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Evaluation of the Swim-Bed Attached-Growth Process for Nitrification of Hanoi Groundwater Containing High Levels of Iron

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Abstract

A swim-bed attached-growth bioreactor (BF reactor) using a novel acryl-fiber biomass carrier (Biofringe: BF) for treating Hanoi groundwater, which is polluted by high levels of ammonium, has been developed. The swim-bed technology is aerobic and combines the advantages of fix-bed attached-growth processes, which can retain high amounts of slowly growing nitrifiers, and moving-bed attached-growth processes, which avoid clogging problems. Experiments were conducted in 7.7-*l* reactors, using synthetic Hanoi groundwater, containing 30 mg-N/*l* of ammonium. Two reactors (BF1 and BF2) were used to investigate the ammonium removal capacities; BF1 was fed influent containing 5 mg/*l* of iron and BF2 was fed influent without iron. Maximum ammonium removal rates of 0.24 and 0.48 kg-N/m^3/d, corresponding to hydraulic retention times (HRTs) of 3 and 1.5 hours were achieved for BF1 and BF2, respectively and nitrification efficiencies are close to 98% for the both reactors. The ferrous form of iron was oxidized to the ferric form as a hydroxide ($Fe(OH)_3$), which was mostly washed out. This resulted in a high iron removal efficiency (98%) with effluent suspended solid (3-6 mg/*l*) containing a low volatile suspended component (20%). Nitrification efficiency decreased sharply due to a decrease in temperature from 25 to 15℃, but efficiency quickly recovered following 1 day of operation, demonstrating that the attached-immobilized nitrifiers in BF reactor were able to adapt to the decrease in temperature. Nitrifying bacteria communities from BF reactors were investigated with rRNA-based molecular techniques, and ammonia oxidizers as *Nitrosomonas* were found in both reactors. Ammonia oxidizers identified as a *Nitrosospira* sp. were also found in BF2.

Key words: Ammonium removal, biofringe, groundwater, iron concentration, nitrification, swim-bed

INTRODUCTION

Hanoi is the second largest city of Vietnam and has an increasing water demand for domestic and industrial uses due to rapid development and urbanization. Hanoi city produces about 500,000 m^3/d of potable water from eight water treatment plants using groundwater drawn from 10 well fields. All of this groundwater is from a deep aquifer and often contains high concentrations of iron (1-25 mg/*l*) due to naturally anaerobic con-

ditions in the aquifer. The results of water quality assessments carried out by different organizations showed that Hanoi groundwater was heavily polluted with ammonium concentration ranged from trace to 30 mg-N/*l*[1, 2]. In addition, the effectiveness of ammonium removal efficiencies is very low in most of the water treatment plants that apply aeration, sedimentation, filtration and chlorine disinfections[2].

Ammonium does not have direct relevance to health and no health-based guidelines have been proposed. Although the threshold odor concentration of ammonium is approximately 1.5 mg/*l* and a taste threshold of 35 mg/*l* has been proposed. Ammonium present in water can easily be converted to nitrite and nitrate, which are associated with serious environmental and human health problems. Thus, in order to meet the maximum acceptable concentrations of nitrate (50 mg NO_3^-/*l*) and nitrite (3 mg NO_2^-/*l*) set by WHO[3], ammonium must be removed. The maximum acceptable concentration of 1.5 mg-N/*l* for ammonium in drinking water, set as the Vietnamese standard based on WHO guidelines is commonly exceeded in most parts of Hanoi.

There are various kinds of ammonium removal processes for water and wastewater treatment such as biological treatment, chemical treatment using ammonia stripping, ion exchange and chlorine dosing. Among these methods, biological treatment is widely adopted due to its being efficient, inexpensive and easy to maintain. The biological method is also relatively free of unwanted by-products.

For biological nitrogen removal, the traditional method combines aerobic nitrification by autotrophic organisms and anoxic denitrification by heterotrophic organisms[5]. In the fist step, ammonium is oxidized to nitrate via nitrite, and in second step nitrate is reduced to nitrogen gas (N_2) via nitrite. New biological processes for ammonium removal have been developed recently, in which shortcuts in the nitrification-denitrification process are used, such as denitrification via nitrite instead of nitrate in the Sharon process[6], anaerobic oxidation of ammonium with nitrite to N_2 by autotrophic bacteria in the anammox process[7], or completely autotrophic removal of nitrogen over nitrite in the Canon[8] and SNAP process[9]. However, all of these new processes have difficulties such as controlling nitrite formation (Sharon) or requiring NH_4^+/NO_2^- ratio (Anammox) and have been reported as applicable process for the separated treatment of ammonium-rich wastewater, where a significance amount of carbon sources can be saved. The traditional biological nitrification-denitrification process is still considered as the best for ammonium removal from groundwater for drinking water proposes.

