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Nitrogen Removal from Groundwater Using a Swim-bed Biological Reactor

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Abstract

High nitrate concentrations in drinking water cause a potential risk to public health, especially for infants. In this study, nitrate removal process using a novel acryl resin fiber biofringe (BF) material as biomass carrier and swim-bed technology was performed in a biological denitrifying reactor (DNBF). This process combines many advantages of both fixed-bed and fluidized-bed such as a long sludge retention time, low effluent suspended solids and turbidity, reduced sensitivity to toxic loads, high treatment efficiency and no need for sludge recycle, etc. Denitrification efficiencies of 80~90% at volumetric loading rates of up to 1.44 kg/m^3/d were achieved in this study with the simple operation and easy maintenance of DNBF process. Clear effluent with low SS levels of less than 10 mg/*l* were observed in whole experimental period. Sludge yield of 0.29 g VSS/g NO_3-$N_{removed}$ and the average sludge retention time of 44 days were approximately calculated. The adaptation in denitrification of DNBF to the change of reactor bulk DO level, stirrer speed, C/N ratio and influent NO_3^- concentration were also investigated in this study.

Keywords: biofringe, denitrification, groundwater, nitrification, swim-bed technology

INTRODUCTION

Nitrate is considered to be relatively less toxic for adult but it can cause health problems for infants, especially those under six months of age. Nitrate can easily be converted to nitrite in the environment by bacteria. In infants, nitrate interacts with the hemoglobin in red blood cells, which causes an oxygen deficiency resulting in methemoglobinemia, commonly known as "blue baby syndrome". The Would Health Organization (WHO) has set maximal allowable concentrations of 11.3 mg NO_3^--N/*l* and 0.03 mg NO_2^--N/*l* in drinking water[1]. Hanoi groundwater, the main source for Hanoi water supply, is presently not contaminated with nitrate (nitrate level of about 3 mg/*l*), but contaminated with ammonium at concentrations range from trace to 30 mg/*l*. The heavily polluted areas are located in the southern part of Hanoi[2]. At present, by applying the convention water treatment process of aeration, sedimentation, filtration and clorine disinfections, effectiveness of ammonium removal is very low in water treatment plants where groundwater sources containing high ammonium and iron concentrations[2]. Ammonium is easily converted to nitrite and nitrate through biological nitrification process. In order to meet the WHO standard, nitrogen removal treatment must be applied. Nitrification of ammonium-contaminated Hanoi groundwater was conducted in our previous studies[3, 4]. In this study, biological nitrate removal (deni-

trification) was experimentally studied. Biological denitrification is well known as conventional nitrate removal method. Other nitrate removal methods with different cost levels and removal performances such as ion exchange, reverse osmosis, and electrodialysis can also be used for drinking water. However these methods have several disadvantages such as excessive operational costs, operational limitations, and problem associated with the waste disposal of by-products, which can greatly reduced by the biological denitrification process[5].

Traditional nitrogen removal systems consist of aerobic nitrification by autotrophic organisms and anaerobic denitrification by heterotrophic organisms[5]. In the first step of nitrification, ammonium is finally oxidized via nitrite to nitrate. In the second step of denitrification, nitrate is reduced via nitrite to nitrogen gas N_2. New biological processes for ammonium removal have been developed recently, in which shortcuts in the nitrification-denitrification part are used, such as the denitrification via nitrite instead of nitrate in the Sharon process[6], or ammonium is oxidized anaerobically with nitrite to N_2 by autotrophic bacteria in the anammox process[7], or nitrogen removed over nitrite in the completely autotrophic as Canon process[8] and SNAP process[9]. These new processes have become of interest as the economical nitrogen removal methods. However all of these are applicable only for ammonium-rich wastewater treatment, but all of these new processes also have been reported as applicable process for the separated treatment of ammonium-rich wastewater. Thus, the separate nitrification and denitrification process is still considered the best method for nitrogen removal from ammonium contaminated groundwater.

In the denitrification step, under anoxic condition, nitrate is converted to harmless nitrogen gas by the following steps[10]:

$$NO_3 \rightarrow NO_2 \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Denitrification can be accomplished by both heterotrophic and autotrophic bacteria. In heterotrophic denitrification process, nitrate as the electron acceptor and organic substrate is electron donor. Various organic substrates have been used for biological heterotrophic denitrification processes such as ethanol, methanol, acetic acid or methane, etc, of which ethanol and methanol is widely used. Many studies have been carried out to compare ethanol and methanol as carbon sources for denitrification and ethanol was found to be more effective. Savia et al.[11], Magnus et al. [12], and Sara Hallin[13] reported that efficient denitrification using ethanol was established in a short time than by using methanol. Magnus[12] and Delanghe et al. [14] also reported that denitrification with ethanol is more stable compared to that with methanol. Methanol generally selected in practice because it less expensive[13] and less sludge production, but recently its use has been questioned due to poisonous effects if ingested[15].

Mateju *et al.*[16] reported the stoichiometric relationship of heterotrophic denitrification with ethanol (C_2H_5OH) as carbon source as given by Eq. (1)

$$0.613C_2H_5OH + NO_3^- \rightarrow 0.10C_5H_7NO_2 + 0.7124CO_2 + 0.286OH^- + 0.98H_2O + 0.449N_2 \quad (1)$$

Where $C_5H_7O_2N$ represents is biological cell formula. The substrate (C_2H_5OH) and NO_3^- ratio (g C_2H_5OH /g NO_3^-) is 0.455 or C/N ratio of 1.05 and 10% of applied NO_3-N is used for cell synthesis.

