Reduction of N\textsubscript{2}O Emission from Biological Nitrogen Removal Processes by \textit{Alcaligenes faecalis} Augmentation

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Abstract: Immobilized \textit{Alcaligenes faecalis} gels were augmented into an intermittent aeration reactor treating wastewater to control the emissions of N\textsubscript{2}O gas. An increase in the hydraulic retention time (HRT) in response to an applied loading caused a significant increase in N\textsubscript{2}O production. Through \textit{A. faecalis} augmentation, the N\textsubscript{2}O conversion per influent total nitrogen (T-N) was reduced to 50 % of that in a control reactor. Although dissolved oxygen (DO) affected the N\textsubscript{2}O emission in both the activated sludge and the \textit{A. faecalis}-augmented culture, the emission in the \textit{A. faecalis}-augmented reactor at a low DO level was much lower than that in the control activated sludge reactor.

Keywords: alcaligenes faecalis, heterotrophic nitrifier, nitrogen removal, nitrous oxide, wastewater treatment

Introduction

Nitrous oxide (N\textsubscript{2}O) is an important greenhouse gas that is also a stratospheric ozone-depleting pollutant. Human activity is a significant source of global N\textsubscript{2}O [1], with wastewater treatment systems being a potential anthropogenic source. N\textsubscript{2}O emissions in wastewater treatment systems mainly arise from the removal of nitrogen in both nitrification and denitrification processes [2-4]. N\textsubscript{2}O emitted from wastewater contributes 5.9 % of the total N\textsubscript{2}O budget [5]. Compared with aerobic processes, intermittent aeration processes are alternative methods of operation for both nitrogen removal and N\textsubscript{2}O emission control because intermittent aeration process allows nitrogen removal to be accomplished biologically in a single reactor, in which the anoxic period and aerobic period are alternated [6]. Nitrogen loss from the mixed liquor of an anoxic-aerobic bioreactor could result from simultaneous autotrophic N\textsubscript{2}O production and heterotrophic reduction in the same reactor [10]. Itokawa and coworkers [7] and Park and coworkers [8] reported that complete nitrification and denitrification could minimize N\textsubscript{2}O formation. Truick and Bulmer [9] reduced N\textsubscript{2}O emission through the use of a denitrifier, \textit{Pseudomonas denitrificans}, with perfluorocarbons.

During cyclic alternation of anoxic and aerobic conditions, an emission peak exists for dissolved oxygen (DO) concentrations between 0.2 and 0.6 mg/L [13]. When DO concentrations are lower than this critical value, the degree of nitrification is too low to produce more N\textsubscript{2}O, whereas values higher than the critical concentration facilitate complete nitrification and alleviate N\textsubscript{2}O emission. However, heterotrophic nitrifiers, for example, \textit{A. faecalis} and \textit{Paracoccus denitrificans} (formerly \textit{Thiosphaera pantotropha}), which exhibit both nitrifying and denitrifying functions, have caused much more interest and a great deal of research into their nitrogen removal and N\textsubscript{2}O emission behavior [11,12]. Although heterotrophic nitrification produces approximately the same amount of N\textsubscript{2}O under certain conditions, such as low pH and a high oxygen level, these heterotrophic nitrifiers could denitrify their nitrification products under aerobic conditions [14]. Inamori and coworkers [15] found that \textit{A. faecalis} plays important roles in producing N\textsubscript{2}O in pure cultures and that the introduction of \textit{A. faecalis} in activated sludge systems was a possible method for re-
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Figure 1. Schematic representation of the intermittent aeration reactor.

becoming $\text{N}_2\text{O}$ emission [16].

The objective of this study was to examine the reduction of $\text{N}_2\text{O}$ emission caused by bio-augmentation of the heterotrophic nitrifying bacteria, such as immobilized gel of *A. faecalis* and poly(ethylene glycol) (PEG).

**Experimental**

**Preparation of Microorganism**

*Alcaligenes faecalis* (IFO14479) was enriched in a medium containing 10 g/L polypepton, 2.0 g/L yeast extract, and 1.0 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, with the pH adjusted to 7.0 before sterilization. The condensed biomass was immobilized into PEG gel [19]. The immobilized *A. faecalis* pellet was introduced into a reactor. An activated sludge seed for the test was taken from a municipal wastewater treatment plant.

