Ultrafine Bubble Diffuser for Enhancing Hydrogen-Dependent Denitrification of Groundwater Treatment

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Abstract
Groundwater contamination, with inorganic nitrogen, is very serious problems in developing countries; especially from NO_3-N contamination. To provide good quality of groundwater, a biological process by hydrogenotrophic denitrification (HD) was selected to convert NO_3-N to harmless nitrogen gas. The performance of a suspended sludge reactor was investigated for nitrate removal via HD. Two reactors were set-up, employing different diffusers; one produced ultrafine bubbles using pressure and vibration, while the other used an air stone, a type of diffuser commonly used in treatment systems. The hydrogen supply was controlled at 0.13 g/d for both reactors and NO_3-N concentration was 40 mgN/L. The experimental results show that: 1) Maximum dissolved hydrogen (DH; 1.0 mg/L) and volumetric mass transfer coefficient (K_La; 0.045 s^{-1}) were greater in the ultrafine bubble reactor than in the air stone reactor (0.7 mg/L and 0.002 s^{-1} respectively). 2) The differences in DH and K_La had significant effects on hydrogen dissolubility, biological hydrogen consumption, and nitrogen removal efficiency. The efficiency of the ultrafine bubble reactor was 99%, and 51% of total hydrogen supply was effectively consumed by hydrogenotrophic denitrification. In comparison, the air stone reactor achieved only 20% efficiency and biological consumption of 9.88% of total hydrogen supply. 3) Specific hydrogen consumption was 0.45±0.06 mg H_2/mg N, which was found nearly previous studies. 4) Bacteria in the *Proteobacter* phylum, *Beta Proteobacteria* class and *Thauera sp.* was main represented under ultrafine bubble which were responsible for HD, resulted in the high level of nitrogen removal from the groundwater. In summary, the use of ultrafine bubbles can enhance HD performance and reduce operating costs; such systems are therefore applicable to developing countries.

Keywords
Hydrogenotrophic denitrification; groundwater; ultrafine bubble; air stone; hydrogen consumption; microbial community

INTRODUCTION

Nitrate contamination of groundwater and surface water are serious environmental issues in developing countries such as Nepal, the Philippines, Vietnam, and Thailand (Khatlwada et al., 2002; Tirado, 2007). Such contamination mainly derives from the extensive use of fertilizer, and the discharge of untreated domestic wastewater and sewage waste from increased urbanization (Zhang et al., 2009). The consumption of high-nitrate water causes methemoglobinemia (blue baby disease), which seriously affects infants and pregnant women. Moreover, high nitrate concentration in the human body can form nitrosamines (via nitrite) and other potential carcinogens metabolites (Bouchard et al., 1992). Consequently, the World Health Organization has established guidelines for safe drinking water quality: 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). For groundwater and surface water remediation, there are two common technologies to remove nitrate contamination: physicochemical process (i.e., chemical precipitation, membrane filtration, electro-dialysis and catalysis) and biological process (i.e., heterotrophic denitrification and autotrophic denitrification)

(Karanasios et al., 2010). Physicochemical technologies are limited by high energy consumption, investment cost and brine waste, such that biological technologies are of increasing interest for developing countries (Shrimali and Singh, 2001). Hydrogen-dependent denitrification (termed hydrogenotrophic denitrification; HD) was developed as a biological process for nitrogen removal. In an HD system, facultative bacteria remove nitrate via microbial metabolism under sufficient hydrogen gas and bicarbonate, as shown in Eq. 1. HD has significant advantages over heterotrophic denitrification: no consuming organic, low biomass production, no residual organic carbon, and harmless to humans (Lee and Rittmann, 2002).

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

Previous studies have examined the implementation of HD systems for drinking water. Vasiliadou et al. (2009) developed a packed bed reactor to treat initial nitrate concentration of 80 mg/L, achieving removal efficiency of 90% and maximum removal capacity of 2260 gNO_3-$N/m^3 \cdot d$. Similarly, a packed bed reactor was developed using polyurethane matrixes (Dries et al., 1988). Under initial nitrate concentration of 15 mg/L, the removal rate was around 0.5 mg/L.h. A system proposed by Khanitchaidecha et al. (2012) achieved 96% efficiency under operating conditions of 70 mL/min hydrogen flow. However, the above systems used commercial types of diffuser such as an air stone. The large bubbles generated by these diffusers limit the solubility of hydrogen gas and hence system performance. The low solubility of hydrogen gas and high cost of hydrogen gas supply are the biggest concerns in implementing an HD system; however, excellent bubble dynamics and appropriate engineering design can enhance the solubility and transfer rate of hydrogen gas. Recently, various types of diffusers have been introduced as commercial products for wastewater treatment plants. Common types of diffuser include an air stone, which has significant advantages of being inexpensive, less prone to plugging, and having improved mixing capacity. In this study, another type of diffuser (termed ultrafine bubble) was used as a hydrogen gas diffuser, and its performance was compared to the air stone for removing nitrate from groundwater via hydrogenotrophic denitrification. The groundwater quality of Kathmandu, Nepal was used as the standard for preparing synthetic groundwater. Specific hydrogen gas consumption and the effectiveness of hydrogen gas supply are also discussed.

