

Simultaneous Removal of Ammonium and Nitrate by a Combination of ANAMMOX and Hydrogenotrophic Denitrification

Tatsuru KAMEI¹⁾, Dai NAITOH¹⁾, Wilawan KHANITCHAIDECHA²⁾, Futaba KAZAMA³⁾

1) *Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 4-4-37, Takeda, Kofu, Yamanashi 400-8510, Japan*

2) *Department of Civil Engineering, Faculty of Engineering, Naresuan University, Phitsanulok 65000, Thailand*

3) *International Research Centre for River Basin Environment, University of Yamanashi, 4-4-37, Takeda, Kofu, Yamanashi 400-8510, Japan*

ABSTRACT

Groundwater is often contaminated by ammonium and nitrate, causing problems in developing countries such as Thailand and Nepal. Therefore, development of new methods for removing contaminants from groundwater is necessary. In this study, we investigated the effectiveness of a new treatment system for simultaneous removal of ammonium and nitrate by anaerobic ammonium oxidation (ANAMMOX) and hydrogenotrophic denitrification. Enriched ANAMMOX sludge was cultivated under two conditions (20 mL/min hydrogen gas flow rate to investigate the effectiveness of ANAMMOX and hydrogenotrophic denitrification; and 60 mL/min hydrogen gas flow rate to examine the effect of the presence of nitrite on the activities of both microbes). Simultaneous removal of ammonium and nitrate was successfully detected using the former condition, and 95% of ammonium and 90% of nitrate were successfully removed. Additionally, the maximum nitrogen removal rate was 0.25 kg-N/m³/d, and 89% of the total dissolved nitrogen was removed. Using the latter condition, increases in nitrite removal were observed only during the supply of hydrogen gas. These results showed that a hydrogenotrophic denitrifier coexisted with ANAMMOX bacteria in our enriched sludge and that ANAMMOX and hydrogenotrophic denitrification removed ammonium and nitrate simultaneously. Therefore, this method may represent a novel efficient technique for the removal of contaminants from groundwater.

Keywords: ANAMMOX, hydrogen, hydrogenotrophic denitrification, nitrate removal

INTRODUCTION

Groundwater is a major water resource for many regions. However, many groundwater sources have been contaminated by inorganic nitrogen, especially ammonium and nitrate (Rivett *et al.*, 2008; Umezawa *et al.*, 2009). Such contamination is often prevalent in developing countries such as Thailand, China, Vietnam, India and Nepal (Bouman *et al.*, 2002; Khatiwada *et al.*, 2002; Suthar *et al.*, 2009; Umezawa *et al.*, 2009), and can cause serious health issues. The World Health Organization (WHO) has set standards for the levels of nitrogenous compounds in drinking water, including those for nitrate (11 mg-N/L), nitrite (0.9 mg-N/L) and ammonium (1.5 mg-N/L) (WHO, 2011). While occurrence of health issues have not been reported, high concentrations of ammonium have shown to cause water to smell badly and lead to the production of nitrite or nitrate, whose concentrations should be minimized. Although groundwater is used as a resource for drinking water, detected values for nitrogenous compounds are often higher than WHO standard levels. For example, 20 mg-N/L ammonium and 10 mg-N/L nitrate were detected in Bangkok, Thailand, and Jakarta, Indonesia,

Address correspondence to Tatsuru Kamei, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Email: t.kamei1029@gmail.com
Received May 22, 2014, Accepted October 24, 2014.

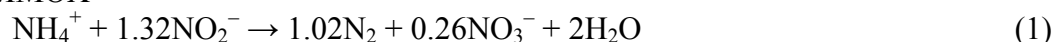
respectively (Umezawa *et al.*, 2009). Moreover, a high concentration of ammonium (62 mg-N/L) was detected in Kathmandu, Nepal (Khatriwada *et al.*, 2002). Therefore, it is imperative to introduce effective systems for the removal of ammonium and nitrate. However, in developing countries where groundwater contamination is often a problem, electricity, funding, and materials for equipment maintenance and operation are limited. Therefore, current treatment systems such as the activated sludge method, which is based on the nitrification and denitrification process, or filtration systems, would not be suitable. Indeed, an appropriate nitrogen removal system suitable for solving groundwater contamination issues in developing countries would have highly efficient removal performance and sustainability.

Anaerobic ammonium oxidation (ANAMMOX) is a well-known microbial process that can remove ammonium and nitrite simultaneously (Mulder *et al.*, 1995; Strous *et al.*, 1998). Equation (1) shows the overall pathway for the removal of these contaminants by ANAMMOX bacteria under anaerobic conditions (Sliekers *et al.*, 2002). Application of ANAMMOX for water treatment expects to reduce operational cost as compared with systems using the nitrification/denitrification method (Sumino *et al.*, 2006; Tsushima *et al.*, 2007). Although groundwater treatment by ANAMMOX seems to be suitable for developing countries, particularly in areas suffering from high ammonium concentration, the system must involve nitrite production and nitrate removal. Recent studies have revealed that the ANAMMOX process can remove nitrate by a combined protocol including a denitrification process (Sumino *et al.*, 2006; Chen *et al.*, 2009; Kumar and Lin, 2010; Wang *et al.*, 2010). According to a previous study, nitrate is partially reduced to nitrite by denitrification. Then, the produced nitrite is removed through the ANAMMOX process. Additionally, the nitrate byproduct from the ANAMMOX process is thought to be removed at the same time. Therefore, using this combination process, ANAMMOX can remove nitrate from the system. Almost all studies combining ANAMMOX with heterotrophic denitrification have been investigating applications for the treatment of sewage water systems. However, in the treatment of groundwater, combination with heterotrophic denitrification is not suitable because groundwater does not contain organic matter to continue “feeding” the heterotrophic denitrification process.

