Effects of Carrier Filling Ratio on Hydrogenotrophic Denitrification (HD) Performance

KENTA SHINODA1, SUPHATCHAI RUJAKOM1, TIPPAWAN SINGHOPON1, RAWINTRA EAMRAT2, TATSURU KAMEI3, and FUTABA KAZAMA4*

1Integrated Graduate School of Medicine, Engineering and Agricultural Sciences, University of Yamanashi /4-4-37 Takeda, Kofu, Yamanashi 400-8510, Japan
2Department of Environmental Science, Faculty of Science, Ramkhamhaeng University /Bangkok, 10240 Thailand
3Department of Life and Environmental Sciences, University of Yamanashi /4-4-37 Takeda Kofu, Yamanashi 400-8510, Japan
4Interdisciplinary Research Centre for River Basin Environment, University of Yamanashi /4-4-37 Takeda, Kofu, Yamanashi 400-8510, Japan

Abstract

The development of a low-cost and efficient hydrogenotrophic denitrification (HD) system for nitrate removal from groundwater is urgently required in developing countries. In the present study, a sponge-based HD reactor was developed to examine the effects of various carrier filling ratios (0%, 10%, 20%, and 30%) on the HD performance. HD reactors with sponges showed higher nitrogen removal capacities than that without sponges. There was no significant difference in the nitrogen removal efficiency at filling ratios of 10%, 20%, and 30%. The NO3-N removal rate varied based on the filling ratio, and reached 382, 470, 548, and 530 g-N/(m3・d) at filling ratios of 0%, 10%, 20%, and 30%, respectively. Furthermore, the attached biomass was considered to play an important role in the enhancement of NO3-N removal. The coexistence of hydrogenotrophic and heterotrophic denitrification activity was observed in the reactors, and there was a strong correlation between total volatile sponge-attached biomass and heterotrophic activity. However, heterotrophic activity accounted for a maximum of only 5.1% of the dissolved inorganic nitrogen removal. The high nitrogen removal rate achieved in this study shows that sponge-based HD reactors can potentially be used for NO3-N removal from groundwater.

Key words: Hydrogenotrophic denitrification, sponge carrier, filling ratio, heterotrophic denitrification, groundwater treatment

INTRODUCTION

Groundwater is one of the main sources of domestic and drinking water in many developing countries, such as Nepal1, Tanzania3, Iran6, and Mexico4. However, groundwater has been known to exhibit elevated nitrate levels, which has drawn widespread attention3-4. Nitrate exposure has been linked to various health effects, and has been associated with methemoglobinemia, gastric cancer, and non-Hodgkin's lymphoma5. Removing contaminants from groundwater is essential to ensuring that it can safely be used for domestic purposes.

Various nitrate removal methods, including physicochemical and biological processes, have been investigated as part of numerous
studies to date. However, the economic conditions that are prevalent in developing countries may not be conducive to certain water treatment practices\(^6\). Therefore, there is a need to develop a water treatment system that is inexpensive, compact, and easy to operate and maintain\(^7\). Biological processes are more suited to these requirements than physicochemical processes, as they are cheaper and simpler to operate\(^8,9\).

Biological denitrification is generally classified as heterotrophic or autotrophic denitrification, depending on the electron donors involved. Heterotrophic denitrification requires organic electron donors to utilize nitrate as electron acceptors\(^9\), whereas autotrophic denitrification requires sulfur-reduced compounds, ferrous iron, hydrogen, pyrite, and arsenite as electron donors\(^10\). The addition of organic carbon for the heterotrophic denitrification can contribute to the deterioration of the treated water quality by causing elevated levels of total organic carbon\(^11\).

The hydrogenotrophic denitrification (HD) process has recently attracted considerable attention as a promising technology for groundwater treatment, and a wide variety of reactors that employ the HD process have been previously reported\(^12-19\). It does not produce toxic waste or require organic carbon, and therefore no secondary treatment is required prior to water use. For application to groundwater treatment under cost and energy constraints, an attached-growth type of reactor that offers a simple configuration and cost-effective operation is more suitable for developing countries than the commonly reported hollow-fiber membrane, gas-permeable membrane, and sequencing batch reactors, which are expensive and require frequent maintenance and post-treatment of the treated water\(^12\). One of the most important features of an attached-growth reactor is the biological carrier, because it influences the bacterial growth, reactor performance, and magnitude of investment\(^20,21\). Among the various biological carriers, sponge carriers are considered to be economical\(^22\). However, the effect of the carrier filling ratio on hydrogenotrophic denitrification has not been well investigated to date. Thus, it is important to study the role of carrier filling ratios in the HD process. The sponges allow for heterotrophic denitrification to occur in the inner side as its porous structure causes the formation of attached-growth biomass, the establishment of the anoxic zone, and carbon storage, which can be utilized for heterotrophic denitrification\(^22-24\). The collaboration of the hydrogenotrophic denitrification and the heterotrophic denitrification can be beneficial in terms of reducing the necessity of H\(_2\) gas supply and faster treatment performance. The coexistence of hydrogenotrophic and heterotrophic denitrification in HD systems has been previously reported\(^12,25\). Heterotrophic denitrification can occur in HD reactors using various organic carbons that derive from bacterial activity inside the reactors, and this heterotrophic activity may be enhanced depending on the carrier filling ratios with the increases in the inner areas. To the best of our knowledge, the effect of carrier filling ratio on the development of heterotrophic activity and the impacts of the heterotrophic activity on HD reactor performance have not elucidated. The clarification of these impacts is necessary for the establishment of a cost-effective and efficient HD reactor.

