Dropping Method for Partial Nitrification of Synthetic Ammonium-contaminated Groundwater

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Abstract

The production of NO₂⁻–N from NH₄⁺–N via partial nitrification is necessary for anaerobic ammonium oxidation (anammox). This study investigated partial nitrification using a dropping reactor that comprised a hanging 10 x 10 x 10 mm polyolefin sponge cube with an effective length of 30 cm. In this present study, a dropping reactor was proposed for the partial nitrification. This configuration simulated a down-flow hanging sponge reactor. The synthetic NH₄⁺–N contaminated groundwater (40 mg/L) was fed at a 1.0–5.0 mL/min flow rate for 287 d. We found that the efficiency of the partial nitrification method increased with an increase in the synthetic groundwater flow rate. The partial nitrification percentage (NO₂⁻–N in effluent) was 11.0–59.5% at 4.0–5.0 mL/min. The amoA gene of ammonia-oxidizing bacteria (AOB) and nxrA gene of nitrite-oxidizing bacteria (NOB) and 16S rRNA genes of Nitrosomonas (AOB) Nitrobacter and Nitrospira (NOB) were detected in the sponge material of the partial nitrification reactor. The AOB and NOB might have contributed to partial and complete nitrification. The results suggested that the proposed partial nitrification method effectively produced NO₂⁻–N from NH₄⁺ in the contaminated groundwater without the high cost of aeration. Therefore, this new method could cost-effectively provide the NO₂⁻ required for anammox.

Keywords: dropping reactor, partial nitrification, NH₄⁺–N, groundwater treatment

INTRODUCTION

Groundwater is an important resource for domestic use and drinking water. However, ammonium nitrogen-contaminated groundwater (NH₄⁺–N) is a major problem in some parts of the world. NH₄⁺–N contamination has adverse effects on odor and taste. High NH₄⁺–N levels are a major factor in reducing the disinfection efficacy of chlorine and other halogens, increasing the risk of pathogen contamination during water treatment and distribution. Therefore, NH₄⁺–N removal before water purification and the water supply chain is important.

The NH₄⁺–N removal from groundwater includes physical methods such as ion exchange and zeolite adsorption and biological methods such as permeable reactive barriers (PRBs) and rapid sand filtration. The zeolite adsorption and ion exchange can efficiently adsorb NH₄⁺–N in groundwater; however, it is necessary to replace the zeolite or filter medium in certain period of time. The biological methods are considered more cost-effective and require lower energy consumption. However, PRBs require large-scale construction, and rapid sand filtration...
cannot completely remove high NH$_4^+$-N concentrations. Therefore, it is important to develop new biological treatment strategies to efficiently remove high NH$_4^+$-N concentrations from groundwater.

Anaerobic ammonium oxidation (anammox) process is well-known as a low-energy consumption and highly efficient N removal technology for N-rich wastewater. However, the anammox process has not been applied to NH$_4^+$-N removal from groundwater due to the prior requirement of partial nitrification to produce NO$_2^-$-N from NH$_4^+$-N.

In conventional partial nitrification, aeration adjustment is necessary to produce NO$_2^-$-N. However, since aeration is not cost-effective due to its high energy consumption, it is important to develop more energy-efficient partial nitrification technologies. Therefore, this study focused on using a dropping reactor for partial nitrification. In this process, porous polyolefin sponge carriers were suspended from the upper part of the reactor, and efficiently transformed NH$_4^+$-N in contaminated groundwater into NO$_2^-$-N and NO$_3^-$-N.

We hypothesized thus that a DHS-like dropping process might be useful for partial nitrification. To the best of our knowledge, this concept had not yet been studied; therefore, we investigated the feasibility of our partial nitrification method, focusing on the influence of the groundwater-dropping flow rate on partial nitrification efficiency.

**MATERIALS AND METHODS**

**NH$_4^+$-N contaminated synthetic groundwater**
Forty mg NH$_4^+$-N/L contaminated synthetic groundwater [0.188 g (NH$_4$)$_2$SO$_4$, 0.5 g NaHCO$_3$, 0.3 g MgSO$_4$·7H$_2$O, 0.027 g KH$_2$PO$_4$, and 0.18 g of CaCl$_2$·2H$_2$O per L] was prepared using tap water. The NH$_4^+$-N and other component concentrations were determined based on the NH$_4^+$-N-contaminated groundwater in the Kathmandu Valley, Nepal.