Heterotrophic biological denitrification has been widely studied, both at lab-laboratory and full-scale application. Different types of denitrification reactor were conducted as: suspended growth, packed or fix bed, and fluidized bed. Different support media were used as biomass carrier such as expanded schist, anthracite, and sand in fixed bed denitrification reactor, sand in fluidized bed reactor, and sodium alginate polymer in immobilized denitrification process[17]. Suspended culture provided higher nitrate removal than biofilms but clarifier for solid-liquid separation and biomass return is needed. Immobilized cells technology in fix bed denitrification reactor offers the advantage in high reaction rate; reduce reactor volume, stable operation without

clarifier and biomass recycle. However there are limitations on the rate of substrate diffution and reaction products through the biomass, which result in the detachment of sludge from the biofilm and reduced cell activity[17]. Clogging problem can also occurs in fix-bed denitrification reactors, thus frequent washing is requited.

In this study, swim-bed technology[18], involving the novel acryl resin fiber material of biofringe (NET Co. Ltd., BF-18) was applied for nitrogen treatment of groundwater. The biofringe material (BF) has a rough texture allowing for attachment of large amounts of biomass. The biofringe with a flexible fringe yarn matrix in a fix position induced by water flow creates a "swimming" motion that enhances mass transfer of nutrients to the attached growth biofilm. This process combines many advantages of both fix-bed and fluidized-bed attached-growth processes such as a long sludge retention time, low effluent suspended solids and turbidity, high treatment efficiency and no need for sludge recycle. This process can reduce sensitivity to the changes of operational conditions such as nitrogen loading rate, oxygen and temperature, etc. It also eliminates head losses with absence of clogging and channeling, which cannot be easily avoided in fix-bed processes. This process is continuously operated without medium or the requirement of screens or traps to prevent washout, which can be difficult to achieve in fluidized bed processes. Two reactors using the BF biomass carrier were used in this study, one for nitrification tests (named NBF) and another for denitrification tests (named DNBF). In this report, we focus on the denitrification process. Ethanol was used as organic substrate for denitrification in this study.

MATERIALS AND METHODS

Experimental system Figure 1 shows a schematic diagram and photographs of the denitrification experimental system used in this study. The main components of the experimental denitrification system consisted of the reactor, influent tank, carbon source and nutrients tanks, stirrer, and peristaltic feed pumps. The reactor used in this study was made from acryl resin and had a diameter of 210 mm and the height to the outlet port was 390 mm with a total volume of 14 *l*. The reactor had two main parts, the central column of 50 mm in diameter and 365 mm in height served as a mixing zone and downdraft section. The mechanical stirrer (FBLM 575 W-A, 3000 rpm) was placed in this zone for mixing as well as providing circulation throughout the reactor. Influent and ethanol-phosphate solutions were introduced within the downdraft section of the mixing zone using peristaltic pumps. The operational temperature of the reactor was maintained at 25℃.

The biological zone in the updraft section contained four double-yarns of BF as biomass carriers. The support filament of 325 mm in length contained 103 fringe yarns for each BF carrier. The total volume parking ratios and specific surface areas of the BF carrier were 0.69 % and 12.53 m^2/m^3, respectively. A pipe of 9 mm in diameter connected to an air pump containing 10 holes of 0.1 mm in diameter was placed in the bottom of the reactor for back washing.

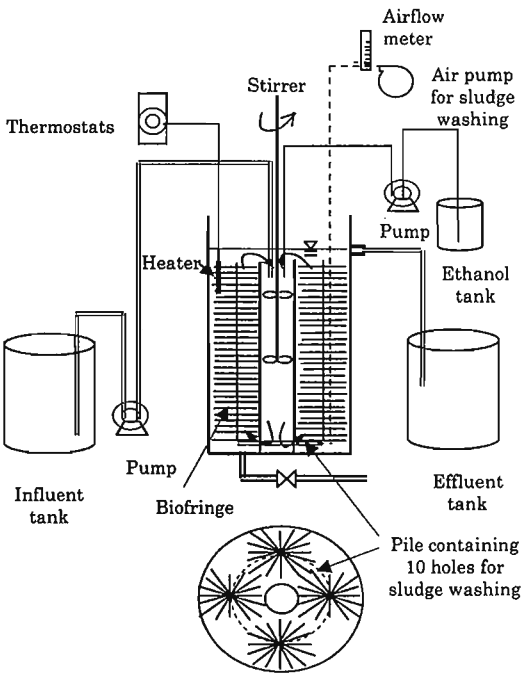

Fig. 1 Schematic diagram of the experimental system

The DNBF reactor was initially seeded with laboratory activated sludge. This sludge had also been used for the NBF reactors, in which oxygen was supply for aerobic growth conditions. Batch denitrification tests were conducted to confirm the denitrifying activity of the seed sludge. A specific denitrification rate of 2.5 mg-N/g-VSS/h was determined demonstrating that denitrifying biomass was present in the seed activated sludge. Figure 2 shows the photographs the BF material, reactor, and the DNBF reactor containing a large amount of sludge.

Experimental procedures For startup of the reactor, 28 g of seed activated sludge was inoculated in the DNBF reactor. Suspended solids (SS) analyses showed 98% and 100% of total sludge had attached on biofringe during 24 and 30 hours of liquid circulation, respectively. After the attachment stage of seed sludge, nitrogen removal experiments combining nitrification and denitrification were initiated. The effluent of the NBF was used as influent for a continuous-flow experiment in the DNBF, as shown in Fig. 3a (an HRT of 10 hours was kept in this period). From day 30, the combined system separated and synthetic influent containing 30 mg NO_3-N/*l* was introduced for the DNBF reactor (Fig. 3b). An HRT of 10 hours was maintained for an initial NO_3^- loading rate of