Therefore, the objective of this study is to develop an efficient HD reactor using sponge material as the biological carrier. Furthermore, the effect of the carrier filling ratio on the HD performance in terms of nitrogen removal efficiency, nitrogen removal rate, bacterial growth, and development of heterotrophic denitrification activity in the sponge-based HD reactor was investigated. In this work, the carrier filling ratio was set based on the percentage of the volume of carrier sponges to the reactor working volume (2 L) in order to represent the amount of sponge carriers. The outcomes of this study will help establish a better HD system for groundwater treatment in developing countries.

**MATERIALS AND METHODS**

**Synthetic groundwater preparation**

The experiments were performed using synthetic groundwater, which was prepared
Effects of Carrier Filling Ratio on Hydrogenotrophic Denitrification (HD) Performance

based on the groundwater quality in Nepal. The synthetic groundwater contained 0.24 g/L of NaNO₃, 0.50 g/L of NaHCO₃, 0.30 g/L of MgSO₄·7H₂O, 0.027 g/L of KH₂PO₄, 0.18 g/L of CaCl₂·2H₂O, and trace elements I and IIₐ. The synthetic groundwater was deoxygenated using argon gas to keep dissolved oxygen concentration below 0.3 mg-O₂/L before supply.

**Sponge-based HD reactor set-up**

Four lab-scale HD reactors were installed, each with a working volume of approximately 2 L. Each reactor was 9 cm in diameter and 31.4 cm in height (Fig. 1). Aqua cube sponges (1 cm × 1 cm × 1 cm) made from polyolefin material were purchased from Sekisui Aqua Systems Co., Ltd, Japan, and placed inside the reactors at filling ratios of 0%, 10%, 20%, and 30% as a carrier material for bacterial attachment (Table 1). In this study, the void ratio of sponges was not considered for the filling ratios. The density of the sponges were approximately 0.035 g/cm³. Twenty sponges were sewn together in a commercial fish line, and each fish line with 20 sponges was suspended from the same level as the outlet. The seed sludge was obtained from a parent HD reactor, which was previously described by Eamrat et al. and acclimated in a reactor. Then, the acclimated HD sludge was added to the HD reactors as volatile suspended solids (VSS) with a dried weight of 0.03 g. The four reactors were continuously operated in parallel at a H₂ gas flow rate of 40 mL/min and a temperature of 32±1 °C. The HRT was varied during the experiment, as shown in Table 2. The liquid inside the reactor was well mixed using a magnetic stirrer, and plastic beads were introduced to the surface of the water inside the reactors to prevent the adverse effects of oxygen.

---

**Table 1** Characteristics of the reactors with various filling ratios. The void ratio of sponges was not considered for the filling ratios.

| Filling ratio (%) | Number of sponges (pieces) | Total weight of sponges (g) |
|-------------------|-----------------------------|-----------------------------|
| Run 1             | 0                           | 0                           |
| Run 2             | 10                          | 200                         | 7.04 |
| Run 3             | 20                          | 400                         | 14.09 |
| Run 4             | 30                          | 600                         | 21.18 |

---

Fig. 1 Schematic of the sponge-based HD reactors.
Determination of nitrate-nitrogen (NO\textsubscript{3}-N) removal, nitrite-nitrogen (NO\textsubscript{2}-N) removal and dissolved inorganic nitrogen removal rate