In the nitrification step, ammonium is converted to nitrite by ammonia oxidizers and then nitrite oxidizers convert nitrite to nitrate under aerobic conditions. The organisms involved are characterized by low specific growth rates[10], thus the retention of biomass in the unit process is an important factor. The swim-bed attached-growth processes, which was defined by Rouse, *et al.*[11], using a novel biofringe (BF) attachment material, was used to evaluate ammonium removal of synthetic Hanoi groundwater in this study. The process is aerobic and combines the advantages of fix-bed attached-growth processes, which can retain large amounts of slowly growing nitrifiers, and moving-bed attached-growth processes, which have no clogging problems. These advantages enable efficient ammonium removal from groundwater containing high levels of iron, where problems due to iron hydroxide precipitation on biomass carriers can be eliminated.

To identify the bacteria responsible for nitrification, many studies have been carried out. *Nitrosomonas* is the most frequently identified genus of ammonia oxidizers. Together with it, other genera like *Nitrosospira* and *Nitrosococcus* are also identified as ammonia oxidizing bacteria[4]. All are generally belonging to the β subclass of the class *Proteobacteria* except for the marine genus *Nitrosococcus*, which belongs to the γ subclass[12]. For the second step of nitrification, the *Nitrobacter* genus, belonging to the α subclass of *Proteobacteria* is the most frequently identified genus. Other

genera like *Nitrospina*, *Nitrococcus* and *Nitrospira* are also identified as nitrite oxidizing bacteria[4]. To investigate the microbial community responsible for nitrification in swim-bed attached-growth reactors, the cloning and sequencing of PCR-amplified partial 16S rDNA genes were used for characterization in this study.

In addition, temperature has been reported as one of the major factors affecting nitrification[5]. Hanoi is located on the northern part of Vietnam and is distinguished as a monsoonal climate region with a hot, rainy season (mid-May to mid-September), and a dry season (mid-october to mid-March). Winter is known as the cold season with many days when the temperature drops below 10 ℃ and the water temperature in treatment plants can often be too low for effective nitrification activity. Thus, the effects of temperature on nitrification in a BF reactor were also investigated in this study.

The objectives of this study were:

- To investigate ammonia removal performance of a BF reactor using the swim-bed attached-growth technology.
- To investigate the influence of iron on nitrification performance in a BF reactor
- To study the impact of temperature on nitrification in a BF reactor
- To identify the dominant bacteria of the biomass off a nitrifying BF reactor.

MATERIALS AND METHODS

Physical description of the experimental system Two reactors (BF1 and BF2) using BF as biomass carrier were used in continuous-flow experiments. Synthetic groundwater containing iron was used as the influent for BF1 and synthetic groundwater without iron was used as the influent for BF2. A schematic diagram of the experimental systems is shown in Fig. 1. Reactors were made of acryl resin and had two main parts, downdraft and updraft sections of 100 × 100 mm and 100 × 25 mm, respectively. Each reactor had a total liquid volume of 7.7 *l* and a height to effluent port of 630 mm with a 530 mm biofringe zone. The biomass carrier was a novel BF material composed of 3-mm diameter fringe yarns (NET Co. Ltd., BF-18) attached to a support filament (Fig. 2). The staple fiber of biofringe yarns is a hydrophilic acrylic composite. The support filament was 530 mm in length and contained 192 fringe yarns with a total biomass carrier volume ratio of 0.5% and specific surface area of 11.74 m^2/m^3. Influent was fed into the updraft section using a peristaltic pump. Air was supplied by an air pump at 2.0 *l*/min to the bottom part of the updraft section using an air diffuser for oxygenating the synthetic groundwater and providing circulation through the reactor. The temperature of the reactors was regulated thermostatically.

Activated sludge obtained from an aerobic basin of a municipal wastewater treatment plant that was cultivated by fill and draw under total oxidation conditions over a period of year in our laboratory using synthetic wastewater mainly composed of meet extract and peptone, was used as seed sludge of this study. In the sludge attachment period, 18.7 g and 18.2 g of activated sludge were attached to the carriers of BF1 and BF2,