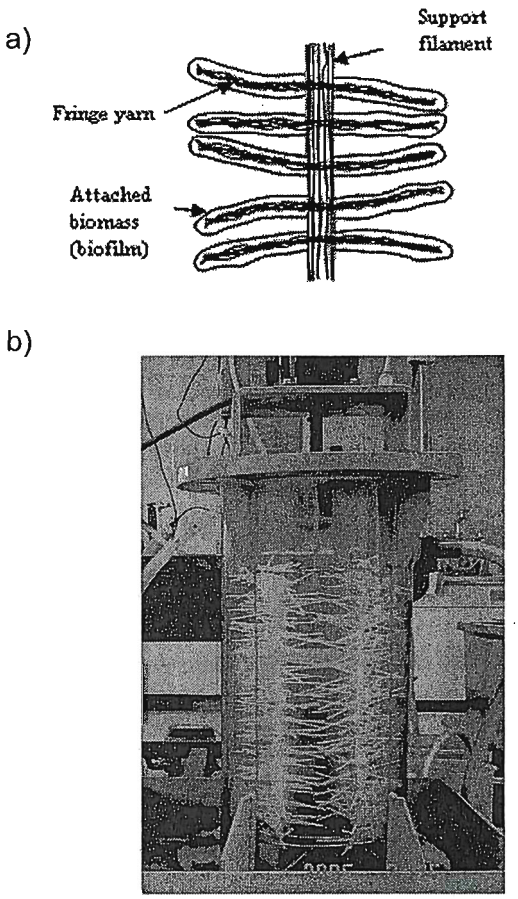

Fig. 2 Photographs of BF material and reactor during experiment

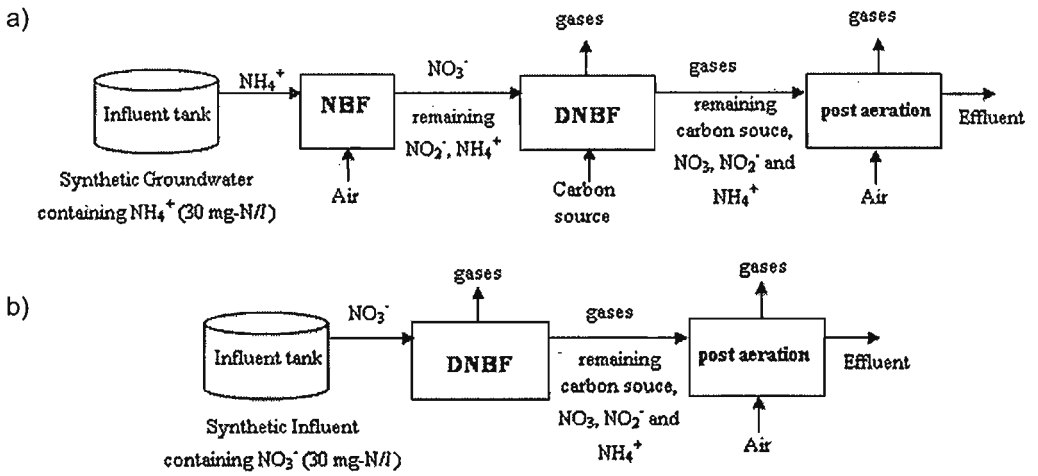

Fig. 3 Schematic diagram of a) the combination of nitrification and denitrification process used NBF and DNBF reactors and b) the denitrification process used DNBF reactor

0.072 kg-N/m³/d. Then, the NO_3^- loading rates were increased in a stepwise manner by decreasing the HRT to evaluate the denitrification capacity of the DNBF reactor. The influent NO_3^- concentration was increased to 50~90 mg-N/L from day 148 to examine denitrification efficiency of the DNBF reactor at a higher NO_3^- contaminated level. The stirrer speed and C/N ratios were changed during the experiment to investigate the optimum conditions and to find the minimum C/N ratio for effective treatment. Influent and effluent samples were taken almost daily for water quality analysis. Effluent SS concentrations were measured for calculation of sludge yield. Post aeration was needed after denitrification in order to remove by-product gases such as nitrogen and hydrogen sulfide. Oxygen supplementation for oxidation of remaining ethanol, NO_2^- and NH_4^+ were also conducted in this step.

Synthetic influents Two media were used in this study. In the first period, NBF effluent was used as influent for the DNBF reactor. Synthetic influent used for the NBF reactor was similar in composition with the polluted groundwater of Hanoi as mentioned in previous study reports[3, 4]. In the second period, the NBF and DNBF reactors were separated and tap water supplemented with 30 mg NO_3-N/*l* was used as synthetic influent for DNBF in order to investigate the nitrogen removal performance of the DNBF reactor. An influent containing a higher NO_3^- concentration of 50~90 mg NO_3-N/*l* was used also in this study. The main components and parameters of influents for the NBF and DNBF reactors are shown in Table 1. An ethanol-phosphate solution was fed separately using a peristaltic pump at C/N ratios in the range of 2.5 to 1. Phosphate was fed as nutrient for biomass growth at P/N ratios of 0.04.

Batch experiments For evaluation of the denitrifying capacity of the seed activated sludge and the activated sludge from the swim-bed attached-growth reactor, batch denitrification experiments were conducted at 25℃ in 1-liter elrenmyer flasks. A specific amount of denitrifying sludge was added to each flask with medium containing 303 mg $NaNO_3$ and 0.26 ml C_2H_5OH 95% (C/N ratio of 2). Tap water was used for dilution to give an initial NO_3^- concentration of 50 mg-N/L. KH_2PO_4 was also added (to 1.0 mg P/*l*) as a nutrient for biomass growth. Anoxic conditions were maintained by purging with argon gas. Shaker mixing was at a speed of 100 rpm at 25℃. Mixed liquor samples were taken every hour and supernatants obtained by centrifugation (3,000 g for 10 min) were used for water quality analyses.

Analytical methods Influent and effluent NO_3^-, NO_2^- and NH_4^+ were analyzed almost daily (at least 2 times a week) for the evaluation of nitrogen removal performance. In addition, total nitrogen (TN) was analyzed frequently for the evaluation of nitrogen removal. According to EPA methods[19], NO_3^- was determined by using the UV spectrophotometer colorimetric brucine method (352.1). According to standard methods[20], NO_2^- was determined by the colorimetric method (4500-NO_2^- B), COD by the closed reflux colorimetric (5220 D) and alkalinity by the titration method (2320 B). Effluent Total-N was determined on well-settled samples, thus not reflecting nitrogen in the biomass or sludge (though not excluding the soluble organic component). By the persulfate method all nitrogen is oxidized to NO_3^-, which was measured using the UV spectrophotometric screening method (4500-NO_3^- B) (the use of an ion analyzer was ineffective in this case due to interference from compounds in the persulfate solution).

