Microbubble Application to Enhance Hydrogenotrophic Denitrification for Groundwater Treatment

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ABSTRACT

The physicochemical and biological characteristics of milli-microbubbles were compared to evaluate their performance on hydrogenotrophic denitrification (HD) for groundwater treatment in remote areas. The hydrogen supply was controlled at 1.14 L/d with 40 mgN/L of NO_3-N. The microbial community structure in two bubble reactors was investigated by high throughput sequencing. Microbubbles enhanced biodegradation in the HD system, providing a maximum nitrogen removal efficiency of 99%. Approximately 50% of total hydrogen was utilized for biological nitrate removal with the highest hydrogen effectiveness achieved at 1.21 g N/g H_2. In contrast, millibubbles achieved less than 10% efficiency and 9.9% of total hydrogen was consumed for biological nitrogen removal. *Thauera* spp., *Hydrogenophaga* spp. and *Rhodocyclaceae* of Proteobacteria phylum were the dominant bacteria in the microbubble reactor, whereas *Methyloversatilis* spp. was dominant in the millibubble reactor, in which a relatively low amount of hydrogen (0.6 mg/L) was dissolved. The differences can be attributed to the higher hydrogen transfer efficiency (45×10^{-3} s^{-1}) and lower rising velocity (0.31 mm/s) of the microbubbles system than the millibubbles system (2×10^{-3} s^{-1} and 480 mm/s). The micro-hydrogen bubble technology affords increased hydrogen effectiveness, reduced energy consumption, and modified system design. Therefore, it is more appropriate for enhancing HD.

1. INTRODUCTION

Groundwater is an important water resource in several developing countries including Nepal, the Philippines, Vietnam, and Thailand, and most residents, especially in remote areas, consume groundwater directly without any treatment (Khatlwada et al., 2002; Tirado, 2007). Therefore, nitrate contamination of groundwater is a serious environmental issue in the above mentioned countries. According to the WHO standard, nitrate-nitrogen (NO_3-N) concentration should not exceed 11 mg/L and nitrite-nitrogen (NO_2-N) should be less than 0.9 mg/L (WHO, 2011), but nitrate contamination ranges from 12 to 60 mg-N/L in these countries. Three significant causes of nitrate contamination are the extensive use of fertilizers, discharge of domestic wastewater, and unsanitary disposal of sewage waste (Khatlwada et al., 2002; Pathak et al., 2009). Moreover, rapid urbanization in recent decades have led to increases in nitrate contamination in terms of concentration values and contaminated areas. The negative effects of nitrate consumption have been manifested in infants and pregnant women as blue baby syndrome (methemoglobinemia). In addition, nitrate can transform to potential carcinogens such as nitrosamines (via nitrite) (Bouchard et al., 1992). Methods of remediating nitrate contamination of groundwater are categorized into physicochemical and biological technologies. Physicochemical methods include chemical precipitation, membrane

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filtration, electro-dialysis, and catalysis. However, limitations of waste treatment, construction cost, and facility maintenance are not conductive to their application in remote areas (Shrimali and Singh, 2001). In contrast, biological methods have advantages of low cost and simple management (Nuclear, 2012). Therefore, they may be applicable to improving groundwater quality in remote areas. Among biological technologies, hydrogenotrophic denitrification (HD) is the most well-known process that leaves no residual organic carbon in the treated water (Karanasios et al., 2010). HD is an autotrophic denitrification process in which hydrogen acts as an electron donor and bicarbonates as carbon source under anoxic conditions. The stoichiometric expression of the HD reaction is shown in Equation (1).

$$NO_3^- + 3.03\ H_2 + H^+ + 0.229\ HCO_3^- \rightarrow 0.48\ N_2 + 3.60\ H_2O + 0.0458\ C_5H_7O_2N \quad (1)$$

However, low solubility, low gas-liquid mass transfer, and high cost of hydrogen gas limit its widespread application. Therefore, advanced technologies such as hollow fiber membranes and gas permeation membranes are applied to the HD system to generate small hydrogen bubbles, increase hydrogen utilization, and eliminate sludge wash-out (Mansell and Edward, 2002). However, membranes are expensive and require frequent cleaning and operation by skillful technicians. Another factor of concern in remote areas is the intermittent supply of electricity. Another means of increasing the efficiency of the HD system and hydrogen gas utilization is to use a gas diffuser to increase the amount of total hydrogen gas supply and concentration of dissolved hydrogen (DH). Various excellent gas diffusers have been used in laboratory and actual wastewater treatment systems. In recent years, the HD system with air stone as hydrogen bubbling diffuser was extensively proposed for nitrate removal. Vasiliadou (2009). developed a packed bed reactor with air stone as gas diffuser to treat an initial nitrate concentration of 80 mg/L, achieving a removal efficiency of 90% and a maximum removal capacity of 2.26 $kgNO_3$-$N/m^3 \cdot d$ (Vasiliadou et al., 2009). Similarly, a system proposed by Khanitchaidecha (2012) achieved 96% efficiency under operating conditions of 70 mL/min hydrogen flow by an air stone diffuser (Khanitchaidecha et al., 2012). Therefore, the air stone is a reliable diffuser appropriate for HD systems in rural areas. However, a large volume of hydrogen gas supply is required to achieve high system performance, which is a drawback of the HD system. The effectiveness of hydrogen gas can be increased by increasing the surface area of bubbles and bubble dynamics through the generation of microbubbles. However, only a few studies have been conducted on the application of microbubbles to enhance the performance of simple HD systems, physicochemical properties, and the bacterial community structure of microbubble systems. In this study, a microbubble generator incorporating an oscillating mesh (named MiBos) was used. This microbubble generator was developed by one of the co-authors of this study, Ito T (Gunma University, Japan). The significant advantage of MiBos is stable generation of microbubbles even with low doses of gas, providing small bubbles that do not negatively affect bacterial cells.