METHODOLOGY

Groundwater preparation

Synthetic groundwater were prepared in accordance with groundwater quality in Kathmandu, Nepal (Chapagain et al., 2010). The chemical constituents were mixed as follows (g/L): 48.5 $NaNO_3$, 0.5 $NaHCO_3$, 0.3 $MgSO_4 \cdot 7H_2O$, 0.03 KH_2PO_4, 0.18 $CaCl_2 \cdot 2H_2O$, and trace elements I and II (Kamei et al., 2015). Nitrate concentration was controlled at 40 mg/L during the experiment. The synthetic groundwater was supplied with argon gas in order to maintain low dissolved oxygen content of 0.3 mg/L before feeding to the reactors. In the batch test to measure specific HD activity and hydrogen requirement, similar groundwater characteristics were prepared.

Reactor set-up and operation

Two laboratory-scale cylindrical reactors were set up (height 28 cm, internal diameter 7 cm, working volume 2 L). One reactor used an ultrafine bubble diffuser for hydrogen, whereas the other used an air stone. Enriched HD sludge (30 mL) were added into both reactors, giving initial sludge concentration of approximately 0.30±0.05 g mixed liquor suspended solids per liter (MLSS/L). The enriched HD sludge was collected from the previous HD reactor, which achieved 90% nitrate removal and operated continuously for 600 days. On the top of the reactors, layers of plastic beads were prepared to reduce the released hydrogen and prevent oxygen penetration from the air. Hydrogen was supplied to the reactors at 1 mL/min from a hydrogen gas generator (HG260, GL

Science, Japan). The synthetic groundwater was continuously fed to the reactors at 4 L/d. Hydraulic retention time was approximately 12 h, and the temperature was controlled by thermostat at32.0±0.5°C. During operation, the liquid and sludge inside the reactors were completely mixed by a magnetic stirrer. The layout of the reactors is illustrated in Fig. 1.

Bubbling diffuser characteristics

Two diffusers were used in this research: ultrafine bubble and air stone. The ultrafine bubble diffuser (trade name MiBos, Japan) employed an oscillating mesh, producing fine bubbles using hydrogen pressure and a vibration system. The air stone was a commercial diffuser of 15 mm diameter and 30 mm length (STARPET, Japan). Average bubble sizes were 25 μm and 1000 μm for the ultrafine bubble and air stone diffusers respectively. The effects of the diffuser properties on the transfer of hydrogen gas to the liquid phase were investigated via batch tests using 1 L cylinder reactor which distilled water set-ups. Hydrogen was supplied via ultrafine bubble or air stone diffuser. Dissolved hydrogen (DH) concentration was frequently measured by a DH meter (ENH-1000, Japan) until DH concentration was stable, and then the hydrogen supply was stopped and continue measured DH until DH reach to 0 mg/L. In addition, temperature and recirculation were controlled to match those of the reactor operation. The volumetric mass transfer coefficient (K_La) of hydrogen gas using both diffusers was calculated from the slope of Eq. 2.

$$ln\left(\frac{C^* - C_L}{C^* - C_s}\right) = - k_La(t - t_s) \tag{2}$$

where; C^* is saturated dissolved hydrogen concentration
C_s is dissolved hydrogen concentration at initial point; t_s
C_L is dissolved hydrogen concentration at time; t

Hydrogenotrophic denitrification sludge activity

Another batch test was set up to examine the activity of initial HD sludge and minimum hydrogen per gram of biological nitrate removed. The collected sludge was washed several times with the synthetic groundwater containing no nitrate. The sludge was then added into 1 mL vials containing the synthetic groundwater (1:1 ratio of liquid:gas phase) with NO_3-N (40 mgN/L) or NO_2-N (40 mgN/L) and plugging with aluminum cap. After supplying the saturated hydrogen, the vials were moved to a temperature-controlled shaker (Bioshaker, BR-43FL). The gaseous hydrogen in the vials was frequently measured and the remaining nitrate and nitrite in the liquid were analyzed.

