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Effect of Ammonium Concentration on the Emission of N_2O Under Oxygen-Limited Autotrophic Wastewater Nitrification

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A significant amount of nitrous oxide (N_2O), which is one of the serious greenhouse gases, is emitted from nitrification and denitrification of wastewater. Batch wastewater nitrifications with enriched nitrifiers were carried out under oxygen-limited condition with synthetic (without organic carbon) and real wastewater (with organic carbon) in order to find out the effect of ammonium concentration on N_2O emission. Cumulated N_2O-N emission reached 3.0, 5.7, 6.2, and 13.5 mg from 0.4 l of the synthetic wastewater with 50, 100, 200, and 500 mg/l NH_4^+-N, respectively, and 1.0 mg from the real wastewater with 125 mg/l NH_4^+-N. The results indicate that N_2O emission increased with ammonium concentration and the load. The ammonium removal rate and nitrite concentration also increased N_2O emission. Comparative analysis of N_2O emission from synthetic and real wastewaters revealed that wastewater nitrification under oxygen-limited condition emitted more N_2O than that of heterotrophic denitrification. Summarizing the results, it can be concluded that denitrification by autotrophic nitrifiers contributes significantly to the N_2O emission from wastewater nitrification.

Keywords: Denitrification, greenhouse gas, nitrification, nitrifiers, nitrous oxide (N_2O), oxygen-limited condition

It is widely accepted that the emission of greenhouse gas by excessive use of fossil fuels causes climate change. Nitrous oxide (N_2O) is one of the greenhouse gases having an approximately 300-fold stronger global warming effect than carbon dioxide, and its contribution is about 9% of total greenhouse gases [5]. Even though N_2O emission from waste and wastewater treatment is only 2.3% of the

total N_2O emission, it occupies a very important position from the aspect of industry category of the N_2O source.

Nitrogen compounds in wastewater are mainly removed by nitrification and denitrification, and it has been known that N_2O is released during the nitrogen transformation [16]. The amount of emitted N_2O is relatively small when compared with the amount of overall nitrogen removed or transformed by microorganisms. It was thought that most of the N_2O is produced through heterotrophic denitrification. Incomplete heterotrophic denitrification of nitrate (NO_3^-) and nitrite (NO_2^-) accumulates N_2O during the denitrification pathway to N_2. However, it has been found that a significant amount of N_2O is also produced by nitrifiers [2, 12]. Some of the N_2O is produced by the incomplete oxidation of hydroxylamine (NH_2OH) during the nitrification [4]. Furthermore, nitrifiers, mostly ammonia oxidizing bacteria (AOB), are known to denitrify nitrite to N_2O, with ammonia or hydrogen as the electron donor, by nitrite reductase and nitric oxide reductase [1, 16].

Dissolved oxygen (DO) is a critical factor in nitrifiers denitrification and the N_2O production is stimulated at low DO [6, 15, 17]. AOB can use nitrite as the electron acceptor for the oxidation of ammonia to NH_2OH to replace oxygen in oxygen-limited condition. It has been reported that about 10% of the ammonium (NH_4^+) load is converted to N_2O when the DO is maintained below 1.0 mg/l [3].

Nitrite is also known to increase N_2O production by nitrifiers denitrification [2]. Nitric oxide (NO) and N_2O were accumulated at high nitrite concentration, and N_2O production increased 4~8 times depending on DO when additional nitrite was injected in the nitrification system [15]. Recently, Kampschreur *et al.* [7] surveyed N_2O emissions from wastewater nitrification and they showed that a very diverse range (0.08~16%) of the influent NH_3-N loads was converted to N_2O-N. The result indicates that various nitrification/denitrification pathways and mechanisms are involved in the N_2O emissions depending on the

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environmental and operational conditions of the wastewater treatment.