Batch experiments were performed at intervals of 15 days, at the end of each phase (on Days 15, 30, 45, and 60) to obtain NO\textsubscript{3}-N removal, NO\textsubscript{2}-N accumulation, and dissolved inorganic nitrogen (DIN: NO\textsubscript{3}-N + NO\textsubscript{2}-N) removal rates as well as biomass concentrations. The rates were determined from linear regressions based on the results of the experiments. Before the batch experiments, synthetic groundwater was fed into the reactors with an HRT of less than 1 h for more than 12 h without H\textsubscript{2} gas supply to completely replace the water inside the reactor. The supply of the synthetic groundwater was then stopped, and the batch experiments began with the start of the supply of H\textsubscript{2} gas. Samples were obtained at regular intervals from each reactor. After completion of the batch experiments, the biomass amounts were determined. Mixed liquor volatile suspended solids (MLVSS), volatile sponge-attached biomass (VSAB), total volatile sponge-attached biomass in the reactor (TVSAB), and total biomass (TB) in the reactor were obtained, which is further described in Analytical Methods.

Heterotrophic denitrification activity test

Batch experiments were performed at the end of the operation (on Day 60) in the same manner as described above, but without H\textsubscript{2} gas supply. Argon gas was supplied into the reactors for 5 minutes right before the batch experiments to decrease O\textsubscript{2} concentrations. The heterotrophic denitrification rates were obtained from the linear regressions.

Analytical Methods

Samples of the influent and effluent were collected and immediately filtered using 0.45-μm membrane filters. The samples were stored in a freezer at -18°C until water quality analysis could be performed. The NO\textsubscript{3}-N and NO\textsubscript{2}-N concentrations were determined by ultraviolet spectrophotometric screening and colorimetric methods, respectively, using a UV-1800 spectrophotometer (SHIMADZU, Japan), in accordance with the standard method\textsuperscript{26}. MLVSS analysis was performed according to the standard method. VSAB was obtained by squeezing each sponge carrier\textsuperscript{22,27}, and measured in the same manner as MLVSS. TVSAB was estimated by multiplying the VSAB by the number of sponges in the respective reactor and dividing the product by the reactor volume. The TB was estimated as the sum of the suspended and attached biomass.

Calculations

The performance of the HD reactors was evaluated on the basis of the nitrogen loading rate, nitrogen removal rate, and nitrogen removal efficiency, which were calculated using Equations (1)–(3).

\[
\text{Nitrogen loading rate} (\text{NLR}) \ [\text{g-N/(m}^3\text{-d)}] = \frac{\text{NO}_3^-\text{N}_{\text{IN}} \times \text{Flow rate}[\text{L/d}]}{\text{Reactor volume} [\text{m}^3]} \tag{1}
\]

\[
\text{Nitrogen removal rate} (\text{NRR}) \ [\text{g-N/(m}^3\text{-d)}] = \frac{\text{NO}_3^-\text{N}_{\text{IN}} - (\text{NO}_3^-\text{N}_{\text{EFF}} + \text{NO}_2^-\text{N}_{\text{EFF}}) \times \text{Flow rate}[\text{L/d}]}{\text{Reactor volume} [\text{m}^3]} \tag{2}
\]

\[
\text{Nitrogen removal efficiency } (\%) = \frac{\text{Nitrogen removal rate} [\text{g-N/(m}^3\text{-d)}]}{\text{Nitrogen loading rate} [\text{g-N/(m}^3\text{-d)}]} \times 100 \tag{3}
\]

where NO\textsubscript{3}-N\textsubscript{IN}, NO\textsubscript{3}-N\textsubscript{EFF}, and NO\textsubscript{2}-N\textsubscript{EFF} represent the influent NO\textsubscript{3}-N, effluent NO\textsubscript{3}-N, and effluent NO\textsubscript{2}-N concentrations, respectively. In this study, ammonium-nitrogen
concentration was not considered as the concentration was negligible (less than 0.5 mg-N/L).

**Statistical analysis**

The mean and standard deviation (SD) of the nitrogen removal efficiencies of all runs during each operational phase and the biomass amount at the end of each phase were calculated. A one-way analysis of variance (ANOVA), followed by post-hoc Tukey’s test were used to confirm significant differences among four groups with different filling ratios. A p value less than 0.05 was considered statistically significant. The data were processed using the statistical analysis software package SPSS v.22 (IBM Corp., Armonk, NY, USA).