Table 1 Composition of synthetic influents for NBF and DNBF reactors

Component and parameter	Influent for NBF	Influent for DNBF	
		I*	II
NO_3^- (mg N/*l*)	0.6-3	20-30	30; 50~90
NO_2^- (mg N/*l*)	0	0-3	0
NH_4^+ (mg N/*l*)	30	0-3.5	0
pH	7.5-8.4	6.8-7.5	7.1-7.6
DO (mg /*l*)	7-8.5	4.0-6.0	7.0-8.5
Alkalinity (mg-$CaCO_3$/*l*)	220-250	20-80	70-90

* BF effluent

NH_4^+ was determined by the OPP method[21]. A UV-visible spectrophotometer (U-2010, Hitachi, Japan) was used for absorption measurements. Reactor pH was monitored by using a pH meter (IM-22P; TOA Electronics, Ltd., Tokyo, Japan) and DO by a DO meter (OM 51; Horiba, Ltd., Kyoto, Japan). The effluent suspended solids (SS), mixed liquor suspended solids (MLSS) and volatile suspended solid (VSS) were measured weekly for the evaluation of sludge retention capacity using standard methods[20]

RESULTS AND DISCUSIONS

Kinetic analysis Batch experiments were conducted to determine the kinetic parameters of the denitrifying sludge. Two sludge samples were taken from DNBF reactor on days 5 and 45 (HRT, 10 hours). The Michaelis-Menten kinetic model was applied. Fig. 4 shows the Lineweaver-Burk's plots used for the determination of the kinetic constants for denitrification. From these plots, the specific maximum denitrification rate (V_m) and saturation constant (K_m) were determined to be:

V_m= 0.44 mg-N/mg VSS/d and K_m= 25.1 mg NO_3-N/l for the sludge sample of day 5

V_m= 0.76 mg-N/mg VSS/d and K_m= 4.06 mg NO_3-N/l for the sludge sample of day 45

A much higher maximum denitrification rate and lower saturation constant were obtained for the denitrifying sludge which had a longer acclimation time in the swim-bed DNBF reactor, thus demonstrating a higher affinity of the DNBF sludge for the substrate.

Denitrification performances Operational conditions for the DNBF process are shown in Table 2. The whole experimental period was divided into 11 runs. Fig. 5 shows the changes in HRT, VLR, influent and effluent nitrogen concentrations, and denitrification efficiencies for the DNFB reactor during

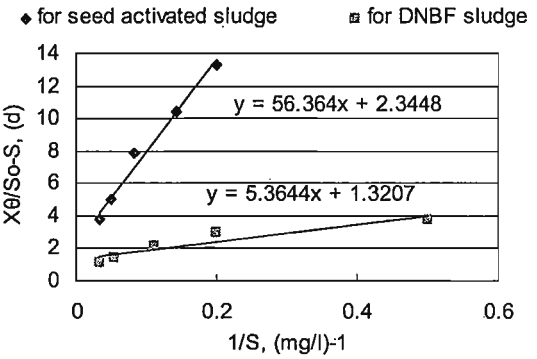

Fig. 4 Linerweaver-Burk's plots for determination of kinetic coefficients K_m and V_m for seed activated sludge and DNBF sludge

Table 2 Operating conditions for DNBF reactor (averages)

Run	Days of operation (d)	Inf. NO_3-N conc. (mg/l)	HRT (h)	VLR* (kg NO_3-N/m^3/d)	Stirrer speed (rpm)	Reactor bulk DO (mg/l)	C/N ratio
A	(1-29)	23-29	10	0.072	1600	1.2~1.5	2~2.5
B	(30-35)	30	10	0.072	1600	1.2~1.5	2~2.5
C	(36-49)	30	10	0.072	2000	4.0~5.2	2~2.5
D	(50-59)	30	10	0.072	1600	0.2~1.2	2~2.5
E	(60-78)	30	7-3	0.10-0.24	1600	0.2~1.2	2
F	(79-119)	30	3-1.5	0.24-0.48	1000	0~0.5	2
G	(120-133)	30	1	0.72	1000	0~0.5	2
H	(134-147)	30	0.5	1.44	1000	0~0.5	2
I	(148-155)	50	1	1.2	1000	0~0.5	2
K	(156-160)	60	1	1.44	1000	0.3~0.7	2
L	(161-165)	90	1	2.16	1000	0.3~0.7	2
M	(166-170)	30	1	0.72	1000	0.3~0.7	1
N	(171-173)	30	1	0.72	1000	0.3~0.7	0.8

* VLR: Volumetric loading rate

Runs A-G. An HRT of 10 hours corresponding to a volumetric loading rate (VLR) of 0.072 kg-N/m^3/d was maintained during Runs A-D.

In Run A, the DNBF reactor was fed nitrified influent (NBF effluent). In this period, the NBF reactor had just restated and was operated at a short HRT of 5 hours for supplying 1.4 *l*/h to the DNBF reactor (DNBF operated at HRT of 10 hours). Nitrification efficiencies of about 80 and 90% and high effluent NH_4^+ concentrations of 2~4.3 mg/*l* were obtained for the NBF reactor in this period.