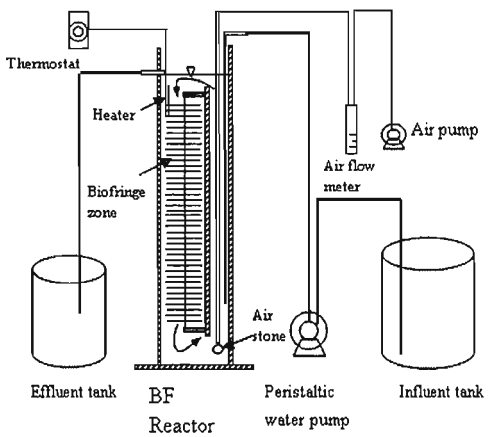

Fig. 1 Schematic diagram of experimental system

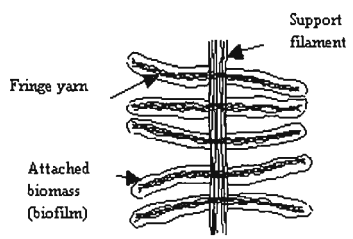

Fig. 2 Configuration of biomass carrier

respectively, during 30 hours of aerobic liquid circulation, which amounted to approximately 2.4 g-MLSS/$l_{reactor}$ and 1.9 g-VSS/l (80%). Influent was feed continuously at a flow rate of 0.43 l/h, corresponding to a hydraulic retention time (HRT) of 18 hours. HRT was reduced in a stepwise manner to determine the maximum acceptable loading rate for effective ammonium removal. The operational conditions for BF1 and BF2 are detailed in Table 1.

Synthetic groundwater composition In this study, synthetic ammonium-polluted groundwater was prepared base on Hanoi groundwater composition, which was reported by Hanoi Clean Water Business Company[1]. Table 2 shows the chemical composition of this synthetic groundwater, containing a maximum ammonium concentration of 30 mg-N/l. Iron in the form of $FeSO_4 \cdot 7H_2O$ was added to the influent of BF1. The pH of the synthetic groundwater ranged between 7.8 and 8.3.

Table 1 Operational conditions for BF1 and BF2

Run (d)	HRT (h)	VLR* (kg NH_4-N/m^3/d)	Iron conc. (mg/l)	Temperature (℃)
BF1				
A (0-45)	18-4	0.04-0.18	2	25
B (46-54)	4	0.18	5	25
C (55-73)	3	0.24	5	25
D (74-87)	2	0.36	5	25
E (88-99)	2.5	0.29	5	25
F (100-120)	3	0.24	5	25
G (121-137)	3	0.24	5	15
H (138-150)	3	0.24	10	15
BF2				
A (0-50)	18-3	0.04-0.24	0	25
B (51-78)	2.5	0.288	0	25
C (79-106)	2	0.36	0	25
D (107-140)	1.5	0.48	0	25
E (141-158)	1	0.72	0	25
F (159-207)	2	0.36	0	25
G (208-230)	2	0.36	0	15

*: Volumetric loading rate

MPN test for enumerating of nitrifying bacteria in sludge The most-probable-number (MPN) analysis using microtiter plates[13] was used to enumerate nitrifying bacteria in the sludge. The mineral media used for ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) were prepared after Boer *et al.*[14]. Biomass was taken from the reactors with a total mixed liquor volume of 50 ml for each sample, which was divided into two portions, one for SS and VSS determination and one for MPN testing. Nitrite was removed from the samples by centrifuging (3,000 rpm; 10 min) and washing with sterile distilled water until the supernatant gave negative reaction with nitrite reagent. An ultrasonic homogenizer (US-150T, Nissei Co. Ltd.) was used to homogenize biomass for the MPN test (5 W output, 1 min). Diphenylamine in H_2SO_4 of 40 μl-0.2% was used to indicate the presence of AOB by determining the presence of nitrite or nitrate. A blue color reaction indicated that nitrite or nitrate had formed for a positive score. Griess-Ilosvay reagent of 40 μl was used to determine the presence of nitrite in the medium for NOB. A red color reaction indicated the presence of nitrite for negative score. After incubation (21-30 days at 28 ± 2℃), MPN was read from positive score results and calculated with a dilution factor[14] and reported as MPN/mg-VSS.

Table 2 Composition of synthetic groundwater (mix in tap water)

Composition	Concentration (mg/l)	Source	Composition	Concentration (mg/l)	Source
NH_4-N	30	NH_4Cl	Ca	25	$CaCl_2 \cdot 2H_2O$
NO_3-N	3.2	$NaNO_3$	Mg	13	$MgCl_2 \cdot H_2O$
SO_4^{2-}	2.8	tap water	Na	35	tap water
SiO_2	30.9	tap water	K	5.7	tap water
Fe (II)	0-10	$FeCl_2$	Alkalinity	200 – 250 (as $CaCO_3$)	$NaHCO_3$