Bacteria community

DNA was extracted from 0.1 g (wet weight) of the suspended solid samples from ultrafine bubble and air stone reactors using the FastDNA® Spin Kit for Soil (MP-Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol. The 16S rRNA gene was amplified by PCR with the universal primer set, 515F (5'- GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') under the following conditions: 94℃ for 3 min, followed by 30 cycles of 94 ℃ for 45 min, 50 ℃ for 1 min and 72 ℃ for 1.5 min and a final elongation at 72℃ for 5 min. The amplified metagenomic sequencing was performed using the Illumina MiSeq platform. The classification of the sequences and annotation of output data were completed with QIIME software (ver. 1.9.0) into operational taxonomic units (OUTs) in MOTHUR according to the MiSeq standard operation procedure (SOP). These analyses were performed by a commercial sequencing service (FASMAC Co., Ltd. Atsugi, Japan).

Analytical methods

Water samples were collected from the synthetic groundwater (inlet) and treated water (outlet), then filtered 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, 1998). Ultraviolet spectrophotometric screening was used for nitrate measurement, and the colorimetric method was used for nitrite measurement using an UV spectrophotometer (UV-1800, Japan). In situ pH and dissolved hydrogen were measured using a pH meter (Horiba, B712) and DH meter (ENH-1000, Japan). Gaseous hydrogen was analyzed using micro-gas chromatography (Varian 490 GC, Netherlands), and reactor performance was calculated using Eqs. 3–5.

Nitrogen loading rate (g/d·m^3) = (Influent NO_3-N × Flow rate)/Volume (3)
Nitrogen removal rate (g/d·m^3) = (Influent NO_3-N- Effluent NO_2-N × Flow rate)/Volume (4)
Nitrogen removal efficiency (%) = (Nitrogen removal rate/Nitrogen loading rate) × 100 (5)

RESULTS AND DISCUSSION

Volumetric mass transfer coefficient of diffusers

From Fig. 2a, at the same hydrogen flow rate of 1 mL/min, the ultrafine bubble reactor contained higher dissolved hydrogen (DH) concentration than the air stone reactor at various times; the DH was 0.72 mg/L for ultrafine bubble at 15 min, compared with 0.29 mg/L for the air stone. After 2 h, maximum DH was observed for both diffusers, at 1.00 and 0.67 mg/L for ultrafine bubble and air stone, respectively. After the hydrogen supply was stopped at hour 2, DH gradually decreased in both reactors. The decay rate in the ultrafine bubble reactor was 1.04 mg/L-h, which was slightly lower than that of air stone reactor (1.12 mg/L-h). This led to longer retention of DH in the ultrafine bubble reactor (of 18 h). Conversely, using the ultrafine bubble diffuser, the gas was mostly transferred to the water and DH can maintain long time in the system. From Fig. 2b, the volumetric transfer coefficients of hydrogen gas for the ultrafine bubble and air stone diffusers were calculated via the slope of $\ln(C^{*}- C_L)/(C^{*} - C_s)$ versus t. The transfer coefficient (K_La) refers to the ability to transfer hydrogen gas to liquid phase, which depends on the size of bubble (Cruz et al., 1999; Painmanakul et al,. 2009). The K_La of the ultrafine bubble reactor was 0.045 s^{-1}, which was approximately 20 times greater than that of the air stone (K_La of air stone was 0.002 s^{-1}). This result confirmed that ultrafine bubble shows greater solubility in water faster that air stone bubble. As, the reactor with ultrafine bubble diffuser might achieve greater nitrate removal than the reactor using the air stone, due to higher K_{La} and longer period of DH retention during operation.