In this study, we developed a groundwater treatment system combining ANAMMOX and hydrogenotrophic denitrification. Hydrogenotrophic denitrification is an autotrophic denitrification process often used for nitrate removal from groundwater or drinking water (Smith *et al.*, 1994; Chan *et al.*, 1999; Lee and Rittman, 2002; Szekeres *et al.*, 2002; Smith *et al.*, 2005; Zhang *et al.*, 2009; Khanitchaidecha *et al.*, 2012; Khanitchaidecha and Kazama, 2012). Hydrogenotrophic denitrification is able to remove nitrate in the form of nitrogen gas through reduction to nitrite, nitric oxide, and nitrogen dioxide, similar to other denitrification processes. Equations (2), (3), and (4) show the nitrogen pathway from nitrate to nitrogen gas without production of nitric oxide or nitrogen dioxide (Lee and Rittman, 2002). Since hydrogen gas is harmless and easy to remove, secondary treatment for removing the electron donor can be omitted. Additionally, the supply of hydrogen gas for maintaining the hydrogenotrophic denitrification process would allow the inside of the reactor to remain anaerobic, which should be better for promoting the stability of the ANAMMOX process. Therefore, the use of combined ANAMMOX and hydrogenotrophic denitrification seems to be

suitable for groundwater treatment. The overall ammonium and nitrate removal process performed through the combination of ANAMMOX and hydrogenotrophic denitrification was calculated from equations (1) and (2), and the entire reaction is shown in equation (5). Nearly equimolar amounts of ammonium and nitrate were removed, and production of residual nitrate was avoided, according to these equations.

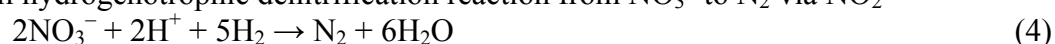
ANAMMOX



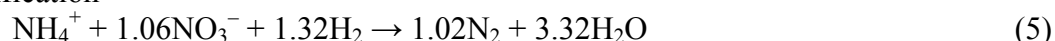
Hydrogenotrophic denitrification



Overall hydrogenotrophic denitrification reaction from NO_3^- to N_2 via NO_2^-



Simultaneous removal of ammonium and nitrate by ANAMMOX and hydrogenotrophic denitrification



However, there is little information about the effectiveness of a combined nitrogen removal system involving ANAMMOX and hydrogenotrophic denitrification. Therefore, in this investigation, we attempted to establish a new nitrogen removal system by incubating ANAMMOX sludge enriched from activated sludge under conditions in which hydrogen gas was supplied, along with ammonium and nitrite or nitrate.

MATERIALS AND METHODS

Enriched ANAMMOX sludge

The ANAMMOX sludge used in this investigation was enriched on non-woven fabric from activated sludge, which was collected from a wastewater treatment plant in Japan. The collected activated sludge had been cultivated for more than 500 days in a fixed-bed reactor placed in an incubator maintained at 35°C. Synthetic medium was prepared as described below and was used for cultivation. The sludge removed ammonium and nitrite simultaneously, with a maximum nitrogen removal rate of 2.8 kg-N/m³/d and maximum removal efficiencies of 97% and 99% for ammonium and nitrite, respectively. Additionally, the existence of *Candidatus Brocadia sinica*, a species known to exist within ANAMMOX sludge (Oshiki *et al.*, 2011), was observed in the enriched sludge by clone library analysis, which was conducted before starting all experiments.

Synthesized inorganic medium

Synthesized inorganic medium was prepared as previously reported (van de Graaf *et al.*, 1996), except that tap water was used. The concentrations of supplements into the medium were as follows: 500 mg/L NaHCO₃, 300 mg/L MgSO₄·7H₂O, 180 mg/L CaCl₂·2H₂O, 27.2 mg/L KH₂PO₄, and trace elements I and II. Trace element I contained 5 g/L EDTA and 5 g/L FeSO₄. Trace element II contained 15 g/L EDTA, 0.43 g/L

$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.24 g/L $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1 g/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.25 g/L $\text{CuSO}_4 \cdot 6\text{H}_2\text{O}$, 0.22 g/L $\text{MnCl}_2 \cdot 5\text{H}_2\text{O}$, 0.22 g/L $\text{NaMoO}_4 \cdot 5\text{H}_2\text{O}$, 0.19 g/L $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, 0.21 g/L $\text{NaSeO}_4 \cdot 10\text{H}_2\text{O}$, and 0.014 g/L H_3BO_4 . Ammonium and nitrite or nitrate were supplied by adding $(\text{NH}_4)_2\text{SO}_4$, NaNO_2 , and/or NaNO_3 , respectively. The concentration of each nitrogen source was changed based on each experimental set up. The dissolved oxygen concentration (DO) of synthesized medium was set below 0.3 mg/L by bubbling argon gas.