**RESULTS AND DISCUSSION**

**Nitrogen removal efficiency under different HRTs**

Table 3  Summary of reactor performance during each phase.

| Phase | Run 1 | Run 2 | Run 3 | Run 4 |
|-------|-------|-------|-------|-------|
| I     | 96.8 ± 2.8 | 99.0 ± 0.9 | 99.4 ± 0.4 | 99.5 ± 0.4 |
| II    | 97.9 ± 1.0 | 98.7 ± 0.4 | 99.0 ± 0.3 | 99.3 ± 0.3 |
| III   | 77.9 ± 9.4  | 96.1 ± 1.6  | 97.7 ± 0.7  | 98.0 ± 0.3  |
| IV    | 37.5 ± 6.5  | 50.2 ± 11.3 | 41.7 ± 18.0 | 42.3 ± 16.4 |

Table 3 summarizes the nitrogen removal efficiencies under various filling ratios in different phases. There were no obvious differences in the average nitrogen removal efficiencies of Runs 1–4 during Phases I and II. High average nitrogen removal efficiencies of 96.8–99.5% were observed in Runs 1–4 during these phases. However, the nitrogen removal efficiency of Run 1 was significantly lower (p < 0.05) than those of Runs 2–4 during Phase III, indicating that the addition of sponge carriers to the reactor can enhance the nitrogen removal capacity. During Phase IV, the average nitrogen removal efficiencies reduced considerably because of NO₂-N accumulation in the reactors. The concentrations of accumulated NO₂-N reached 15.2, 21.8, 28.7, and 25.0 mg-N/L in Runs 1, 2, 3, and 4, respectively. The slowed-down HD process can be explained by an inappropriately low HRT, which reduced the contact time between the substrate and the bacteria responsible for the microbial activity.

Thus, the performance of the HD reactor with sponge carriers is better than that of the HD reactor without sponge carriers. However, the filling ratio has little effect on the nitrogen removal efficiencies as long as an appropriate HRT is maintained. Furthermore, the shortest HRT that the sponge-based HD reactor can maintain was determined to be 4 h.

**Changes in NO₃-N removal, NO₂-N accumulation, and DIN removal rate with different carrier filling ratios**

Figure 2 shows the variation in the NO₃-N removal rate, NO₂-N accumulation rate, and DIN removal rate of each reactor, as determined via the batch experiments at the end of each operational phase. NO₃-N removal rate demonstrated an increasing trend in all runs over the course of the experiment. The highest NO₃-N removal rate observed in this study was 547.7 g-N/(m³·d) on Day 60 in Run 3, followed by 529.8, 469.7, and 382.0 g-N/(m³·d) in Runs 4, 2, and 1, respectively, on Day 60. Run 3 always exhibited the highest NO₃-N removal rate among the four runs, followed by Runs 4, 2, and 1 after Day 15. In contrast, the trend of variation in DIN removal rate differed from that variation in NO₃-N removal rate; the DIN removal rate in all runs dramatically decreased on Day 60. This is due to an increase in NO₂-N accumulation rate. Regardless of filling ratio, the NO₂-N accumulation rate increased in all runs. However, a decrease in the NO₂-N removal rates was not observed on Day 60, which indicates that the change in HRT may exert a greater influence on the NO₂-N reduction process than the NO₃-N reduction process. The highest DIN removal rate of 347.2 g-N/(m³·d) was achieved on Day 45 in Run 3, followed by Runs 4, 2, and 1, with DIN removal rates of 331.1, 293.8, and 191.1 g-N/(m³·d), respectively. The DIN removal
rates on Day 60 were 166.2, 186.4, 108.0, and 148.2 g-N/(m³·d) in Runs 1, 2, 3, and 4, respectively. NO₂-N was not detected in Runs 2–4 until Day 60. The NO₂-N accumulation rates on Day 60 were 215.8, 283.3, 439.6, and 381.6 g-N/(m³·d) in Runs 1, 2, 3, and 4, respectively.

Variations in biomass amount with different carrier filling ratios

The biomass of the sponge-based HD reactor was broadly classified into two groups: biomass attached to the sponges and biomass suspended in the reactor. VSAB and TVSAB represent the attached biomass whereas MLVSS represents the suspended growth biomass. TB is the sum of TVSAB and MLVSS and shows the total biomass present in the reactors.

Table 4 summarizes the VSAB in Runs 2–4, that is, the amount of biomass per gram of sponge. VSAB gradually built up in all runs and reached 0.31, 0.31, and 0.26 g-VSS/g-sponge in Runs 2, 3, and 4, respectively, on Day 60. Statistical analysis indicated that there was no significant difference in the VSAB in Runs 2–4.

Figure 3 depicts the MLVSS, TVSAB, and TB over the course of the experiment in all runs. The MLVSS in Run 1 continuously increased over the course of the entire operation, reaching 3.1 g-VSS/L on Day 60, whereas that of Run 4 increased slightly, reaching 0.31 g-VSS/L, which is one-tenth of the value attained in Run 1. The MLVSS in Runs 2 and 3 gradually increased until Day 45 and then reduced on Day 60. For suspended sludge, higher filling ratios...
Effects of Carrier Filling Ratio on Hydrogenotrophic Denitrification (HD) Performance

resulted in lower MLVSS values (Run 1 > Run 2 > Run 3 > Run 4 on Day 60).