The objective of this study is to investigate the applicability of the micro-hydrogen bubble technology to enhancing HD performance, and consequently develop a simple operative, cost effective, and highly effective hydrogen system for groundwater treatment. Micro-hydrogen bubbles produced with the MiBos diffuser were compared with milli-hydrogen bubbles generated by the air stone. The nitrogen removal performance, effluent quality (NO_3-N and NO_2-N concentration), hydrogen effectiveness, biological hydrogen consumption and microbial community in the two systems were investigated. Lastly, the hydrogen gas rate transfer coefficient (K_La) and rising velocity of the hydrogen bubbles were compared to explain the difference in the two hydrogen bubble processes for nitrate removal from groundwater via HD.

2. METHODOLOGY

2.1 Diffuser characteristic

Two types of hydrogen-bubble diffusers were used in this study: an air stone (STARPET, Japan) of 15×30 mm diameter was used to produce hydrogen gas in the first HD reactor, and a microbubble generator incorporated with an oscillating mesh (MiBos) was used in the second reactor. The average bubble size in the first reactor was approximately $2.20\pm0.25\times10^3$ μm,

categorized as millibubbles. The average bubble size in the second reactor was about 25±13 µm, categorized as microbubbles (Takahashi, 2005). The physicochemical characteristics including total gas-liquid mass transfer coefficient (K_La) and rising velocity of hydrogen bubble were calculated using Equation (2) and Equation (3) (Stenstrom, 2007; Ghosh, 2009). Argon gas was supplied into 1 L of distilled water to remove dissolve oxygen (DO) in water and DO concentration was monitored by a DO meter until it reached lower than 0.3 mg/L. Subsequently, hydrogen gas was continuously supplied at 1 mL/min via the two diffusers. In-situ DH concentration was frequently measured by a DH meter (ENH-1000, Japan) until it reached the steady state. After that, the hydrogen gas supply was stopped immediately. The decreasing DH concentration was measured until it reached 0 mg/L. During the experiment, the temperature was controlled at 32±0.5 °C by a thermostat and liquid circulation was kept at 150 rpm using a magnetic stirrer, which was also applied to the mixed liquid. The total gas-liquid mass transfer coefficient can be determined as follows:

$$\ln\frac{(C^* - C_L)}{(C^* - C_S)} = -K_La(t - t_S) \quad (2)$$

K_La = total gas-liquid mass transfer coefficient
C^* = dissolved hydrogen concentration at saturation concentration (mg/L)
C_L = dissolved hydrogen concentration at time (mg/L)
C_s = dissolved hydrogen concentration at start point (mg/L)
t = time (min)

$$V = \frac{1}{18} \times \frac{gd^2}{v} \quad (3)$$

V = the rising velocity (m/s)
g = gravitational acceleration (m/s^2)
d = bubble diameter (m)
v = kinematic viscosity of water (m^2/s)

2.2 Reactor setup and operation

Two laboratory-scale cylindrical reactors were set up (height 31.4 cm, internal diameter 9 cm, working volume 2 L). One reactor used a MiBos diffuser, whereas the other used an air stone diffuser. Enriched HD sludge from a previous study was added into both reactors which was obtained from a reactor with high nitrogen removal efficiency (90% of total nitrogen removal and no nitrite accumulation) that operated for over 600 days. A nitrogen loading rate of 80 g-N/m^3/day, hydraulic retention time of 24 h, hydrogen gas flow rate of 40 mL/min, dissolved hydrogen concentration of 1.5±0.1 mg/L and temperature of 32±0.5 °C were maintained in the reactor (Eamrat et al., 2017). The enriched HD sludge was add to a reactor until the concentration of the mixed liquid suspended solid (MLSS) was 0.30±0.05 g/L. Synthetic groundwater was prepared based on the groundwater quality of Kathmandu, containing approximately 40 mg-N/L of nitrate. The chemical detail consisted of 0.24 g/L of $NaNO_3$, 0.5 g/L of $NaHCO_3$, 0.3 g/L of $MgSO_4{\cdot}7H_2O$, 0.027 g/L of KH_2PO_4, 0.18 g/L of $CaCl_2{\cdot}2H_2O$ and Trace element I and II. Moreover, the synthetic groundwater was supplied with argon gas to maintain the dissolved oxygen concentration (0.3±0.1 mg/L) before continuously fed to the reactors at 4 L/d, the hydraulic retention time was 12 h and solid retention time was 12 h. The water temperature was controlled at 32±0.5 °C. Hydrogen was supplied to the reactors with the lowest flow rate of 1 mL/min from a hydrogen gas generator (HG260, GL Science, Japan) via the two diffusers. One reactor used a MiBos as diffuser, whereas the other used an air stone as diffuser. The surfaces of the reactors were covered with plastic beads to prevent oxygen penetration from the air. During operation, the liquid and sludge inside the reactors were completely mixed by a magnetic stirrer. A schematic diagram of reactor operation is illustrated in Figure 1. To investigate the performance of the micro- and milli-hydrogen bubble reactors, nitrogen removal efficiency, biological hydrogen gas consumption by denitrifiers and hydrogen effectiveness were calculated using Equation (4)-(7).