Ammonium is the substrate of nitrification and the electron donor for the denitrification of nitrite by nitrifiers. As the nitrification and the autotrophic denitrification can also be affected by free ammonia (NH_3) inhibition [9, 10], ammonium concentration exerts a significant impact on N_2O production in wastewater treatment. However, very few studies have been carried out on this aspect. Kampschreur et al. [7] mentioned that an increase in N_2O production was observed upon an ammonium pulse in aerobic condition in their study on NO emission.

The objective of this study was to estimate the effect of ammonium concentration on N_2O emission by nitrifiers under oxygen-limited condition. For this purpose, batch nitrifications were carried out with enriched nitrifiers at different ammonium concentrations in oxygen-limited condition to encourage nitrifiers denitrification. Cumulated N_2O emission and N_2O yields were analyzed to relate N_2O emission to ammonium concentration.

MATERIALS AND METHODS

Batch Nitrification Reactor for N_2O Emission

The batch reactor (1 l) was tightly sealed with a rubber cap to prevent gas leak from the reactor, and 20 ml/min air was supplied to the reactor to keep dissolved oxygen low and to enhance the accuracy of N_2O emission measurement. Compressed air was provided to the reactor through a needle valve and a flow meter for the control of air flow. Off-gas was injected to a gas chromatography apparatus for N_2O measurement and a bubble flow meter was used for the measurement of off-gas flow rate, as shown in Fig. 1. Enriched nitrifying sludge in the laboratory was used for the experiment. The nitrifying
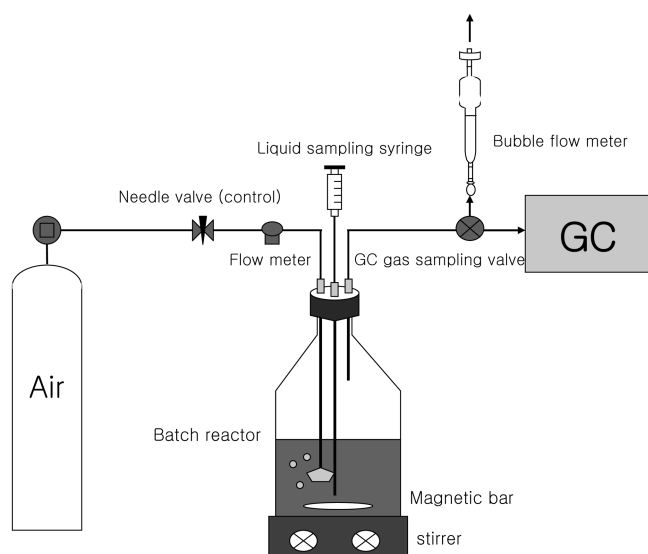

Fig. 1. Schematic diagram of the experimental set-up of the batch reactor for estimation of N_2O emission during nitrification.

sludge was incubated in a laboratory sequencing batch reactor (SBR) for more than 3 months by providing ammonium (240 mg/l NH_4^+-N) in the mineral medium as the only energy source, and ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) were the dominant microorganisms in the SBR. Nitrifying bacteria were harvested after the settling period of the SBR and 350 ml of the nitrifying bacteria was transferred to the batch reactor to make mixed liquor suspended solids (MLSS) of about 3,500 mg/l. Enriched nitrifying bacteria occupied at least 30% of the total bacteria in the sludge, as confirmed by fluorescence in situ hybridization. Detailed protocols and hybridization probes used for the identification and quantification of nitrifying bacteria can be found elsewhere [8]. The nitrifying sludge was stirred and aerated for 2 h to remove residual ammonium completely. After the aeration, 50 ml of synthetic wastewater containing ammonium was injected to the reactor to make a total volume of 400 ml, and compressed air (20 ml/min) was provided for nitrification.

