their concentrations should be maintained as low as possible to achieve a high nitrification efficiency by keeping the pH within the neutral range of 7~8 [1].

Nitrogen Removal and N₂O Emission in SND-SBR

In this study, the SND-SBR removed ammonium through simultaneous nitrification and denitrification by intermittent aeration. Wastewater was fed to the SND-SBR for 440 min, and air and nitrogen were intermittently supplied every 10 min to provide a microaerobic condition during the 445 min of operation. Fig. 3 shows the profiles of a cycle of the SND-SBR in steady state. The NH₄⁺-N mass did not significantly change throughout the operation cycle. It was 300 mg at the start of the operation and decreased slightly to 286 mg at the end of the SND-SBR cycle (Fig. 3A). NO₂⁻-N increased somewhat, while NO₃⁻-N decreased during the cycle. In one cycle of the SND-SBR, 432 mg of NH₄⁺-N was fed to the reactor. The nitrification efficiency of the SND-SBR was about 87%, which was estimated from the NH₄⁺-N concentrations of the influent (240 mg/l) and the effluent (31.8 mg/l) [(240 - 31.8)/240 × 100(%) = 87%]. The total nitrogen removal efficiency reached 85% [(240 - 36.2)/240 × 100(%) = 85%]. The SND-SBR showed almost the same efficiency of total nitrogen removal as the CNV-SBR, even though the nitrification efficiency was somewhat lower than the CNV-SBR.

Fig. 3B shows the accumulated amounts of N₂O-N emitted from the SND-SBR during the anoxic and the aerobic phases, which were measured every 10 min. The accumulated N₂O-N emitted from the SND-SBR cycle was 26.2 mg, which consisted of 13.3 mg from the aeration-off period and 12.9 mg from the aeration-on period. The aeration-off period emitted slightly more N₂O than the aeration-on period. The amounts of N₂O-N emitted during the aeration-on and aeration-off periods were similar. This was in contrast to the result of the CNV-SBR operation, which showed significant differences between the anoxic and aerobic phases. The N₂O stripping effect was likely negligible since we provided the same flow rate of air and N₂ in the aeration-on and aeration-off periods. In addition, dissolved N₂O was also maintained very low (0.001~0.004 ml N₂O/l) in the SND-SBR. The total N₂O-N yield based on the input NH₄⁺-N was 6.1% (26.2/432 × 100(%) = 6.1%). Based on the yield, the SND-SBR emitted about 5 times more N₂O than the CNV-SBR.

