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Characteristics of N_2O release from fluidized media type BNR processes and identification of N_2O sources

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ABSTRACT

The effects of C/N ratios and fluidized media on N_2O emissions were investigated during the conventional biological nitrogen removal (BNR) process for wastewater treatment containing high levels of nitrogen. Low concentration of N_2O emissions was maintained at around 50~100 ppm when the C/N ratio was 3. However higher amounts of N_2O (maximum rate 800 ppm) were released from denitrification, when the C/N ratio was 1.5 and 1, compared to when the ratio was 3. The influence of oxygen concentration on N_2O release as well as its sources was investigated using a nitrogen isotope (^{15}N) tracer method. Our results indicated that around 50% of the released N_2O was originated from denitrification under aerobic conditions. Under aerobic conditions, the proportion and amount of N_2O emissions from denitrification increased with a decrease in dissolved oxygen (DO). In micro-aerobic conditions, 98.6% of N_2O emission was originated from denitrification. This result implies that an anoxic zone was present even in aerobic reactors and that reactors with low DO tended to have larger anaerobic zones.

Keywords: Nitrous oxide (N_2O); Nitrification; Denitrification; ^{15}N-Isotope; C/N ratio

1. Introduction

Climate change conventions intended to address that the problem of global warming influences the global economy in many ways. Among greenhouse gases such as CO_2, CH_4, N_2O and CFCs, much more concern and interest have concentrated on N_2O because of its high global warming potential (GWP) of 320 [1,2].

Although the atmospheric concentration of N_2O gas is only 310 ppb, it is very stable in the troposphere. Wastewater treatment systems emit large amounts of N_2O, comprising approximately 20% of total anthropogenic sources [3]. Hence, the characterization of N_2O sources and development of control technologies for this gas are crucial.

Nitrous oxide gas is produced as a byproduct of biological nitrification and intermediate product of denitrification processes in wastewater treatment systems [4]. N_2O gas production from the nitrification process is simplified as a mechanism shown in the Fig. 1 and the factors influencing N_2O production include oxygen concentration, temperature, pH, concentration of ammonia, and accumulation of nitric oxide [5,6].

Benckiser et al. [7] reported that when organic material inputs into a nitrification tank were increased, N_2O emissions also increased due to the accumulation of ammonia and nitrite ions caused by retardation of autotrophic nitrification. Therefore, organic matter

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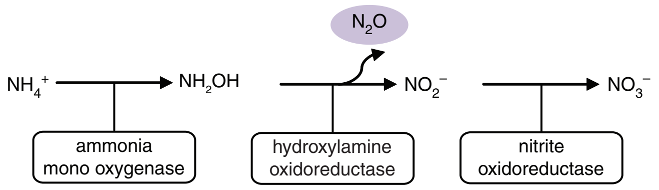

Fig. 1. Biological nitrification process.

content and nitrogen concentration in influent waters are important factors affecting N_2O emissions.

As nitrification is an aerobic process that is strongly influenced by oxygen content, the rate of nitrification and the proportion of N_2O gas in products of nitrification are subject to change with oxygen content [8]. Focht and Chang [5] reported that soil N_2O emissions were enhanced when the oxygen supply was insufficient and that maximum N_2O gas emission was obtained when soil had both aerobic and anaerobic conditions.

The following mechanism shown in the Fig. 2 explains N_2O emissions in the biological denitrification process. The factors influencing this mechanism include the C/N ratio, anoxic level, pH, temperature, BOD, NO_x, inhibitors [9,10,11].

Complete denitrification includes the consecutive conversion (referred to as Path-1) of nitrate (NO_3^-) and nitrite (NO_2^-) into N_2 gas via nitric oxide (NO) and nitrous oxide (N_2O), and the direct conversion (referred to as Path-2) of nitric oxide (NO) into N_2 gas without going through the nitrous oxide (N_2O) phase. When denitrifiers, which are heterotrophic organisms, are under low organic matter conditions, the process of Path-1 prevails and the denitrification process stops at the phase of N_2O production.