Experimental setup for measuring the simultaneous removal of ammonium and nitrate

Enriched ANAMMOX sludge on non-woven fabric (W 50 mm × D 5 mm × H 160 mm) was collected and added into a rectangular reactor with a 4.2-L working volume. New non-woven fabric was also added into the reactor for the attachment of bacteria. The top of the reactor was opened to avoid causing a hydrogen gas explosion, and plastic balls were floated within the reactor to prevent oxygen penetration from the air. A schematic diagram of the experimental setup is shown in Fig. 1. The experimental conditions were as follows: water temperature maintained at 35°C using a thermostat, continuous mixing at 100 rpm by a magnetic stirrer, and 7.0-h hydraulic retention time (HRT). Since ANAMMOX bacteria are easily deactivated from the effect of pH, two experimental conditions were tested: with pH control (Run 1) or without pH control (Run 2). For Run 1, the pH was maintained at around 7.8 – 8.2, that was in optimum pH range for ANAMMOX species of *Candidatus Brocadia sinica* (Oshiki *et al.*, 2011), by supplying carbon dioxide gas connected to a pH controller. For elucidating the efficiency of that combined process, ammonium and nitrate concentrations were set at 40 mg-N/L which was a higher concentration than that of actual groundwater. Synthesized medium, as well as hydrogen gas produced from a hydrogen gas generator (HG 260, GL Sciences, Japan), was continuously supplied from the bottom of the reactor. The flow rate of hydrogen gas was set at 20 mL/min. After 30 days of continuous operation, a batch experiment was conducted to examine the chronological changes in the concentration of each inorganic nitrogenous compound. The experimental conditions in each run were the same as that in continuous experiment, but pH was not controlled to examine pH changes in Run1.

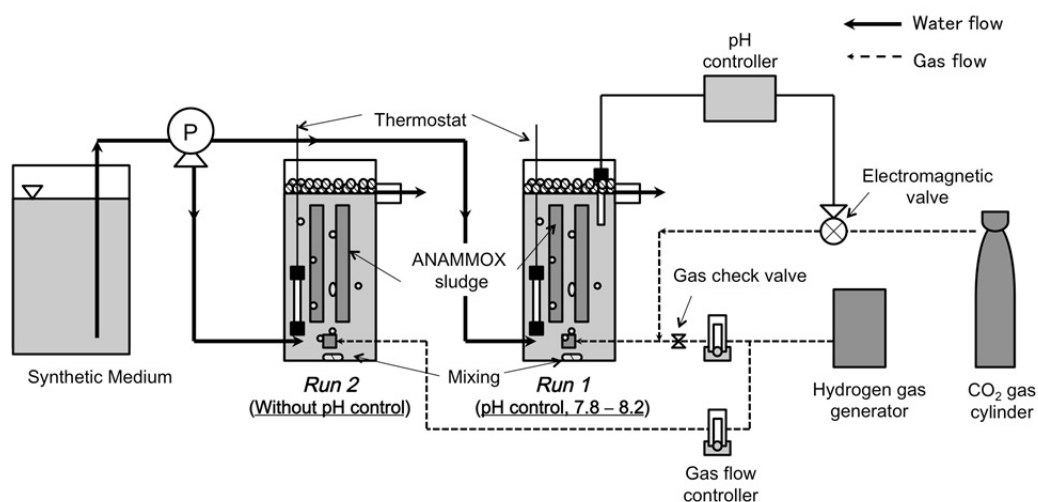


Fig. 1 - Schematic diagram of the experimental setup.

Effects of the hydrogen gas supply with ammonium and nitrite on ANAMMOX and hydrogenotrophic denitrification

In order to examine the change in activities of both ANAMMOX bacteria and hydrogenotrophic denitrifier in the presence of ammonium and nitrite with hydrogen gas supply. Enriched ANAMMOX sludge accumulated on non-woven fabric (W 50 mm × D 5 mm × H 160 mm) was continuously incubated in a new rectangular reactor having a 1.5-L working volume. The nitrogen compounds of ammonium and nitrite were added into the synthesized medium and concentrations were set at around 20 – 60 mg-N/L, respectively. The experimental conditions were set at optimum for each bacterium and the details were as follows: 35°C water temperature suitable for ANAMMOX bacterial growth, continuous mixing at 100 rpm by a magnetic stirrer, and 6.3-h HRT. Since high flow rate of hydrogen gas supply enhanced hydrogenotrophic denitrification activity (Khanitchaidecha and Kazama, 2012), the hydrogen gas flow rate was changed to 60 mL/min which was three times higher than that of the previous experiment. For elucidating pH variation, pH was not controlled. After 70 days of operation, batch experiments were conducted to examine the chronological changes in the concentration of each nitrogenous compound and pH and to elucidate the microbial activities existing in the reactor. The supply conditions of hydrogen and argon gases were examined. The experimental conditions were set the same as in the continuous experiment, but the concentrations of ammonium and nitrite were set at 20 mg-N/L.

