NOVEL HIGH RATE NITRIFICATION TREATMENT OF AMMONIUM POLLUTED HANOI GROUNDWATER

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ABSTRACT

This study demonstrates the use of an inexpensive and durable acryl-resin fiber (BL) material as a biomass carrier for the nitrification treatment of Hanoi groundwater polluted with ammonium. The biomass retention capacity of BL for nitrifying activated sludge was determined to be 6,200 g-MLSS/m^3 and the effluent from the nitrifying reactor packed with BL material was free of SS. Results of continuous-flow nitrification experiments using the BL material consistently demonstrated a high rate of nitrification. The reactor achieved 95~99% of nitrification efficiencies at ammonium loading rates up to 0.75 g-N/l.d. The influent iron, alkalinity concentrations, pH and HRT were all correlated with the nitrification efficiencies. For effective nitrification treatment, these operational parameters must keep as free of influent iron; influent alkalinity of 200~230 mg/l; pH of 6.8~7.2; and an HRT of greater than 1 hr. The observed sludge yield (Y_{obs}) was determined to be 0.067 g-VSS/g-NH_4-N removed. The microbial community in the nitrifying sludge attached on BL material was examined. Three protozoa species of *Aspidisca* sp., *Euglypha* sp., *Philodina* sp. and a lineage of *Nitrosomonas oligotropha* were identified as the predominant protozoa and bacterium in the nitrifying sludge.

KEYWORDS

groundwater, ammonium, nitrification, biomass carrier, acryl-resin

INTRODUCTION

Groundwater is used as the major source for the public water supply system of Hanoi, which is distributed through eight major water treatment plants (WTPs) and a number of small water supply stations. The system has a capacity of about 450,000 m^3/d for a population of 1.4 million in urban districts [1]. In recent years, ammonium contamination in the groundwater of Hanoi has become an increasing problem for water quality [2-4]. The most heavily polluted areas are located mainly in the southern part of the city, where NH_4-N concentrations range from 20- 30 mg/L [5]. Presently, all WTPs employ the same conventional water treatment process consisting of only iron removal and chlorination, thus the treated water is frequently in violation of both the Vietnamese drinking water standard and the WHO guideline for ammonium in drinking water of 1.5 mg-N/L. Efficiencies of ammonium removal are very low at the WTPs where the source groundwater has high

concentrations of ammonium and iron [6]. Thus, the development of technically and economically favorable ammonium removal system including biological nitrification treatment is required. Biological nitrogen removal process is regarded to be an economical water treatment.
This study focuses on biological treatment of groundwater contaminated with ammonium and presents the results of efficient nitrification treatment using a novel acryl-resin fiber (BL) material as a biomass carrier.

MATERIALS AND METHODS

Synthetic groundwater

Based on the analytical results of groundwater qualities in Hanoi area[5], the synthetic groundwater used in this study was artificially prepared and its composition was shown in Table 1.

Table 1 Composition of the synthetic groundwater

Composition	Concentration (mg/l)	Source
NH_4- N	30	NH_4Cl
NO_3- N	3.2	$NaNO_3$
TOC	3.2	$C_6H_{12}O_6$
SO_4^{2-}	2.8	tap water
SiO_2	30.9	tap water
Fe(II)	0~18	$FeCl_2$
Ca	25	$CaCl_2.2H_2O$
Mg	13	$MgCl_2.6H_2O$
Na	35	tap water
K	5.7	tap water
Alkalinity	100~250 (as $CaCO_3$)	$NaHCO_3$

Cultivation of nitrifying activated sludge

Nitrifying activated sludge was prepared by fill and draw cultivation under total oxidation conditions for more than one year using synthetic wastewater. The concentrated synthetic wastewater consisted of (g/l tap water) pepton 60, meat extract 40, and $NaHCO_3$ 21, which corresponded to the COD_{cr} concentration of 100 g/l. In addition, the following inorganic salts were added to the substrate with concentrations of (mg/l) NaCl 2, KCl 2.8, $CaCl_2$ 2.8, $MgSO_4 \cdot 7H_2O$ 4.