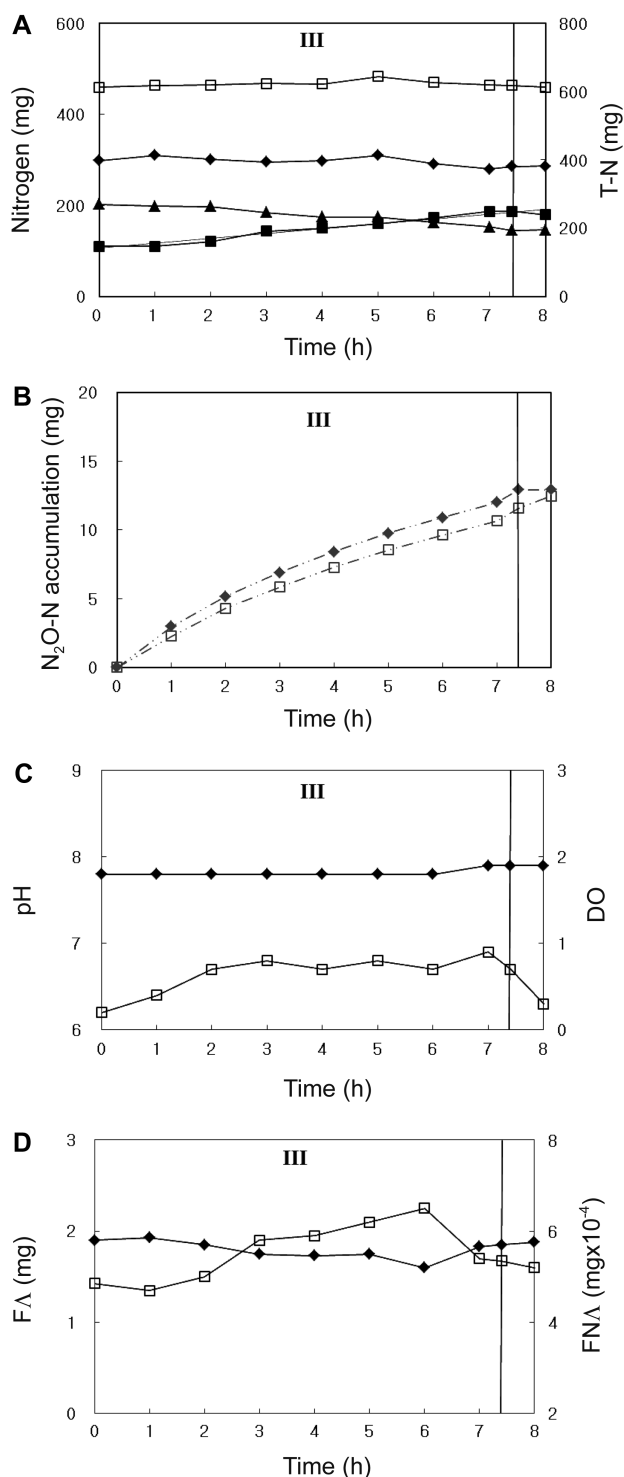


Fig. 3. N_2O emission from the SND-SBR during simultaneous nitrification and denitrification.

(A) Nitrogen concentration profiles in the SND-SBR (\blacklozenge : $\text{NH}_4^+\text{-N}$; \blacksquare : $\text{NO}_2^-\text{-N}$; \blacktriangle : $\text{NO}_3^-\text{-N}$; \square : Total N; III: microaerobic (aeration on/off) condition). (B) Accumulated $\text{N}_2\text{O-N}$ emission from the SND-SBR (\blacklozenge : aeration-off period; \square : aeration-on period). (C) Profiles of the pH and DO in the SND-SBR (\blacklozenge : pH; \square : DO). (D) Profiles of free ammonia (FA) and free nitrous acid (FNA) in the SND-SBR (\blacklozenge : free ammonia; \square : free nitrous acid).

Fig. 3C shows the pH and the average dissolved oxygen profiles for the SND-SBR. The pH remained constant during the cycle. The average dissolved oxygen was maintained between 0.5 and 1.0 mg/l, which corresponds to the microaerobic condition and is appropriate for simultaneous nitrification and denitrification. Fig. 3D shows the profiles of FA and FNA in the SND-SBR. The FA and FNA concentrations remained at almost the same level as the pH, and the concentrations of $\text{NH}_4^+\text{-N}$ and $\text{NO}_2^-\text{-N}$ did not change significantly during the SND-SBR operation, as shown in Fig. 3A and 3C. The concentrations of FA and FNA were between 1.5–2.0 mg/l and $5.0\text{--}6.0 \times 10^{-4}$ mg/l, respectively, and the FNA during the SND-SBR operation was about 3 times higher than that of the CNV-SBR operation.

DISCUSSION

N_2O Emission in Nitrification

Most of the N_2O emission from wastewater treatment plants occurs during wastewater nitrification and denitrification. The factors affecting N_2O emission during nitrification and denitrification were investigated during both CNV-SBR and SND-SBR operations. Dissolved oxygen and nitrite are known to exert significant effects on N_2O emission during wastewater nitrification [12, 17, 22]. N_2O emission increased at a low dissolved oxygen concentration owing to autotrophic denitrification by AOB, which uses nitrite as the electron acceptor instead of oxygen for the oxidation of ammonia [2]. It has been reported that about 10% of input $\text{NH}_4^+\text{-N}$ was emitted as N_2O when the dissolved oxygen was lower than 1.0 mg/l [5]. Therefore, it is important to keep the dissolved oxygen level high enough for wastewater nitrification to reduce N_2O emission. Nitrite is also known to accelerate N_2O emission when the nitrite level increases in wastewater nitrification, since AOB reduces nitrite to N_2O .

Dissolved oxygen in the aerobic phase in the CNV-SBR was higher than that of the SND-SBR, which is important in regard to not limiting wastewater nitrification (Fig. 2B and Fig. 3C). The nitrite concentration in the SND-SBR was much higher than that of the CNV-SBR. When considering the concentrations of dissolved oxygen and nitrite in the SBRs, it is likely that the SND-SBR had the potential to emit more N_2O than the CNV-SBR. The experimental results also support this hypothesis, since CNV-SBR emitted 1.6 mg of $\text{N}_2\text{O-N}$, while the SND-SBR emitted 12.9 mg of $\text{N}_2\text{O-N}$ during the aeration-on period.