Hydrogenotrophic denitrification sludge activity

The activity of hydrogenotrophic denitrification sludge was investigated via specific hydrogen consumption, which means the amount of hydrogen consumed in order to remove 1 g of nitrate. During batch tests, the concentrations of hydrogen gas and nitrate were frequently measured at various sludge contents and the results are presented in Fig. 3. The amount of nitrate removal was positively correlated with hydrogen gas consumption, and the observed trend was very similar to the theoretical value (calculated from Eq. 1). According to these results, specific hydrogen consumption was 0.45±0.06 mg H_2/mg N. Generally, there are two main conversion steps (nitrate to nitrite and nitrite to nitrogen gas) for hydrogenotrophic denitrification. The first conversion of nitrate to nitrite consumed around 0.16±0.04 mg H_2/mg N, and the later stage of nitrite conversion to nitrogen gas consumed 0.29±0.09 mgH_2/mg N. The specific hydrogen consumption in this study agreed with the values reported in the literature: 0.14–0.17 mg H_2/mg N for converting nitrate to nitrite, 0.21–0.26 mg H_2/mg N for converting nitrite to nitrogen gas (Lee and Rittmann, 2002; 2003), and 0.35–0.43 mg H_2/mg N for complete nitrate removal (Ergas and Reuss, 2001; Ghafari, et al., 2009; Lee and Rittmann, 2002, 2003; Mansell and Schroeder, 2002). These results confirm that this microorganism was hydrogenotrophic denitrification, with nitrate removal rate of 3.55±0.92 mg/MLSS·h and hydrogen consumption rate of 1.61±0.61 mg/MLSS·h (data not shown).

Performance of hydrogenotrophic denitrification reactor

This section discusses the long-term operational performances of the reactors fitted with ultrafine bubble or air stone diffuser. The hydrogen supply was controlled at 1 mL/min (or 0.13 g/d) for ultrafine bubble and air stone reactors. The ultrafine bubble reactor achieved 40% nitrogen removal efficiency on the first day, compared with only 8% for the air stone reactor (Fig. 4). The effluent nitrate and nitrite concentrations were 22 and 1 mg/L for the ultrafine bubble reactor, and 36 and 0.4 mg/L for the air stone reactor. The performance of the ultrafine bubble reactor continued to increase, reaching 99% at 9 days. At peak performance, the nitrate and nitrite concentrations were less than 1 mg/L, which meet the standards for safe drinking water (WHO, 2011). On the other hand, the performance of the air stone reactor was slightly increased and reached the maximum of 20% reactor increased only slightly over time, reaching 20% in 14 days, despite sufficient supply of hydrogen (1.44 L/d; 0.13 g/d) to both reactors. However, excellent nitrogen removal efficiency was found when using ultrafine bubble diffuser. This presented that the ultrafine bubble generated the small size of hydrogen bubble which easy to dissolve and consume by microorganisms. According to specific hydrogen consumption and reactor performance, biological hydrogen consumption for hydrogenotrophic denitrification was calculated and presented in Fig. 5. The difference between total hydrogen supply and biological hydrogen consumption was assumed to be the unused hydrogen that was released to the air. It can be seen that in the first day of operating the ultrafine bubble reactor, 0.027 g/d of hydrogen was effectively consumed for nitrogen removal (21.15% of total supply), while 0.103 g/d of hydrogen was released (78.85% of total supply). However, in subsequent days, biological hydrogen consumption gradually increased to 0.065 g/d (51% of total supply). Similarly, the amount of hydrogen released declined to 0.065 g/d (49% of total supply). These findings indicate the effectiveness of hydrogen consumption during operation. In the air stone reactor, 0.013 g/d (9.88% of total supply) of hydrogen was consumed biologically and the release of hydrogen was 0.117 g/d (90.12 % of total supply) throughout the operation. In summary, the use of an ultrafine bubble diffuser enhanced reactor performance for nitrogen removal and the effectiveness of hydrogen consumption, 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 compared to previous studies using sequencing batch reactor, attached growth reactor, biofilm reactor and packed bed reactor (see Table 1). The ultrafine bubble reactor achieved excellent efficiency of greater than 90%, as in previous studies. Significantly, the hydrogen flow rate of 0.13 g/d was relatively low, which resulted in the high hydrogen effectiveness of 1206.15 mg N/g H_2. Therefore, the use of an ultrafine bubble diffuser is an option for enhancing the performance of a hydrogenotrophic denitrification reactor.