Biomass carrier

Novel BL material (NET. Co. Ltd, Hygo, Japan) was used for immobilization of nitrifying activated sludge. BL material is light in weight, and inexpensive and durable. Two strips of the BL material (Fig. 1) each with a one-sided surface area of 300×450 mm and a weight of 32.7 g were used as biomass carriers in this study. With a width of 15 mm, the BL strips had a total effective volume of 4.05×10-3 m^3 and a bulk density of 16,000 g/m^3. The BL strips were folded in to 4 layers and set symmetrically on two sides of the reactor using aluminum frames (see Fig. 2).

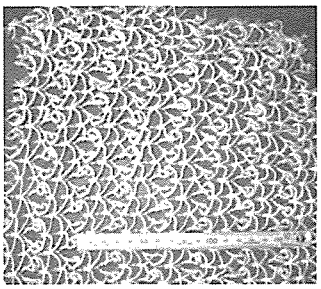


Fig. 1 Photograph of BL

Reactor description and operation

Fig. 2 shows the schematic diagram of nitrifying reactor used in this research.. The 5-l reactor was made of PVC and the influent was fed by using peristaltic pump. BL strips were set symmetrically in the reactor. Air was supplied from the bottom of reactor at flow rate of 0.5~1.5 l/ min.

The reactor was initially inoculated with 15 g of acclimated nitrifying activated sludge. This seed sludge completely attached to the BL materials within 2 hoours of gentle aeration. The influent NH_4-N concentration was maintained at 30 mg/l and the hydraulic retention time (HRT) was varied from 24 h to 0.5 h by changing the influent flow rate.

Analytical methods

The pH and DO were measured by using a Mettler Toledo-320 pH meter (Switzerland) and UC-12 Digital DO/ O2/ Temp. Meter (TOA, Ltd., Japan), respectively. Analyses of NH_4-N, NO_3-N, NO_2-N, MLSS, SS and alkalinity were performed according to Standard Methods for the examination of Water and Wastewater [7].

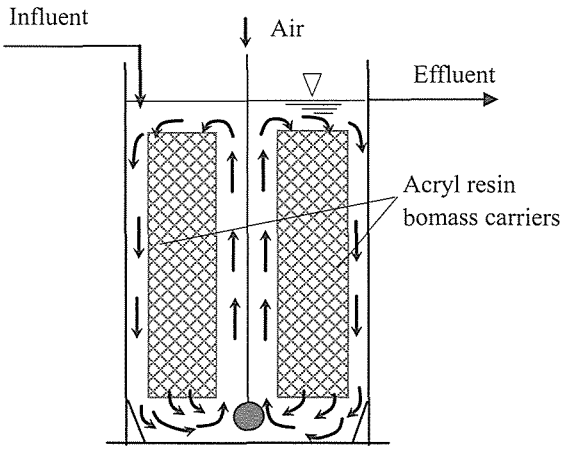


Fig. 2 Schematic diagram of the experimental system

Identification of bacterial community in nitrifying sludge

Microbial community in a well-established nitrifying sludge on BL material was investigated using 16S rDNA comparative sequence analysis to determine the phylogenetic identities of the dominant members of the microbial community. For DNA extraction and PCR amplification of 16S rDNA, two samples (each of 100 μl) of sludge with the influent ammonium concentrations of 10 and 30 mg/l, which were termed samples A and B, were taken from the reactor.

DNA extraction and PCR amplification of 16S rDNA

Total genomic DNA was extracted from the samples using the Isoplant kit (Nippongene, Tokyo). All PCRs were conducted with an equimolar mixture of two forward primers (CTO189fM (5'-GGAGRAAAGCAGGGGATCG-3'), CTO189fC (5'- GGAGGAAAGTAGGGGATCG-3') and a reverse primer CTO654r (5'-CTAGCYTTGTAGTTTCAAACGC-3'). The forward primers CTO189fM and CTO189fC were synthesized separately (concentration of 10 pmol/μl for each primer) and collectively referred to as CTO189fMC. These primers are designed to amplify partial 16S rDNA sequences (465-bp) from ammonium-oxidizing bacteria belonging to proteobacterium β-subdivision. The ability of this primer set was tested experimentally with 100 ammonia oxidizer-like 16S rDNA clones representing the currently recognized sequence clusters [8].