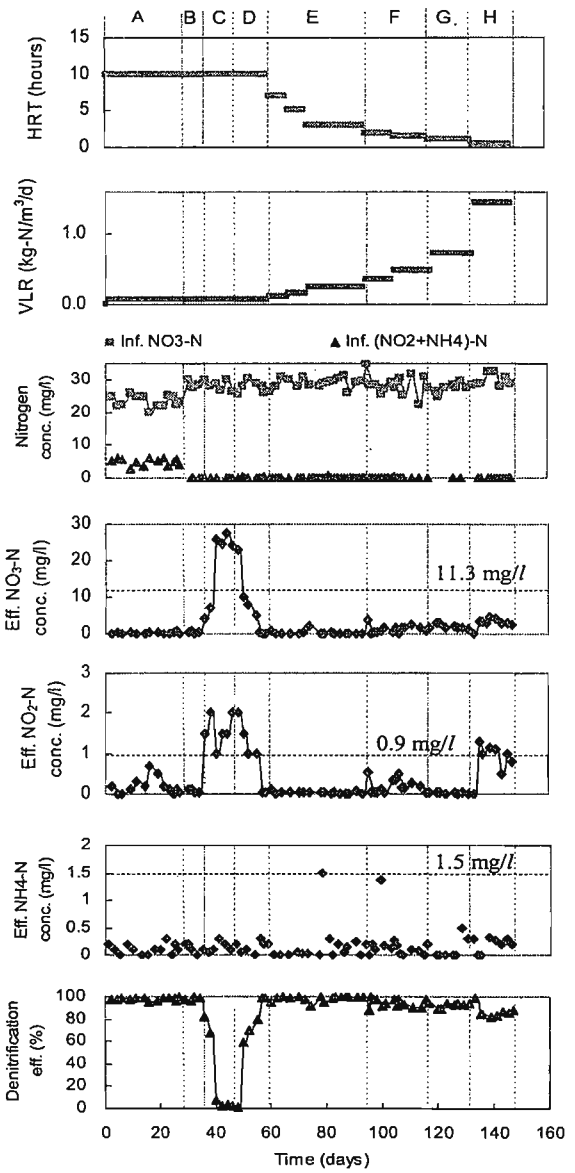

Fig. 5 Changes in HRT, VLR, influent and effluent nitrogen concentrations and denitrification efficiencies for DNBF during Runs A-H

The DNBF process influent contained 23-26.5 mg NO_3-N/*l* and 2.5~6 mg (NO_2+NH_4)-N/*l*. Influent DO and pH levels were in range of 4~6 mg/*l* and 6.8~7.4, respectively. The stirrer speed was set at 1,500 rpm and the observed bulk DO concentrations were 1.2~1.5 mg/*l* for the DNBF reactor during this run. The results showed that effluent NO_2-N, NO_3-N, NH_4-N (Fig. 5) and TN for the DNBF process were close to zero during this run demonstrating that nitrogen was effectively removed and both nitrification and denitrification occurred in the DNBF reactor. Denitrification efficiencies remained high at 98~100% with a high bulk DO of 1.2~1.5 mg/*l* while previous studies reported that denitrification rate decreased to zero when DO reach 1.0 mg/*l*[8]. These different results can be explained by using the theoretical assumption which is presented in Fig. 6a. A large sludge amount was retained on biofringe creating a thick biofilm with the thickness ranging from 2 to 15 mm. Denitrifying biomass was also formed as flock with flock size in the range of 2~12 mm (Fig. 7). The previous studies have been demonstrated that DO concentrations in the biofilm strongly depend on the bulk DO concentrations, thickness of biofilm and diffusion factor. DO levels in the biofilm decrease from outside to inside layers of the biofilms or flocks, clusters. Nitrification can occur in the outside layers (aerobic zone) of the biofilm where oxygen is available and denitrification can occur in the deeper layers (anoxic zone) of the biofilm, where DO is close to zero.

From day 30, the NBF and DNBF reactors were separated, and a medium consisting of 30 mg NO_3-N/*l* was used as influent for the DNBF reactor. Influent DO and pH levels were in the ranges of 7.0~8.5 mg/*l* and 7.1~7.6, respectively. The DNBF reactor obtained high denitrification efficiencies of 98~100% in Run B. From day 36 the stirrer speed was increased to 2,000 rpm to improve circulation of water through the biological

zone (Run C). Bulk DO concentrations in the biological zone increased to 4.0~5.2 mg/*l* during this run. Denitrification efficiencies decreased sharply during this run and dropped to zero at days 44-48. As shown in Fig. 6b, the high bulk reactor DO levels resulted in enlargement of the aerobic zones, which led to the anoxic zones becoming smaller and the denitrification becoming weaker. From these results, it was found that denitrification was inhibited at DO concentration of 4 mg/*l* and stopped when DO concentrations reach to 5 mg/*l* in the swim-bed DNBF reactor. High stirrer speed resulting on higher water flow and cycle was a reason for the higher bulk DO levels in the DNBF reactor due to oxygen diffusion from the air. High water flow speed through the biological zone at high stirrer speeds is also a reason for high DO levels in the biofilm due to the high diffusion of oxygen. At stirrer speed of 1500 rpm, water flow velocity thought the BF zone (updraft section) was about 12 cm/s and that was 22 cm/s at stirrer speed of 2000 rpm. When the stirrer speed was reduced again to about 1,500 rpm from day 50, the bulk DO concentration decreased to less than 1.2 mg/*l* in Run D and nitrogen removal efficiencies increased sharply to 99% at day 56. Then NO_3^- VLRs were increased stepwise to 0.72 kg-N/m^3/d corresponding to an HRT as short as one hour (Runs E, F and G). High denitrification efficiencies of 90~100% were obtained in these Runs. Very small decrease in denitrification efficiencies occurred when the VLR was increased sharply. This demonstrated that DNBF denitrifying bacteria were in sufficient number and quickly adapted to a sharp increase in VLR.

Denitrification efficiencies decreased to 80~90% in Run H when the VLR was increased to an extremely high level of 1.44 kg-N/m^3/d (HRT was 0.5 hour), which resulted in higher effluent TN levels of 3~6 mg/*l*, but these values were still below the maximal acceptable nitrogen concentration for drinking water[1]. Fig. 8 shows the nitrogen removal rate as a function of loading rate. These results were high in comparison to that of other drinking water denitrification processes shown in Table 3.