Ammonium (NH_4^+-N) concentration in the batch reactor was varied to 50, 100, 200, and 500 mg/l with the synthetic wastewater and an equivalent amount of bicarbonate ($NaHCO_3$) was also added to keep the pH at 7.0~7.5. The synthetic wastewater also contained mineral compounds as follows (mg/l): $MgSO_4 \cdot H_2O$ (2), KCl (3), $NaHPO_4 \cdot 12H_2O$ (12), $CaCl_2 \cdot 2H_2O$ (3), KH_2PO_4 (4), $FeCl_3 \cdot 6H_2O$ (1). As the synthetic wastewater did not contain any organic compounds, the growth of heterotrophic bacteria and heterotrophic denitrification are very limited. For the real wastewater sludge, reject water (which contained 125 mg/l NH_4^+-N and 700 mg COD) from a municipal wastewater treatment plant was used. An equivalent amount of $NaHCO_3$ to NH_4^+-N was added to the wastewater to prevent pH decrease by the nitrification. All the batch nitrification and N_2O emission measurements were carried out in duplicate and the average values were used for the analysis.

Analytical Methods

Liquid samples were filtered through a 0.2 μm syringe filter before the analysis. NH_4^+-N was analyzed by an ion chromatography (ICS-1500, Dionex) equipped with a cationic column (CS15, Dionex), and 10 mN H_2SO_4 + 9% acetonitrile was used as the eluent. NO_2^--N and NO_3^--N were analyzed by an ion chromatography (DX-500, Dionex) equipped with an anionic column (AS14, Dionex), and 0.5 M Na_2CO_3 + 0.5 M $NaHCO_3$ was used as the eluent. MLSS and MLVSS in the reactor were measured after drying the solids in the oven at 100~110°C for 2 h and heating at 550°C for 15 min. N_2O in the off-gas was measured by a gas chromatography (6890, Agilent) with a HP-FFTP column and an electron capture detector at the oven temperature of 50°C, injector at 100°C, and detector at 250°C. N_2 was used as the carrier gas.

Nitrogen mass balance was performed based on the measured values of nitrogen compounds (NH_4^+-N, NO_2^--N, NO_3^--N, N_2O-N) and the estimated nitrogen mass of the nitrifying bacteria.