$$\text{Nitrogen removal efficiency (\%)} = \frac{\text{Nitrogen removal rate } [g \cdot N/(m^3 \cdot d)]}{\text{Nitrogen loading rate } [g \cdot N/(m^3 \cdot d)]} \times 100 \quad (4)$$

$$\text{Nitrogen loading rate } [g \cdot N/(m^3 \cdot d)] = \frac{\text{Influent nitrate } [g \cdot N/L] \times \text{Flow rate } [L/d]}{\text{Reactor volume } [m^3]} \quad (5)$$

$$\text{Nitrogen removal rate } [g \cdot N/(m^3 \cdot d)] = \frac{(\text{Nitrate + Nitrite removed})[g \cdot N/L]) \times \text{Flow rate } [L/d]}{\text{Reactor volume } [m^3]} \quad (6)$$

$$\text{Hydrogen effectiveness } [\text{mg} \cdot \text{N/g} \cdot \text{H}_2] = \frac{\text{Nitrogen removal rate } [\text{g·N/(m}^3\text{·d)}] \times \text{Reactor volume } [\text{m}^3]}{\text{Total hydrogen supply volume } [\text{g·H}_2\text{/d}]} \quad (7)$$

2.3 Analytical method

Water samples were collected from the synthetic groundwater (inlet) and treated water (outlet), then filtered through a 0.45 µm membrane filter and kept in the sampling bottles for water quality analysis. The concentrations of nitrate and nitrite were measured in accordance with the standard method for water and wastewater analysis (APHA et al, 2012). Ultraviolet spectrophotometric screening was used for nitrate measurement, and the colorimetric method was used for nitrite measurement using a UV spectrophotometer (UV-1800, Japan). In situ pH and DH were measured using a pH meter (Horiba, B712) and a DH meter (ENH-1000, Japan).

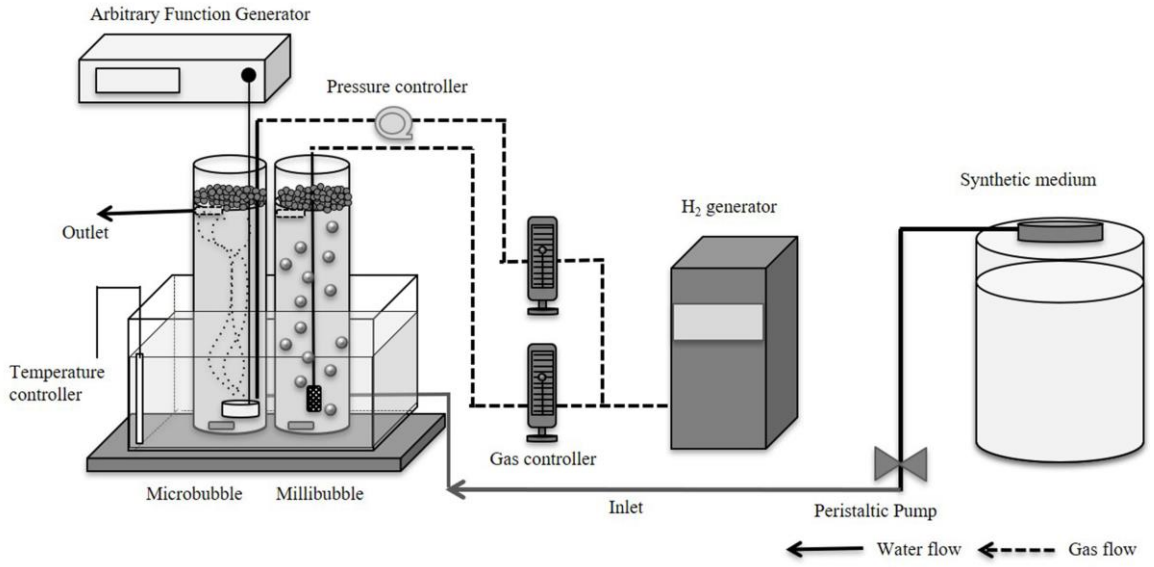

Figure 1. Layout of laboratory-scale hydrogenotrophic denitrification reactors

2.4 DNA extraction, PCR and Illumina next-generation sequencing analysis

To identify the microbial community in micro- and milli- hydrogen bubble reactors, sludge samples of about 0.1 g (wet weight) were collected and examined using the Illumina Next Generation Sequencing method. The total DNA in each sample was extracted using a FastDNA® Spin Kit for soil (MP-Biomedicals, Santa, CA, USA) according to the manufacturer's protocol. The V4 hypervariable region of the 16S rRNA gene was selected for polymerase chain reaction (PCR). The primers were the universal primer set; 515F (5′-GTGCCAGCM GCCGCGGTAA-3′) and 806R (5′-GGACTACHVG GGTWTCTAAT-3′). The 25 µL of PCR reaction mixture comprised 12.5 µL of sybrII, 0.1 µL of forward primer, 0.1 µL of reverse primer, 10.3 of water and 2 µL of DNA extracted sample. The PCR protocol consisted of denaturation at 94 °C for 30 s, followed by 40 cycles of denaturing at 94 °C for 15 s, annealing at 55 °C for 30 s, extension at 72 °C for 30 s, and the final elongation at 72 °C for 5 min. The amplicons from all samples were sent out for pyrosequencing using the Ilumina MiSeq platform of a commercial sequencing service (FASMAC Co., Ltd. Atsugi, Japan).