Hanaki et al. [9] reported that N_2O emissions were related to the C/N ratio and that there were higher N_2O emissions under low carbon conditions. Based on these results, it was speculated that the C/N ratio of influent water is one of the most important factors influencing N_2O emissions in the BNR process. Byproducts accumulated when free oxygen was present in the denitrification tank due to interference with the synthesis and activities of reductase [12]. In this case, as nitrite reductase which involves the reduction reaction of N_2O into N_2 is easily influenced by oxygen, the ratio of $N_2O/(N_2+N_2O)$ increased with an increase in oxygen concentration [5,9].

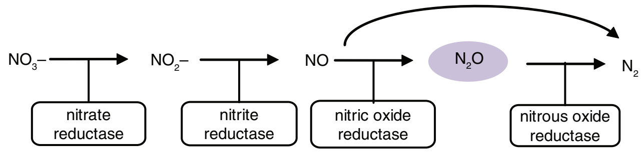

Fig. 2. Biological denitrification process.

A recent study reported that higher N_2O generation generally corresponded with higher nitrite concentrations, but also that many competing and parallel nitrogen transformation reactions occur [13]. Adouani et al. [14] studied NO_2 accumulation and showed that NO_2^- concentrations had a direct impact on N_2O emissions and that a significant amount of NO was correlated to significant emission of N_2O.

While much research has been devoted to developing biological nitrogen removal systems using different types of media to prevent the loss of nitrifiers and facilitate nitrification process, few studies have been implemented characterizing the factors influencing N_2O emissions when various media are used in the systems. It is speculated that sources and paths of N_2O emissions change when media are filled in the BNR process, because aerobic and anaerobic conditions are created outside and inside the media, respectively, and result in simultaneous nitrification and denitrification. However, it has not yet been clearly identified how much of and from which process the N_2O gas is produced [15]. Stevens et al. [16] assessed the contribution of N_2O emissions to nitrification and denitrification using a ^{15}N isotope, and Khalil et al. [17] also used a ^{15}N isotope to identify the sources of N_2O, depending on the partial pressure of oxygen. Hwang and Hanaki [18] demonstrated that biological denitrification was a main source of released N_2O even when the oxygen of the bulk atmosphere was as high as 15% by applying a tracer method using a ^{15}N-isotope into an oxygen controllable reactor with artificial refuse.

This study investigated the impact of various C/N ratio on N_2O characteristics and production caused by nitrification and denitrification in the BNR (biological nitrogen removal) process applied fluidized media for nitrogen removal from high nitrogen contained wastewater, We also studied N_2O occurrence pattern and source by effect of DO concentration using ^{15}N-stable isotope tracer method in the pathway of biological nitrogen removal.

2. Experimental methods

2.1. Continuous BNR process

The BNR process used in this study was a bench scale A/O process composed of nitrification and denitrification reactors, and the working volume of each reactor was 4ℓ. Experiments were performed by using an A/O process with porous cubic sponge media (6 mm×6 mm×6 mm) in the nitrification reactor(the 'biofilm' reactor), shown in Fig. 3.

The main component of the porous cubic sponge media was polyurethane, and the media were packed into the nitrification reactor at a packing rate of 20% (v/v).

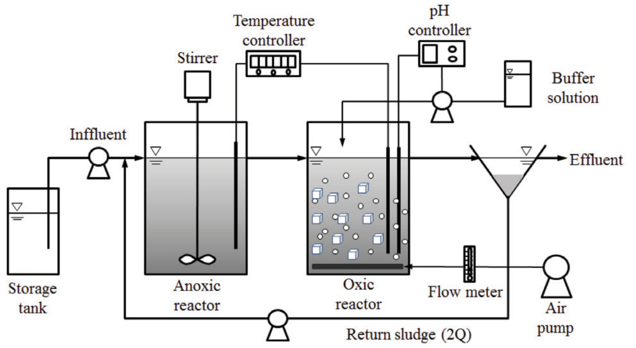

Fig. 3. Schematic diagram of the bench scale BNR process (biofilm reactor).