RESULTS AND DISCUSSION

N_2O Emission During Batch Nitrification

Nitrite and ammonium as well as dissolved oxygen are known to affect N_2O emission during nitrification [16]. It has been reported that N_2O emission during nitrification is
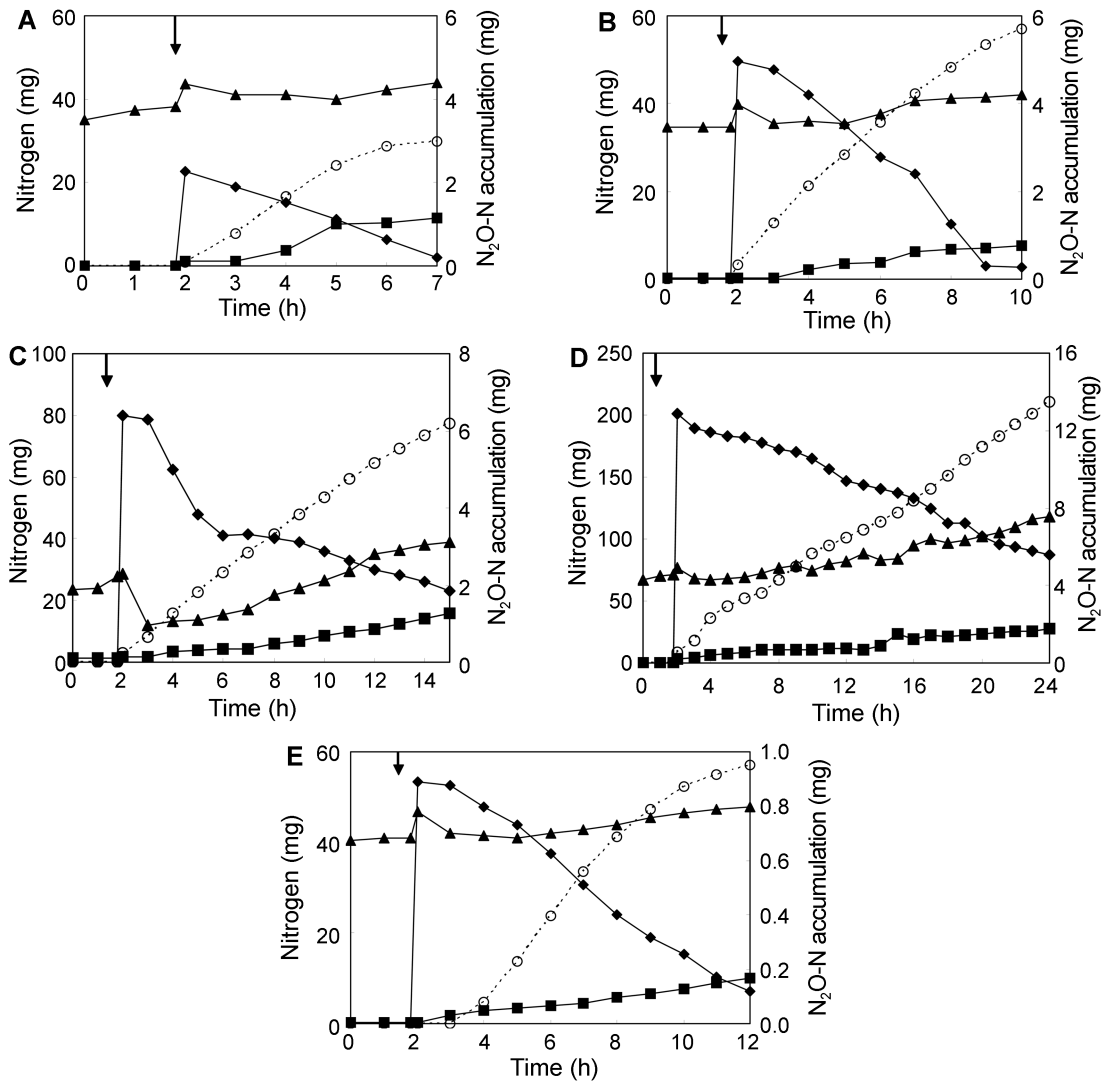
Fig. 2. N₂O accumulation and nitrogen component profiles during the batch nitrification with 50 (**A**), 100 (**B**), 200 (**C**), 500 (**D**) mg/l NH₄⁺-N, and real wastewater (**E**) containing 125 mg/l NH₄⁺-N (◆: NH₄⁺-N; ■: NO₂⁻-N; ▲: NO₃⁻-N; ○: N₂O-N; ↓ : NH₄⁺-N injection).

mainly resulted from the oxidation of ammonium with nitrite as the electron acceptor in oxygen-limited or absent conditions [10, 16]. In this study, the effect of ammonium concentration on N₂O emission during nitrification was investigated with enriched nitrifiers.

Fig. 2A–2E show the profiles of nitrogen compounds during nitrification with different ammonium concentrations [50 (A), 100 (B), 200 (C), 500 mg/l NH₄⁺-N (D), and 125 mg/l NH₄⁺-N of real wastewater (E)]. All the nitrogen compounds in Fig. 2 are shown in quantitative nitrogen mass in the reactor for nitrogen mass balance, and N₂O-N was the cumulated mass in the off-gas. The initial NO₃⁻-N was due to the transferred nitrifying sludge from the mother reactor (SBR). When ammonium was injected into the batch reactor, the NO₂⁻ and NO₃⁻ concentrations increased by the oxidation of ammonium. N₂O emission also began with the injection

of ammonium, and seems to be directly related to the oxidation of ammonium.