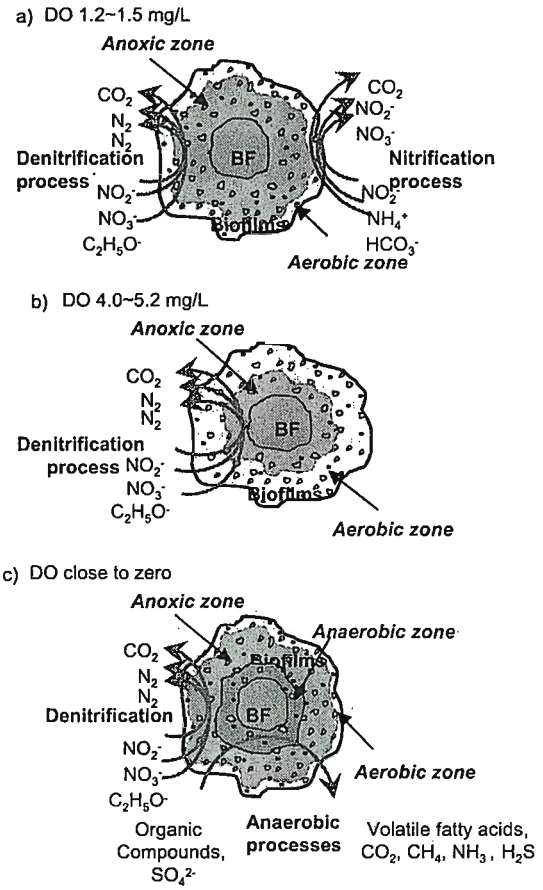

Fig. 6 Biological reaction within bacterial biofilms

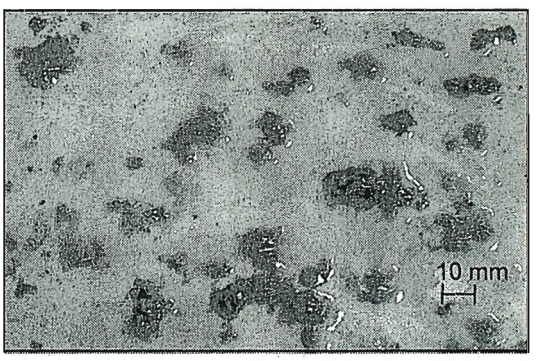

Fig. 7 Photograph of sludge detached from the DNBF biomass in flock shape

Accumulation of excess sludge resulted in an enlargement of the anaerobic zone. This phenomenon resulted in the production of hydrogen sulfide smell and increases in effluent NH_4^+ concentration as well as COD removal (Fig. 6c). By using air back-washing (20 *l*/min for 1min), most of the sludge detached from the BF material and then reattached while some washed out of the reactor. Denitrification efficiencies decreased 2~4% on the day of sludge washing and then again increased indicating that the reattached sludge was sufficient for denitrification process at the applied VLR.

From day 148, in order to examine the adaptation of the DNBF reactor to treating higher NO_3^- contaminated groundwater, the HRT was kept at 1 hour and influent NO_3^- concentration was increased to 50~90 mg-N/*l* (Run I, K, L). High denitrification efficiencies of 90~95% at VLR of 1.2 kg-N/m^3/d were achieved during Run I. During Run K, when the loading rate was 1.44 kg-N/m^3/d (NO_3^- concentration was 60 mg-N/*l*), denitrification efficiencies of 88~90% were achieved, with effluent TN in a range of 6~7 mg/*l*. These results demonstrated that for the same loading rate of 1.44 kg/m^3/d, a higher influent NO_3-N concentration and longer HRT or contact time (HRT of 1 hour versus 0.5 hour) did not have much influence on denitrification efficiency. These results demonstrated that the DNBF process had a high adaptability for treating higher nitrate concentration influent. At a high loading rate of 2.16 kg-N/m^3/d during Run L, denitrification efficiencies decreased to 82~86%, resulting on high effluent TN levels of up to 11.6~16.0 mg-N/*l*. Effluent NO_3^- and NO_2^- were 5.7~6.9 and 4.6~10.3 mg-N/*l*, respectively. Higher nitrogen removal rates of 1.73~1.84 kg-N/m^3/d were achieved in this run, but effluent TN concentrations exceed the maximum contaminated limit for TN in drinking water. Thus a longer HRT is required.

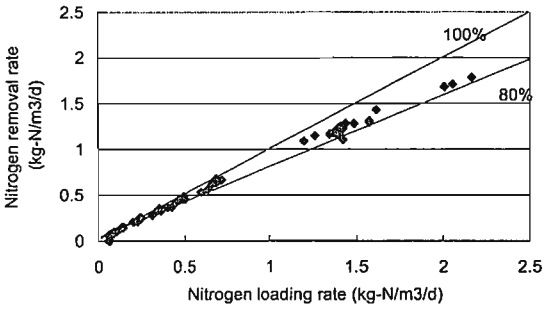

Fig. 8 Nitrogen removal rate as a function of loading rate

DO influence As mentioned in the previous section, the DO concentration in the DNBF reactor affected denitrification rate. The denitrification rate decreased sharply and even stop when reactor bulk DO increased to 4~5.2 during Run C. At DO concentrations of 1.2~1.5 mg/*l* during Run A, B, D, E and 0.3~0.7 mg/*l* during Run H~L, effective denitrification could be achieved with high denitrification efficiencies and high NO_3^- loading rates up to 1.44 kg-N/m^3/d. At very low DO concentrations of closed to zero and excess sludge accumulated during Run F, anaerobic reaction occurred resulting on the production of hydrogen sulfide smell and

Table 3 Comparison of denitrification performance for drinking water production in different systems

System	Electron donor	Removal rate (kg NO_3-N/m^3/d)	Temp. (℃)	Reference
Fluidized-bed Bioreactor	Hydrogen	0.34	18-23	25
Fixed-bed Bioreactor	Ethanol	0.75	12	26
Fixed-bed Bio-electrochemical reactor	Hydrogen	0.25	25	27
Immobilized Bioreactor	Starch	0.46		28
Membrane Bioreactor	Ethanol	0.30	25	32
Swim-bed DNBF reactor	**Ethanol**	**1.84**	**13**	**This study**

increases in effluent NH_4^+ and COD consumption.