An artificial substrate composed of K_2HPO_4 (1.5 g/ℓ), $MgSO_4.7H_2O$ (0.05 g/ℓ), $FeSO_4.7H_2O$ (0.02 g/ℓ), $CaCl_2$ (0.04 g/ℓ), $MnSO_4.H_2O$ (0.05 g/ℓ), KCl (0.07 g/ℓ), NH_4HCO_3 (3.4 g/ℓ), CH_3COOH (4.0 g/ℓ) was used in this study.

The anoxic reactor (denitrification reactor) was agitated slowly with a stirrer for stable denitrification, and for the nitrification in the biofilm reactors, conditions were maintained as follows: DO: 3~4 ppm, pH: 7~8, temp.: 25±0.5°C, MLSS: 2,000~2,500 mg/ℓ, HRT: 1 day. The influent and return sludge were transported via a peristaltic metering pump, and the ratio of return sludge was set to 2Q (influent flow). The ammonia concentration was fixed as 400 mg/ℓ to investigate the effects of the C/N ratio on the pattern of N_2O emissions. The C/N ratio was maintained as 3 until 16th day, then as 1.5 from the 17th to 48th days and as 1.0 from the 49th to 90th days.

2.2. N_2O gas measurement

The N_2O gas was measured using gas chromatography (GC) following the headspace method, and the pretreatment procedure for the headspace method was as follows: 37 mℓ of liquid sample from the headspace in the reactor was injected into a 50 mℓ vial and 0.1 mℓ of 5% (w/v). Hibitane (Chlorohexidine Gluconate) was added to prevent additional microbial N_2O production.

The vial was sealed airtight after the headspace gas in the vial was substituted with He gas. The vial was placed in an incubator and maintained at 25°C after hand-shaking 10 times to ensure gas-liquid equilibrium in the vial. The 4 mℓ of headspace gas in the vial was collected with a gastight-syringe and the concentration of N_2O was measured with GC (Younglin Co., M600D, Korea) equipped with a pulsed discharge detector. The packing material for the GC column was a molecular sieve 13X (injection temp.: 80°C, oven temp.: 80°C, detector temp. 100°C) and the carrier gas was He with a flow rate of 50 mℓ/min. The Nessler method was used to analyze the ammonia nitrogen. The concentrations of NO_2^--N and NO_3^--N were measured using an ion chromatograph (IC; JL science Co., Korea), and the analyses of other parameters were carried out according to the standard methods [19].

2.3. ^{15}N-stable isotope tracer method

When the operation of the BNR process was over, some of the sponge media in the nitrification reactor were collected and then filled in the 3 L batch reactor at a packing rate of 20% (v/v). The composition of the artificial substrate in the influent was the same as that in the BNR process. The initial conditions for batch operation included an ammonia concentration of 300 mg/ℓ, a C/N ratio of 1.0, temperature of 25°C and pH 7.0±0.2. In order to investigate the effects of DO on the pattern and the source of released N_2O from the nitrification reactor, DO concentrations were adjusted to 0~0.5, 1.0, 2.0, 3.0, and 4.0 mg/ℓ.

The initial concentrations of NO_2^--N and NO_3^--N were approximately 0 mg/ℓ and 70 mg/ℓ, respectively. NO_3-N was produced using potassium nitrate (KNO_3; Shoko Co., Japan), labeled with 30 atom % of ^{15}N, which was used to trace the source of released N_2O from the nitrification reactor. Therefore, initial NO_3^--N concentrations according to molecular weight were 49 mg/ℓ of $^{62}(NO_3^-)$-^{14}N and 21 mg/ℓ of $^{63}(NO_3^-)$-^{15}N.

The main pathways of N_2O production from nitrification and denitrification, and the conceptual principles of the ^{15}N-stable isotope tracer method are shown in Fig. 2. When the nitrogen in the NO_3^- is a mixture of ^{14}N and ^{15}N with a mass base ratio of $^{14}NO_3^-$: $^{15}NO_3^-$ = a : b, the mass of N_2O emitted from denitrification can be $^{44}(N_2O)$, $^{45}(N_2O)$, $^{46}(N_2O)$, and the amount of emission of each N_2O will be proportional to the square of the mass ratio (a:b), i.e., $^{44}(N_2O)$:$^{45}(N_2O)$:$^{46}(N_2O)$ = a^2:2ab:b^2. On the other hand, the N_2O emission from nitrification occurs only in the form of $^{44}(N_2O)$, and is not influenced by the initial concentration of NO_3^- in the sample (Fig. 4).