3. RESULTS AND DISCUSSION

3.1 Performance of the two reactors for hydrogenotrophic denitrification

Two-hydrogen bubble processes with millibubbles from the air stone and microbubbles using MiBos as diffusers were operated to evaluate the nitrogen removal performance, hydrogen effectiveness, and biological hydrogen consumption via hydrogen oxidizing denitrification. Hydrogen gas was continuously supplied into reactors and controlled at 1 mL/min (or 1.14 L/d). The in-situ dissolved hydrogen (1.4-1.5 mg/L) in the micro-reactor was similar to that in literature reporting 1.44 mg/L of hydrogen gas in water at 32 °C. DH was slightly lower than the theoretical value in the milli-

reactor (1.2 mg/L). Figure 2 shows two phases of the experiments adaptation and reaction periods. During the adaptation period (0-9 days), significant fluctuations in the amounts of NO_3-N and NO_2-N were observed in the milli-reactor. Approximately 10-15 mg-N/L of nitrate in the synthetic groundwater was removed, and then converted to nitrite. The highest nitrite accumulation was found to be 10 mg/L in day four of the operation. The overall nitrogen removal efficiency was approximately zero. After adaptation periods, the nitrogen removal efficiency was increased to 20% (nitrite removal). Moreover, DH concentration was suddenly decreased from 1.2 mg/L to 0.5 mg/L, although hydrogen gas was continuously supplied to the reactor. The dropping DH concentration implies that the amount of hydrogen gas transferring to the liquid phase was lower than that consumed by denitrifying bacteria. In the micro-hydrogen bubble reactor, nitrite was also found in the effluent from nitrate conversion in the adaptation period. However, some nitrate could be completely converted to nitrogen gas and released to the air. The performance of the micro-hydrogen bubble reactor continuously increased to 99% after the adaptation period (Figure 2(b)). At peak performance, the nitrate and nitrite concentrations were less than 1 mg/L, which meet the standards for safe drinking water. Moreover, DH was found to be in the range of 1.4-1.5 mg/L during the experiment. All above results show that the micro-hydrogen bubble reactor offers effective denitrification and high DH (greater dissolution in water and longer persistence).

