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Factors affecting nitrous oxide production: a comparison of biological nitrogen removal processes with partial and complete nitrification

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Abstract

Nitrous oxide (N_2O) emission from biological nitrogen removal (BNR) processes has recently received more research attention. In this study, two lab-scale BNR systems were used to investigate the effects of various operating parameters including the carbon to nitrogen (C/N) ratio, ammonia loading, and the hydraulic retention time on N_2O production. The first system was operated in a conventional BNR mode known as the Ludzack–Ettinger (LE) process, consisting of complete denitrification and nitrification reactors, while the second one was operated in a shortcut BNR (SBNR) mode employing partial nitrification and shortcut denitrification, which requires less oxygen and carbon sources. As the C/N ratio was decreased, a significant increase in N_2O production was observed only in the anoxic reactor of the LE process, indicating that N_2O was released as an intermediate of the denitrification reaction under the carbon-limited condition. However, the SBNR process did not produce significant N_2O even at the lowest C/N ratio of 0.5. When the SBNR process was subjected to increasing concentrations of ammonia, N_2O production from the aerobic reactor was rapidly increased. Furthermore, the increasing production of N_2O was observed mostly in the aerobic reactor of the SBNR process with a decline in hydraulic retention time. These experimental findings indicated that the increase in N_2O production was closely related to the accumulation of free ammonia, which was caused by an abrupt increase of the ammonium loading. Consequently, the partial nitrification was more susceptible to shock loading conditions, resulting in a high production of N_2O, although the SBNR process was more efficient with respect to nitrogen removals as well as carbon and oxygen requirements.

Abbreviations: BNR – biological nitrogen removal; C/N – carbon to nitrogen ratio; FA – free ammonia; HRT – hydraulic retention time; LE – Ludzack–Ettinger; SBNR – shortcut biological nitrogen removal.

Introduction

Biological nitrogen removal (BNR) is widely used as an add-on process to many biological treatment systems worldwide, since it is commonly applicable to wastewater streams containing various nitrogen sources. The BNR consists of the two-step process, nitrification and denitrification. Nitrification is the reaction where ammonia (NH_4^+-N) in the influent stream is oxidized to nitrite (NO_2^--N) and subsequently oxidized to nitrate (NO_3^--N) as energy sources to nitrifying bacteria; therefore, the concentration of dissolved oxygen (DO) should be maintained at an appropriate level in nitrification reactors (Brindle et al. 1998). Biological denitrification takes place in an anoxic zone where nitrate is finally reduced to nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) in the absence of oxygen. Since nitrate is used as an electron acceptor for the denitrification reaction, a sufficient amount of carbon is required to achieve effective nitrogen removals (Louzeiro et al. 2002). As a result, maintaining the DO level for the nitrification as well as supplying external carbon sources to the denitrification can be common engineering challenges.

In order to enhance BNR performance, many processes have been developed by modifying the conventional process. The conventional BNR process, known as the Ludzack–Ettinger (LE) process, is operated in an anoxic–aerobic sequence in which the nitrate produced in the second (aerobic) reactor is fed to the first (anoxic) reactor along with the influent wastewater. In this reactor configuration, the denitrification can be limited greatly in the anoxic reactor of the conventional process depending on the sludge recycle ratio. Therefore, most efforts to modify the conventional process have been devoted to improve the system configuration to more effectively feed nitrate to the anoxic zone. Another system modification to solve the engineering challenges is the shortcut biological nitrogen removal (SBNR) process in which ammonia is partially oxidized to nitrite and the nitrite is directly reduced to dinitrogen (N_2). The SBNR uses a shortcut microbial pathway instead of following the complete oxidation via NO_3^--N in the overall reaction. The SBNR theoretically requires 25% less oxygen for the nitrification and 40% less carbon source for the denitrification; therefore, it can be an economically feasible alternative to the conventional process (Police et al. 2002; Schmidt et al. 2003; Tseng et al. 1998). In addition, it was reported that the rate of nitrite reduction was approximately four times faster than that of nitrate reduction in the denitrification reaction (Chung & Bae 2002).

In addition to the engineering feasibility of the BNR processes, the nitrous oxide (N_2O) emission from the processes has drawn more research interest, since N_2O is known to be green house gas. Although N_2O is emitted in smaller quantities than other greenhouse gases such as CO_2 and CH_4, it has a long lifetime in the atmosphere and a relatively large energy absorption capacity per molecule, resulting in a high global warming potential about 300 times that of CO_2 (Choudhary et al. 2002; Seinfeld & Pandis 1997). The emission of N_2O from BNR processes is one of the most important anthropogenic sources including cultivated soils and biomass burning (Mosier et al. 1999). Figure 1 illustrates the proposed microbial pathway of nitrogen metabolisms and indicates that N_2O can be produced by both the nitrification and denitrification reaction (Wrage et al. 2001). N_2O is released from the nitrification reaction as a byproduct of ammonium oxidation under aerobic conditions by nitrifiers (Poth et al. 1985), while N_2O is an intermediate of the denitrification reaction where nitric oxide (NO) is reduced to N_2.

The N_2O production in BNR processes is sensitive to operating conditions (Focht 1974; Wrage et al. 2004) such as DO concentrations. The SBNR process has, in particular, a high potential for N_2O production (Schulthess and Gujer 1996, since it needs to be operated at relatively high pHs (8.0–8.4) and low DO concentrations (1–2 mg l^{-1}). However, few studies have investigated how the SBNR process affects the biochemical mechanisms of N_2O production. Integrated studies, which focus on linking biological nitrogen removals to N_2O production from modified BNR processes, are therefore required to better understand biochemical N_2O production mechanisms and to develop more environmentally friendly BNR processes.