Bacteria Community

The microbial communities in both systems were analyzed by next generation sequencing method. The qPCR results showed an existence of $2.82 \times 10^6 \pm 0.30$ copies/μL in ultrafine bubble and 1.60 ± 0.10 copies/μL in air stone. Taxonomic composition on the relative abundance was summarized at the phylum, family and genus levels as shown in Fig6 (a) and (b). The results show that there was a minor difference at the phylum level; *Proteobacter* (83.66%) was the predominant phylum, followed by *Bacteroidetes* (9.25%) and *Firmicutes* (2.90%) of the bacteria detected in ultrafine bubble, whereas *Proteobacter* (76.23%), *Planctomycetes* (5.95%) and *Chloroflexi* (0.99%) of the bacteria detected in air stone. The main difference between two systems which was found in dominant genus. *Thauera sp.* was predominant genus for 29.35%, *Rhodocyclaceae* (26.13%) *and Hydrogenophaga sp.* (8.47%) which found in ultrafine bubble supply. Previous studies reported that dominant phylum of bacteria community in autotrophic denitrification was *Proteobacter* including class of *Beta, Gamma* and *Alpha Proteobacteria*. *Thauera sp*. was one of genus which found in autotrophic denitrification and heterotrophic denitrification system as certified amphitrophy denitrifiers (Xu et al., 2015 and Mao et al., 2013). Therefore, *Thauera sp*. might be responsible for HD processes under less hydrogen supply by ultrafine bubble diffuser. In the other hand, the majority as air stone consisted of *Methyloversatilis sp.* (25.96%), *Thauera sp.* (13.79%) and *Hydrogenophaga sp.* (8.48%). Air stone operation was found low nitrogen removal efficiency as compared with ultrafine bubble due to insufficiently H_2 in the system. Therefor, *Methyloversatilis sp.* was main dominant. *Methyloversatilis sp.* was found in sediment, Lake and denitrification process (Smalley et al., 2015). It should be highlight that the use of an ultrafine bubble diffuser can enhance majority of microbial community to hydrogen-oxidizing bacteria under less H_2 supply. *Thauera sp*., *Rhodocyclaceae and Hydrogenophaga sp.* might be response for HD under less hydrogen condition and the excellent performance on nitrogen removal.

CONCLUSION

This research compared the performance of ultrafine bubble and air stone diffusers for nitrate removal from drinking water. The ultrafine bubble diffuser achieved higher dissolved hydrogen of 1.0 mg/L (air stone diffuser 0.7 mg/L); and volumetric mass transfer coefficient of hydrogen gas of approximately 0.005 s^{-1}, which was much greater than that from the air stone (0.002 s^{-1}). These results induced high solubility of hydrogen and effective hydrogen consumption for biological nitrate removal in the ultrafine bubble reactor. The ultrafine bubble reactor achieved excellent nitrogen removal efficiency of 99% at the steady state; around 0.065 g/d of hydrogen was utilized for biological nitrate removal (total hydrogen supply was 0.13 g/d). In comparison, the air stone reactor achieved less than 20% efficiency and 0.015 g/d biological consumption of hydrogen. Moreover, bacteria community was analyzed by next generation sequencing method. Main relative abundance of microbial community was difference based on bubble size and H_2 sufficient. *Thauera sp*., *Rhodocyclaceae and Hydrogenophaga sp.* was predominant genus in ultrafine bubble, whereas *Methyloversatilis sp., Rhodocyclaceae and Hydrogenophaga sp.* was predominant genus in air stone. Therefore, the use of an ultrafine bubble diffuser is an option for enhancing the performance of a hydrogenotrophic denitrification reactor.

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REFERENCES

Bouchard, D.C., Williams, M.K., Surampalli, R.Y. 1992 Nitrate contamination of groundwater: sources and potential health effects. *American Water Works Association* **28**(9), 85-90.

Cruz, A.J.G., Silva, A.S., Araujo, M.L.G.C., Giordano, R.C., Hokka, C.O. 1999 Estimation of the volumetric oxygen transfer coefficient (KL_a) from the gas balance and using a neural network technique. *Chemical engineering* **16**(2), 179-183.

Ergas, S.J., Reuss, A.F. 2001 Hydrogenotrophic denitrification of drinking water using a hollow fibre membrane bioreactor. *Water Supply Research Technology AQUA* **50**(3), 161-171.

Ghafari, S., Hasan, M., Aroua, M.K. 2009 Effect of carbon dioxide and bicarbonate as inorganic carbon source on growth and adaptation of autohydrogenotrophic denitrifying bacteria. *Hazardous Materials* **162**(2-3), 1507-1513.

Karanasios, K.A., Vasiliadou, I.A., Pavlou, S., Vayenas, D.V. 2010 Hydrogenotrophic denitrification of potable water: A review. *Hazardous Materials* **180**(1-3), 20-37.