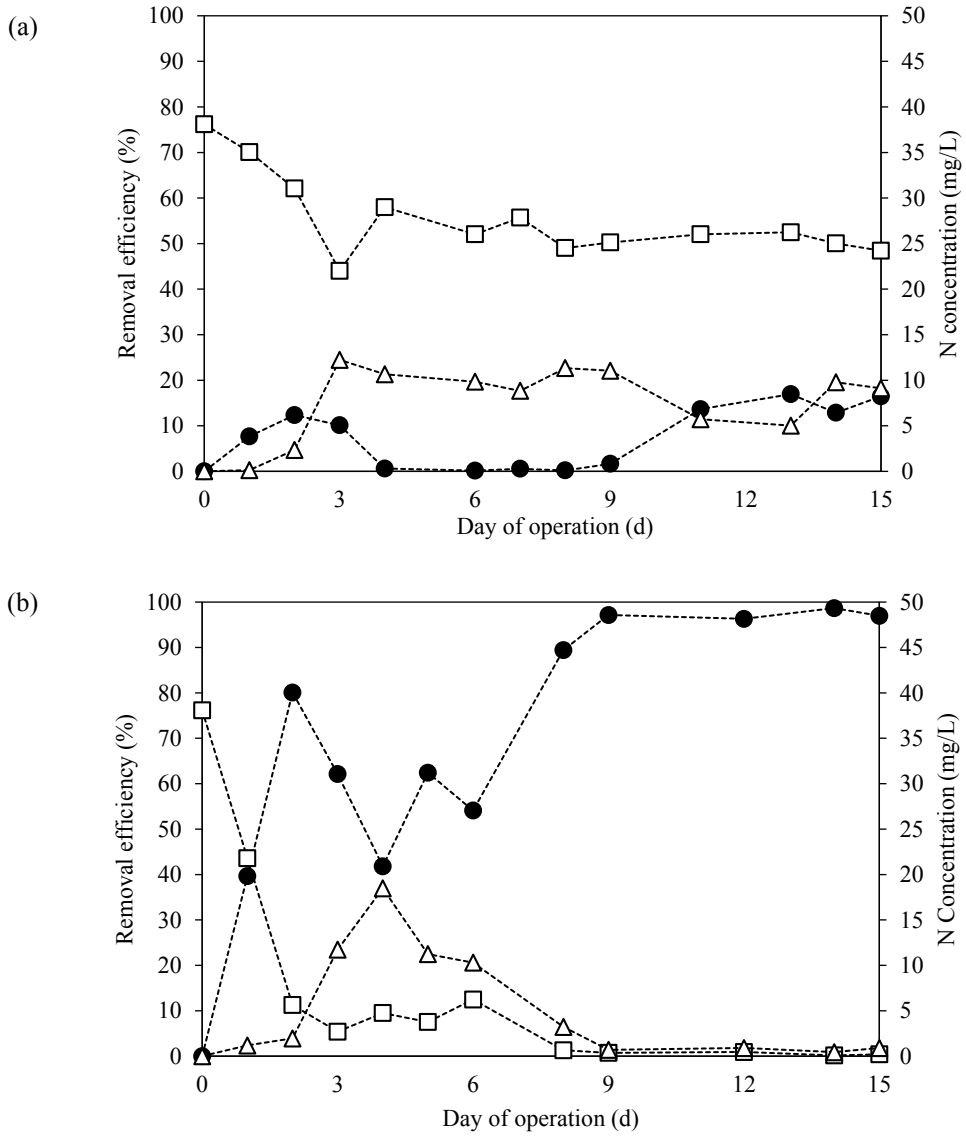

Figure 2. Performance of two systems in term of nitrogen removal efficiency and effluent concentration by (a) millibubble reactor and (b) microbubble reactor [●=Removal efficiency (%); □=NO_3-N concentration (mg/L); Δ=NO_2-N concentration (mg/L)]

According to the nitrogen removal performance, the percentage of biological hydrogen gas consumption by denitrifiers was calculated (Figure 3). The specific hydrogen consumption was calculated based on the stoichiometry of the denitrification reaction with hydrogen gas as the electron donor (Equation (1)). Here, each mole of nitrate reduces to nitrogen gas per 3.03 mole of hydrogen gas consumed. Consequently, 0.43 g of hydrogen gas was consumed in order to remove 1 g of NO_3-N. Figure 3 shows the differences between total hydrogen supply; biological hydrogen consumption was assumed to be the unused hydrogen that was released to the air. Biological hydrogen consumption was less than 10% in the milli-hydrogen bubble reactor, showing that hydrogen gas was mainly released to the air. Although, the biological denitrification consumption was low, the hydrogen effectiveness of the milli-hydrogen bubble reactor was 197 mg-N/g-H_2. On the other hand, in the micro-hydrogen bubble reactor, hydrogen consumption was found to be around 25% during the adaptation period and the value increased to around 50% after the adaptation period. These findings indicate that the effectiveness of hydrogen during operation was 1.21 g-N/g-H_2, which was higher than that of the milli-hydrogen bubble reactor by about 6 times. In summary, micro-hydrogen bubbles can enhance reactor performance for nitrogen removal and hydrogen effectiveness, leading to a low-cost treatment system that might be affordable for developing countries. The overall performance of the two suspended growth reactors in this study was also compared to that of previous studies using sequencing batch reactor, attached growth reactor, biofilm reactor and packed bed reactor. Reactor configuration and diffuser types affect nitrogen removal efficiency and hydrogen gas effectiveness. In an attached growth reactor, a packed bed reactor with air stone for hydrogen bubbling achieved high removal efficiency under short hydraulic retention time as compared with the MiBos diffuser. However, a large volume of hydrogen gas is supplied into the air stone system making the hydrogen gas effectiveness low. For the MiBos diffuser, microbubble technology can enhance the nitrogen removal efficiency and hydrogen gas effectiveness as compared to the air stone (Table 1). The micro-hydrogen bubble reactor achieved excellent efficiency of greater than 90%. Therefore, the micro-hydrogen bubble reactor offers potential for enhancing the performance of a hydrogenotrophic denitrification reactor.