**Experimental System**

The continuous reactors possessing submerged membrane filter [20] were operated in a 4-L working volume, as shown in Figure 1. Two parallel reactors were used to investigate the characteristics of $\text{N}_2\text{O}$ emission. An immobilized *A. faecalis* pellet was added into one reactor with a 10% volume ratio. The other reactor was operated with suspended activated sludge as a control. The experiments consisted of two stages. The first stage of operation was performed with different hydraulic retention times (HRTs) of the system (from 12 to 36 h). Intermittent aeration was undertaken to perform the nitrification and denitrification processes. The operating cycle consisted of a 60-min aerobic phase and a 30-min anoxic phase. At an HRT of 24 h, a track study was conducted in the course of an anoxic-aerobic cycle. The second stage of the experiment was conducted to examine the response of systems to DO variations at the end of the experiment under three different DO conditions.

**Wastewater Composition**

The reactor was operated using domestic wastewater taken from the influent of the municipal wastewater treatment plant. Domestic wastewater stored at 5 °C was fed to reactors at room temperature (20 °C). Throughout the experiment, the pH of the wastewater was maintained at ca. 7.0. The composition of the wastewater was 100~120 mg/L $\text{BOD}_5$, 40~45 mg/L $\text{T-N}$, and 5~6 mg/L $\text{T-P}$. Nitrogen ions in the wastewater consisted of ca. 20~25 mg/L of oxidized nitrogen and less than 0.3 mg/L of oxidized nitrogen.

**Analytical Methods**

The concentration of nitrogen in the influent and effluent of the reactor was analyzed using automated colorimetric methods on a TRAACS-800 instrument [17]. The TOC (Total Organic Carbon) was analyzed by a TOC Analyzer (TOC-500, Shimadzu Co.). $\text{N}_2\text{O}$ gas samples were injected into a gas chromatography equipped with an electron-capture detector (ECD) and a column packed with Poropak Q (Shimadzu Co.). The length of the packed column was 1.8 m. The temperatures of the detector and oven were maintained at 340 and 80 °C, respectively. Argon containing methane (5%) was supplied as a carrier gas; the column flow rate was adjusted to 40 mL/min.

**Results and Discussion**

**Reactor Performance and Effects of HRTs**

After start-up operation of a month, the mixed liquor-suspended solids (MLSS), except for the pellet, were maintained constantly at ca. 3,000 mg/L. Most of the organics and $\text{NH}_4^+$ nitrogen were oxidized as a result of the nitrification in all of the reactors at 3 mg/L DO. The total nitrogen removal efficiency ranged from 40 to 55%.

Figure 2 shows that the $\text{N}_2\text{O}$ conversion rates varied with respect to the HRTs in both the control and augmentation reactors. The $\text{N}_2\text{O}$ conversion rate of the control reactor was calculated to be 0.6% of the influent nitrogen at 36 h of HRT; it increased to 8.4% at 12 h of HRT. The transformation of the influent total nitrogen into $\text{N}_2\text{O}$ was influenced by the nitrogen loading due to the increased HRT. Zheng and coworkers (1994) also reported that the retention time promoted $\text{N}_2\text{O}$ production in response to both nitrifying and denitrifying activated sludge [18].

The $\text{N}_2\text{O}$ conversion rate of the augmentation reactor with a 10% volume ratio of immobilized *A. faecalis* pel-
Figure 2. N₂O conversion plotted with respect to the HRT in the control (○) and augmentation (●) reactors. The control reactor was operated with suspended activated sludge; the augmented reactor incorporated an immobilized *A. faecalis* pellet into the activated sludge culture with 10% volume ratio.