TVSAB also increased gradually in Runs 2 - 4, reaching a maximum of 1.1, 2.2, 2.8 g-VSS/L in Runs 2, 3, and 4, respectively, on Day 60. The values were, however, significantly different (p < 0.05) for each filling ratio. Thus, higher TVSAB levels can be achieved as the filling ratio in the HD reactors increases.

For TB in the reactors, higher filling ratios cause faster accretion during the first 30 days. However, the value in Run 3 surpassed that in Run 4 on Day 45 and remained the highest among the four filling ratios on Day 60. Although Run 3 exhibited the highest TB on Days 45 and 60, it decreased on Day 60. This phenomenon was also observed in Run 2, as well as in previous research. This is presumed to be a result of biomass degradation, which is further discussed in the next section. On Day 60, the TB reached 3.7 g-VSS/L (Run 3), followed by 3.1 g-VSS/L (Runs 1 and 4), and 2.8 g-VSS/L (Run 2). Although the TB of Run 1 was higher than that of Run 2 and was nearly the same as that of Run 4, the NO₃-N removal rate of Run 1 was lower than that of Run 2 and Run 4 (Fig. 2), implying that the attached biomass plays a significant role in the enhancement of NO₃-N removal in the sponge-based HD reactors.

**Evaluation of heterotrophic denitrification in the absence of H₂ gas supply**

Figure 4 depicts the variation in nitrogen concentrations during the batch experiment conducted without H₂ gas supply to
understand the heterotrophic denitrification activity inside the reactors. It is apparent that higher nitrogen removals were observed in HD reactors with higher filling ratios. After 24 h, 41.6 % of nitrogen was heterotrophically removed in Run 4, which was the highest nitrogen removal efficiency. This was followed by Runs 3, 2, and 1, which demonstrated nitrogen removal efficiencies of 33.0%, 11.6%, and 8.4% respectively. The heterotrophic denitrification rates were 3.0, 4.2, 13.1, and 17.0 g-N/(m³·d) in Runs 1, 2, 3, and 4, respectively. Run 1 showed the lowest heterotrophic activity among the four conditions although its total biomass was higher than that of Run 2, indicating that the heterotrophic activity was greater in attached biomass than in suspended biomass. Although heterotrophic NO₃⁻-N removal is much slower than hydrogenotrophic NO₃⁻-N removal, higher filling ratios increase the heterotrophic activity in the sponge-based HD reactors.

A high correlation between TVSAB and heterotrophic denitrification rate was observed (Fig. 5). Higher sponge filling ratios were found to create favorable conditions for the development of heterotrophic activity inside the reactors. As can be seen in Figure 3-c, Run 4 exhibited the largest total attached biomass, resulting in the highest heterotrophic denitrification activity among the four conditions, as the denitrification performance relies on the magnitude of the bacterial population. The occurrence of heterotrophic denitrification has been observed in the inner sponges in sponge-based systems, such as moving bed reactors. In moving bed reactors, aerobic conditions were established on the surface of the sponges...
while a dissolved oxygen gradient existed in the interior of the sponges. In addition, the carbon sources derived from the interior can be utilized. In the present study, it is speculated that a hydrogen gradient was established between the surface and the interior of the sponges, and heterotrophic activity subsequently developed using the carbon sources. A wide variety of potential carbon sources are believed to exist in the HD reactors. For instance, the sources could include the hydrolysis and bacterial degradation inside the reactor^{12} as well as cell components and soluble microbial products (SMP)^{13}. SMP is classified into utilization-associated products (UAP) and biomass-associated products (BAP). UAP is related to substrate metabolism and biomass growth, whereas BAP is related to biomass decay^{30}. The bacterial community inside the HD reactor is not solely autotrophic; heterotrophic organisms can be digested, leading to the generation of volatile fatty acids (VFAs)^{13}. Extracellular polymeric substances (EPS) are metabolic products excreted as a result of microbial activities. They are responsible for aggregate formation^{25}. These sources can serve as carbon sources for heterotrophic activity. It is noteworthy that all the HD bacteria are mixotrophic and can behave both autotrophically and heterotrophically, depending on the conditions^{30}.

Thus, the addition of sponge carriers enhances the development of heterotrophic denitrification in the HD reactors; however, the contributions to the DIN removal rate under $H_2$ supply are 1.3, 1.4, 3.8, and 5.1% in Runs 1, 2, 3, and 4, respectively.