In this study, the effects of operating conditions including the carbon to nitrogen (C/N) ratio, ammonia loading and the hydraulic retention time (HRT) on the N_2O production were investigated using a lab-scale SBNR system (i.e., a partial oxidation BNR). A conventional, complete oxidation BNR system operated in the LE configuration was also tested under identical operating conditions to those of the SBNR for comparison.

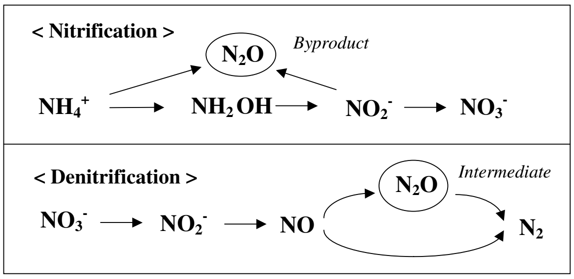

Figure 1. Reaction pathways of N_2O production in the nitrification and the denitrification process.

The main objective of this study was to provide a process control strategy to minimize N_2O production while maintaining effective nitrogen removal.

Materials and methods

System configurations and experimental conditions

The two identical lab-scale BNR systems used in this study consisted of an anoxic (denitrification) reactor, an aerobic (nitrification) reactor and a settling tank, with working volumes of 3, 6, and 3 l, respectively (Figure 2). Both SBNR and LE processes were initially seeded with activated sludge obtained from a wastewater treatment plant in Yongin, Korea. To provide a basis for comparison, a set of operating parameters was established as the control condition used throughout this study (Table 1). The control condition was selected based on operating parameters reported in the literature (Anthonisen et al. 1976; Bae et al. 2002a, 2002b; Garrido et al. 1997; Prakasam & Loehr, 1972) and parameters showing near-optimal performance in another BNR system operated in our laboratory. At the control condition, the DO concentration in the aerobic reactor of the SBNR process was controlled in a range of 1–2 mg l^{-1} in order to increase the concentration of nitrite (NO_2^--N), while the DO in the aerobic reactor of the LE process maintained at values greater than 3 mg l^{-1} for complete nitrification. The HRT was maintained at 2 days at the control condition, and the concentrated sludge from the settling tank was returned to the aerobic reactor of each BNR process at the recycling flow rate of 6 l/day and the suspended solid concentration of approximately 5000 mg/l. The chemical composition of the synthetic wastewater used at the control condition was K_2HPO_4 0.94 g l^{-1}, $MgSO_4 \cdot 7H_2O$ 0.5 g l^{-1}, $FeSO_4 \cdot 7H_2O$ 0.02 g l^{-1}, $CaCl_2$ 0.04 g l^{-1}, $M_mSO_4 \cdot H_2O$ 0.05 g l^{-1}, KCl 0.07 g l^{-1}, NH_4HCO_3 3.39 g l^{-1}, and CH_3COOH (as the sole carbon source) 4.0 g l^{-1} for the SBNR and 6.0 g l^{-1} for the LE. During this study, one operating parameter was altered according to the experimental design described below, while other operating parameters remained constant.

Since the reaction rate of denitrification relies on available carbon sources, a C/N ratio can be the most important operating parameter in both SBNR and LE processes. In order to better understand organic carbon availability at a given nitrogen concentration, we introduced an observed C/N ratio and defined it as the ratio of concentrations of soluble organic carbon in terms of COD and oxidized nitrogen species available for denitrification (Equation (1)). As the control condition, the SBNR system received 600 mg NH_4^+-N l^{-1} at a C/N ratio of 2, and the LE system was operated at an inlet ammonium concentration of 600 mg l^{-1} and a C/N ratio of 3.

$$\text{C/N ratio} = \frac{\text{Soluble COD (mg l}^{-1})}{NO_2^-(\text{mg}-\text{N l}^{-1}) + NO_3^-(\text{mg}-\text{N l}^{-1})} \quad (1)$$

For acclimation, each BNR process was initially operated for 160 days under the control condition.

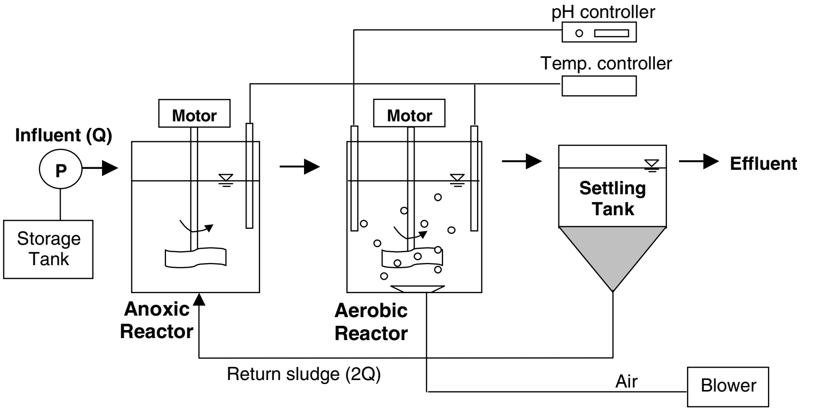

Figure 2. Schematic of the lab-scale biological nitrogen removal (BNR) systems. The configurations of both conventional (LE) and modified (SBNR) processes are identical.