Fig. 2A shows the nitrification of wastewater containing 50 mg/l NH₄⁺-N. After 5 h of wastewater injection, 91% of the NH₄⁺-N was removed and 3.0 mg of N₂O-N was emitted while 4.0 mg of NO₃⁻-N and 11.2 mg of NO₂⁻-N were increased after the ammonium injection. After 8 h of wastewater nitrification with 100 mg/l NH₄⁺-N, 94% of the NH₄⁺-N was removed and 5.7 mg of N₂O-N was emitted while 7.2 mg of NO₃⁻-N and 9.2 mg of NO₂⁻-N were increased after the injection (Fig. 2B). Fig. 2C shows the nitrification of 200 mg/l NH₄⁺-N. Ammonium removal efficiency reached 71% in 13 h, and 6.2 mg of N₂O-N was emitted while 14.5 mg of NO₃⁻-N and 14.6 mg of NO₂⁻-N were increased. Fig. 2D shows the nitrification of 500 mg/l NH₄⁺-N and 56% of the inlet ammonium was removed in

22 h. During the nitrification, 13.5 mg of N_2O-N was emitted and 46.2 mg of NO_3^--N and 34.8 mg of NO_2^--N were increased. The results showed that N_2O-N emission increased with the NH_4^+-N concentration of the synthetic wastewater. Fig. 2E shows the nitrification of real wastewater containing 125 mg/l NH_4^+-N. For the 10 h of nitrification, 86.5% of NH_4^+-N was removed increasing 6.5 mg of NO_3^--N and 12.5 mg of NO_2^--N. Conversely, the real wastewater emitted only 1.0 mg of N_2O-N. The amount of N_2O-N emitted in the real wastewater nitrification was significantly lower than that of the synthetic wastewater, which does not have organic carbons.

Nitrification rate did not increase proportionally with the NH_4^+-N concentration. The overall nitrification rates of Fig. 2A−2E were 4.1, 5.9, 4.4, 5.1, and 4.6 mg/h NH_4^+-N, which are similar to one another despite of different ammonium concentrations. The reason seems to be the limited supply of oxygen to the batch reactors, and the nitrification is controlled by the oxygen. Longer reaction time is needed at higher NH_4^+-N concentrations for complete nitrification. DO was maintained less than 1.0 mg/l throughout the batch nitrification (data not shown).

The cumulated N_2O-N emissions of the synthetic wastewater in Fig. 2A−2D were 3.0, 5.7, 6.2, and 13.5 mg at the NH_4^+-N loads of 20, 40, 80, and 200 mg, respectively. The total amount of N_2O emission was gradually increased with the ammonium load or the initial ammonium concentration. In the case of the real wastewater (Fig. 2E), 1.0 mg of N_2O-N was emitted at the NH_4^+-N load of 50 mg, which is significantly lower than those of the synthetic wastewater. The main difference of the synthetic and the real wastewater is that the real wastewater has organic components whereas the synthetic wastewater does not. Therefore, it is thought that the presence of organic components affects the emission of N_2O during the nitrification. However, the reason why organic components lower N_2O emission during nitrification is not clear and it has not been reported yet. It can be postulated that heterotrophic denitrification may also occur at low DO in the presence of organic components by simultaneous nitrification and denitrification. The N_2O and nitrite produced during the nitrification could be reduced to N_2 by heterotrophic denitrification in the real wastewater. Nitrite reduction could decrease N_2O production by nitrifiers denitrification [2]. Lemaire *et al*. [11] compared N_2O emission during denitrification with a real raw wastewater and a synthetic wastewater, which had methanol, and they reported that addition of raw wastewater led to immediate reduction and depletion of N_2O emission. The result implies that mixed carbon sources in real wastewater reduce N_2O emission in denitrification.

N_2O Yield and Emission Rate
Relatively small amounts of NO_2^--N and NO_3^--N were found in the reactor after the nitrification compared with

the removed NH_4^+-N, as shown in Fig. 2. It seemed that some of the oxidized nitrogen was removed by denitrification. Simultaneous nitrification and denitrification (SND) may occur at low DO when organic components are available as an electron donor [13]. Some organics can be available from the sludge even though no external organic components are supplied in Fig. 2A−2D. It can also be confirmed by the presence of N_2 and NO by nitrogen mass balance showing that denitrification was going on in the batch reactor [11].