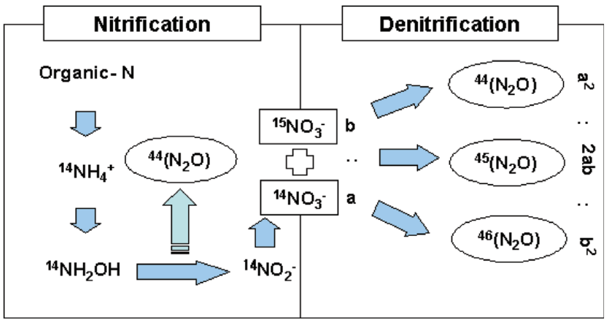

Fig. 4. Conceptual diagram of the ^{15}N-stable isotope tracer method for the evaluation of N_2O sources and contribution to the N_2O production between nitrification and denitrification.

As such, if isotope analysis of N_2O shows that the mass ratio of $^{44}(N_2O)$:$^{45}(N_2O)$:$^{46}(N_2O)$ corresponds to the ratio of a^2: 2ab: b^2, the emitted N_2O is believed to be produced solely by denitrification, while only the form of $^{44}(N_2O)$ is detected, the emitted N_2O is thought to be solely from nitrification. Hence, the sources and contributions to the N_2O production can be evaluated using the isotopic mass analysis of N_2O using the ^{15}N-stable isotope.

$$a = \frac{^{46}A}{2b}, b = \sqrt{^{46}A} \tag{1}$$

$$A_N = {}^{44}A - a^2, A_{DN} = {}^{46}A + {}^{46}A + a^2 \tag{2}$$

$$D_N(\%) = \frac{A_N}{A_N + A_{DN}} \times 100, D_{DN}(\%) = \frac{A_{DN}}{A_N + A_{DN}} \times 100 \tag{3}$$

^{44}A, ^{45}A, ^{46}A: GC/MS peak area of N_2O isotopes

A_N: N_2O peak area due to nitrification

A_{DN}: N_2O peak area due to denitrification

D_N: the % contributions of nitrification to the N_2O production

D_{DN}: the % contributions of denitrification to the N_2O production

In order to evaluate the amounts of N_2O produced and to identify the sources of N_2O according to DO, the batch reactor was prepared and N_2O gas was measured using the headspace method mentioned above. The nitrogen isotopes in the N_2O were analyzed with GC/MS (Shimadzu, GCMS-QP2010, Japan) and the conditions for isotope analysis are shown in Table 1.

3. Results and discussion

3.1. Effects of the C/N ratio on N_2O production

Fig. 5 shows the changes in denitrification efficiency and COD/NO_X-N ratio when the C/N ratio in the influent varied from 3, 1.5, and 1. The COD/NO_X-N ratio is the concentration ratio of the inflowing NO_X-N (NO_2^--N and NO_3^--N) and organic matter. The theoretical COD/NO_X-N ratio required for NO_3^--N to be transformed into N_2 gas using the chemical equation by Matějů et al. [20] is 3.74 (g COD/g NO_3^--N). We restate that the organic matter must be supplied in sufficient amounts to maintain the COD/NO_X-N ratio above 3.74 for complete denitrification.

Table 1
Analytical conditions of GC/MS for N_2O measurement

Parameter	Condition
Column flow	He, 1.8 mℓ/min
Injection mode	Split, 1 : 2
Column	HP-PLOT 30 m(L)×0.32 mm(I.D.) × 20 µm (film thickness)
Column temp.	35°C (Start 5 min) → (20°C /min) → 150°C (End)
Ion source temp.	200°C
Interface temp.	250°C
Ionization	EI, 70 eV
Mass range	TIC, *m/z*: 10~50