Table 1. Operating parameters at the control condition and resulting concentrations of nitrogen species

Parameters	SBNR process		LE process	
	Anoxic	Aerobic	Anoxic	Aerobic
Inlet NH_4^+-N concentration (mg l^{-1})	600		600	
Hydraulic retention time (day)	2		2	
C/N ratio (–)	2	–	3	–
Dissolved oxygen (mg l^{-1})	0	1–2	0	> 3.0
pH	7.5–8.0	8.0–8.4	7.5–8.0	8.0–8.4
Temperature (°C)	25	30	25	30
MLSS (mg l^{-1})	2000–2500	2000–2500	2000–2500	2000–2500
NH_4^+-N concentration (mg l^{-1})	105[a] (80–130)[b]	8 (1–20)	100 (80–140)	7 (1–10)
NO_2^--N concentration (mg l^{-1})	0.1 (0–5)	80 (20–100)	0 (ND)[c]	0.5 (0–5)
NO_3^--N concentration (mg l^{-1})	1.0 (0–5)	25 (5–45)	0.5 (0–10)	150 (100–180)

[a] The average concentration determined during the initial acclimation period.
[b] The concentration range determined during the initial acclimation period.
[c] Below detection limit.

Following the initial acclimation period, three experimental parameters were sequentially changed (Table 2). First, the C/N ratio supplied to each BNR process was modified as listed in Table 2 (Run 1), while other operating parameters were identical to the control condition. The second experiment (Run 2) was designed to investigate the effect of ammonium (NH_4^+-N) loading on the nitrogen removals and N_2O production. During Run 3, the hydraulic retention time was sequentially changed, while other operating parameters including the C/N ratio and the inlet NH_4^+-N concentration remained the same as the control condition. Between each experiment, all operating parameters were changed back to the control condition at least for 5 days to minimize a carry-over effect from the previous change.

Analytical methods

N_2O production was measured using the headspace method. A 37 ml of the liquid sample taken from each reactor were added into a 53 ml serum vial containing 3 ml of 5% chlorohexidine gluconate as a microbial inhibitor. The vial was vigorously mixed for 1 min and allowed to stand for 1 h to let the headspace equilibrate with the liquid phase. 4 ml gas samples were withdrawn from the headspace using a gas-tight syringe and injected into a gas chromatograph (M600D, Younglin Co., Korea) equipped with a pulse discharge detector (PDD) and a capillary column (Mole Sieve 13X, Resttek, USA). Helium was used as a carrier gas at a flowrate of 50 ml/min, and an isothermal condition was used at an oven temperature of 80 °C.

Table 2. Experiment conditions used in this study

Run No.	Parameter changed	Days	SBNR process	LE process
Run 1 (Days 160–250)	C/N ratio (–)	160–174	2.0	3.0
		175–187	1.0	2.0
		188–223	0.5	1.0
		224–240	2.0	3.0
Run 2 (Days 290–333)	Inlet ammonium concentration (mg-N l^{-1})	290–298	600	600
		299–305	700	700
		306–328	800	800
		329–333	600	600
Run 3 (Days 334–390)	Hydraulic retention time (day)	334–363	1.0	1.0
		364–379	0.5	0.5
		380–390	2.0	2.0

The temperature of the injection port was 80 °C and the detector temperature was held at 100 °C.

Liquid samples were periodically collected and analyzed for total ammonia, nitrite and nitrate. The concentrations of nitrite (NO_2^--N) and nitrate (NO_3^--N) were measured using an ion chromatograph (Model 9600, Younglin Co., Korea) equipped with a UV detector. A spectrophotometer (Model DR400, HACH Co., USA) and a NH_3-N assay kit (HACH Co., USA) were used to determine the total ammonia concentration. In this study, a free ammonia concentration is calculated by the following equation (Anthonisen et al. 1976) using the pH of the liquid sample and the measured total ammonia concentration:

$$\text{Free ammonia conc. (mg l}^{-1}\text{ as NH}_3) = \frac{17}{14} \times \frac{\text{TA} \times 10^{\text{pH}}}{\frac{K_b}{K_w} + 10^{\text{pH}}} \qquad (2)$$

where TA is the total ammonia concentration including the ammonium ion and the free ammonia (mg-N l^{-1}), K_b is the ammonia equilibrium constant, and K_w is the water dissociation constant. Other analyses for soluble chemical oxygen demand (COD), DO concentration, pH and suspended solid (SS) were determined according to Standard Methods (1998).

Results

Prior to starting a series of experiments, each process was operated for 160 days for acclimation under its control condition described above. With the control condition, the LE process showed a near complete oxidation of the ammonium introduced as well as an effective denitrification at an overall nitrogen removal efficiency of 73.8% (Table 1). Meanwhile, the overall nitrogen removal efficiency of the SBNR process was 81.2% which was slightly higher than that of the LE process. A significant accumulation of nitrite (NO_2^-) was not observed in the LE process, whereas the nitrite concentration was maintained high in the aerobic reactor of the SBNR process due to its partial oxidation mechanism. Based on the experimental results obtained from the control condition, a series of experiments was performed at modified operating parameters.

Run 1: The effect of C/N ratio

During Run 1, C/N ratios were sequentially modified in each BNR process to determine how the amount of carbon available for the denitrification affected the N_2O production. Figure 3 summarizes changes in N_2O concentration produced from the anoxic and the aerobic reactor in each process over the 80 days of operation following the acclimation period. The aerobic (nitrification) reaction did not produce a significant amount of N_2O in either process at the C/N ratios tested herein. In addition, there were no significant increases in N_2O production even at the lowest C/N ratio of 0.5 in the anoxic reactor of the SBNR process. The LE process, in contrast, showed a rapid increase in N_2O production only from the anoxic reactor with an average concentration of 130 ppm when the C/N ratio was reduced from 2 to 1.