Water sampling and analysis

Influent and effluent water samples were collected and filtered using a 0.45-μm pore-sized membrane filter. Samples were then frozen and stored until water quality analysis. Ammonium was analyzed using the phenate method (JWWA, 1993). Nitrite and nitrate were analyzed by colorimetric and ultraviolet spectrophotometric screening methods, respectively (APHA, 1998). The nitrogen loading rate (NLR) and nitrogen removal rate (NRR) were calculated from equations (6) and (7). In this study, *Inf.DIN* represented the summation of ammonium, nitrite, and nitrate concentrations in influent water and ΔDIN represented the differences of summation of ammonium, nitrite, and nitrate concentrations in influent and effluent water.

$$NLR [kg-N/m^3/d] = \{Inf.DIN [kg-N/L] \times Flow\ rate [L/d]\} / Working\ Volume [m^3] \quad (6)$$

$$NRR [kg-N/m^3/d] = \{\Delta DIN [kg-N/L] \times Flow\ rate [L/d]\} / Working\ Volume [m^3] \quad (7)$$

RESULTS AND DISCUSSION

Examination of the simultaneous removal of ammonium and nitrate

Variations in the concentrations of nitrogenous compounds and pH in influent and effluent water are shown in Fig. 2. Since operational error that increased DO in the synthetic medium occurred on day 7, decreased ammonium and increased nitrate concentrations in influent water were observed. During the initial reaction period from days 1 to 3, both the ammonium and nitrate concentrations decreased sharply, with the nitrite concentration reaching below 1 mg-N/L in the effluent water from both reactors. These results indicated that partial reduction of nitrate to nitrite, and removal of ammonium and nitrite produced by ANAMMOX process simultaneously occurred. The effluent pH in Run 2 also increased from 8.0 to 8.5 at the same time. After these periods,

the concentrations of all nitrogenous compounds in the effluent water were increased in both reactors. However, removal of ammonium, nitrate, and nitrite was only detected in Run 1. The maximum removal efficiencies of ammonium and nitrate in Run 1 were around 95% and 90%, respectively. Although nitrate removal and nitrite production were both detected, stagnation of ammonium removal was detected in Run 2 until the end of the experiment.

The trends for NLR and NRR are shown in Fig. 3. Increase and decrease of NRRs were found in both reactors from days 1 – 5, after which the NRR of Run 1 increased, shifting from an initial value of 0.04 to a maximum of 0.25 kg-N/m³/d. The removal efficiencies of all dissolved inorganic nitrogen (DIN) were 89% at this time. In contrast, Run 2 did not exhibit an increase in NRR after day 10 and only reached a maximum NRR of 0.06 kg-N/m³/d, which was around four times lower than that of Run 1.

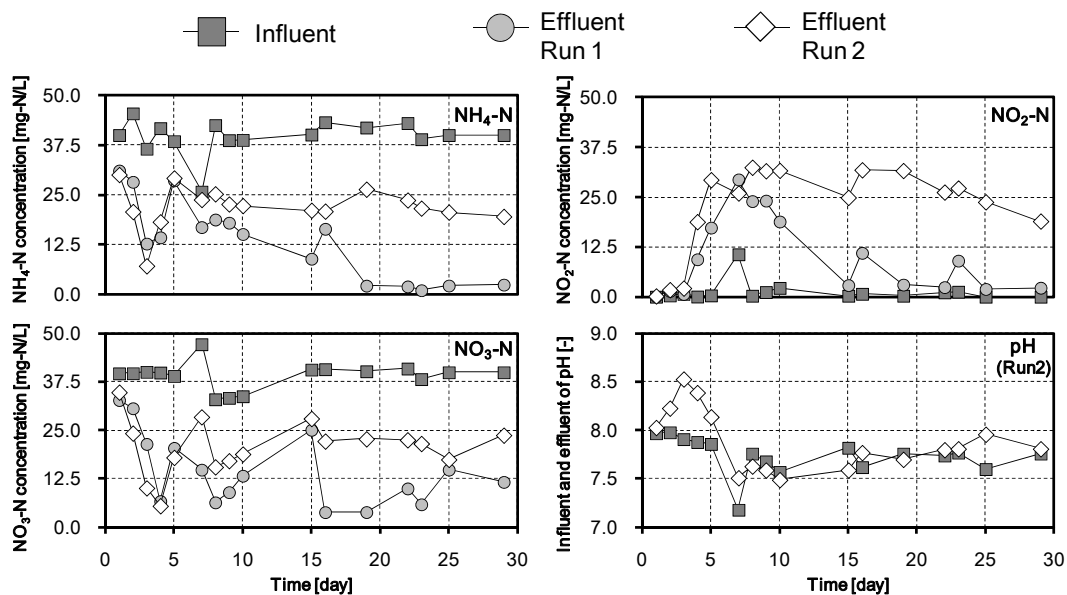


Fig. 2 - Variations in the concentrations of nitrogenous compounds and pH.