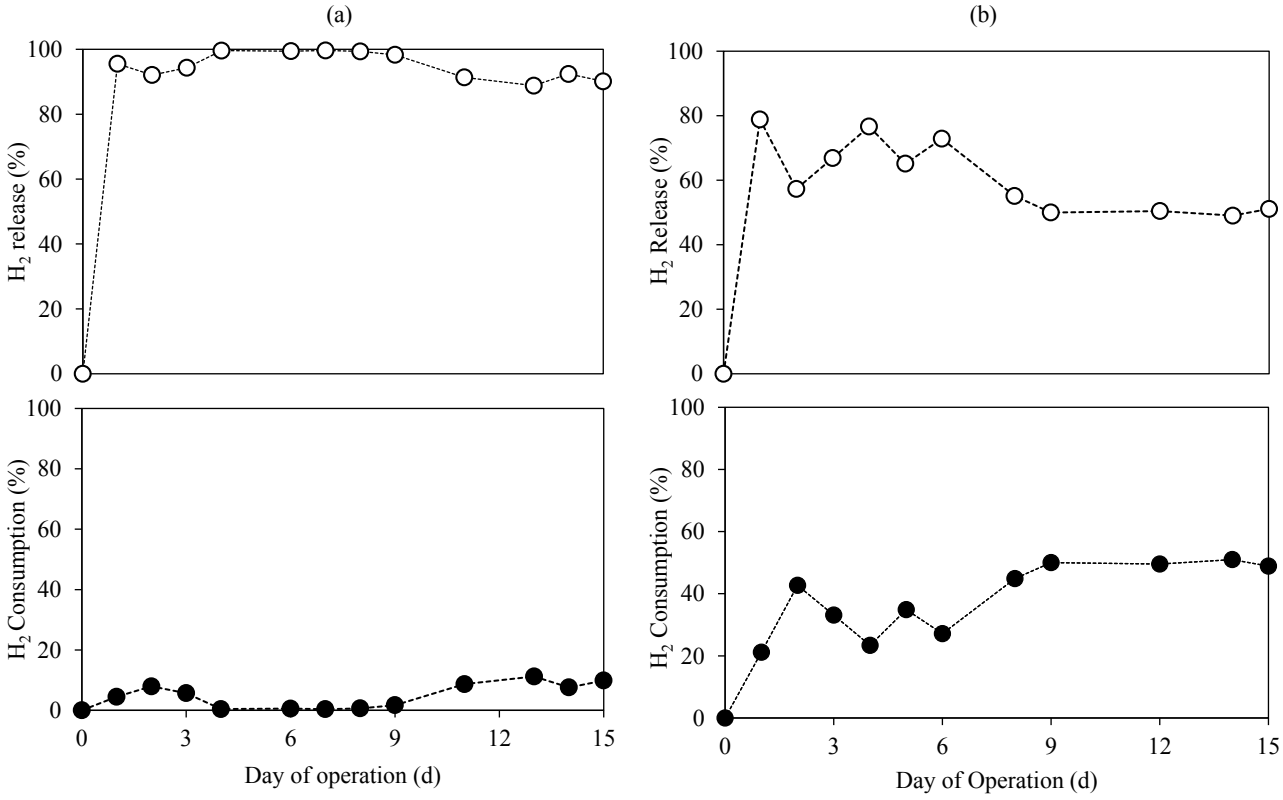

Figure 3. Percentage of biological hydrogen consumption for denitrification with two bubbles reactors; (a) millibubble reactor and (b) microbubble reactor

Table 1. Performance of HD systems in the literature

Reactors	Diffuser types	HRT (h)	H_2 supply (g/d)	Removal efficiency (%)	H_2 effectiveness (g-N/g-H_2)	References
Sequencing batch reactor	Bubble stone	480	2.14	100	0.12	Mousavi et al. (2013)
Sequencing batch reactor	Commercial bubble stone	3	1.34	100	0.01	Ghafari et al. (2009)
Attached growth reactor	Air stone	2.5	9.00	96	0.06	Khanitchaidecha et al. (2012)
Packed bed reactor	Fixed nozzles	1.0	11.57	82	0.34	Vasiliadou et al. (2009)
Packed bed reactor	Aquarium diffusing stone	2.0	12.86	80	0.04	Lee et al. (2010)
Suspended growth reactor	Air stone	12.0	0.13	16	0.20	This study
Suspended growth reactor	MiBos	12.0	0.13	98	1.21	This study

3.2 Mechanism of physical properties by micro-hydrogen bubbles

The difference in nitrogen removal efficiency and hydrogen dissolution between two reactors with milli-hydrogen bubble from Air stone and micro-hydrogen bubble from MiBos reactors is clarified in this section. Physical properties such as total gas-liquid mass transfer coefficient (K_La) and rising velocity of hydrogen bubbles generated from the two diffusers are summarized in Table 2. The increasing rate of DH concentration in the microbubble reactor was found to be faster than that in the millibubble reactor because of the transfer of most microbubbles to the surrounding liquid, large driving force, and low rising velocity. The transfer coefficient (K_La) refers to the ability to transfer hydrogen gas to the liquid phase, which depends on the size of bubble (Cruz et al., 1999; Painmanakul et al., 2009). As such, the K_La of the microbubble reactor was 45×10^{-3} s^{-1}, which is approximately 22.5 times greater than that of the millibubble reactor (2×10^{-3} s^{-1}). Micro-hydrogen gas transfer rateto dissolved hydrogen was faster than millibubble hydrogen gas. Moreover, the DH concentration arising from microbubbles persists about 10 times longer in the liquid phase than that arising from millibubbles under the same hydrogen supply amount. Therefore, microbubbles can enhance dissolved hydrogen for an extended duration, which has the potential to enhance nitrate removal via hydrogenotrophic denitrification. Moreover, the rising velocity of bubbles was found to be approximately 0.31 mm/s and 480 mm/s for the microbubble and millibubble reactors, respectively (Equation 3). Low rising velocity of microbubble caused bubbles to gradually shrink in water and ultimately disappear by dissolution. Therefore, the rate transfer of hydrogen gas to liquid phase was also related with rising velocity. Therefore, microbubbles of hydrogen were easily dissolved and consumed by microorganisms.