**Set up of the partial nitrification process with dropping reactor**
A polyolefin sponge (10 × 10 × 10 mm; Sekisui Aqua System Company, Osaka, Japan) was used as a bacterial carrier in the dropping reactor. Approximately 30 sponge cubes were connected by a nylon thread to form a hanging sponge unit with an effective length of 30 cm (Fig. 1). Four sponge units were prepared. The sponge units were incubated overnight in 600 mL 1:5 v/v activated sludge-tap water suspension, with approximately 2.4 g/L mixed liquor volatile suspended solids (MLVSS) as the bacterial load.

![Schematic diagrams of sponge carrier and reactor system. (a) Schematic diagram of sponge unit; (b) Schematic diagram of reactor system. The cylinder-type reactor was not closed at the top, the sponge unit was fixed to a rod, and the rod was fixed to the reactor. Since the circumference of the fixed rod was open, gas was exchanged between atmosphere and inside of reactor.](image-url)
inoculum (collected from a municipal wastewater treatment plant in Kofu, Yamanashi, Japan.) Subsequently, 4 sponge units were suspended in a single cylindrical reactor (Fig. 1).

Operational conditions of the dropping reactor The dropping reactor hanging 4 sponge units was operated at room temperature (24–26°C) for 287 d. The synthetic groundwater was supplied from the top of the reactor using a peristaltic pump at a 1.0 mL/min flow rate (d 1 to 118; unstable setup phase, and d 119 to 173; phase 1), 2.0 mL/min (d 174 to 198; phase 2), 3.0 mL/min (d 199 to 241; phase 3), 4.0 mL/min (d 242 to 266; phase 4), and 5.0 mL/min (d 267 to 287; phase 5). The NH₄⁺ content was measured using indophenol method, while the NO₂⁻ and NO₃⁻ contents were measured by ultraviolet spectrophotometric screening and colorimetric methods, respectively, using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

MLVSS analysis was performed using the standard method. The sludge adhering to the sponge was collected by crushing the sponge according to the method reported in a previous study. DNA extraction from sponge carrier and quantification of functional genes After 287 d, the sludge on the sponge carriers was collected from four sponge units at 0 (top), 10, 20, and 30 (bottom) cm from the top of the sponge unit. The DNA of the sludge was extracted using the FastDNA SPIN Kit for Soil (MP Biomedicals, USA). The bacterial 16S rRNA, ammonia monoxygenase (amoA), NOB nitrite oxidoreductase (nxrA), nitrite reductase (nirK and nirS), and N₂O reductase (nosZ) genes were quantified by real-time quantitative polymerase chain reaction (RT-qPCR) in a Thermal Cycler Dice Real-Time System II (Takara Bio Inc., Shiga, Japan). The qPCR mixture (25 µL) contained 2 µL template DNA, 12.5 µL TB Green Premix Ex Taq II (Tli RNase H Plus) (Takara Bio), 0.5 µL each forward and reverse primer (0.5 µM), and 9.5 µL ultrapure water. 314F–534R, Amo589f-Amo718r, F1norA-R1norA, nirK583F-nirK909R, nirS634F-nirS836R, and nosZ1527F-nosZ1773R were used to amplify the bacterial 16S rRNA, amoA, nxrA, nirK, nirS, and nosZ genes, respectively. The thermal conditions for qPCR analysis were 95°C for 30 s, followed by 40 cycles at 98°C for 5 s, annealing temperature for 50 s (bacterial 16S rRNA gene, 60°C; amoA gene, 56°C; nxrA gene, 58°C; nirK gene, 63°C; nirS gene, 57°C; nosZ gene, 58°C), and an extension at 72°C for 1 min, followed by a dissociation stage (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s). Genomic DNA from Escherichia coli JM109 (Takara Bio) was used as the standard for bacterial 16S rRNA gene. The synthetic plasmid carrying the target sequence of amoA, nxrA, nirK, nirS, or nosZ gene was used as the standard for each gene. Ten-fold serial dilutions of standard samples were subjected to qPCR. The range of qPCR recovery efficiencies for all genes were 0.90 to 0.99. All qPCR assays were duplicated.

Phylogenetic bacterial community analysis The extracted bacterial DNA samples in the microbial community were analyzed using Illumina MiSeq 16Sr RNA sequencing (Illumina, San Diego, CA, USA). The V4 region of the 16S rRNA gene was amplified by PCR using the universal primers 515F (5'–ATGTGCCAGCMGCCGCGGTAA–3') and 806R (5'–BGGACTACHVGGGTWTCTAAT–3'). The PCR amplicons were sequenced on an Illumina MiSeq Sequencer (Illumina). Sequencing and sequence-read analyses were conducted using FASMAC (Kanagawa, Japan).