As the C/N ratio decreased, both nitrite and nitrate concentrations in the anoxic reactor of each BNR process increased due to the limitation of carbon sources for denitrification as shown in Figure 4. A significant accumulation of nitrite and

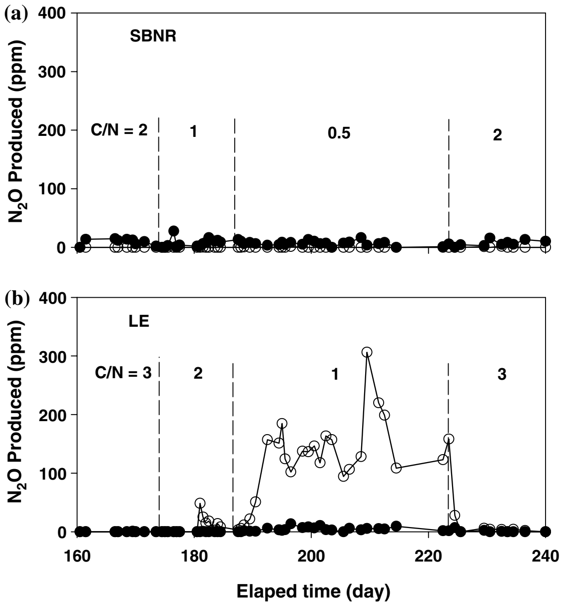

Figure 3. Nitrous oxide concentrations measured from the anoxic (○) and the aerobic reactor (●) of the SBNR (a) and the LE process (b) during Run 1 where the C/N ratio was sequentially changed as indicated.

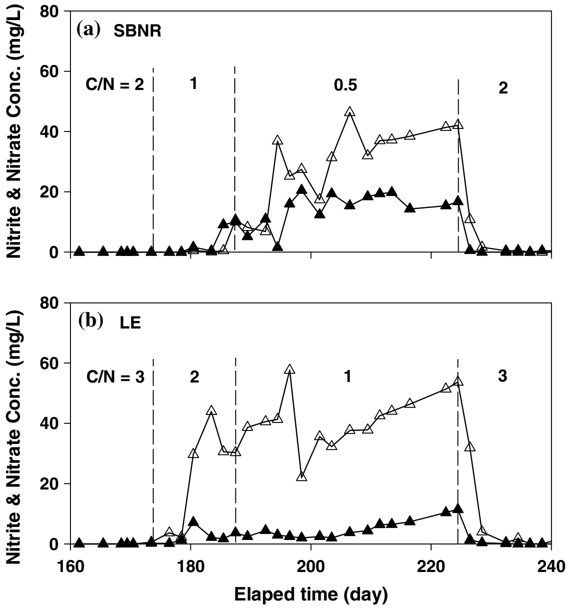

Figure 4. Changes in nitrite (▲) and nitrate (△) concentrations in the anoxic reactor of the SBNR (a) and the LE process (b) during Run 1 as a function of the C/N ratio as indicated.

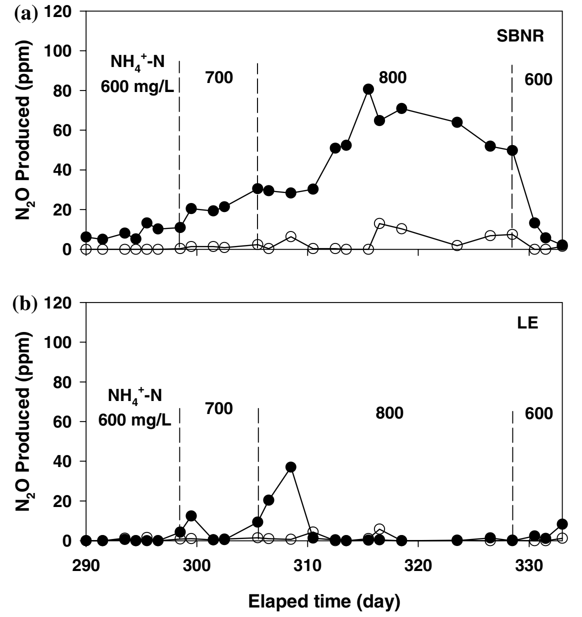

Figure 5. Nitrous oxide concentrations measured from the anoxic (○) and the aerobic reactor (●) of the SBNR (a) and the LE process (b) during Run 2 where the inlet ammonia concentration was sequentially changed.

nitrate occurred at the C/N ratio of 0.5 in the SBNR process, but the accumulation started even when the C/N ratio was reduced to 2 in the LE process. As the C/N ratio was changed back to the control conditions (i.e., the C/N ratio of 2 for the SBNR, and 3 for the LE process), the nitrite and nitrate previously accumulated were rapidly utilized, and their concentrations dropped to those measured during the initial acclimation period.

Run 2: The effect of ammonium (NH_4^+-N) loading

The ammonium (NH_4^+-N) loading to both processes was changed stepwise during Run 2 (days 290–333). Figure 5 shows changes in N_2O produced from the anoxic and the aerobic reactors in each process. In the SBNR process, the N_2O concentration produced from the aerobic reactor increased as a function of the inlet NH_4^+-N concentration, yielding the maximum N_2O concentration of 80.6 ppm. Meanwhile, in the LE process, temporal increases of N_2O production were observed when the inlet NH_4^+-N concentrations were increased to 700 and 800 mg-N l^{-1}, respectively, and the N_2O concentrations dropped in a few days to the values observed at the control condition.