N_2O Emission in Denitrification

Unlike nitrification, the presence of dissolved oxygen during denitrification stimulates N_2O emission, because N_2O reductase of denitrifying bacteria is inhibited by oxygen [13]. It is also possible that high nitrite concentrations or nitrite injection increase NO or N_2O emission, as the

denitrification rate did not increase promptly. In addition, wastewater with a low COD/N ratio also results in N_2O emission owing to the shortage of electron donors for denitrification. Hanaki *et al.* [7] reported that wastewater with a low COD/N ratio of 1.5 emitted about 10% of the input nitrogen as N_2O during denitrification. Itokawa *et al.* [10] also reported that 20~30% of input nitrogen was converted to N_2O in an intermittently aerated bioreactor when the COD/N ratio in the wastewater was less than 3.5. In this experiment, the wastewater had a COD/N ratio of 3.56, which is higher than the theoretical ratio needed for complete denitrification. In the denitrification (anoxic) phase of the CNV-SBR, most of the nitrate was denitrified without the accumulation of nitrite, even though 3.9 mg of N_2O -N was emitted (Fig. 2A). In contrast, for the SND-SBR, some of the carbon source was inevitably consumed by aerobic respiration, which decreased the available COD for denitrification. This process emitted 13.3 mg of N_2O -N during the aeration-off period, which was approximately 3.4 times more than the amount emitted during the CNV-SBR process. The results of N_2O emission of the two SBRs support the hypothesis that a low COD/N ratio increases N_2O emission during wastewater denitrification. It has also been argued that nitrite accumulation is the direct reason for N_2O emission other than a low COD/N, which leads to nitrite accumulation [7]. However, it is not easy to clearly identify a single factor for N_2O emission during the reactor operations. It seems that both nitrite and a low COD/N contribute to N_2O emission. Further studies are needed to clearly identify individual effects on N_2O emission during denitrification.

N_2O Emission in Simultaneous Nitrification and Denitrification

Simultaneous nitrification and denitrification has been developed for nitrogen removal so that existing facilities can be used without the need to construct baffles [22]. However, the results of this study showed that the SND-SBR emitted more N_2O than the CNV-SBR. In the presence of oxygen, denitrification will deteriorate and N_2O emission will increase in the SND-SBR as N_2O reductase is inhibited. Furthermore, electron donors for heterotrophic denitrification are more limited in the SND-SBR than the CNV-SBR, since the carbon source is consumed not only for denitrification but also aerobic oxidation. Thus, careful consideration should be applied when adopting simultaneous nitrification and denitrification technology for nitrogen removal, because it may increase N_2O emission. In addition, it has also been reported that a rapid change in the operation condition can increase N_2O emission, such as abrupt changes in ammonium load [22], oxygen limitation [12], and nitrite concentration [18]. Nitrifying bacteria and denitrifying bacteria may increase N_2O emission owing to the time required to adapt to a changed environment. From

this aspect, the SND-SBR may be more susceptible to changes in operation condition than the CNV-SBR. Therefore, this may also be the reason for the higher N_2O emission in the SND-SBR than the CNV-SBR.

The effects of FA and FNA on N_2O emission have not been clearly identified. Both FA and FNA are known to inhibit AOB and nitrite oxidizing bacteria (NOB) [1], and a recent study showed that FNA has a greater effect on N_2O emission than FA [20, 21]. In this study, FA and FNA levels of the SBRs were too low to inhibit AOB. However, the FNA concentration was higher during the SND-SBR operation than the CNV-SBR operation, which may have had some effect on NOB. However, their inhibitory effects on N_2O emission are thought to be very limited, because NOB do not directly participate in N_2O production [23]. However, inhibition of NOB may slow down nitrite oxidation to nitrate accumulation during nitrification, and the accumulated nitrite can enhance N_2O emission during nitrification and denitrification.

In summary, a comparative study was performed to estimate N_2O emission during nitrification, denitrification, and simultaneous nitrification and denitrification from the nitrogen-removing sequencing batch reactors (CNV-SBR and SND-SBR). Simultaneous nitrification and denitrification produced the highest N_2O emission yield (6.1%), which was followed by denitrification (0.9%) and nitrification (0.4%). The microaerobic condition of the SND-SBR seemed to be the main cause of N_2O emission. The low dissolved oxygen of the microaerobic condition inhibited N_2O reductase and stimulated autotrophic denitrification. Thus, appropriate dissolved oxygen level is critical for reducing N_2O emission during nitrification and denitrification at wastewater treatment plants.

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REFERENCES

1. Anthonisen, A. C., R. C. Loehr, T. B. S. Prakasam, and E. G. Srinath. 1976. Inhibition of nitrification by ammonia and nitrous acid. *J. Water Pollut. Control* **48**: 835–852.
2. Bock, E., I. Schmidt, R. Stuvén, and D. Zart. 1995. Nitrogen loss caused by denitrifying *Nitrosomonas* cells using ammonium or hydrogen as electron donors and nitrite as electron acceptor. *Arch. Microbiol.* **163**: 16–20.