Phylogenetic analysis of 16S rDNA from biofilm bacteria Total genomic DNA was extracted from the biofilm and purified by using Isoplant kit (Nippon gene, Tokyo) following the manufacturer's instruction. To analyze the microbial community of the sludge from BF reactors, PCR was performed to amplify a part of 16S rDNA sequence with the bacterial primers and the extracted DNA. The primers of 16S6F[15] (5'-GGAGAGTTAG ATCTTGGCTCAG-3'), UB338 I[16] (5'-GCTGC CTCCCGTAGGAGT-3') and EUB338 I[17] (5'-GCAGCCACCCGTAGGTGT-3') were used. KOD-plus, a DNA polymerase (TOYOBO Inc., Osaka, Japan) were used for PCR. Thermal cycling consisted of 8 cycles each of 15 s at 94℃, 2 s at 55℃ and 30s at 68℃. The obtained products were inserted into *Hinc*II site of pBluescript II SK (+) (Toyobo, Osaka) and transformed *E. coli* 10B. For grouping of the cloned insert DNAs, those were re-amplified by PCR with the same primers, and the products were digested with two restriction enzymes, *Hae* III/*Taq* I (incubated at 65℃ for 1 hour), electrophoressed, and the resultant DNA fragment patterns were exploited for grouping the clones (RFLP method).

For sequencing of the cloned DNA, RV primer (5'-CAGGAAACAGCTATGAC-3') specific for the outside sequence of multicloning site of the vector was used. Plasmid of sequence grade was prepared by using QIAprep Spin Miniprep Kit (QIAGEN) according to the manufacturer's instructions. NCBI BLAST (http://www.ncbi.nlm.nih.gov/) program was used for analyses of DNA sequence and homology searches.

Analytical methods The effluent suspended solids (SS), mixed liquor suspended solids (MLSS) and volatile suspended solids (VSS) concentrations were quantified weekly for the evaluation of sludge retention capacities using Standard Methods[18].

Influent and effluent NH_4^+, NO_2^- and NO_3^- were analyzed frequently for evaluation of NH_4^+ removal performance. NO_3^- was determined by using the UV spectrophotometer screening method, NO_2^- and alkalinity were determined by the colorimetric method and the titration method, respectively[18]. NH_4^+ was determined by the OPP method[19] and measured by UV-visible spectrophotometer (U-2010, Hitachi, Japan). Reactor pH, temperature, DO, and flow rate were monitored by using a pH meter (IM-22P; TOA Electronics, Ltd., Tokyo, Japan), DO meter (OM 51; Horiba, Ltd., Kyoto, Japan) and flow meter (KOFLAC), respectively.

RESULTS AND DISCUSSION

Nitrification performance Changes in influent and effluent concentrations of NH_4-N, NO_2-N and NO_3-N for the BF1 reactor are shown in Fig. 3. Volumetric loading rate (VLR) and ammonium removal efficiency for the BF1 reactor are shown in Fig. 4. Influent iron was 2 mg/*l* during the start-up period and then increased to 5 mg/*l* from day 46. High nitrification and ammonium removal

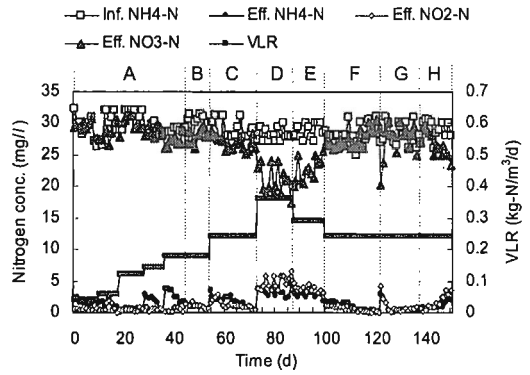

Fig. 3 Changes in influent NH_4-N, loading rate and effluent NH_4-N, NO_2-N, NO_3-N in BF1

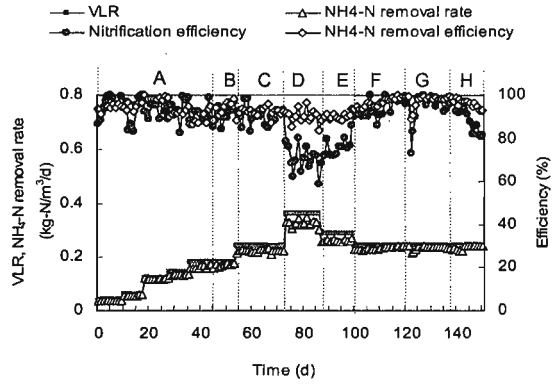

Fig. 4 Changes in loading rate, nitrification efficiency and nitrogen removal rate in BF1

efficiencies of 95-100% were maintained for 73 days in BF1 until the HRT was reduced to 2 hours. These results indicate that the nitrification process using BF material as biomass carrier was not affected by 5 mg/*l* of influent iron with a VLR of 0.24 kg NH_4-N/m^3/d.