Either nitrite accumulation or free ammonia concentration can be a reason for the increase in N_2O production from the aerobic reactor of the SBNR process (Beline et al. 1999; Weon et al. 1999). Figure 6 shows the nitrite accumulation in terms of the fraction of the total oxidized nitrogen (i.e., $C_{NO_2^-\text{-N}}/[C_{NO_2^-\text{-N}} + C_{NO_3^-\text{-N}}]$ in this simplified experimental condition). As indicated in Figure 6, the nitrite accumulation was in the range of 57–73% in the aerobic reactor of the SBNR process, whereas the nitrite accumulation always remained low at less than 6% during Run 2 of LE operation. In addition, the free ammonia concentrations in the aerobic reactor of the SBNR process were maintained at a much higher level than those found in the LE process at all the C/N ratios throughout the Run 2 operation. It is interesting to note that the days when the N_2O production significantly increased were closely related to the points where the free ammonia concentrations were generally greater than 4 mg l^{-1} in the aerobic reactor of the SBNR.

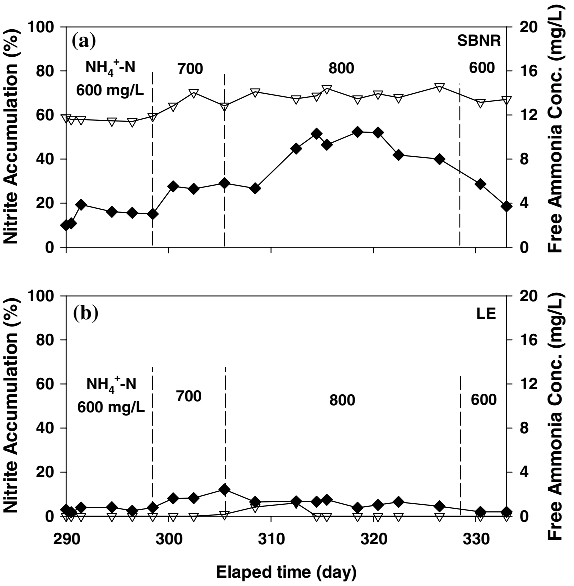

Figure 6. Changes in nitrite accumulation (▽) and free ammonia concentration (◆) in the aerobic reactor of the SBNR (a) and the LE process (b) during Run 2 as a function of the inlet ammonia concentration. The nitrite accumulation is defined as the nitrite fraction of the total oxidized nitrogen.

Run 3: The effect of hydraulic retention time (HRT)

The effect of HRT on N_2O production was examined on days 334–390 (Run 3) as illustrated in Figure 7. Since the HRT was sequentially reduced at the same inlet NH_4^+-N concentration (i.e., 600 mg l^{-1}) during Run 3, the inlet NH_4^+-N loading was correspondingly increased. In the aerobic reactor of the SBNR process, the N_2O production rapidly increased right after the HRT decreased from 2 to 1 day, and then gradually declined. In the same manner, a rapid increase in N_2O production was also observed when the HRT was reduced to 0.5 day in the SBNR process.

The rapid increase in N_2O production is reflected by the changes in free ammonia concentration, similar to the results observed during Run 2. In the SBNR process, the significant increase of the N_2O production was determined when the free ammonia concentration increased to values greater than 4 mg l^{-1} (Figure 8). Even though such a high increase of N_2O production was not observed in the LE process, the increase in free ammonia concentration closely matched with the points at which the measured N_2O concentration increased.

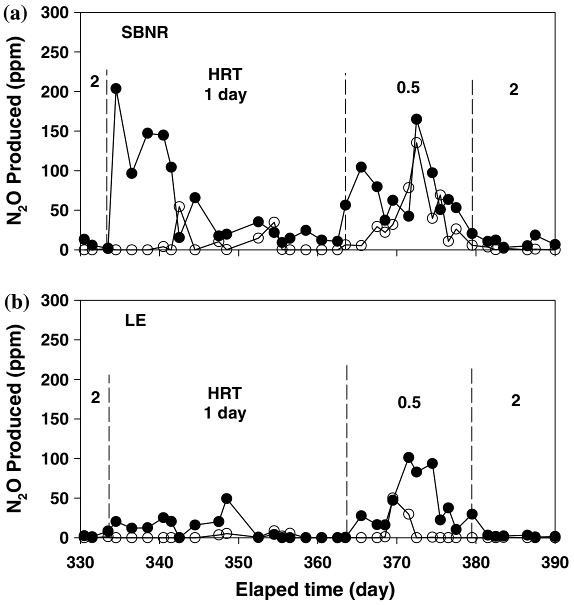

Figure 7. Nitrous oxide concentrations measured from the anoxic (○) and the aerobic reactor (●) of the SBNR (a) and the LE process (b) during Run 3 where the hydraulic retention time was sequentially changed.

Discussion

The overall nitrogen removal efficiency was higher in the SBNR process under the control condition

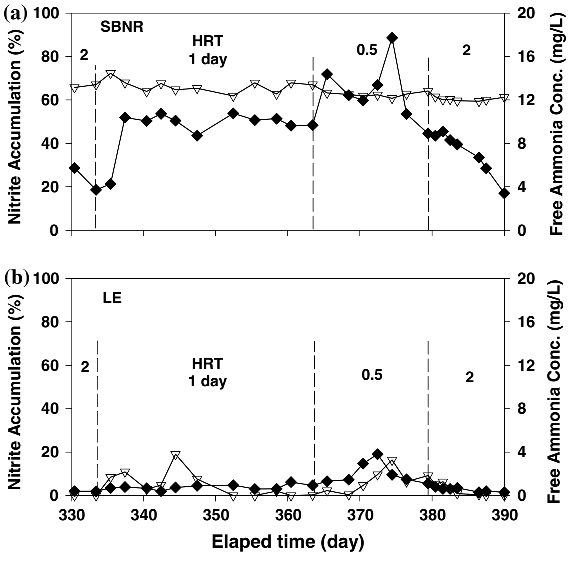

Figure 8. Changes in nitrite accumulation (▽) and free ammonia concentration (◆) in the aerobic reactor of the SBNR (a) and the LE process (b) during Run 3 as a function of the hydraulic retention time.