Khanitchaidecha, W., Shakya, M., Tatsuru, K., Kazama, F. 2012 NH4-N removal through nitrification and hydrogenotrophic denitrification in simple attached growth reactors. *Water, Air and Soil Pollution* **223**(7), 3939-3953.

Khatlwada, N.R., Takizawa, S., Tran, T.V.N., Inoue, M. 2002 Groundwater contamination assessment for sustainable water supply in Kathmandu valley, Nepal. *Water science and Technology* **46**(9), 147-154.

Kamei T, Naitoh D, Khanitchaidecha W, Kazama F. 2015 Simultaneous removal of ammonium and nitrate by a combination of ANAMMOX and hydrogenotrophic denitrification. *Water Environment Technology* **13**(2), 167–178.

Lee, K.C., Rittmann, B.E. 2002 Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water. *Water Research* **36**(8), 2040-2052.

Lee, K.C., Rittmann, B.E. 2003 Effect of pH and precipitation on autohydrogenotrophic denitrification using the hollow-fiber membrane-biofilm reactor. *Water research* **37**(7), 1551-1556.

Mansell, B.O., Schroeder, E.D. 2002 Hydrogenotrophic denitrification in microporous membrane bioreactor. *Water research* **36**(19), 4683-4690.

Mao Y, Xia Y, Zhang T. 2013 Characterization of Thauera-dominated hydrogen-oxidizing autotrophic denitrifying microbial communities by using high-throughput sequencing. *Bioresource Technology* **128**, 703-710.

Mousavi, S., Ibrahim, S., Aroua, M.K. 2013 Effects of operational parameters on the treatment of nitrate-rich wastewater by autohydrogenotrophic denitrifying bacteria. *Water and Environment* **28**(3), 556-565.

Smalley, N. E., Taipale, S., De Marco, P., Doronina, N. V., Kyrpides, N., Shapiro, N.,Kalyuzhnaya, M. G. 2015 Functional and genomic diversity of methylotrophic Rhodocyclaceae: Description of Methyloversatilis discipulorum sp. nov. *Systematic and Evolutionary Microbiology* **65**(7), 2227–2233.

Painmanakul, P., Wachirasak, J., Jamnongwong M., Herbrard, G. 2009 Theoretical prediction of volumetric mass transfer coefficient (KL_a) for designing an aeration tank. *Engineering* **13**(3), 13-28.

Shrimali, M., Singh, K.P. 2001 New methods of nitrate removal from water. *Environmental Pollution* **112**(3), 351-359.

Tirado, R. 2007 Nitrate in drinking water in the Philippines and Thailand. Greenpeace Research Laboratories Technical note 12/2007(GRL-TN-10-2007), November 2007.

Vasiliadou, I.A., Karanasion, K.A., Pavlou, S., Vayenas, D.V. 2009 Experimental and modelling study of drinking water hydrogenotrophic denitrification in packed-bed reactor. *Hazardous materials* **165**(1-3), 812-824.

WHO. 2011 Guidelines for drinking water quality, 4th edition. Publication of world health organization.

Zhang, Y., Zhong, F., Xia, S., Wang, X., Li, J. 2009 Autohydrogenotrophic denitrification of drinking water using a polyvinyl chloride hollow fiber membrane biofilm reactor. *Hazardous Materials* **170**(1), 203-209.

Table 1. Performance of HD systems in the literature

Reactor	**Diffuser Type**	**Media for microorganisms attachment**	**Influent NO_3-N (mg/L)**	**HRT (h)**	**H_2 supply (g/d)**	**Removal efficiency (%)**	**H_2 effectiveness (mgN/mg H_2)**	**Reference**
Sequencing batch reactor	Bubble stone	No	1000	480	2.14	100	116.82	Mousavi, Ibrahim, & Aroua (2013)
Sequencing batch reactor	Commercial bubble stone	No	20	3	1.34	100	12.38	Ghafari et al. (2009)
Attached growth reactor	Air stone	Yes	20	2.5	9.00	96	58.88	Khanitchaidecha et al. (2012)
Packed bed reactor	Fixed nozzles	Yes	340	1.0	11.57	82	347.20	Vasiliadou et al. (2009)
Packed bed reactor	Aquarium diffusing stone	Yes	20	2.0	12.86	80	41.38	Lee et al. (2010)
Glass bead biofilm reactor	Pressurized gas tank	Yes	150	16.0	0.64	100	316.41	Park et al. (2005)
Suspended growth reactor	Air stone	No	40	12.0	0.13	16	196.92	This study
Suspended growth reactor	Ultrafine bubble	No	40	12.0	0.13	98	1206.15	This study