From day 74, HRT was reduced to 2 hours, corresponding to a VLR of 0.36 kg NH_4-N/m^3/d while influent iron was maintained at 5 mg/*l*. Ammonium removal and nitrification efficiencies decreased to 84 and 60%, respectively, on day 87 and did not improved until the VLR was reduced. Nitrite and ammonium were accumulated in this stage (Fig. 3, Run D). Nitrification efficiencies gradually increased at an HRT of 2.5 hours (Fig. 4, Run E), but full nitrification was restored only when the HRT was increased again to 3 hours (Fig. 4, Run F). The decrease in nitrification efficiency may have been due to the insufficient amounts of nitrifying bacteria in the reactor under the high ammonium loading rate.

Fig. 5 and 6 show the nitrification performance for the BF2 reactor. Full nitrification was sustained until the HRT was reduced to 1.5 hours, corresponding to a VLR of 0.48 kg NH_4-N/m^3/d. Nitrification efficiencies and ammonium removal efficiencies were depressed on the first few days of each Run and then gradually increased to 95-100% in Runs B, C, and D (Figs. 5 and 6) suggesting there were not enough nitrifying organisms to cope with the increases in loading. Nitrite accumulated at the early stage of each Run and then gradual decreased, which might have been due to the growth of nitrite oxidizers or the reduction in toxic free ammonia following acid production by ammonium oxidizers[17]. Results also showed that increases in ammonium removal efficiencies were faster than increases in nitrification efficiencies in all Runs, which can be explained by the higher cell yield of ammonium oxidizing bacteria compared with that of nitrite oxidizers. Nitrification efficiencies and ammonium removal efficiencies decreased and did not improve at an HRT of 1 hour, corresponding to a very high NH_4-N loading rate of 0.72 kg/m^3/d. Both nitrification efficiencies and ammonia removal

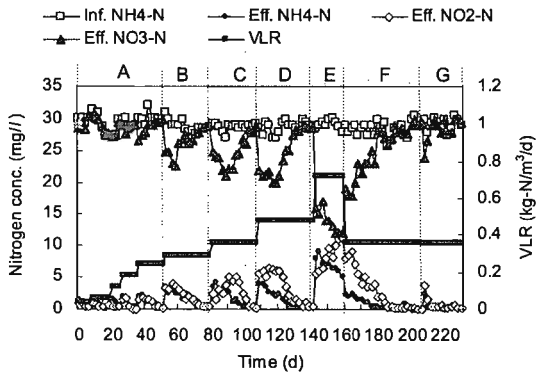

Fig. 5 Changes in influent NH_4-N, loading rate and effluent NH_4-N, NO_2-N, NO_3-N in BF2

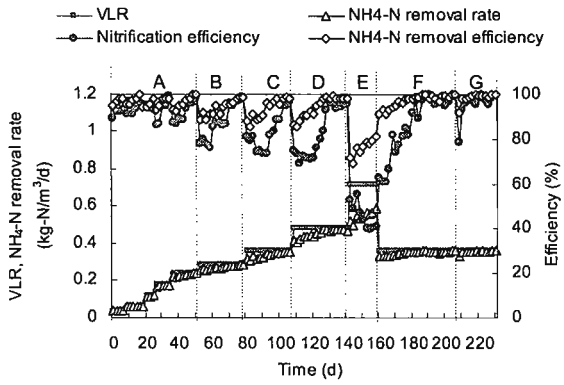

Fig. 6 Changes in loading rate, nitrification efficiency and nitrogen removal rate in BF2

efficiencies decreased sharply under this operational condition and high concentrations of ammonium and nitrite accumulated in the effluent. Also the high influent flow rates during short HRT periods caused an increase in washout of nitrifiers leading to reduce sludge retention times. These results clearly showed that the nitrification capacity in BF1 with the presence of influent iron was lower than that of BF2.

By keeping the air flow rate at 2.0 *l*/min, a bulk DO of above 5 mg/*l* was maintained through all operational periods in BF1 and BF2. Synthetic groundwater alkalinity of 200-240 mg-$CaCO_3$/*l* was sufficient for alkalinity requirement by the nitrification process in both reactors keeping reactor pH levels in a range of 7-7.5, which is within the optimal

range for nitrifiers[20].

Nitrifying bacteria Two sludge samples were taken from the BF1 and BF2 reactors under stable operational conditions with an HRT of 3 hours in BF1 and 2 hours in BF2 for MPN tests and DNA sequence analyses.