employed in this study (see Table 1), indicating that the partial oxidation and short-cut denitrification process provides better performance in terms of removing nitrogen more effectively and requiring less carbon sources and oxygen supply. However, the partially oxidized condition in the SBNR process resulted in an accumulation of intermediates such as nitrite and a higher possibility of N_2O production as well. The experimental findings herein, as summarized in Figure 9, indicate that the N_2O production significantly increased from the aerobic reactor of the SBNR with the increasing inlet ammonia loading as well as the increasing HRT. In contrast, the increase in C/N ratio only affected the N_2O production in the anoxic reactor of the LE process, which utilizes the complete oxidation and denitrification mechanism.

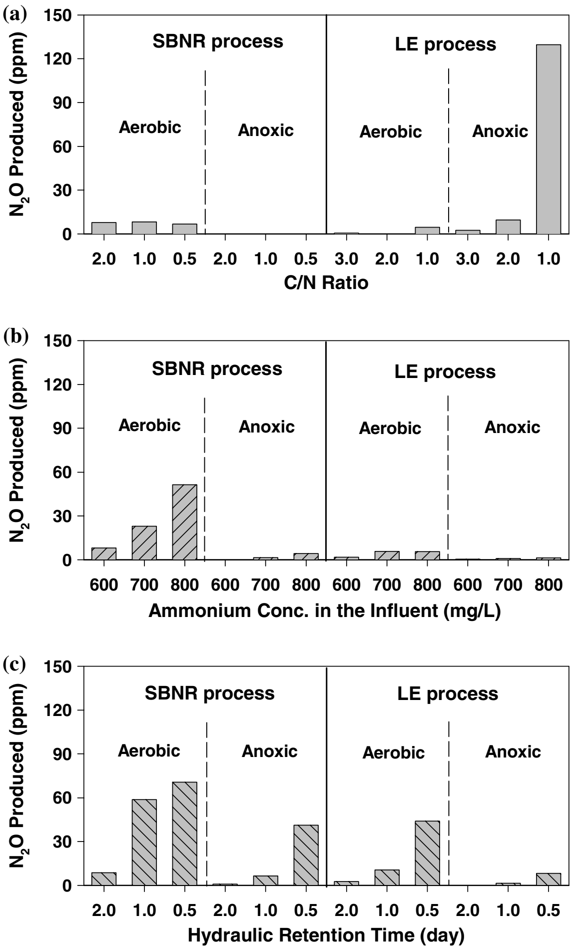

Figure 9. The average N_2O production as a function of operating parameters examined in each reactor of the BNR processes.

The increase in N_2O production at the C/N ratio of 1 in the denitrification reaction of the LE process implies that the final step of the denitrification reaction in the conventional process can not fully occur under this carbon-limited condition. This was consistent with the experimental results reported by Hanaki et al. (1992) who showed an increased N_2O production in a denitrification process when the C/N ratio rapidly dropped. Itokawa et al. (2000) demonstrated that 20–30% of the nitrogen introduced to a BNR process was converted to N_2O when a C/N ratio of below 3.5 was maintained in a denitrification reactor under a steady-state condition. Mean-while, an insignificant increase in N_2O production from the anoxic reactor of the SBNR indicates that the relative amount of available carbon source is not a critical factor for the N_2O production in the SBNR process which requires less carbon for denitrification. It is also clear that the lowered C/N ratio had a little effect on the N_2O production from the aerobic reaction in each process, implying that the NH_4^+-N introduced was almost completely oxidized to NH_2OH without producing the byproduct, N_2O (Figure 1).

The relatively low C/N ratio, at which the SBNR process eventually yielded the significant increase of nitrite and nitrate concentrations in the anoxic reactor, indicates that the BNR process employing the partial nitrification can maintain more stable denitrification than the LE process. Biochemical reactions below can be useful to explain why the nitrite denitrification can more effectively reduce oxidized nitrogen to the final product (i.e., N_2) at the low C/N ratio.

$$6NO_2^- + 3CH_3OH + 3CO_2 \rightarrow 3N_2 + 6HCO_3^- + 3H_2O$$

$$6NO_3^- + 5CH_3OH + CO_2 \rightarrow 3N_2 + 6HCO_3^- + 7H_2O$$

Assuming no carbon assimilation for cell growth, the nitrate denitrification theoretically needs 2.86 g-COD/g-N, whereas 40% less carbon sources is required for the nitrite denitrification at a value of 1.72 g-COD/g-N (US EPA 1993). As a result, the SBNR that utilizes nitrite as a starting compound for the reaction can complete the denitrification steps at a low C/N ratio. This result is consistent with the experimental finding that no significant production of N_2O was observed, even when the nitrite and nitrate concentrations increased under the C/N ratio of 0.5 in the SBNR process.

Nitrite accumulation has been believed to be one of the main factors for an increase in N_2O production from the nitrification reaction in BNR processes (Beline et al. 1999; Gejlsbjerg et al. 1997; Goreau et al. 1980; Park et al. 2000). In this study, a significant production of N_2O was also observed with the increasing ammonia loading in the inlet stream when the nitrite accumulation was high in the range of 57–73% in the aerobic reactor of the SBNR process. Nevertheless, the nitrite accumulation was remained high in the same range when the rate of N_2O production dropped back to its low level from the aerobic reactor of the SBNR (see Figures 5(a) and 6(a), Figures 7(a) and 8(a)). As Gejlsbjerg et al. (1997) found that the presence of ammonia enhanced N_2O production when nitrite concentrations were high, both nitrite accumulation and ammonia concentrations should be taken into account to obtain an insight into how the byproduct is produced from the nitrification process.