3. Burgess, J. E., B. B. Colliver, R. M. Stuetz, and T. Stephenson. Dinitrogen oxide production by a mixed culture of nitrifying bacteria during shock loading and aeration. *J. Ind. Microbiol. Biotechnol.* **29**: 309–313.
4. Colliver, B. B. and T. Stephenson. 2000. Production of nitrogen oxide and dinitrogen oxide by autotrophic nitrifiers. *Biotechnol. Adv.* **18**: 219–232.
5. Goreau, T. J., W. A. Kaplan, S. C. Wofsy, M. B. McElroy, F. W. Valois, and S. W. Watson. 1980. Production of nitrite and nitrogen oxide (N₂O) by nitrifying bacteria at reduced concentrations of oxygen. *Appl. Environ. Microbiol.* **40**: 526–532.
6. Grady, C. P. L., G. Daigger, and H. C. Lim. 1999. *Biological Wastewater Treatment, Second Edition, Revised and Expanded*. Dekker, NY, United States.
7. Hanaki, K., Z. Hong, and T. Matsuo. 1992. Production of nitrous oxide gas during denitrification of wastewater. *Water Sci. Technol.* **26**: 1027–1036.
8. Hooper, A. B. and K. R. Terry. 1979. Hydroxylamine oxidoreductase of *Nitrosomonas*: Production of nitric oxide from hydroxylamine. *Biochim. Biophys. Acta* **571**: 12–20.
9. IPCC. 2006. *IPCC Guidelines for National Greenhouse Gas Inventories*. IGES, Japan.
10. Itokawa, H., K. Hanaki, and T. Matsuo. 1996. Nitrous oxide emission during nitrification and denitrification in a full-scale night soil treatment plant. *Water Sci. Technol.* **34**: 277–284.
11. Kaempfer, H., G. Daigger, and C. Adams. 2000. *WEFTEC 2000, Annual Conference & Exposition on Water Quality and Wastewater Treatment*, 73rd, Anaheim, CA, United States.
12. Kampschreur, M. J., N. C. G. Tan, R. Kleerebezem, C. Picoreanu, M. S. M. Jetten, and M. C. M. van Loosdrecht. 2008. Effect of dynamic process conditions on nitrogen oxides emission from a nitrifying culture. *Environ. Sci. Technol.* **42**: 429–435.
13. Kampschreur, M. J., H. Temmink, R. Kleerebezem, M. S. M. Jetten, and M. C. M. van Loosdrecht. 2009. Nitrous oxide emission during wastewater treatment. *Water Res.* **43**: 4093–4103.
14. Kim, D. J. and Y. Kim. 2011. Effect of ammonium concentration on the emission of N₂O under oxygen-limited autotrophic wastewater nitrification. *J. Microbiol. Biotechnol.* **21**: 988–994.
15. Mao, J., X. Q. Jiang, L. Z. Yang, J. Zhang, Q. Y. Qiao, C. D. He, and S. X. Yin. 2006. Nitrous oxide production in a sequence batch reactor wastewater treatment system using synthetic wastewater. *Pedosphere* **16**: 451–456.
16. Otte, S., N. G. Grobben, L. A. Robertson, M. S. M. Jetten, and J. G. Kuenen. 1996. Nitrous oxide production by *Alcaligenes faecalis* under transient and dynamic aerobic and anaerobic conditions. *Appl. Environ. Microbiol.* **62**: 2421–2426.
17. Stensel, H. D. 2001. *Conference Proceedings - Water Environment Federation Annual Conference & Exposition*, 74th, Atlanta, GA, United States.
18. Tallec, G., J. Garnier, G. Billen, and M. Gossais. 2006. Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: Effect of oxygenation level. *Water Res.* **40**: 2972–2980.
19. Tchobanoglous, G., F. L. Burton, and H. D. Stensel. 2003. *Wastewater Engineering Treatment and Reuse, 4th Ed.* McGraw-Hill, NY, United States.
20. Vadivelu, V. M., Z. Yuan, C. Fux, and J. Keller. 2006. The inhibitory effects of free nitrous acid on the energy generation and growth processes of an enriched *Nitrobacter* culture. *Environ. Sci. Technol.* **40**: 4442–4448.
21. Vadivelu, V. M., Z. Yuan, C. Fux, and J. Keller. 2006. Stoichiometric and kinetic characterisation of *Nitrobacter* in mixed culture by decoupling the growth and energy generation processes. *Biotechnol. Bioeng.* **94**: 1176–1188.
22. Wilderer, P. A., R. L. Irvine, and M. C. Goronszy. 2001. *Sequencing Batch Reactor Technology, Scientific and Technical Report No. 10*. IWA Publishing, UK.
23. Wrage, N., G. L. Velthof, M. L. van Beusichem, and M. Oenema. 2001. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Biochem.* **33**: 1723–1732.
24. Zheng, H., K. Hanaki, and T. Matsuo. 1994. Production of nitrous oxide gas during nitrification of wastewater. *Water Sci. Technol.* **30**: 133.