From the nitrogen mass balance of the sealed batch nitrification reactor, it was possible to determine the fate of ammonium during the nitrification and the amount of nitrogen converted to gaseous compounds other than N_2O. Possible gaseous nitrogen compounds other than N_2O are NO, N_2, and NH_3. NO and N_2 are thought to be derived from the denitrification, and NH_3 is from the stripping of the wastewater. The amount of gaseous NH_3 transferred by stripping is thought to be very small, however, as the pH was maintained at neutral (7.0~7.5) condition and the air flow rate was low (20 ml/min). Therefore, gaseous NH_3 was assumed negligible for the nitrogen mass balance.

For the mass balance, NH_4^+-N, NO_2^--N, and NO_3^--N were measured from the liquid phase and N_2O-N was measured from the off-gas. Ammonium nitrogen assimilated to biomass during the nitrification was also estimated by the following equation [14]:

$$1.02\ NH_4^+ + 1.89\ O_2 + 2.02\ HCO_3^- \rightarrow 0.021\ C_5H_7O_2N + 1.06\ H_2O + 1.92\ H_2CO_3 + 1.00\ NO_3^-$$
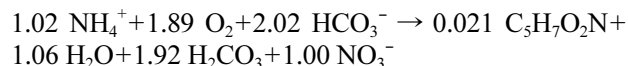
The yield or conversion of emitted N_2O-N from wastewater reported in the literature varies significantly depending on the wastewater composition and operation condition [7]. In this study, the N_2O-N conversion and yield were defined by the amount of N_2O-N emission divided by input and removed NH_4^+-N loads, respectively.

In the case of 50 mg/l NH_4^+-N (Fig. 2A), the cumulative N_2O-N from the off-gas was 3.0 mg, and the other gaseous nitrogen compounds (NO, N_2) were estimated to be 2.1 mg from the mass balance. About 13% of the input NH_4^+-N was converted to N_2O-N, and about 15% of the removed NH_4^+-N was converted to N_2O-N. As the NH_4^+-N removal efficiencies were different from the batches, N_2O-N yield based on the removed NH_4^+-N seemed to be more reasonable to quantify and analyze the nitrogen flux to N_2O-N during nitrification. The average N_2O-N emission rate was 0.6 mg/h. The data from Fig. 2B−2E could also be used to calculate the yield and N_2O-N emission rate by the above method (Fig. 3 and 4).

Fig. 3 shows the cumulative N_2O-N emission, and N_2O-N conversion and yield based on the input and removed NH_4^+-N of the wastewater. The cumulative amounts of N_2O-N of the synthetic wastewater were 3.0 (A), 5.7 (B), 6.2 (C), and 13.5 mg (D), and it increased with NH_4^+-N. In real
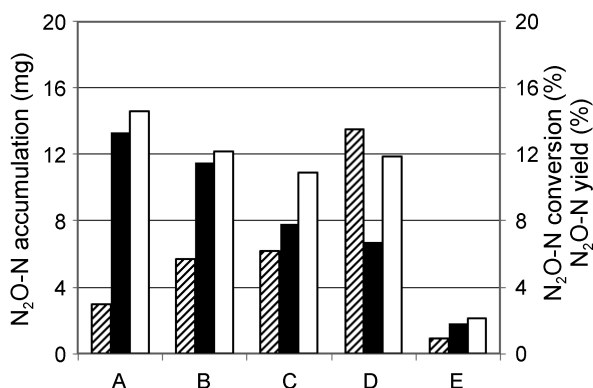
Fig. 3. N₂O production profile of the NH_4^+-N concentration [▨: N₂O-N accumulation; ■: N₂O-N conversion (N₂O-N_{acc}/NH_4^+-N_{in}); □: N₂O-N yield (N₂O-N_{acc}/NH_4^+-$N_{removed}$)].

wastewater, the cumulative N₂O-N was only 1.0 mg, and it was much lower than that of the synthetic wastewater.

N₂O-N conversions based on input NH_4^+-N were 13.3%, 11.5%, 7.8%, and 6.7% at the NH_4^+-N concentrations of 50, 100, 200, and 500 mg/l NH_4^+-N, respectively. The conversion decreased with NH_4^+-N concentration as the nitrification efficiency also decreased with the input NH_4^+-N concentration owing to the oxygen limitation. For the real wastewater, only 2.2% of the input NH_4^+-N was converted to N₂O-N, and it was much lower than that of the synthetic wastewater.