Microbial mechanisms in the nitrification pathway and/or interactions between ammonia oxidizing bacteria and nitrite oxidizing bacteria can be a more plausible reason for the increase in aerobic N_2O production. One of the common ammonia oxidizing bacteria, *Nitrosomonas europaea*, generates a high flux of N_2O production at DO concentration of less than 2.1 mg l^{-1} (Dundee et al. 2001). Therefore, in the aerobic reactor of the SBNR process where the DO was maintained below the reported value, ammonia oxidizing bacteria would have a high potential to produce the byproduct, N_2O. Moreover, nitrite oxidizing bacteria are known to be more susceptible to the free ammonia than ammonia oxidizers, and the inhibitive effect becomes more severe as the free ammonia concentration increases. Weon et al. (1999) reported that free ammonia concentrations of 1–40 mg l^{-1} did not inhibit ammonia oxidizers, but nitrite oxidizers received a serious inhibitive effect at free ammonia concentrations greater than 1 mg l^{-1}. As shown in Figure 6, the free ammonia concentrations in the aerobic reactor of the SBNR process were maintained at a much higher level than those found in the LE process throughout the operation. Based on the inhibition threshold values suggested by Weon et al (1999), it can be said that only the nitrite oxidizers in the SBNR process were experiencing a possible inhibition. Consequently, the high concentration of free ammonia inhibited the nitrite oxidizer, resulting in the nitrite accumulation, and the N_2O production increased proportionally to the concentration of free ammonia.

The response of N_2O production was prompt with the decreasing HRTs in the SBNR process. These findings are consistent with experimental results reported by Hanaki et al. (1994) who observed that a BNR process experienced a higher N_2O production as its HRT was suddenly shortened. In contrast, the N_2O production was less sensitively affected in the LE process with the shortened HRTs. These results imply that the partial nitrification reaction employed in the SBNR process be most susceptible with respect to N_2O production to the HRT perturbation. The increase in free ammonia concentration was one of the primary reasons for the rapid increase in N_2O production as shown in Figures 7(a) and 8(a).

Interestingly, a pattern of the N_2O increase and gradual decline after the change in operating parameter was observed. For instance, when the HRT subjected to the SBNR process was shortened to 1 day, the N_2O production was rapidly increased greater than 200 ppm, but eventually decreased and dropped to less than 20 ppm after 10 days under the same operating parameters (Figure 7(a)), even though the free ammonia concentration remained high. The same pattern was also observed in the LE process when the HRT was changed to 0.5 day. These results imply that the high free ammonia concentration triggered the inhibitory effect on nitrite-oxidizing microorganisms resulting in the increase of N_2O production, but the nitrite oxidizers could be gradually adapted to the unfavorable condition and overcome the inhibition. Thus, the gradual adaptation should be taken into account when estimating the contribution of N_2O produced from the BNR processes studied herein to the global greenhouse effect. Further investigation of microbial inhibition is required to better quantify the N_2O production under perturbed operating conditions in BNR processes.

Conclusions

In this investigation, the potential of N_2O production was determined in BNR processes with different reaction mechanisms. As the C/N ratio of the LE process decreased, a significant increase in N_2O production was observed in the anoxic reactor, indicating that N_2O was incompletely reduced to N_2 and released as a reaction intermediate under the carbon-limited conditions. In contrast, the SBNR process did not produce significant N_2O even at the lowest C/N ratio of 0.5. However when the ammonium concentration in the inlet stream was increased, the SBNR process showed a rapid increase in N_2O production from the aerobic reactor. The changes in free ammonia concentration closely reflected the N_2O production from the aerobic reactor of the SBNR process, since a build-up of free ammonia can cause a severe inhibition to nitrite oxidizing bacteria, resulting in a byproduct release from the nitrification reaction. In addition, the decline in HRT yielded the increasing production of N_2O mostly from the aerobic reactor of the SBNR process. The increasing concentration of free ammonia was also responsible for the increase in N_2O production with decreasing HRTs.

The SBNR process, which employs the partial nitrification and the shortcut denitrification reaction mechanisms, is more efficient with respect to nitrogen removals as well as carbon and oxygen requirements. Nevertheless, the partial nitrification could be more easily inhibited by the increased ammonia loading and system perturbation, resulting in a high production of N_2O. In contrast, the conventional process produces less N_2O from the nitrification reaction at the changed operating condition, but the denitrification is the reaction that releases more N_2O under a carbon-limited condition. Consequently, care should be given when constructing and/or operating BNR processes in order to balance efficient nitrogen removals with minimal N_2O production, particularly when key operating parameters tested in this study significantly vary with time. Furthermore, an integrated study is required to quantitatively determine the conditions that could cause the microbial inhibition since many factors including the free ammonia concentration and the gradual adaptation should be taken into account.