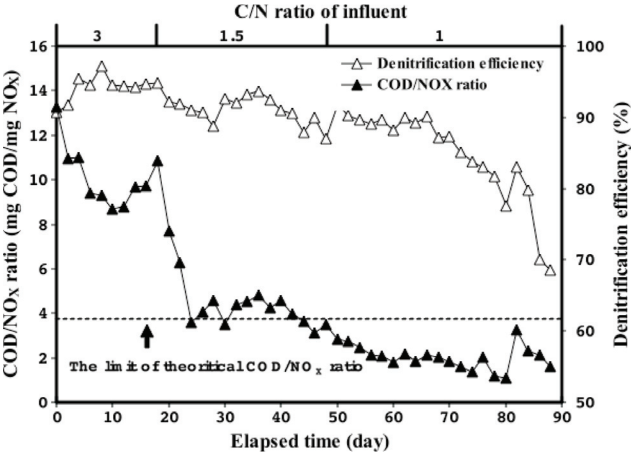

Fig. 5. Effects of the C/N ratio on COD/NO_X-N ratios and denitrification efficiency in an anoxic reactor (here, NO_X-N = NO_2^--N + NO_3^--N).

$$0.819CH_3COOH + NO_3^- \rightarrow 0.068C_5H_7NO_2 + HCO_3^- + 0.301CO_2 + 0.902H_2O + 0.466N_2 \tag{4}$$

When the C/N ratio of the influent water was changed from 3 to 1.5, the COD/NO_X-N ratio was sharply reduced from 10.7 to 3.8 (mg COD/mg NO_x). There was no change in denitrification efficiency because the COD/NO_X-N ratio was still higher than 3.74. Whereas, when the C/N ratio of the influent water was reduced from 1.5 to 1, the COD/NO_X-N ratio in the denitrification tanks of the biofilm reactors dropped below 3.74, resulting in a sharp reduction in the denitrification efficiency to 70%.

A supply of organic carbon sufficient to donate electrons is needed for efficient biological nitrogen removal, because nitrification and denitrification can be interrupted otherwise. In reality, overall nitrogen removal efficiency is easily decreased because of lowered denitrification rates due to accumulation of nitrite and nitrate ions resulting from small supply of organic carbon sources.

When the C/N ratio of the influent water was 3 and 1.5, nitrite ions did not accumulate in the denitrification tank in the anoxic reactors. However, nitrite ions were found in the denitrification tank when the C/N ratio was 1 (Fig. 6). This indicates that the denitrification process was hindered when the C/N ratio was 1. This also implies that the minimum C/N ratio of the influent water for complete

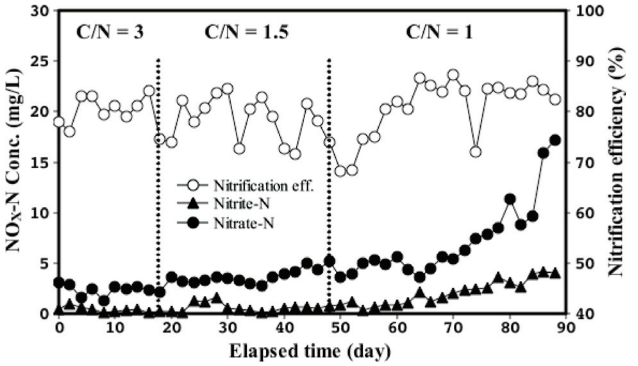

Fig. 6. Change in nitrite and nitrate concentrations in the anoxic reactors and the nitrification efficiency with different influent C/N ratios.

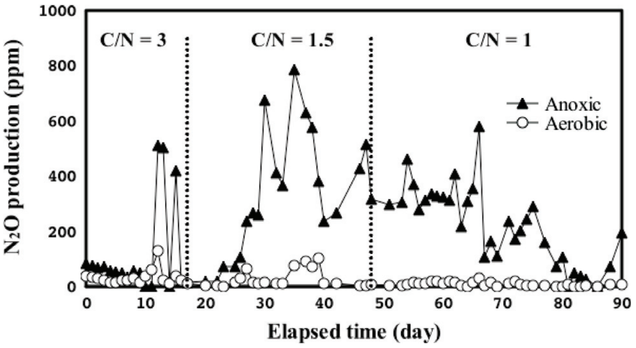

Fig. 7. Effect of the C/N ratio on nitrous oxide production from the biofilm reactors.