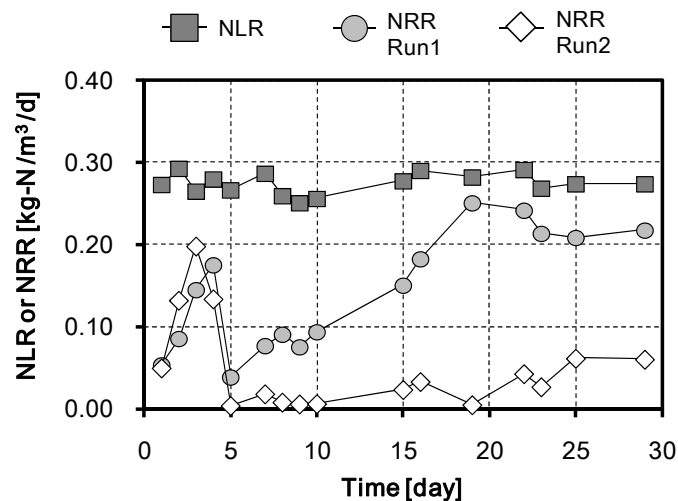


Fig. 3 - Trends for NLR and NRR in Runs 1 and 2.

The results of batch experiment are shown in Fig. 4. In this set of experiments, pH was not controlled. Nitrate removal and nitrite production were examined for both conditions. In the case of Run 2, nitrate removal reached 77% within 8 h of operation, and the nitrite concentration was increased by 37 mg-N/L at most. However, ammonium was not removed under these conditions, and the maximum removal efficiency for ammonium was 27%. In contrast, ammonium and nitrite removal were clearly detected in Run 1. The removal efficiencies of ammonium and nitrate after 7 h of operation, which was the same retention time as for the continuous experiment, were 90% and 100%, respectively. By 8 h of operation, ammonium removal efficiency was 91%. The ratio of ammonium to nitrate removal for each sampling time was calculated (Fig. 5). High correlations were observed, and the ratio was $\text{NH}_4\text{-N}:\text{NO}_3\text{-N} = 1:1.20$. The observed value was similar to the theoretical value of $\text{NH}_4\text{-N}:\text{NO}_3\text{-N} = 1:1.06$ from equation (5). These results suggested that combined nitrogen removal by ANAMMOX and hydrogenotrophic denitrification may have occurred in Run 1.

Both runs showed the same tendency that NRRs increased and decreased at the initial phase (days 0 – 5) of continuous experiment. These data suggested that ANAMMOX bacteria may have been deactivated due to changes in the condition inside the reactor. In the case of Run 2, the pH was increased and eventually reached 8.5 (Fig. 2). While this value does not strictly indicate that inhibition occurred in case of *Candidatus Brocadia sinica*, the threshold for inhibition may have changed due to effects on operational conditions for cultivation (Jin *et al.*, 2012). Since recovery of NRR and ammonium removal were not observed in both the continuous and batch experiments after pH increase (Figs. 3 and 4), it was assumed that ANAMMOX bacteria might be inhibited by pH increase at the initial phase of Run 2. To obtain stable performance of simultaneous ammonium and nitrate removal, pH control should be conducted. Although NRR recovered until the end of continuous experiment, Run 1 also showed the same tendency even in the optimum pH condition. So, other reasons such as microbial changes in the ANAMMOX sludge might also give some negative effects on the performance of the combined process. Therefore, further microbial investigation should be conducted.

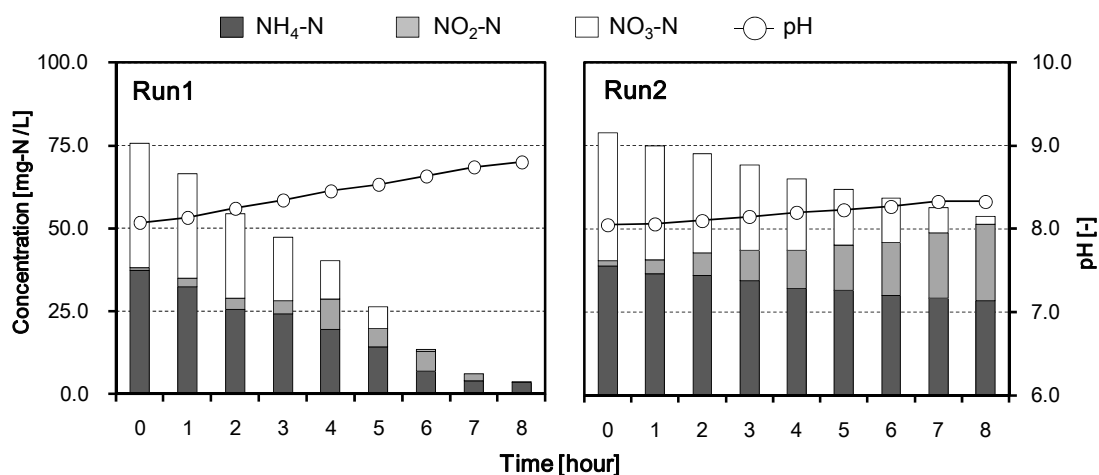


Fig. 4 - Variations in the concentrations of each nitrogenous compound and the pH value of the reaction solution in batch experiments.