N₂O-N yields based on removed NH_4^+-N of the synthetic and the real wastewaters were 10.9~14.6% and 3.7%, respectively (Fig. 3). The yields were higher than the conversions, as the smaller denominators (reacted NH_4^+-N instead of input NH_4^+-N) are used. Kampschreur et al. [7] summarized the N₂O-N conversion results reported in the literature. In most cases, synthetic wastewater showed higher N₂O-N conversion (%) based on nitrogen load than the real wastewater. The N₂O-N yield could provide information about the production source of N₂O, as the yield depends largely on the nitrogen metabolic pathway.
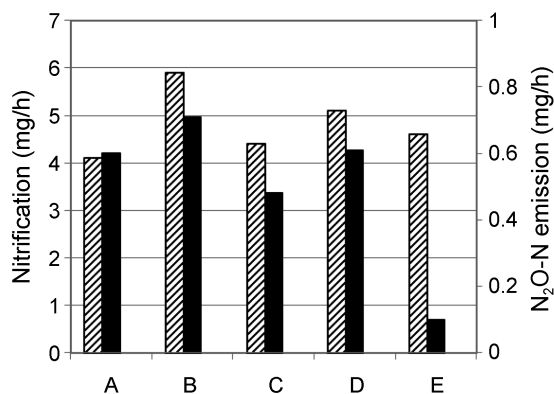
For our purpose, the N₂O-N yield gives more valuable information than the conversion.

The nitrification (NH_4^+-N removal) rates had relatively similar values (average 4.9 mg/h) as they were limited by the oxygen supply, as shown in Fig. 4. In synthetic wastewater, N₂O-N emission rates were relatively the same and the average emission rate was 0.60 mg/h even though the highest was 0.71 mg/h at 100 mg/l NH_4^+-N and the lowest was 0.48 mg/h at 200 mg/l NH_4^+-N. The nitrification rate of the real wastewater was 4.6 mg/h, which is similar to that of the synthetic wastewater. However, its N₂O-N emission rate was 0.1 mg/h, which is significantly lower than that of the synthetic wastewater.

Factors Affecting N₂O Emission

It is thought that ammonia concentration and the load do not have direct effect on the N₂O-N emission rate. However, a higher nitrification rate of the synthetic wastewater yielded more N₂O-N emission, as shown in Fig. 5. It means an increased nitrification flux produces more N₂O-N in autotrophic nitrification.

The major difference of the synthetic wastewater and the real wastewater used in this study is whether they have organic compounds or not. SND can occur in the presence of organics and oxygen-limited concentration. The real wastewater used in this study had enough COD for denitrification. Therefore, the nitrification of the real wastewater at low DO had a sufficient condition for SND.

Heterotrophic denitrification may yield less N₂O-N emission per denitrified nitrogen than the nitrifiers denitrification. Tallec et al. [15] reported that about 58~83% of N₂O was emitted through nitrifiers denitrification, and the other N₂O (17~42%) was emitted by heterotrophic denitrification under nitrification inhibition condition. The result supports the above hypothesis and provides a clue for why the real
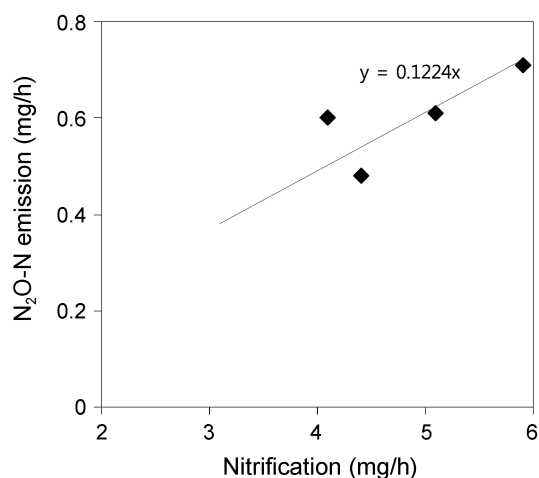

Fig. 4. Nitrification and N₂O generation rates of various concentrations of ammonia wastewater. (▨: Nitrification rate; ■: N₂O rate).