denitrification is approximately 1.5. On the other hand, the optimum COD/NO_X-N ratio for denitrification varied depending on relative concentrations of organic matter, nitrite and nitrate. Chiu and Chung [21] observed that the optimum COD/NO_X-N ratio decreased with the increasing nitrate concentration. These results could be explained by the fact that the change in the COD/NO_X-N ratio influenced microbial metabolic pathways and patterns for substrate competition. This may explain the various optimum C/N ratios reported for different BNR systems, such as activated sludge, RBC, A_2O processes.

Effects of the C/N ratio on N_2O emissions from denitrification and nitrification tanks are shown in Fig. 7. Higher N_2O emissions (maximum rate 800 ppm) were observed from the denitrification tank when the C/N ratio was 1.5 and 1, compared to when the ratio was 3. This was probably due to the fact that insufficient organic carbon sources interfered denitrification process. The low concentration of N_2O emissions was maintained at around 50~100 ppm when the C/N ratio was 3. In this condition, there were several short-duration peaks of N_2O concentration, as high as 500 ppm. These peaks could be explained by the fact that the filled media facilitated nitrification, which produced sufficient NO_x, resulting in higher denitrification loads in the anoxic tank. The C/N ratio in the influent water needed to reduce N_2O production to be over 1.5, indicating the importance of a sufficient supply of organic carbon sources to suppress N_2O production in the denitrification process.

3.2. Source of N_2O production in anoxic conditions

We set the isotopic ratio of the initial substrate (NO^{3-}-N) as $^{14}NO_3^-$-N:$^{15}NO_3^-$-N = 7:3 to trace the contributions of nitrification and denitrification to N_2O emissions in anoxic conditions when the media was filled. The results of the isotopic analysis of N_2O production in anoxic conditions are shown in Table 2. In the initial stage of the reaction, no N_2O was produced, probably because complete denitrification was not obstructed. The N_2O emissions and the accumulation of NO_2^--N were observed 2 hours after the reaction was initiated, probably due to the fact that a lack of organic matter hindered denitrification. The GC/MS analysis results of N_2O gas showed that the ratio of $^{44}N_2O$:$^{45}N_2O$:$^{46}N_2O$ was similar to the theoretical ratio of 1:0.86:0.18, indicating that most of the N_2O originated from denitrification.

Table 2
^{15}N-isotope analysis to trace sources of N_2O emissions in the denitrification process

	Reaction time (hr)					
	0	1	2	3	4	5
NO_3^--N (as 30% of ^{15}N, mg/ℓ)	136	78	61	44	38	33
NO_2^--N (mg/ℓ)	0	0.1	1.3	2.3	3.6	4.2
N_2O production (ppm)	ND	ND	30.4	27.6	43.6	49.3
$^{44}N_2O$: $^{45}N_2O$: $^{46}N_2O$	–	–	1:0.89:0.19	1:0.93:0.20	1:0.85:0.18	1:0.94:0.21
Reference	$^{44}N_2O$: $^{45}N_2O$: $^{46}N_2O$ = 1: 0.86: 0.18					

ND: Not Detected

3.3. Source of N_2O production in aerobic conditions

When the DO concentration was 0~0.5 mg/ℓ, 268 ppm of N_2O was produced, of which 99% originated from denitrification (Fig. 8 and 9). This is explained by the fact that a low concentration of oxygen in the reaction tank retarded nitrification and the presence of a small amount of oxygen also obstructed denitrification, resulting in the large emission of N_2O. Similar results were also found by Hong et al. [8] who observed that conditions with amounts of DO less than 0.5 mg/ℓ in the activated sludge increased N_2O production, while N_2O emissions were rather reduced when the DO was lower than 0.1 mg/ℓ, because nitrification was difficult to occur in low DO conditions.

As the DO concentration increased, the amount of N_2O production dramatically decreased and the fraction of N_2O production was mostly contributed to nitrification process (Figs. 8 and 9). This was due to the fact that sufficient dissolved oxygen expanded the aerobic conditions where more N_2O was produced through nitrification.