Table 2. Summary of the comparison of the physical properties between microbubbles and millibubbles

Diffusers	Bubble types	Flow rate (mL/min)	Total H_2 supply (mL)	Bubble diameter (μm)	K_La (s^{-1})	Rising velocity (mm/s)
Air stone	Millibubble	1	5	$2.2\pm0.25\times10^3$	2×10^{-3}	480
MiBos	Microbubble	1	5	25±13	45×10^{-3}	0.31

3.3 Microbial community

After the comparison of biological and physicochemical performance of the two different bubble processes (micro-hydrogen bubble and milli-hydrogen bubble), the bacterial community was further analyzed with high throughput sequencing of 16S rRNA gene of bacteria. Sludge samples from both milli- and micro-bubbles reactors were taken on the 15th day of operation for identifying abundant microbial communities. As shown in Figure 4, the total of identified bacteria phyla for both samples were 18. *Proteobacteria* was the most predominant phylum (83%) with some *Bacteroidetes* (9%) and *Firmicutes* (3%) in the microbubble reactor (Figure 4(a)), while

the milli-hydrogen bubble reactor was mainly represented by *Proteobacteria* (76%) with some *Bacteroidetes* (12%) and *Planctomycetes* (6%) as shown in Figure 4(b). Within *Proteobacteria, Betaproteobacteria* (60-71%) was the most predominant class instead of *Alphaproteobacteria* (12%) in both samples, while *Grammaproteobacteria* (4%) was found only in the milli-hydrogen bubble reactor. In a previous study, *Proteobacteria*, *Firmicutes*, *Bacteroidetes and Planctomycetes* were detected in autohydrogenotrophic denitrification and denitrification (Wang et al., 2015). Juretschko (2002) demonstrated that the phylum *Planctomycetes* was detected in nitrifying-denitrifying activated sludge from an industrial sewage treatment plant (Juretschko et al., 2002). Moreover, *Firmicutes* was reported to be predominant in autotrophic denitrification biocathode and hydrogenotrophic denitrification under thermophilic (30 °C) conditions (Wang et al., 2015; Xiao et al., 2015). *Bacteroidetes* was found to be dominant in prior nitritation and partial nitritation processes (Chen et al., 2016). From the present study, it is demonstrated that the microorganisms in both reactors were detected in the denitrification and the microorganisms could use hydrogen gas as the electron donor for nitrate removal. Conventionally, the bacterial community structures from two samples were further analysed at the family and genus levels (Figure 5). *Thauera* spp., Rhodocyclaceae, and *Hydrogenophaga* spp. belonging to Betaproteo-bacteria were highly enriched in the microbubble reactor and accounted for 29.3, 26.1, and 8.5% of the total bacteria, respectively. On the contrary, *Methyloversatilis* spp. (25.9%), *Thauera* spp. (13.8%), and *Hydrogenophaga* spp. (8.5%) were abundant in the milli-hydrogen bubble reactor. In a previous study, *Thauera* spp. and *Hydrogenophaga* spp. were detected in a hydrogenotrophic denitrification bioreactor for nitrate removal and *Thauera* spp. was found to have potential for nitrate removal under low hydrogen supply (Eamrat et al., 2017; Mao et al., 2013; Chen et al., 2015). *Methyloversatilis* spp. was recognized to enable heterotrophic denitrification, consuming organic carbon (i.e., methanol) as carbon source for nitrate removal (Sun et al., 2016). The role of *Methyloversatilis* spp. in denitrification was classified (Mustakhimov et al., 2013); the bacteria was discovered in the methylotrophy metabolic pathways during the transformation of nitrate to nitrite under anoxic conditions. Related with the reactor performance, the low dissolved hydrogen of 0.5 mg/L in the milli-hydrogen bubble reactor resulted in insufficient hydrogen for complete hydrogenotrophic denitrification, and thus heterotrophic denitrification occurred and became dominant. It can be summarized that nitrate removal in the micro-hydrogen bubble reactor occurred through hydrogenotrophic denitrifiers, whereas that in the milli-hydrogen bubble reactor was enabled by a combination of hydrogenotrophic denitrifiers and heterotrophic denitrifiers.