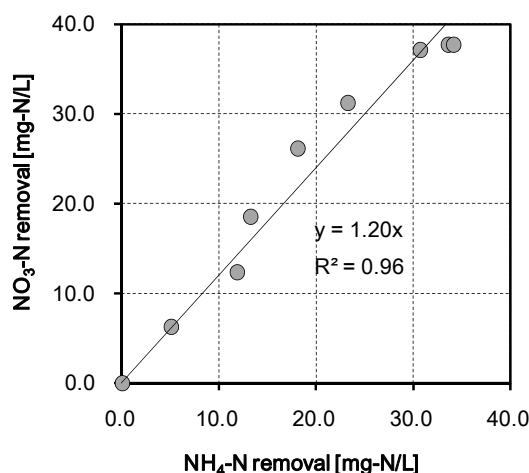


Fig. 5 - Correlation between the removal of ammonium and nitrate in the batch experiment in Run 1.

The result of batch experiment in Run 1 revealed that nitrate removal was more efficient than ammonium removal (Fig. 5). In the combined process, enhancement of nitrate removal induces increase in nitrite production and ammonium removal as shown in equations (1), (2) and (5). However, residual ammonium was observed, and even nitrite concentration was below 1 mg-N/L by the end of the experiment. These data indicated that nitrite was more efficiently removed. Therefore, it was assumed that nitrite removal by hydrogenotrophic denitrification may have also occurred with the combined process.

Effects of the hydrogen gas supply with ammonium and nitrite on ANAMMOX and hydrogenotrophic denitrification

We examined how microbial activities are changed under coexisting ammonium and nitrite with hydrogen gas supply. The average NLR, NRR, and pH in each experimental period (11 – 30, 31 – 50, and 51 – 70 days) are shown in Fig. 6. Because ammonium was released from sludge, the average NRR and NLR were not calculated for the first 0 – 10 days. There was a gradual increase of NRR during each period, reaching a maximum of 0.10 ± 0.02 kg-N/m³/d during the period of 51 – 70 days. At the same time, the nitrite removal efficiency reached around $76\% \pm 24\%$, and the average pH increased from 8.8 ± 0.2 to 9.2 ± 0.5 . Interestingly, changes in water quality were also associated with changes in the color of the sludge. During the initial phase, the sludge was red in color while accumulation of brown sludge with a concomitant decreased in red sludge was observed.

After the continuous experiment, batch experiments were conducted to clarify the microbial activities existing in the reactor under the supply of hydrogen or argon gas (Fig. 7). The type of gas supplied affected nitrite removal. Nitrite removal was only detected when hydrogen gas was used. Under these conditions, the nitrite removal efficiency reached 100% within 4 h. An increase in pH was also detected from 7.8 to 9.3, concurrent with nitrite removal. Moreover, almost 52% of all DIN was removed when hydrogen gas was supplied. In contrast, simultaneous ammonium and nitrite removal were slightly detected only when argon gas was supplied. Furthermore, DIN removal efficiency reached a maximum of 12%. The experimental results indicated that

hydrogenotrophic denitrifier originally existed in our enriched ANAMMOX sludge and it increased and turned to dominant microbial activity during the continuous experiment. ANAMMOX process was inhibited or decreased when compared with hydrogenotrophic denitrifier, but it still existed in the reactor at end of the experiment.

The experimental results showed that nitrite competition might have occurred between ANAMMOX and hydrogenotrophic denitrifier. Both microbes can use nitrite as a nitrogen source (Mulder *et al.*, 1995; Smith *et al.*, 2005). The growth rates calculated from nitrite utilization of each microbe were 0.455 – 0.917 L/h for hydrogenotrophic denitrifier (Vasiliadou *et al.*, 2006a; Vasiliadou *et al.*, 2006b; Vasiliadou *et al.*, 2009) and 0.0041 L/h for *Candidatus Brocadia sinica* (Oshiki *et al.*, 2011). Although the species of hydrogenotrophic denitrifier were not clearly identified in our case, the growth rates were expected to be 100-fold different. Thus, nitrite is used by hydrogenotrophic denitrifier. Additionally, the pH is increased by this process according to equation (3). Average pH in the continuous experiment reached a maximum of 9.3 which was over the optimum value for ANAMMOX bacteria. Because of both reasons, ANAMMOX bacterial activity might have been inhibited. Therefore, we assumed that the control of the activity of nitrite removal by hydrogenotrophic denitrifier is critical for obtaining stable performance of the combined system.

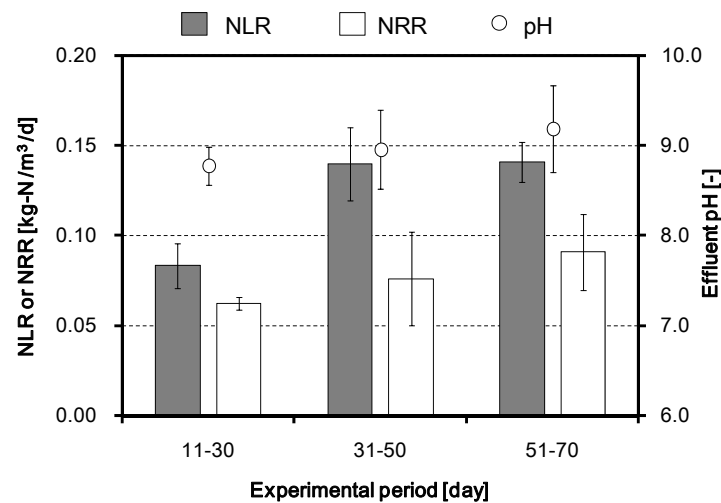


Fig. 6 - Trends for the average NLR, NRR, and pH during each experimental period. (Error bars represent standard deviations.)