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References

American Public Health Association, American Water Works Association, and Water Environment Federation (1998) Standard Methods for the examination of water and wastewater, 20th edn. Washington, DC

Anthonisen AC, Loehr RC, Prakasam EG & Srinath TBS (1976) Inhibition of nitrification by ammonia and nitrous acid. J. Water Pollut. Control Fed. 48(5), 835–852

Bae W, Baek SC, Chung JW & Lee YW (2002a) Optimal operational factors for nitrite accumulation in batch reactors. Biodegradation 12: 359–369

Bae W, Ko GB, Hong SH & Lee YW (2002b) Leachate treatment using shortcut biological nitrogen removal process. J. Korean Solid Waste Eng. Soc. 19(2), 192–198

Beline F, Martinez J, Chadwick D, Guiziou F & Coste CM (1999) Factors affecting nitrogen transformations and related nitrous oxide emissions from aerobically treated piggery slurry. J. Agric. Eng. Res. 73: 235–243

Brindle K, Stephenson T & Semmens MJ (1998) Nitrification and oxygen utilization in a membrane aeration bioreactor. J. Membrane Sci. 144: 197–209

Choudhary MA, Akramkhanov A & Saggar S (2002) Nitrous oxide emissions from a New Zealand cropped soil: tillage effects, spatial and seasonal variability. Agric. Ecosys. Environ. 93: 33–43

Chung JW & Bae W (2002) Nitrite reduction by a mixed culture under conditions relevant to shortcut biological nitrogen removal. Biodegradation 13: 163–170

Dundee L & Hopkins DW (2001) Different sensitivities to oxygen of nitrous oxide production by *Nitrosomonas europaea* and *Nitrosolobus multiformis*. Soil Biol. Biochem. 33: 1563–1565

Focht DD (1974) The effect of temperature, pH and aeration on the production of nitrous oxide and gaseous nitrogen: a zero-order kinetic model. Soil Sci. 118(3), 173–179

Garrido JM, Benthum van WAJ, Loosdrecht van MCM & Heijnen JJ (1997) Influence of dissolved oxygen concentration on nitrite accumulation in a biofilm airlift suspension reactor. Biotech. Bioeng. 53(2), 168–178

Gejlsbjerg B, Frette L & Westermann P (1997) Dynamics of N_2O production from activated sludge. Water Res. 32(7), 2113–2121

Goreau TG, Kaplan WA, Wofsy SC, McElroy MB, Valois FW & Watson SW (1980) Production of NO_2^- and N_2O by nitrifying bacteria at reduced concentrations of oxygen. Appl. Environ. Microbiol. 40: 526–532

Hanaki K, Hong Z & Matsuo T (1992) Production of nitrous oxide gas during denitrification of wastewater. Water Sci. Technol. 26(5–6), 1027–1036

Hanaki K, Hong Z & Matsuo T (1994) Production of nitrous oxide gas during nitrification of wastewater. Water Sci. Technol. 30(6), 133–141

Hynes RK & Knowles R (1984) Production of nitrous oxide by *Nitrosomonas europaea*: effects of acetylene, pH, and oxygen. Canadian J. Microbiol. 30: 1397–1404

Itokawa H, Hanaki K & Matsuo T (2000) Nitrous oxide production in high-loading biological nitrogen removal process under low COD/N ratio condition. Water Res. 35(3), 657–664

Louzeiro NR, Mavinic DS, Oldham WK, Meisen A & Gardner IS (2002) Methanol-induced biological nutrient removal kinetics in a full-scale sequencing batch reactor. Water Res. 36(11), 2721–2732

Mosier A, Kroeze C, Nevison C, Oenema O, Seitzinger S & Cleemput Ovan (1999) An overview of the revised 1996 IPCC guidelines for national greenhouse gas inventory methodology for nitrous oxide from agriculture. Environ. Sci. Policy 2: 325–333

Park KY, Inamori Y, Mizuochi M & Ahn KH (2000) Emission and control of nitrous oxide from a biological wastewater treatment system with intermittent aeration. J. Biosci. Bioeng. 90(3), 247–252

Pollice A, Tandoi V & Lestingi C (2002) Influence of aeration and sludge retention time on ammonium oxidation to nitrite and nitrate. Water Res. 36: 2541–2546

Poth M & Focht DD (1985) 15N kinetic analysis of N_2O production by Nitrosomonas europaea: an examination of nitrifier denitrification. Appl. Environ. Microbiol. 49: 1134–1141

Prakasam TBS & Loehr RC (1972) Microbial nitrification and denitrification in concentrated waste. Water Res. 6: 859–869

Schmidt I, Sliekers O, Schmid M, Bock E, Fuerst J, Kuenen JG, Jetten MSM & Strous M (2003) New concepts of microbial treatment processes for the nitrogen removal in wastewater. FEMS Microbiol. Rev. 27(4), 481–492

Seinfeld JH & Pandis SN (1997) Atmospheric Chemistry and Physics. John Wiley and Sons, New York

Tseng C-C, Potter TG & Koopman B (1998) Effect of influent chemical oxygen demand to nitrogen ratio on a partial nitrification/complete denitrification process. Water Res. 32: 165–173

U.S. EPA (1993) Manual Nitrogen Control, EPA/625/R-93/010. U.S. Environmental Protection Agency, Washington

Schulthess van R & Gujer W (1996) Release of nitrous oxide (N_2O) from denitrifying activated sludge: verification and application of a mathematical model. Water Res. 30(3), 521–530

Weon SY, Kim MS & Lee SI (1999) Effect temperature and free ammonia concentration on nitrification of enriched nitrifiers. J. Korean Soc. Environ. Eng. 21(9), 1613–1620

Wrage N, Velthof GL, Laanbroek HJ & Oenema O (2004) Nitrous oxide production in grassland soils: assessing the contribution of nitrifier denitrification. Soil Biol. Biochem. 36(2), 229–236

Wrage N, Velthof GL, Beusichem van ML & Oenema O (2001) Role of nitrifier denitrification in the production of nitrous oxide. Soil Biol. Biochem. 33: 1723–1732