Temperature influence Denitrification was affected by temperature, though it is less sensitive in comparison with that of nitrification. As similar to other biological process, optimum temperature for denitrification is in range of 30~35℃. At low temperatures, denitrification decreased markedly due to the higher oxygen solubility, thus decreasing the biological denitrification rate. Gauntlett and Craft reported that, with every 10℃ increased in temperature, a doubling of denitrification rate is possible[22]. The DNBF reactor temperature was kept at 25℃ through most of the study. During Runs K, L, M and N, at high loading rates of 1.44~2.16 kg-N/m^3/d (30~90 mg influent NO_3-N/*l*, HRT 1 hour), the temperature in the reactor was reduced to 12~13℃ due to very cold weather even though the heater was set at 25~30℃. Reactor bulk DO levels were in a range of 0.5~0.7mg/*l* during these runs and no decreases in denitrification efficiencies were observed. High denitrification efficiencies of 90% were still achieved during Runs K, M and N.

Low sludge growth rate caused by low temperature might not affect denitrification rate because the BF material can retain a large amount of denitrifying biomass. As mentioned before, a large amount of sludge attached on biofringe was composed of thick biofilms, and DO concentrations in denitrification zone within biofilm was not the same as reactor bulk DO concentrations. Thus, denitrification was not inhibited by a higher DO levels in the DNBF reactor due to low operational temperature condition. These results demonstrated that effective denitrification could be achieved even at a low temperature of 12℃ using the DNBF process.

Carbon to nitrogen (C/N) ratio and COD consumption C/N ratio was maintained at a high value of 2~2.5 throughout most of time of the experiment to ensure that organic substrate was always available (as shown in Table 2). Changes in influent and effluent NO_3-N and COD concentrations during the DNBF experimental runs are shown in Fig. 9. High organic substrate degradations of 60~90% were observed during Runs A, B, and D, with mg COD consumption per mg of NO_3-N denitrified to nitrogen gas were calculated to be 5.0 to 6.8 and the C_{used}/N ratios ranging from 1.41 to 1.64 were estimated during these Runs (1 mg C was approximately 3.9 mg COD). These values were high in comparison with the calculated value from the stoichiometry of the denitrification process (Eq. 4-1), which shows that denitrification of 1 mg NO_3-N to nitrogen gas consume 1.05 mg C. These were also higher than the results reported in previous studies[10, 26, 27]. The COD removals remained at 40~50% during days 38~42 (Run C), when the reactor bunk DO reach to about 5 mg/*l* and denitrification decreased to zero. This clearly showed that organic carbon acted as an electron donor for nitrate reduction as well as a source of cellular substrate for biological respiration in anoxic denitrification and it also influenced the depletion of oxygen in the DNBF reactor (aerobic activity). The high stirrer speed of 1,500~2,000 rpm could be the only reason for oxygen diffusion from the air. This explanation was strongly confirmed when stirrer speed was reduced to 1,000 rpm and COD degradation subsequently decreased to 38~46% during Run E. COD consumption per mg of NO_3-N denitrified to nitrogen gas was calculated to be 3.4 to 4.2 during this period. Then these values again increased to 5.0~6.2 during days 70~74, which might be because the COD degradation increased due to anaerobic biological activity, as noted in the previous section. After sludge

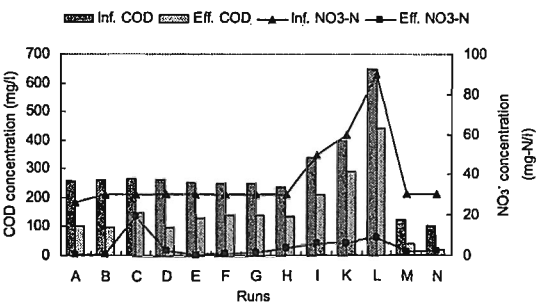

Fig. 9 Changes in influent and effluent COD and NO_3-N during experiment for the DNBF reactor

washing, sludge was partly washed out, these values again decreased. During Runs I, K and L, at high loading rate of 1.2~2.16 kg-N/m³/d, the COD consumptions per mg of NO_3-N denitrified to nitrogen gas were 2.7~3.2 mg, approximately. The C consumption per mg NO_3-N denitrified to nitrogen gas were calculated to be 0.7 to 0.93 during Runs F-L. Changes in mg COD/mg NO_3-$N_{removed}$ ratio and bulk DO for the DNBF reactor are shown in Fig. 10. The mg C consumed per mg NO_3-N denitrified to nitrogen gas were calculated to be 0.7 to 0.93 during Runs F-L.

Very high denitrification efficiencies of 80~100% resulting in low effluent TN levels of 0~6mg/*l* in the effluent were obtained throughout the experiment for treating 30 mg NO_3-N/*l* influent with high NO_3^- VLR of up to 1.44 kg-N/m³/d. These values were much lower compared to the WHO maximum allowable nitrate level (11.3 mg-N/*l*), indicating that the lower C/N ratio is acceptable. Lower C/N ratio of 1 and 0.8 were applied during Runs M and N for determining the lowest acceptable C/N ratio in continuous denitrification experiment for treating nitrified Hanoi groundwater. The influent contained 30 mg NO_3-N/*l* and HRT was 1 hour during these Runs. Denitrification efficiencies of about 91 and 88% were observed for Runs M and N, respectively. The effluent NO_3^- and NO_2^- concentrations of about 2.7~3.3 and 0.01~0.15 mg-N/*l*, respectively, during these runs, were still much lower in comparison to the maximum allowable level set by WHO, so the applied C/N ratio could be more reduced.