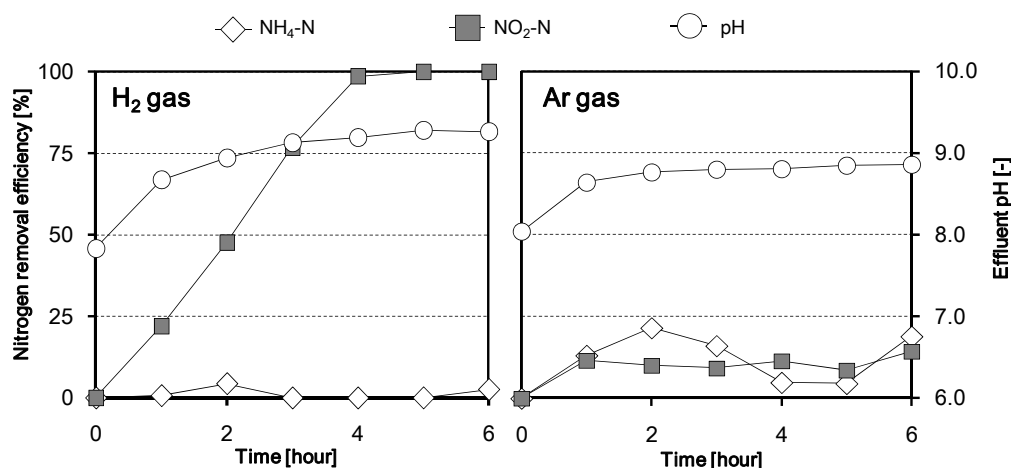


Fig. 7 - Effects of different gas supplies on short-term changes in water quality.

CONCLUSIONS

ANAMMOX sludge was cultivated with supplied hydrogen gas to clarify whether or not it was feasible to simultaneously remove ammonium and nitrate by combined ANAMMOX and hydrogenotrophic denitrification. Our data demonstrated that simultaneous removal of ammonium and nitrate was possible using existing ammonium and nitrate while supplying hydrogen gas at 20 mL/min. A maximum NRR of 0.25 kg-N/m³/d was detected in the pH-controlled reactor. The maximum removal efficiencies for ammonium and nitrate were 95% and 90%, respectively. However, pH increase and nitrite removal were enhanced when ANAMMOX sludge was cultivated in the presence of ammonium and nitrite with hydrogen gas supply at 60 mL/min. These results suggest that the combined denitrification method is suitable for removing ammonium and nitrate simultaneously, and therefore, this is a novel system for groundwater treatment. For a stable performance of the combined system, the control of the nitrite removal activity of hydrogenotrophic denitrification and maintenance of pH suitable for ANAMMOX may be needed. Further studies are necessary to determine the optimal operational parameters for avoiding nitrite competition or to clarify microbial changes.

ACKNOWLEDGEMENT

The authors wish to thank, “Project for Hydro-Microbiological Approach for Water Security in Kathmandu Valley, Nepal” under Science and Technology Research Partnership for Sustainable Development (SATREPS) program for providing financial assistance to carry out the research work. SATREPS is a collaborative program between the Japan Science and Technology Agency (JST) and the Japan International Cooperation Agency (JICA).

REFERENCES

- APHA (1998) Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association/American Water Works Association/Water Environment Federation, New York, USA.