MPN tests showed that the MPNs of ammonia oxidizers and nitrite oxidizers were 3.28×10^9 and 4.6×10^9 $gVSS^{-1}$, respectively in the BF1 nitrifying biofilm, and 3.36×10^9 and 4.7×10^{10} $gVSS^{-1}$, respectively, for the BF2 reactor. These results showed that the numbers of nitrite oxidizers in BF1 was lower compared to those in BF2, while there was not much difference in the numbers of ammonia oxidizers; thus it was clear that nitrite oxidizers were inhibited due to the accumulation of ferric iron on BF in BF1 reactor. In addition, nitrite oxidizers number was higher comparing to ammonia oxidizers number in both reactors, which might be closely relate with the low effluent nitrite observed during long stable operational periods.

Clone analysis After digestion of the cloned DNA with *Hae* III and *Taq* I restriction endonucleases, clones were classified according to their RFLP patterns. Thirteen clones were found in the BF1 biomass and eighteen in the BF2 biomass. Homology search results using the Blast search program and the DDBJ registered accession numbers (16 sequences) are listed in Tables 3 and 4. BF1 sample (higher content of iron) has lower population of nitrifying bacteria as compared with BF2 sample. These results show that the precipitated ferric iron within the biomass inhibited growth of nitrifying bacteria, resulting in the lower ammonium removal rate for BF1. From this analysis, only one clone (8% of the total) from BF1 was identified as an ammonia-oxidizing bacteria related to *Nitrosomonas* sp. AL212 (AB 000699, 98% of identity), while five clones (28%) from BF2 were identified as ammonia-oxidizing bacteria. Three clone sequences from BF2 were related to *Nitrosomonas oligotropha* (AF272422, 98 % of identity), one was related to *Nitrosomonas* sp. R5c88 (AF386750, 95 %) and one was related to *Nitrosospira* sp. Nsp65 (AY123813, 98%). *Nitrosomonas* sp. AL212 is related to *Nitrosomonas oligotropha* and is commonly found in freshwater habitats or in cultures with low ammonium concentrations, relating to typical nitrifying bacteria with high substrate affinity. These results agree well with batch experimental results, which have carried out in the previous period of this study[21], showing that saturation constant

Table 3 Homology search results for BF1 clones (13 clones were found, 1 clone (8%) was identified as ammonia-oxidizing bacteria)

Clone type	Accession number	Bacterial strain showed the highest homology	Number of clones	Identities
1	AB222586	· Uncultured yard-trimming-compost bacterium clone S-48 16S rRNA gene (AY095415)	3	98%
2	AB222587	· Unidentified bacterium clone GLB-5 16S rRNA gene (AY345575)	2	97%
3		· Bacterium Ellin5265 16S rRNA gene (AY234616)	2	100%
4	**AB222588**	**· Nitrosomonas sp. AL212 16S rRNA gene (AB000699)**	**1**	**98%**
5	AB222589	· Agricultural soil bacterium clone SC-I-55 16S ribosomal RNA gene (AJ252641)	1	98%
6	AB222592	· Uncultured Acidobacterium group bacterium clone N12.63WL 16S ribosomal RNA gene (AF431532)	1	91%
7	AB22590	· Uncultured bacterium PHOS-HE34 16S ribosomal RNA gene (AF314422)	1	96%
8	AB222591	· Uncultured bacterium clone D8A_5 16S rRNA gene (AY768825)	1	97%
9		· Uncultured bacterium gene for 16S ribosomal RNA (AB089944)	1	100%

Table 4 Homology search results for BF2 clones (18 clones were found, and 5 clones (28%) were identified as ammonia-oxidizing bacteria)

Clone type	Accession number	Bacterial strain showed the highest homology	Number of clones	Identities
6	AB222592	· Uncultured Acidobacterium group bacterium clone N12.63WL 16S rRNA gene (AF431532)	1	91%
10	AB222593	· Uncultured bacterium gene for 16S ribosomal RNA (AB118914)	1	95%
11	**AB222594**	**· Nitrosomonas oligotropha 16S rRNA gene (AF272422)**	**3**	**98%**
12	AB222595	· Uncultured bacterium clone HP1A94 16S rRNA gene (AF502208)	2	98%
13		· Uncultured bacterium clone S4A5 16S ribosomal RNA gene (AY382154)	2	100%
14	**AB222596**	**· Nitrosomonas sp. R5c88 16S ribosomal RNA gene (AF386750)**	**1**	**95%**
15	**AB222597**	**· Nitrosospira sp. Nsp65 16S rRNA gene (AY123813)**	**1**	**98%**
16		· Xanthomonas axonopodis gene for 16S ribosomal RNA (AB101447)	1	100%
17	AB222598	· Trojanella thessalonices 16S ribosomal RNA gene (AF069496)	1	96%
18		· Uncultured bacterium gene for 16S ribosomal RNA (AB089944)	1	100%
19	AB222599	· Parvulomonas gwangyangenesis gene for 16S ribosomal RNA (AY682384)	1	89%
20	AB222600	· Uncultured bacterium clone H29K gene for 16S ribosomal RNA (AY395182)	1	95%
21		· Uncultured Acidobacteria bacterium gene for 16S ribosomal RNA (AY899797)	1	100%
22	AB222601	· Unidentified bacterium clone LWSR-73 gene for 16S ribosomal RNA (AY345534)	1	94%