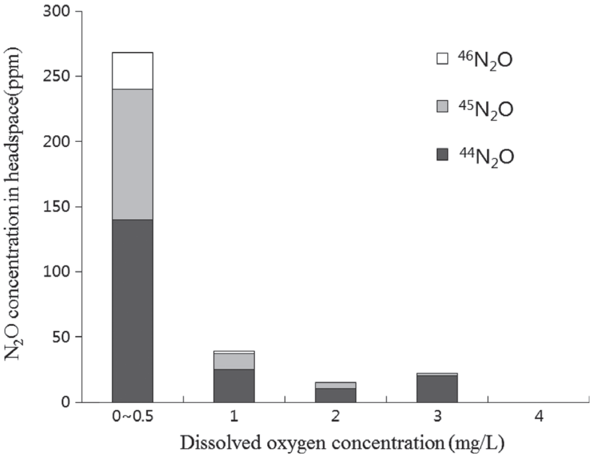

Fig. 8. Effects of dissolved oxygen concentration on the production and isotopic composition of N_2O under aerobic conditions.

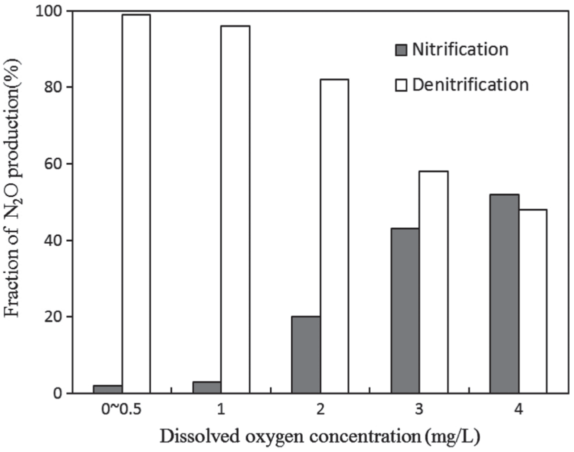

Fig. 9. Effects of dissolved oxygen concentration on the sources of N_2O emissions under aerobic conditions.

Fig. 9 also showed that more than 50% of the emitted N_2O gas was from the denitrification process when the media was filled, even in conditions with DO concentrations of 2~4 mg/ℓ, which is sufficient for biological nitrification. This indicates that the filled media could increase nitrification efficiency and N_2O emissions at the same time because it stimulated both nitrification and denitrification. This is probably due to the fact that oxygen depletion inside the media and insufficient organic matter supply served as suitable conditions for partial denitrification.

4. Conclusions

In this study, we investigated the effects of C/N ratios on N_2O emissions from nitrification and denitrification tanks in the BNR process. The major findings are as follows:

1. Changes in C/N ratio in the influent significantly influenced the denitrification process. When the COD/NO_X-N ratio is close to the theoretical optimum of 3.7 or lower, more N_2O was produced due to retardation of the denitrification process. When the input load of organic matter was constant and the NO_X-N increased due to nitrification, especially high levels of N_2O gas were emitted in the denitrification tank. Hence, lower COD/NO_X-N ratios may explain the hindrance of complete denitrification. A supply of sufficient organic carbon was an important factor to keep more theoretical optimum COD/NO_X-N ratio for ensure complete denitrification to restrain N_2O production in the BNR system applied fluidized media.
2. The ^{15}N tracer method showed that more N_2O gas was emitted under anoxic conditions, and that 99% of this emission was attributed to the denitrification process. As DO increased, N_2O emissions were reduced and the nitrification process prevailed. However, even in aerobic conditions where high levels of DO, 4 mg/ℓ, were observed, 50% of the emitted N_2O originated from denitrification, probably because there are anaerobic or anoxic zones inside the media, even in aerobic conditions and as the DO decreases, the zones tend to be larger, which caused a large amount of N_2O emissions from partial denitrification. The improvement of mass transfer coefficient of media and operation performance of the system are required to transfer oxygen into the inside of the media for reducing N_2O production in the BNR system applied fluidized media.

Acknowledgements

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