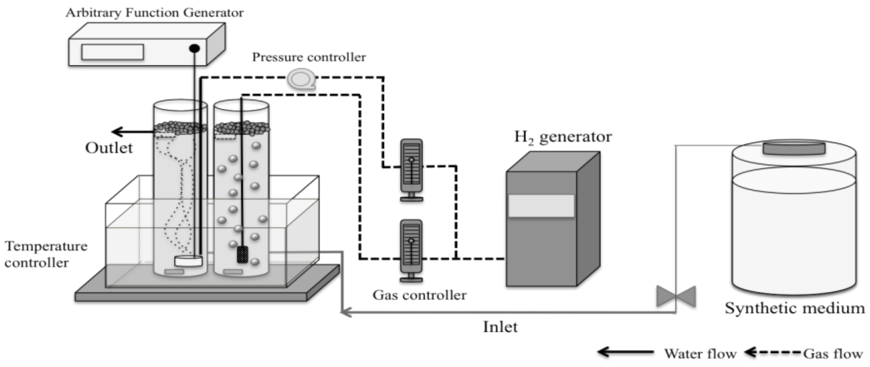
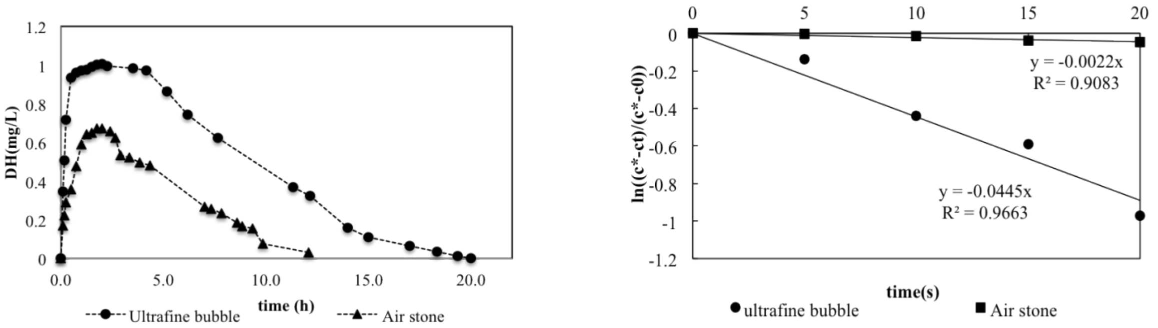


Figure 1. Layout of laboratory-scale hydrogenotrophic denitrification reactors



(a) **(b)**

Figure 2. (a) Profile of dissolved hydrogen concentrations; (b) logarithm function of dissolved hydrogen and time

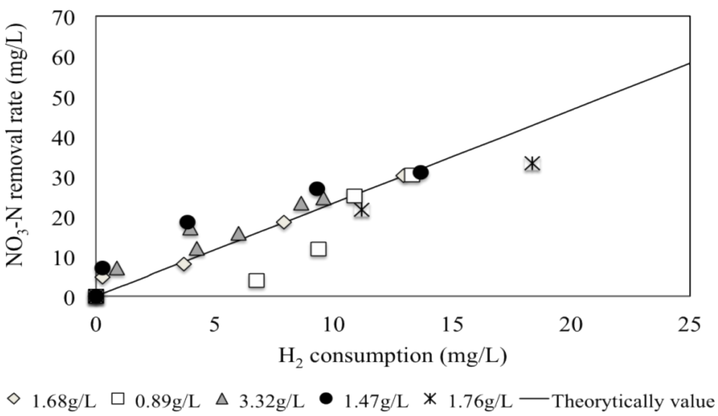


Figure 3. Correlation of H_2 consumption and nitrate removal at various biomass concentrations