Figure 4. N₂O emission plotted with respect to the DO level in the control (○) and augmentation (●) reactors. The control reactor was operated with suspended activated sludge; the augmented reactor incorporated an immobilized *A. faecalis* pellet into the activated sludge culture with 10% volume ratio.

let was 50% of that of the control reactor, but the trend of the N₂O emission with respect to an increase in the HRT was similar to that of the control reactor. N₂O is a by-product of microbial metabolism; its transmission and emission can be affected by differences in the uptake efficiencies of the microbial species. Growth in a suspension such as activated sludge could not offer a high biomass retention or the intimate coexistence of nitrifiers and denitrifiers. The reduction of N₂O emissions in the augmentation reactors resulted from the reduction of intermediate formation and the ease of consumption of N₂O. A lower production of intermediates could facilitate the reduced N₂O emission because allowing simultaneous nitrification and denitrification represses N₂O emission. Therefore, this result implies that bio-augmentation, i.e., the existence of heterotrophic nitrifiers, has great potential for the reduction of N₂O emissions.

**Track Study for Cyclic N₂O Emission**

In the course of intermittent aeration, the N₂O emissions created a pattern exhibiting cyclic behavior. Figure 3 shows the N₂O production and DO in the intermittent reactor at 24 h HRT. In the control reactor, a great deal of N₂O was emitted during the early stage of the aerobic phase in an anoxic-aerobic cycle. A DO range from 0.5 to 1.0 mg/L was maintained at the beginning of the aerobic phase and then rose to sufficiently high levels from 3 to 4 mg/L, with the duration time (15 to 30 min) depending on the carbon-to-nitrogen ratio in the influent. A low DO concentration of a certain critical range of ca. 0.5 mg/L promoted N₂O production by nitrifiers in activated sludge. Oxygen deficiency in the reactor favored an increase in the N₂O emissions; thus, the incomplete nitrification and denitrification under low DO conditions may inactivate N₂O reductase and promote the formation of NO. However, in the augmentation reactor, heterotrophic nitrifiers also have a denitrifying function at low DO levels, so successive denitrification of N₂O was continued by the same microorganisms, leading to a simultaneous reduction of N₂O emission.

**Effect of DO Levels**

At the end of the experiment, the continuous operation of the reactors at three different DO levels was performed to confirm the effect of DO on the N₂O emission.
Two of the reactors had N₂O emission patterns that varied according to the DO levels in the reactor, as shown in Figure 4. Only a small amount of N₂O was detected in each reactor under anoxic conditions of less than 0.2 mg/L DO. On the other hand, the control reactor showed a significant N₂O emission at 0.6 mg/L DO, most likely due to insufficient nitrification and accumulation of the intermediate. Oxygen limitation significantly impacted N₂O emission in the control reactor because an autotrophic nitrifier such as *Nitrosomonas europaea* could produce N₂O under oxygen stress conditions through so-called nitrifier denitrification [19]. In the augmentation reactor, the findings support the hypothesis that it is possible to reduce N₂O emission through simultaneous N₂O production and reduction. In contrast to the situation at low DO conditions, the N₂O emissions in the two reactors under high DO conditions (3 mg/L) were almost similar because heterotrophic nitrifiers also produce significant amounts of N₂O [13].

**Conclusions**

Nitrous oxide (N₂O) gas was emitted during nitrogen removal from domestic wastewater. It can be emitted as a by-product of the nitrogen removal process for wastewater. Intermittent aeration reactors fed with domestic wastewater were operated to investigate the effect of bioaugmentation for nitrous oxide emission. An immobilized gel of *A. faecalis* was added into intermittent aeration reactors treating wastewater to control the emissions of N₂O gas. The immobilized gel of *A. faecalis* reduced N₂O emission. The N₂O conversion rate in the control reactor was only 0.6 % of the influent total nitrogen (T-N) at 36 h of HRT, but it increased to over 8.4 % at 12 h of HRT. Although the immobilized *A. faecalis* gel-augmented reactor showed a similar pattern according to HRT, the amount of emitted N₂O was half of that from the control reactor. The higher N₂O emission rate in the control reactor might have been caused by a low DO level in the early aerobic period. Continuous operation under different DO conditions implied that the aerobic conditions of a lative lower DO could enhance N₂O emission in the control reactor.

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**References**