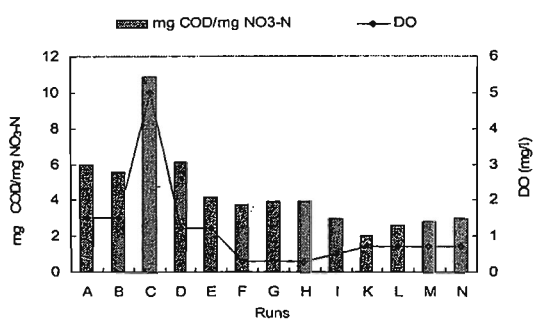

Fig. 10 Change in COD/mg NO_3-N removed and bulk DO for the DNBF reactor

Alkalinity production and pH Influent alkalinity levels were in a range of 20~90 mg-$CaCO_3$/*l* and effluent alkalinities were between 90 and 190 mg-$CaCO_3$/*l* and the reactor pH levels changed from 7.1 to 8.1 during Runs A~H, which is within the optimum range for denitrification[29]. No pH adjustment was requited for efficient denitrification in the DNBF reactor. Considering that 7.1 mg alkalinity was consumed for 1 mg of NH_4-N oxidized as the results found for nitrification study[3], alkalinity production per mg of nitrate nitrogen reduced during Run A was 3.5 mg $CaCO_3$ and during Runs B~E, these values were estimated to be in the range of 3~4 mg. These values were in accordance to that of other studies[30], which have shown that 3.5 mg alkalinity as $CaCO_3$ is produced per mg NO_3-N reduced to nitrogen gas. High NO_3^- influent concentrations of 60~90 mg/*l* resulted on high effluent alkalinity ranging from 250 to 280 mg/*l* during Runs K and L, respectively. Reactor pH levels increased up to 9.1 during these Runs. No significant reduction on nitrogen removal rates during these runs with high denitrification efficiencies of up to 90 and 86% were observed during Runs K and L, respectively, at loading rates as high as 1.44~2.16 kg/m³/d.

Effluent SS, sludge yield, and sludge withdrawal Clear effluent with very low SS concentrations of 0~5 mg/*l* were obtained with nitrate VLRs up to 0.48 kg-N/m³/d. Effluent SS levels increased slightly to less than 10 and 15 mg/*l* at VLRs of 0.72 and 1.44 kg-N/m³/d, respectively during Runs G and H. These results demonstrated that the BF biomass carrier could retain high amounts of sludge. The end product of nitrogen gas escaped as gas bubbles, which bound to the suspended sludge and caused sludge to rise to the surface of the reactor. This sludge was estimated to be 0~5 mg per litter of influent and needed to be removed frequently only during Runs F, G and H. Bulk MLSS concentrations during experiment were approximately the same as the SS concentrations in the effluent.

After a long operational period of 140 days, the effluent SS levels decreased to less than

5 mg/*l* event at high VLRs of 1.44 kg-N/m^3/d and short HRT of one hour, and sludge on the water surface of the reactor was close to zero. Even without occasional back washing, no smell of hydrogen sulfide (H_2S) occurred and effluent ammonium levels closed to zero during this runs. These results demonstrated that sludge growth might be close to a stationary phase in this period. A total sludge amount in the DNBF reactor of 148 g was estimated at day 180 and the observed sludge yield (Y_{obs}) of 0.29 g VSS/g NO_3-N removed was estimated for the first 180 days using Eq. 2. The average sludge retention time (θ) of 44 days was also estimated in this period using Eq. 3 when sludge yield Y and decay rate k_d were 0.8 and 0.04 1/d, respectively, which were typical value for denitrifying sludge[32]. Further research is needed to investigate the denitrifying sludge growth rate as well as the sludge withdrawal rate under different operational conditions.

$$Y_{obs} = \frac{(C_t - C_o) + \sum_{i=1}^{n} C_i + \sum_{i=1}^{t} (C_{eff})_i}{\sum_{i=1}^{t} S_i} \quad (2)$$

$$Y_{obs} = \frac{Y}{1 + k_d \theta_c} \quad (3)$$

where:

Y_{obs} observed sludge yield coefficient (g-VSS/g-NO_3-N removed)
$(C_{eff})_i$ amounnt of suspended solid in effluent (g-VSS)
C_t biomass in the reactor at time t (g-VSS)
C_o biomass in the reactor at time zero (g-VSS)
$\sum_{i=1}^{n} C_i$ withdrawal total sludge in n times during experiment (g-VSS)
S_i amount of NO_3^- removed (g- N)
t operation time (d)

CONCLUSIONS

An attached-growth DNBF swim-bed reactor was shown to be effective for achieving high nitrogen removal performances. Very low effluent TN levels of less than 2 mg N/*l* were obtained even at a high VLR of 0.72 kg/m^3/d, corresponding to an HRT of only 1 hour with an influent of 30 mg/*l* NO_3-N. Even at an extremely high VLR of 1.44 kg/m^3/d, high denitrification efficiencies of 80 ~90% were obtained with effluent TN concentration of about 3~6 mg/*l*, which are well below the maximum allowable level for nitrogen in drinking water. The BF biomass carrier also offered a big advantage with respect to sludge retention capacity, which was demonstrated in low effluent SS levels even at an HRT as short as one-half hour.

The BF material is a hydrophilic acrylic composite that is light weight, inexpensive and durable; thus the BF swim-bed attached-growth process is easy to operate and maintain and is economically favorable. The NBF-DNBF system appears to be a good choice for removing ammonium from Hanoi groundwater. Also, as on extension of preliminary observations in this research, the possibility of using the swim-bed technology for denitrification at much higher loading rates (higher nitrate concentrations) conducive to industrial applications would be of value.

Finally, while simultaneous nitrification and denitrification with concurrent BOD removal were demonstrated here, the possibility of meaningfully using these reactions in a single unit process would be a stimulating avenue of study.

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