(K_m) 2.19 mg NH_4-N/L was determined. *Nitrosomonas* sp. R5c88 was also identified as a bacterium responsible for ammonia oxidation in freshwater environments[22]. These ammonia-oxidizing genera are reported to growth effectively at low ammonium concentrations[23]. *Nitrosospira* sp. Nsp65, identified in this study, is closely related to *Nitrosospira* sp. and commonly forms monospecies clusters and has been reported as a dominant strain in a nitrifying fluidized bed reactor[24]. No clones relating to nitrite-oxidizers were found, but MPN tests results showed that large numbers of nitrite oxidizers were contained in the sludge. This could be because there might have been unidentified nitrite oxidizers under the unique conditions found in a BF reactor. One clone related to an uncultured bacterium PHOS-HE34 (16S rRNA gene (AF314422)) was found in BF1 and 3 clones related to uncultured bacterium HP1A94 (16S rRNA (AF502208)) and *Xanthomonas axonopodis* (16S rRNA gene (AB101447)) were found in BF2. These clones had also been found in the ammonium containing wastewater activated sludge process[25]. From clone sequence analyses and literature surveys, a suggestion could be made that there were unidentified nitrifying bacteria in the sludge of the BF reactor.

SS and VSS analysis Effluent SS and VSS concentrations for the BF1 reactor ranged from 3 to 10 mg/*l* and 0.6 to 2 mg/*l*, respectively, corresponding to VSS content of 20%. The VSS contents of sludge detached from the BF1 and BF2 were 60-70%, and 75-80%, respectively. Influent iron for BF1 reactor was fixed at 5 mg/*l*, but the effluent level remained very low at 0.03 to 0.2 mg/*l*. It was pointed out that ferrous iron was oxidized to ferric iron, which precipitates as iron oxide or iron hydroxide ($Fe(OH)_3$) then washes out. The small amount of ferric iron might have remained in the reactor and attached to the biomass, which might account for the lower VSS content of BF1.

Iron influence on nitrification process

High nitrification efficiencies of 95-100% were maintained in BF1 at HRTs greater than 3 hours (Figs. 3 and 4). These results demonstrate that simultaneous nitrification and iron removal is possible in a BF reactor. Only small amounts of precipitated ferric iron remained attached on the BF biomass carrier due to the unique characteristic of swim-bed technology. By a gradual stepwise decrease in HRT, higher nitrification capacity of BF1 will be achieved. However, the results also showed clearly that there was a negative influence on nitrifying performance by the presence of iron in the synthetic groundwater. Fig. 7 shows the comparison of nitrification capacities between BF1 and BF2. This could be due to the lower population in BF1 biomass in comparison to that of BF2.

Influent iron was increased to 10 mg/*l* at day 138 (BF1, Run H), after which nitrification efficiencies and ammonium removal rates gradually decreased. VSS determination from biomass taken from BF1 in this stage showed a relatively low VSS value of 50%. This suggests that a higher amount of precipitated ferric iron was attached on the BF. The decrease in nitrification efficiencies in this Run (BF1, Run H-Fig. 5) might cause the decrease of nitrifying bacteria due to the biofilm occupation by attached ferric iron on BF. Ferrous iron oxidation could also occur on the surface of nitrifying biomass, which results in an oxygen deficiency in the biofilm.

In an effort to remove precipitated ferric iron from the attached biomass, influent was stopped and the air flow rate was increased to 8 *l*/min for detaching biomass from biofringe. Then the air flow rate was reduced to 2 *l*/min as before for biomass reattachment. After 8 hours of reattachment, part of the biomass did not reattach and was washed out from the reactor when influent was again supplied. Effluent VSS analysis showed low level of 25% in this phase, it was pointed out that precipitated ferric iron was mainly washed out in effluent. By using this backwashing method, precipitated ferric iron can be removed from the biofilm. However, nitrification and ammonium removal efficiencies were decreased due to biomass lost. Subsequently, the reactor required 16 days to reach to 90% of nitrification efficiency and 95% of ammonium removal efficiency, even though influent iron was again reduced to 5 mg/*l* in this run (data not shown). These results demonstrate that nitrification can be inhibited at iron concentration of 10 mg/*l* due to precipitated ferric iron accumulation in biofilm. However, precipitated iron can be removed from a BF reactor by using higher flow rates through BF zone. By applying this backwashing method nitrification inhibition due to precipitated ferric iron might be reduced. Greater amounts of iron could be removed simultaneous and a higher ammonium removal rate could be achieved.