**Comparison of reactor performances and operating conditions**

Table 5 summarizes the operational conditions and the results of various HD reactors developed for groundwater treatment to compare their performance. In the present study, the nitrogen removal efficiency of the sponge-based HD reactor matched the nitrogen removal efficiency achieved in most previous studies. However, there was a considerable difference in the nitrogen removal capacity in terms of NRR. The sponge-based HD reactor exhibited an NRR as high as 209.9 g-N/(m$^3$·d), which was higher than the NRR values of previously

| Reactor                  | Carrier                  | Packing ratio (%) | NO$_3^-$N (mg$^{-}$N/L) | H$_2$ flow rate (mL/min) | H$_2$ pressure (MPa or atm) | HRT (h) | Reactor volume (L) | Efficiency (%) | NRR (g-N/(m$^3$·d)) | Reference |
|-------------------------|--------------------------|-------------------|--------------------------|--------------------------|-----------------------------|---------|-------------------|----------------|---------------------|-----------|
| Attached growth         | Polyolefin               | 0 %               | 40                       | 40                       | 6                           | 2       |                  | 97.9           | 157.0               | This study |
|                         |                          | 10 %              | 4                        | 4                        |                             |         |                  | 96.1           | 205.9               |           |
|                         |                          | 20 %              | 4                        | 4                        |                             |         |                  | 97.7           | 209.2               |           |
|                         |                          | 30 %              | 4                        | 4                        |                             |         |                  | 98.0           | 209.9               |           |
| Suspended growth        | Fiber                    | 40                | 40                       | 15 mL/min                | 12                          | 2       |                  | 86.4–96.5       | 77.2                | 8         |
| Attached growth         | Fiber                    | 660 cm$^2$        | 20                       | 50–70 mL/min             | 6.7                         | 3       |                  | 96.4           | 69.1                | 12        |
| Suspended growth membrane| Fiber                   | 48                | DH1.6                    | 12                       | 7                           | 100     |                  | 37.7           | 13                  |           |
| Bio-ceramsite           | Ceramsite                | Packed (50 cm)    | 30                       | 0.01 MPa                 | 24                          | 2.3     |                  | 96.2           | 28.9                | 14        |
| Submerged membrane      | Hollow fiber membrane    | 940 cm$^2$        | 25                       | DH1.6                    | 3                           | 5.6     |                  | 100            | 200                 | 15        |
| Membrane biofilter      | Hollow fiber             | 86.2 cm$^2$       | 10                       | 0.05 MPa                 | 0.5–4                       | 0.024   |                  | 97             | 384                 | 16        |
| Hollow fiber            | Polypropylene hollow fiber| 0.37 m$^2$       | 65–200                   | 0.3–0.6 atm              | 4–30                        | 1.2     |                  | 100            | 59–770              | 17        |
| Fluidized-bed biofilm   | using solid-polymer      | 2.2 L             | 20–90                    | 1                        | 2.2                          | 100     |                  | 2160           |                    | 18        |
| Unsaturated-flow        | Plastic biofilm          | 3.4 L             | 25                       | DH1.5                    | 430 mL/min                  | 8.7     |                  | 100            | 2100                | 19        |
reported reactors. Although the unsaturated flow pressurized reactor can achieve a high NRR of 2100 g-N/(m\(^3\)・d), the reactor requires frequent cleaning\(^{19}\). Moreover, the reactor itself is more complex than the sponge-based HD reactor. The fluidized-bed reactor also demonstrated a high NRR. However, the upflow velocity in a this reactor must be sufficiently high to maintain efficient fluidization inside the reactor, which may result in insufficient nitrogen removal\(^{31}\).

Hollow fiber membrane and membrane biofilter reactors offer the advantages of high \(H_2\) utilization coupled with acceptable nitrogen removal, but the investment in infrastructure is high and membrane fouling is a concern\(^{31}\). The sponge-based HD reactor developed in this study can address these concerns because it has a simple design, uses low-cost materials, and does not need to be cleaned very frequently. Thus, the sponge-based HD reactor is suitable for groundwater treatment in developing countries. In order to put the sponge-based HD reactor into practical application in developing countries, the efficient \(H_2\) gas utilization and the minimization of \(H_2\) gas supply should be further studied due to the cost and low solubility of \(H_2\) gas.