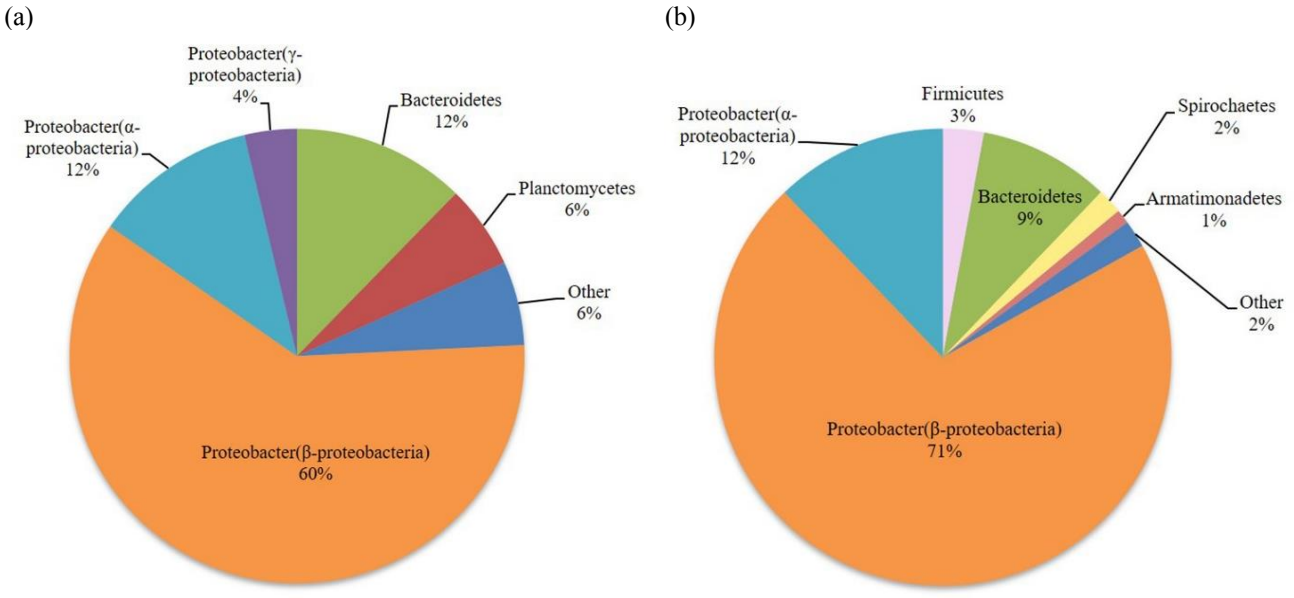

Figure 4. Relative abundance values at the phylum levels with two hydrogen-bubble systems; (a) millibubble system and (b) microbubble system.

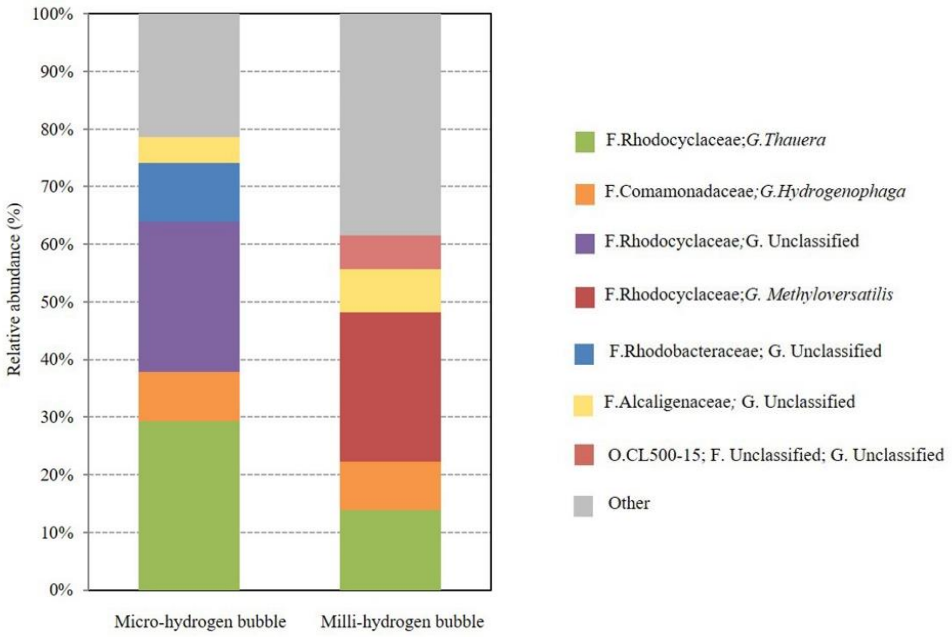

Figure 5. Relative abundance values at the family and genus levels with two hydrogen bubble systems during microbubble system and millibubble system; The abundance values lower than 5% were included in "other" group.

4. CONCLUSIONS

This research compared the performance of micro-hydrogen bubbles (mean bubble size of 25±13 μm) and milli-hydrogen bubbles (mean bubble size of $2.20\pm0.25\times10^3$ μm) generated from MiBos and Air stone for nitrate removal from groundwater. The micro-hydrogen bubble reactor performed better than the milli-hydrogen bubble reactor by achieving excellent nitrogen removal efficiency and increased hydrogen effectiveness. The nitrogen removal efficiency reaching 99 % and around 50% of total hydrogen was utilized for biological consumption, which increased the hydrogen effectiveness to reach 1.21 g-N/g-H_2. In comparison, the milli-hydrogen bubble reactor achieved less than 10% efficiency and biological consumption accounted for 9.9% of total hydrogen at the same dose of hydrogen supply with the micro-hydrogen bubble reactor. The different results demonstrate that microbubbles have high dissolution ability, faster hydrogen mass transfer efficiency (45×10^{-3} s^{-1}), and low velocity (0.31 mm/s) as compared with milli-hydrogen bubbles (2×10^{-3} s^{-1} and 480 mm/s). Physicochemical properties significantly affected the microbial community. *Thauera* spp., *Hydrogenophaga* spp. and Rhodocyclaceae belonging to Betaproteobacteria, which can use nitrate as the electron accepter and hydrogen as electron donor under anaerobic conditions, were enriched in the micro-hydrogen bubble reactor, enabling hydrogenotrophic denitrification. In the milli-hydrogen bubble reactor, insufficient hydrogen caused *Methyloversatilis* spp. to become dominant instead of *Thauera* spp., *Hydrogenophaga* spp., and Rhodo-cyclaceae, thus, both heterotrophic and hydrogenotrophic denitrification possibly occurred. However, other factors including system designs and long-term operation should be further studied before applying the HD system to the treatment of contaminated water.

5. ACKNOWLEDGEMENTS

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