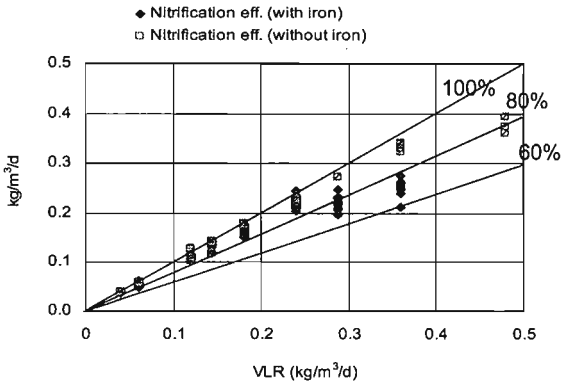

Fig. 7 Nitrification rate as function of loading rate for BF1 and BF2

Effect of temperature on nitrification

Temperature is regarded to be the major factor affecting nitrification. Various studies have reported that the overall nitrification rate decreases with decreasing temperature and the effects of temperature on nitrification rate and growth of nitrifying bacteria have been studied by many researchers[26-28]. Eqs. 1 and 2 show the temperature dependency of specific growth rate of nitrifying bacteria reported by Downing *et al,* and US EPA, respectively[27]. The percent decrease in nitrification rate is the same as the percent decrease in growth rate by the line relationship as Eq. 3[28].

$$\mu = 0.18 \times e^{0.12\,(T-15)} \qquad (1)$$

$$\mu = 0.47 \times e^{0.09\,(T-15)} \quad (2)$$

$$\mu_{max} = \frac{Y \times (-\Delta N/\Delta t)}{X_a} \quad (3)$$

where

μ specific growth rate of nitrifying bacteria, 1/d

μ_{max} maximum specific growth rate of nitrifying bacteria, 1/d

Y yield of nitrifying bacteria, g-VSS/g-NH_4^+-N removed

T temperature in the reactor, ℃

$-\Delta N/\Delta t$ nitrification rate g-N/l/d

X_a concentration of nitrifying oxidizers in the reactor, g-VSS/l

To study the influence of temperature on nitrification rate in a BF reactor, the temperature was reduced from 25℃ to 15℃ during Run G in both experimental reactors. Nitrification efficiencies for BF1 and BF2 decreased sharply to 73 and 78%, respectively. But these nitrification efficiencies quickly recovered to 92-98% after one day then remained stable in both reactors. No decrease in nitrification efficiencies due to the temperature decreased from 25℃ to 15℃ was observed, while it could be decreased 69% and 59% by using the equations 1 and 2, respectively. This could be explained that the increase of bulk reactor DO concentration under low temperature (theory on equilibrium DO concentration) lead to the increase of active nitrification zones. The temperature change also influences on substrate diffusion and transport. In addition, the clone analysis showed that *Nitrosospira* is present in cluster formation in the BF reactor. A previous study also showed that cluster formation might provide protection against hazardous environmental factors[24]. No negative impact due to temperature decrease to low value of 15℃ was observed in BF reactors. This could be the strong advantage of BF reactor for nitrification.

CONCLUSIONS

The conclusions can be drawn from this study as follow:

1. Large amounts of nitrifying biomass were maintained in the BF reactors allowing for 95 % nitrification efficiency under a high VLR of 0.48 kg NH_4-N/m^3/d (BF2).
2. Nitrification and iron removal were successfully performed simultaneously in a BF reactor with VLRs up to 0.24 kg NH_4-N/m^3/d and influent iron concentration of 5 mg/l without clogging problems.
3. Higher iron and ammonium levels could be simultaneously removed if ferric iron attached on biofilm is removed frequently. Further development for effective removal method of attached iron on BF is need.
4. The lower VSS content observed from BF1 biomass showed the precipitation of ferric iron on BF, which associated with lower biomass and nitrifying organism on BF carriers.
5. A reduction in nitrification efficiency occurred due to the sudden decrease in temperature from 25 to 15℃, but was quickly restored after only one day. No negative impact due to temperature decrease to low value of 15℃ was observed in BF reactors.
6. Clones closely related to the ammonia oxidizer *Nitrosomonas* sp. AL212 were identified in BF1 and *Nitrosomonas oligotropha*, *Nitrosomonas* sp. R5c88 and *Nitrosospira* sp. Nsp65 were presented in BF2. These strains commonly appear in freshwater aquatic habitats and under environmental condition with low ammonia concentrations.

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