**CONCLUSIONS**

A sponge-based HD reactor was developed for \(NO_3^-\)N removal from groundwater. The results indicated that HD reactors with sponges (10%, 20%, and 30%) demonstrated considerably higher nitrogen removal capacities than those without sponges (0%). However, there was no significant difference in nitrogen removal efficiencies for filling ratios of 10%, 20%, and 30%, where the efficiencies reached 96.1–99.4% at HRTs of 4–24 h. The \(NO_3^-\)N removal rate varied as a function of the filling ratio, and reached 382.0, 469.7, 547.7, and 529.8 g-N/(m\(^3\)・d) for filling ratios of 0%, 10%, 20%, and 30%, respectively, on Day 60. The attached HD biomass was considered to play a significant role in the enhancement of \(NO_3^-\)N removal. The coexistence of hydrogenotrophic and heterotrophic denitrification activity was observed in the reactor. The addition of sponges into the HD reactor enhanced the development of the heterotrophic activity, and a strong correlation was found between TVSAB and heterotrophic denitrification activity. However, heterotrophic denitrification accounted for a maximum DIN removal of 5.1%, compared to DIN removal by hydrogenotrophic denitrification. The high NRR obtained in this study implied that the sponge-based HD reactor demonstrates potential for groundwater treatment in developing countries.

**ACKNOWLEDGEMENTS**

This research was financially supported by (1) the Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA), Science and Technology Research Partnership for Sustainable Development (SATREPS), and (2) Japan Society for the Promotion of Science (JSPS) KAKENHI, Japan, Grant Number 19J13824. We would like to thank Editage (www.editage.com) for English language editing.

**REFERENCES**

1) Shakya, B. M., Nakamura, T., Kamei, T., Shrestha, S. D., Nishida, K.: Seasonal groundwater quality status and nitrogen contamination in the shallow aquifer system of the Kathmandu Valley, Nepal. Water, 11, 2184 (2019)

2) Elisante, E., Muzuka, A. N. N.: Occurrence of nitrate in Tanzanian groundwater aquifers: A review. Appl. Water Sci., 7, 71–87 (2017)

3) Qasemi, M., Afsharnia, M., Farhang, M., Bakhshizadeh, A., Allahdadi, M., Zarei, A.: Health risk assessment of nitrate exposure in groundwater of rural areas of Gonabad and Bajestan, Iran. Environ. Earth Sci., 77, 551 (2018)

4) Fabro, A. Y. R., Ávila, J. G. P., Alberich, M. V. E., Sansores, S. A. C., Camargo-Valero, M. A.: Spatial distribution of nitrate health risk associated with groundwater use as drinking water in Merida, Mexico. Appl. Geogr., 65, 49–57 (2015)

5) Ward, M. H., Jones, R. R., Brender, J. D., de Kok, T. M., Weyer, P. J., Nolan, B. T., Villanueva, C. M., van Breda, S. G.: Drinking water nitrate and human health: An
updated Review. Int. J. Environ. Res. Public Health, 15, 1–31 (2018)

6) Shrestha, K. B., Thapa, B. R., Aihara, Y., Shrestha, S., Bhattarai, A. P., Bista, N.: Hidden cost of drinking water treatment and its relation with socioeconomic status in Nepalese urban context. Water, 10, 607 (2018)

7) Shrestha, K. B., Kamei, T., Shrestha, S., Aihara, Y., Bhattarai, A. P., Bista, N., Thapa, B. R., Kazama, F., Shindo, J.: Socioeconomic impacts of LCD-treated drinking water distribution in an urban community of the Kathmandu Valley, Nepal. Water, 11, 1323 (2019)

8) Eamrat, R., Tsutsumi, Y., Kamei, T., Khanitchaidecha, W., Tanaka, Y., Kazama, F.: Optimization of hydrogenotrophic denitrification behavior using continuous and intermittent hydrogen gas supply. J. Water Environ. Technol., 15, 65–75 (2017)

9) Rezvani, F., Sarrafzadeh, M.–H., Ebrahim, S., Oh, H–M.: Nitrate removal from drinking water with a focus on biological methods: A review. Environ. Sci. Pollut. Res., 26, 1124–1141 (2019)

10) Capua, F. D., Papirio, S., Lens, P. N. L., Esposito, G.: Chemolithotrophic denitrification in biofilm reactors. Chem. Eng. J., 280, 643–657 (2015)

11) Khanitchaidecha, W., Shakya, M., Kamei, T., Kazama, F.: NH₃-N removal through nitrification and hydrogenotrophic denitrification in simple attached growth reactors. Water Air Soil Pollut., 223, 3939–3953 (2012)

12) Khanitchaidecha, W., Kazama, F.: Hydrogenotrophic denitrification in an attached growth reactor under various operating conditions. Water Sci. Technol.: Water Supply, 12, 72–81 (2012)