- Bouman B. A. M., Castaneda A. R. and Bhuiyan S. I. (2002) Nitrate and pesticide contamination of groundwater under rice-based cropping systems: past and current evidence from the Philippines. *Agric. Ecosyst. Environ.*, **92**(2), 185-199.
- Chan C. C., Tseng S. K. and Huang H. K. (1999) Hydrogenotrophic denitrification with immobilized *Alcaligenes eutrophus* for drinking water treatment. *Bioresour. Technol.*, **69**(1), 53-58.
- Chen H., Liu S., Yang F., Xue Y. and Wang T. (2009) The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal. *Bioresour. Technol.*, **100**(4), 1548-1554.
- Jin R. C., Yang G. F., Yu J. J. and Zhen P. (2012) The inhibition of the Anammox process: a review. *Chem. Eng. J.*, **197**(1), 67-79.
- JWWA (1993) Standard Method for Examination of Water. Japan Water Works Association, Tokyo, Japan, pp.188-189. (in Japanese)
- Khanitchaidecha W. and Kazama F. (2012) Hydrogenotrophic denitrification in an attached growth reactor under various operating conditions. *Water Sci. Technol.: Water Supply*, **12**(1), 72-80.
- Khanitchaidecha W., Shakya M., Kamei T. and Kazama F. (2012) NH₄-N removal through nitrification and hydrogenotrophic denitrification in simple attached growth reactors. *Water Air Soil Pollut.*, **223**(7), 3939-3953.
- Khatriwada N. R., Takizawa S., Tran T. V. N. and Inoue M. (2002) Groundwater contamination assessment for sustainable water supply in Kathmandu Valley, Nepal. *Water Sci. Technol.*, **46**(9), 147-154.
- Kumar M. and Lin J. G. (2010) Co-existence of anammox and denitrification for simultaneous nitrogen and carbon removal—Strategies and issues. *J. Hazard. Mater.*, **178**(1), 1-9.
- Lee K. C. and Rittman B. E. (2002) Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water. *Water Res.*, **36**(8), 2040-2052.
- Mulder A., van de Graaf A. A., Robertson L. A. and Kuenen J. G. (1995) Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. *FEMS Microb. Ecol.*, **16**(3), 177-184.
- Oshiki M., Shimokawa M., Fujii N., Satoh H. and Okabe S. (2011) Physiological characteristics of the anaerobic ammonium-oxidizing bacterium '*Candidatus Brocadia sinica*'. *Microbiol.*, **157**(6), 1706-1713.
- Rivett M. O., Buss S. R., Morgan P., Smith J. W. and Bemment C. D. (2008) Nitrate attenuation in groundwater: A review of biogeochemical controlling process. *Water Res.*, **42**(16), 4215-4232.
- Sliekers A. O., Derwort N., Gomez J. L. C., Strous M., Kuenen J. G. and Jetten M. S. M. (2002) Completely autotrophic nitrogen removal over nitrite in one single reactor. *Water Res.*, **36**(10), 2475-2482.
- Smith R. L., Buckwalter S. P., Repert D. A. and Miller D. N. (2005) Small-scale, hydrogen-oxidizing-denitrifying bioreactor for treatment of nitrate-contaminated drinking water. *Water Res.*, **39**(10), 2014-2023.
- Smith R. L., Ceazan M. L. and Brooks M. H. (1994) Autotrophic, hydrogen-oxidizing, denitrifying bacteria in groundwater, potential agents for bioremediation of nitrate contamination. *Appl. Environ. Microbiol.*, **60**(6), 1949-1955.
- Strous M., Heijnen J. J., Kuenen J. G. and Jetten M. S. M. (1998) The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic

- ammonium-oxidizing microorganisms. *Appl. Microbiol. Biotechnol.*, **50**(5), 589-596.
- Sumino T., Isaka K., Ikuta H., Saiki Y. and Yokota T. (2006) Nitrogen removal from wastewater using simultaneous nitrate reduction and anaerobic ammonium oxidation in single reactor. *J. Biosci. Bioeng.*, **102**(4), 346-351.
- Suthar S., Bishnoi P., Singh S., Mutiyar P. K., Nema A. K. and Patil N. S. (2009) Nitrate contamination in groundwater of some rural areas of Rajasthan, India. *J. Hazard. Mater.*, **171**(1-3), 189-199.
- Szekeres S., Kiss I., Kalman M. and Soares M. I. M. (2002) Microbial population in a hydrogen-dependent denitrification reactor. *Water Res.*, **36**(16), 4088-4094.
- Tsushima I., Ogasawara Y., Kindaichi T., Satoh H. and Okabe S. (2007) Development of high-rate anaerobic ammonium-oxidizing (anammox) biofilm reactors. *Water Res.*, **41**(8), 1623-1634.
- Umezawa Y., Hosono T., Onodera S., Siringan F., Buapeng S., Delinom R., Yoshimizu C., Tayasu I., Nagata T. and Taniguchi M. (2009) Erratum to "Sources of nitrate and ammonium contamination in groundwater under developing Asian megacities" [Science of the Total Environment **404**(2008) 361-376]. *Sci. Total Environ.*, **407**(9), 3219-3231.
- van de Graaf A. A., de Bruijn P., Robertson L. A., Jetten M. S. M. and Kuenen J. G. (1996) Autotrophic growth of anaerobic ammonium-oxidizing micro-organisms in a fluidized bed reactor. *Microbiol.*, **142**(8), 2187-2196.
- Vasiliadou I. A., Karanasios K. A., Pavlou S. and Vayenas D. V. (2009) Experimental and modelling study of drinking water hydrogenotrophic denitrification in packed-bed reactors. *J. Hazard. Mater.*, **165**(1), 812-824.
- Vasiliadou I. A., Pavlou S. and Vayenas D. V. (2006a) A kinetic study of hydrogenotrophic denitrification. *Process Biochem.*, **41**(6), 1401-1408.
- Vasiliadou I. A., Siozios S., Papadas I. T., Bourtzis K., Pavlou S. and Vayenas D. V. (2006b) Kinetics of pure cultures of hydrogen-oxidizing denitrifying bacteria and modeling of the interactions among them in mixed cultures. *Biotechnol. Bioeng.*, **95**(3), 513-525.
- Wang C. C., Lee P. H., Kumar M., Huang Y. T., Sung S. and Lin J. G. (2010) Simultaneous partial nitrification, anaerobic ammonium oxidation and denitrification (SNAD) in a full-scale landfill-leachate treatment plant. *J. Hazard. Mater.*, **175**(1), 622-628.
- WHO (2011) Guidelines for Drinking-water quality, 4th ed. World Health Organization, Geneva, Switzerland.
- Zhang Y., Zhong F., Xia S., Wang X. and Li J. (2009) Autohydrogenotrophic denitrification of drinking water using a polyvinyl chloride hollow fiber membrane biofilm reactor. *J. Hazard. Mater.*, **170**(1), 203-209.