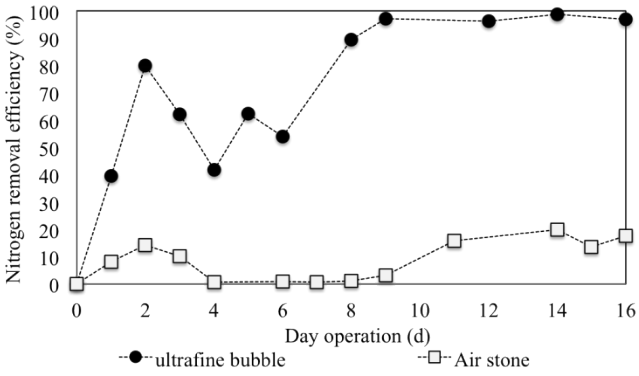
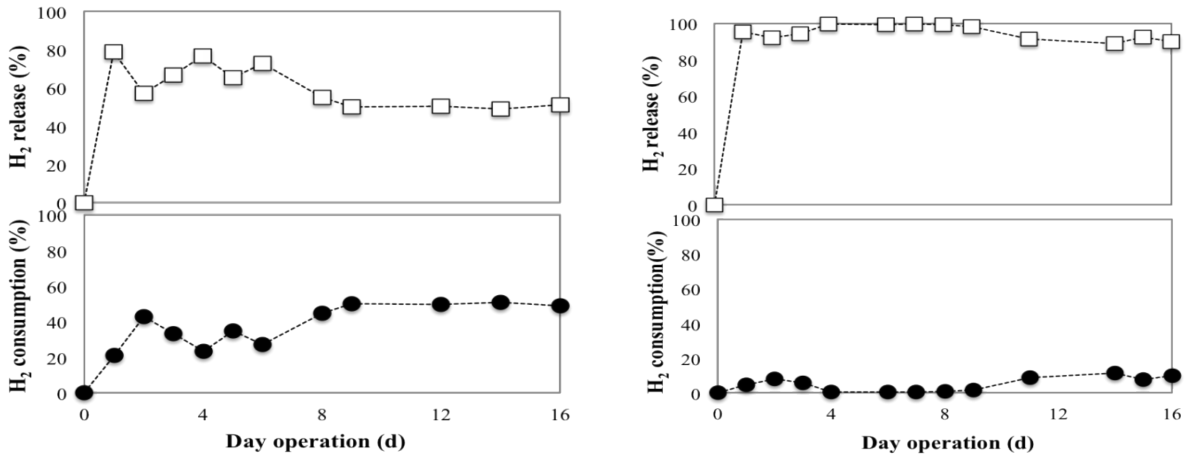
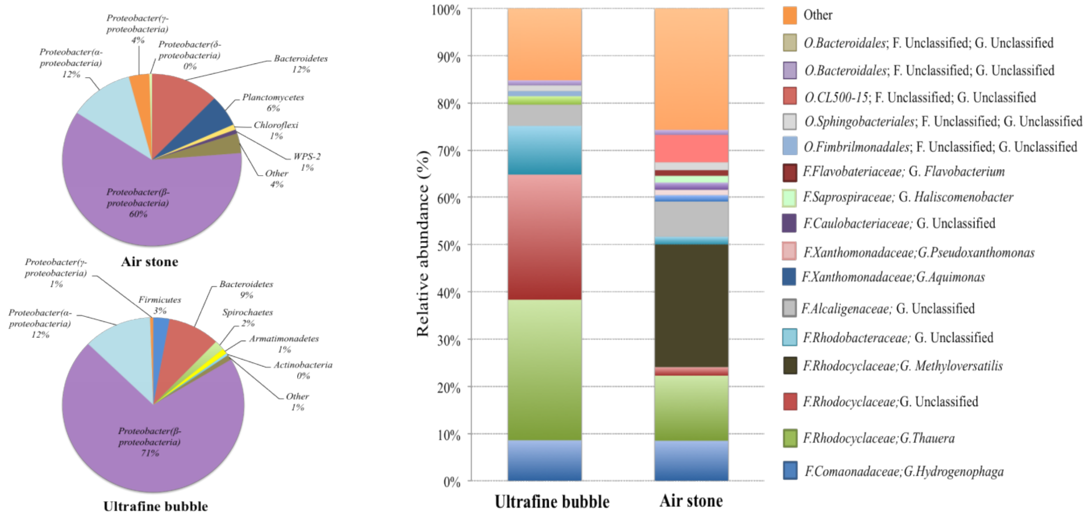


Figure 4. Performance of reactors using ultrafine bubble and air stone diffuser



(a) **(b)**

Figure 5. Variation in H_2 balance between H_2 consumption (%) and H_2 release (%) under (a) Ultrafine bubble system and (b) Air stone system



(a) **(b)**

Figure 6. Relative abundance of microbial community; (a) the phylum level of air stone and ultrafine bubble, (b) the family and genus levels under air stone and ultrafine bubble systems.