Fig. 5. The relationship of nitrification rate and N₂O generation rate during oxygen-limited wastewater nitrification.
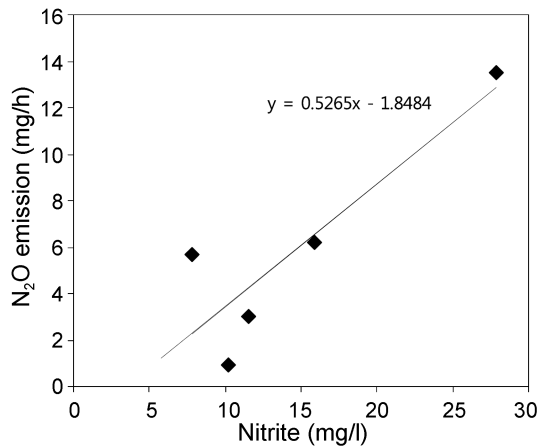
Fig. 6. Effect of nitrite concentration on N_2O generation rate during oxygen limited wastewater nitrification.

wastewater emitted less N_2O than the synthetic wastewater. As the synthetic wastewater lacks organics for heterotrophic denitrification, most N_2O emission is through the autotrophic nitrification and nitrifiers denitrification. However, the real wastewater has a heterotrophic denitrification pathway for N_2O emission as well as autotrophic nitrification and nitrifiers denitrification. The real wastewater heterotrophic denitrifying bacteria can intercept nitrite from the nitrifiers denitrification to N_2, NO, or N_2O by heterotrophic denitrification. However, the heterotrophic denitrification has less N_2O emission than nitrifiers denitrification, as the traditional nitrous oxide reductase genes have not been identified in AOB genomes [10].

Fig. 6 shows the effect of nitrite concentration on N_2O emission based on the end points of batch nitrification. N_2O emission increased with nitrite concentration in both the synthetic wastewater and the real wastewater. The result is in agreement with the hypothesis that nitrite acts as an electron acceptor to produce N_2O both in heterotrophic denitrification and nitrifiers denitrification [16]. It has also been reported that N_2O emission increases with nitrite concentration in nitrification [7, 15]. Low DO accelerates nitrite as an electron acceptor to increase N_2O production, as the available oxygen is scarce.

As ammonium and nitrite are the substrates of the N_2O generation reaction in nitrifiers denitrification, high concentrations of ammonium and nitrite are the necessary condition for N_2O emission in nitrification at low DO and they affect the N_2O emission significantly. Recently, Kim *et al.* [10] also showed that nitrifiers denitrification was the main cause of N_2O emission in nitrification even though they provided about 13 times more oxygen than did this study.

In summary, N_2O emission increased with wastewater ammonium concentration and the load when oxygen was limited. In real wastewater, N_2O emission was significantly lower than that of synthetic wastewater, even though ammonium removal rates were similar. N_2O emission also increased with increased ammonium removal rate and nitrite concentration. Denitrification by autotrophic nitrifiers emitted more N_2O than the heterotrophic denitrification in oxygen-limited condition. N_2O is mainly produced by nitrifiers denitrification with nitrite as the electron acceptor and ammonium with enriched nitrifying sludge as the electron donor in the absence of organic carbons. However, N_2O emission is decreased when organic carbons are available by heterotrophic denitrification to reduce nitrite. Maintaining an appropriate dissolved oxygen level and keeping the nitrite concentration low are the key strategies to reduce N_2O emission from wastewater nitrification. Further analyses of the quantitative contribution of dissolved oxygen and nitrite on N_2O emission in nitrification and denitrification are needed for the minimization of greenhouse gas emissions from wastewater treatment plants.

Acknowledgment

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