13) Mo, H., Oleszkiewicz, J. A., Cicek, N., Rezania, B.: Incorporating membrane gas diffusion into a membrane bioreactor for hydrogenotrophic denitrification of groundwater. Water Sci. Technol., 51, 357–364 (2005)

14) Chen, D., Yang, K., Wang, H., Lv, B.: Nitrate removal from groundwater by hydrogen-fed autotrophic denitrification in a bio-ceramsite reactor. Water Sci. Technol., 69, 2417–2422 (2014)

15) Rezania, B., Oleszkiewicz, J. A., Cicek, N.: Hydrogen-dependent denitrification of water in an anaerobic submerged membrane bioreactor coupled with a novel hydrogen delivery system. Water Res., 41, 1074–1080 (2007)

16) Xia, S., Zhong, F., Zhang, Y., Li, H., Yang, X.: Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor. J. Environ. Sci., 22, 257–262 (2010)

17) Ergas, S. J., Reuss, A. F.: Hydrogenotrophic denitrification of drinking water using a hollow fibre membrane bioreactor. J. Water Supply: Research and Technology-Aqua, 50, 161–171 (2001)

18) Komori, M., Sakakibara, Y.: High-rate hydrogenotrophic denitrification in a fluidized-bed biofilm reactor using solid-polymer-electrolyte membrane electrode (SPEME). Water Sci. Technol., 58, 1441–1446 (2008)

19) Epzstein, R., Beliavski, M., Tarre, S., Green, M.: Pressurized hydrogenotrophic denitrification reactor for small water systems. J. Environ. Manag., 216, 315–319 (2018)

20) Gu, Q., Sun, T., Wu, G., Li, M., Qiu, W.: Influence of carrier filling ratio on the performance of moving bed biofilm reactor in treating coking wastewater. Bioresour. Technol., 166, 72–78 (2014)

21) Quan, F., Yuxiao, W., Tianmin, W., Hao, Z., Libing, C., Chong, Z., Hongzhang, C., Xiuling, K., Xinhui, X.: Effect of packing rates of cubic-shaped polyurethane foam carriers on the microbial community and the removal of organics and nitrogen in moving bed biofilm reactors. Bioresour. Technol., 117, 201–207 (2012)

22) Zhang, X., Chen, X., Zhang, C., Wen, H., Guo, W., Ngo, H. H.: Effect of filling fraction on the performance of sponge-based moving bed biofilm reactor. Bioresour. Technol., 219, 762–767 (2016)

23) Guo, W., Ngo, H.–H., Dharmawan, F., Palmer, C. G.: Roles of polyurethane foam in aerobic moving and fixed bed bioreactors. Bioresour. Technol., 101, 1435–1439 (2010)

24) Lim, J.–W., Seng, C.–E., Lim, P.–E., Ng, S.–L., Sujaari, A.–N.A.: Nitrogen removal in moving bed sequencing batch reactor using polyurethane foam cubes of various sizes as carrier materials. Bioresour.
25) Nguyen, T.-N.-P., Chen, P.-C., Huang, C.: Nitrate removal and extracellular polymeric substances of autohydrogenotrophic bacteria under various pH and hydrogen flow rates. J. Environ. Sci., 63, 50–57 (2018)

26) APHA: American Public Health Association. Standard methods for the examination of water and wastewater. 22th ed. Washington, D. C., USA: American Public Health Association (APHA), American Works Association (AWWA) and Water Environment Federation (WEF) (2012)

27) Nguyen, T. T., Ngo, H. H., Guo, W., Johnston, A., Listowski, A.: Effects of sponge size and type on the performance of an up-flow sponge bioreactor in primary treated sewage effluent treatment. Biore sour. Technol., 101, 1416–1420 (2010)

28) Moussavi, G., Jafari, S. J., Yaghmacian, K.: Enhanced biological denitrification in the cyclic rotating bed reactor with catechol as carbon source. Biore sour. Technol., 189, 266–272 (2015)

29) Dareioti, M. A., Kornaros, M.: Effect of hydraulic retention time (HRT) on the anaerobic co-digestion of agro-industrial wastes in a two-stage CSTR system. Biore sour. Technol., 167, 407–415 (2014)

30) Rezania, B., Oleszkiewicz, J. A., Cicek, N., Mo, H.: Hydrogen-dependent denitrification in an alternating anoxic-aerobic SBR membrane bioreactor. Water Sci. Technol., 51, 403–409 (2005)

31) Karanasios, K. A., Vasiliadou, I. A., Pavlou, S., Vayenas, D. V.: Hydrogenotrophic denitrification of potable water: A review. J. Hazard. Mater., 180, 20–37 (2010)

(Submitted 2020. 7. 20)
(Accepted 2020. 8. 30)