Data and statistical analyses The nitrogen-
loading rate (NLR), ammonium removal, and partial nitrification ratios of the dropping reactor were calculated using equations (1)–(3):

\[ \text{Nitrogen loading rate} = \frac{\text{NH}_4^+ - \text{N}_{\text{influent}} \times \text{Flow rate}}{\text{Reactor length}} \] (1)

Ammonium removal ratio (%) = \[ \frac{\text{NH}_4^+ - \text{N}_{\text{influent}} - \text{NH}_4^+ - \text{N}_{\text{effluent}}}{\text{NH}_4^+ - \text{N}_{\text{influent}}} \times 100 \] (2)

Partial nitrification ratio (%) = \[ \frac{\text{NO}_2^- - \text{N}_{\text{effluent}}}{\text{NH}_4^+ - \text{N}_{\text{influent}}} \times 100 \] (3)

The NH\(_4\)\(^+\)-N\(_{\text{influent}}\), NH\(_4\)\(^+\)-N\(_{\text{effluent}}\), and NO\(_2\)\(^-\)-N\(_{\text{effluent}}\) represented influent water sample of NH\(_4\)\(^+\)-N, effluent water sample of NH\(_4\)\(^+\)-N, and NO\(_2\)\(^-\)-N concentrations, respectively. The pH, DO, and N concentration data were obtained in setup phase, phases 1, 2, 3, 4, and 5. The mean and standard deviation of each value was calculated for each phase. The data of phase 1–5 was used for correlation analysis. Correlation analysis was performed using R statistical software version 3.6.0\(^{23}\). Significant differences between pairs of groups were identified using a Student’s t-test (\(p < 0.05\)).

**RESULTS AND DISCUSSION**

**NH\(_4\)\(^+\)-N transformation ability of the dropping reactor at various flow rates** The performance was evaluated from phase 1 to phase 5 excluding the data during the unstable setup phase (d 1 to 118) in which the N concentrations in effluent fluctuated greatly. The changes in the pH, DO, and N concentrations of the influent and effluent water are shown in Fig. 2. The average pH of the influent and effluent over 287 d were 7.3 ± 0.54 and 7.3 ± 0.44, respectively, and the average DO concentration over the same period were 3.6 ± 1.7 and 5.9 ± 1.2 mg/L, respectively (Fig. 2b). The results indicated that atmospheric oxygen was incorporated into the synthetic groundwater during the dropping process.

In phase 1 (flow rate 1.0 mL/min, NLR 0.17 ± 0.02 g/m\(^2\) day), the NH\(_4\)\(^+\)-N and NO\(_2\)\(^-\)-N concentration in the effluent was undetectable after 119 d. In phase 2 (flow rate 2.0 mL/min, NLR 0.30 ± 0.06 g/m\(^2\) day) and phase 3 (flow rate 3.0 mL/min, NLR 0.51 ± 0.10 g/m\(^2\) day), the NH\(_4\)\(^+\)-N and NO\(_2\)\(^-\)-N concentrations in the effluent were 0.02–23.1 mg/L and 0.04–23.0 mg/L, respectively. In phase 4 (flow rate 4.0 mL/min, NLR 0.75 ± 0.03 g/m\(^2\) day) and phase 5 (flow rate 5.0 mL/min, NLR 0.84 ± 0.24 g/m\(^2\) day), the NH\(_4\)\(^+\)-N and NO\(_2\)\(^-\)-N concentrations in the reactor effluent were 0.05–12.6 mg/L and 3.6–23.0 mg/L, respectively. The NO\(_2\)\(^-\)-N concentration in the effluent was approximately 1.0 mg/L throughout the 287 d experimental period.

The changes in the ammonium removal and partial nitrification ratios are shown in Fig. 3. In phase 1, ammonium removal and partial nitrification were unstable until 138 d; however, after 146 d, the ratios were 99.5–100% and 0–2.1%, respectively; in phases 2 and 3, they were 20.9–99.9% and 0.11–59.5%, respectively, and in phases 4 and 5, they were 66.6–99.9% and 11.0–59.5%, respectively.

Previous studies have demonstrated the partial nitrification in DHS reactors treating wastewater\(^{12,13}\). In this study, the partial nitrification was observed in a DHS-like dropping nitrification reactor treating groundwater. The NH\(_4\)\(^+\)-N concentration in the effluent from the dropping nitrification reactor was lower than in conventional partial nitrification methods\(^{24}\); however, partial nitrification was observed in the dropping reactor without aeration. The dropping process reduced the operational energy requirement compared to conventional partial nitrification methods requiring aeration.

**Effect of DO concentration and flow rate on the partial nitrification** The correlations between the flow rate and DO concentration of effluent water sample, ammonium removal, and partial nitrification obtained in the above experiment are shown in Fig. 4. The DO concentration of effluent water sample increased as the flow rate increased (Fig. 4a). A significant positive correlation was found between flow rate and DO concentration (\(r = 0.80, p < 0.05\)). Previous study reported that the DO concentration in DHS reactor was higher under higher flow rate condition
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Fig. 2  pH, dissolved oxygen, and nitrogen concentration over 287 days. (a) pH; (b) Dissolved oxygen concentration; (c) Nitrogen concentration.

Fig. 3  Ammonium removal and partial nitrification ratios over 287 days.
compared to lower flow rate condition\(^{20} \). In DHS reactor, the oxygen transfer into water from atmosphere is carried out on the surface of sponge carriers. Surface water volume on sponge materials at higher flow rate can be more than that at lower flow rate\(^{25} \). Therefore, oxygen supply ability of DHS can be higher under moderately high flow rate conditions\(^{25} \). Similarly, in this study, the DO concentration in effluent of dropping reactor increased by increasing the flow rate.

Whereas, there was negative correlation between the flow rate and ammonium removal ratio \((r = -0.88, p < 0.05)\) (Fig. 4b). DO concentration increased slightly as the flow rate increased. Thereby, it has possibility that ammonia oxidation was promoted. However, the ammonia removal ratio might decrease due to increase of NLR as the flow rate increased. On the other hands, the partial nitrification ratio increased as the flow rate increased and showed a significant positive correlation with the flow rate and partial nitrification \((r = 0.97, p < 0.05)\) (Fig. 4c). The possible reasons are that 1) ammonia oxidation was promoted with DO concentration increased and 2) nitrite oxidation rate by NOB might be lower compared to ammonia oxidation by AOB due to the competition for DO concentration with AOB. Therefore, NO\(_2\)-N concentration in effluent and partial nitrification ratio in dropping reactor might increase.

**Abundance of functional microbial genes involved in nitrogen transformations and bacterial community structure in the reactor**

The abundance of the 16S rRNA, *amoA*, *nxrA*, *nirK*, *nirS*, and *nosZ* genes in the activated sludge used as a microbial source and the sludge attached to the sponge unit at 0 (inflow point), 10, 20, and 30 cm from the top of the reactor at the end of the experiment is shown in Fig. 5. The

![Fig. 4 Correlation diagrams for three parameters. (a) Correlation between flow rate and dissolved oxygen concentration; (b) Correlation between flow rate and ammonium removal ratio; (c) Correlation between flow rate and partial nitrification ratio. Symbols show averages. Error bars show standard deviations.](image)

![Fig. 5 Abundance of 16S rRNA, *amoA*, *nxrA*, *nirK*, *nirS*, and *nosZ* genes in reactor system at end of operation. Symbols show averages of duplicate experiments. Error bars show standard deviations.](image)
abundance of the 16S rRNA gene in the original activated sludge and the sludge attached to the sponge unit was similar (6.4 × 10^{11}–2.9 × 10^{12} copies/g-MLVSS). The abundance of the AOB amoA gene (6.7 × 10^8 –3.2 × 10^{10} copies/g-MLVSS) in the sludge attached to the sponge unit was one or more orders higher than in the original activated sludge (7.8 × 10^6 copies/g-MLVSS). The abundance of amoA in the sludge attached to the sponge increased from the top to the middle of the reactor. The abundance of the NOB nxrA gene in the sludge attached to the sponge unit (9.0 × 10^{10}–1.5 × 10^{11} copies/g-MLVSS) was 3 to 4 orders higher than in the original activated sludge (2.6 × 10^7 copies/g-MLVSS). These results indicated that over the 287-d dropping nitrification experiment, AOB and NOB were enriched in the sludge attached to the sponge unit. In contrast, the abundance of the nirK, nirS, and nosZ denitrifying bacterial genes in the sludge attached to the sponge unit were similar to or lower than in the original activated sludge.

The bacterial community structures of the sludge attached to the sponge unit were different from the original activated sludge (Fig. 6). Moreover, the bacterial community structures differed from the upper part to the bottom of the sponge unit. *Nitrosomonas* was detected as an AOB from the top to the bottom of sponge unit. *Nitrobacter* was detected as an NOB at the top of the reactor, and *Nitrospira* was detected as an NOB at the top, middle, and bottom of the reactor. The AOB and NOB may contribute to partial nitrification and complete nitrification in the sponge units.

**CONCLUSIONS**

In this study, the partial nitrification was observed by dropping NH_4^+-N contaminated synthetic groundwater into sponge units. We found that the partial nitrification efficiency increased with the water flow rate and that *Nitrosomonas* AOB might contribute to partial nitrification in the reactor. The results suggested that dropping nitrification reactors could be a new, cost-effective partial nitrification method.
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