PCR amplification was performed in 50μl of reaction mixtures containing an appropriate volume of amplification buffer, 10 nmol each of dNTP, 50 nmol $MgSO_4 \cdot 7H_2O$, 10 pmol each of primer CTO189fM and CTO189fC, 20 pmol of primer CTO654r, 20 ng of template DNA, 1 unit of DNA polymerase, KOD-plus (Toyobo, Osaka). The reactions were performed by 2400 GeneAmp PCR System Thermal Cycler (PrekinElmer) consisting of an initial denaturation at 94℃ for 2 min followed by 40 cycles of denaturation at 94℃ for 15 sec with primer annealing at 60℃ for 2 sec and elongation at 60℃ for 40 sec (final elongation extended to 5 min). The amplified PCR products (5μl) were analyzed by gel electrophoresis in a 1.5% agarose gel, and then stained by ethidium bromide.

Restriction fragment length polymorphism (RFLP) and sequence analyses of 16S rDNA

The PCR products were ligated into the TaqI site of pBluescript II SK+ (Toyobo, Osaka), and *E. coli* XL-1 Blue cells (Stratagene, CA, USA) were transformed using the integrated plasmids. Twenty three clones were picked from both samples A and B, and each clone was used as a template for PCR with above mentioned primer set. The PCR products were digested with the restriction enzyme TaqI, and then restriction fragment length patterns were obtained by agarose gel electrophoresis (2% agarose gel). The restriction fragments were categorized into a series of size

classes, and each bacterial taxon was determined to have either a presence or an absence of a restriction fragment, or fragments, in each of these size class categories. Clones showed the same restriction patterns were considered to be of the same bacterial taxon. The representative 16S rDNA clone for each bacterial taxon was sequenced and compared with the sequences of defined phylogenetic affiliations in the database of DNA data bank of Japan (DDBJ). The sequencing reaction was performed using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo), and then analyzed with an autosequencer (ABI prizm 310 genetic analyzer).

RESULTS AND DISCUSSION

Sludge retention capacity

In order to investigate the maximum sludge retention capacity of the BL materials, the BL materials were dipped in nitrifying activated sludge liquor at various initial MLSS concentrations ranging from 1,500 to 5,000 mg/l. The decrease in MLSS concentration with the elapse of time indicates the rate of sludge attachment on BL. Almost all seed sludge, whose initial concentration was below 5,000mg/l, was retained by BL material within 2 hours of aeration (see Fig. 3). However, seed sludge whose initial concentrations above 5,000 mg/l did not attach completely within 2 hours of liquid circulation. Time courses of sludge retention using 2 strips of BL material are shown in Fig. 4. These results indicate that the maximum sludge retention capacity of BL is about 6,200g-MLSS/m^3.

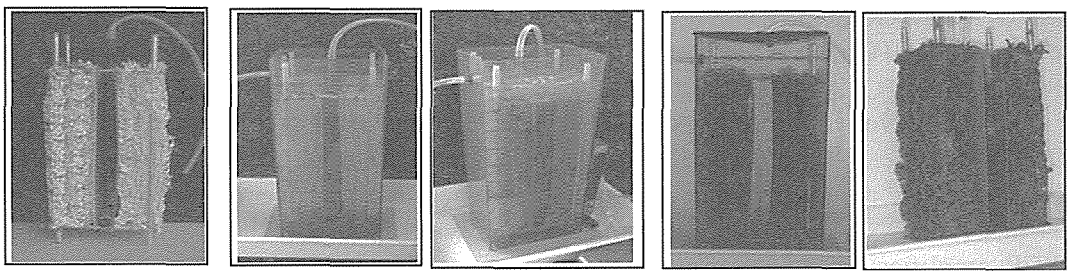


Fig. 3 Photographs of BL material and reactor during sludge attaching experiment
(a)BL material, (b)BL material dipped with nitrifying sludge, (c)starting of aeration, (d)after 2 hours of aeration, (e)BL material with attached nitrifying sludge

Nitrification performance of reactor

After attachment of nitrifying sludge on the BL material, nitrification treatment was started with an influent NH_4-N concentration of 30mg/l, iron of 18 mg/l, and alkalinity of 100 mg/l at a HRT of 24 hours. As shown in Fig. 5(c and d), the effluent NH_4-N concentration was high with only 30% of influent NH_4-N nitrified to NO_3-N and the pH dropped to 4.7. This may have been

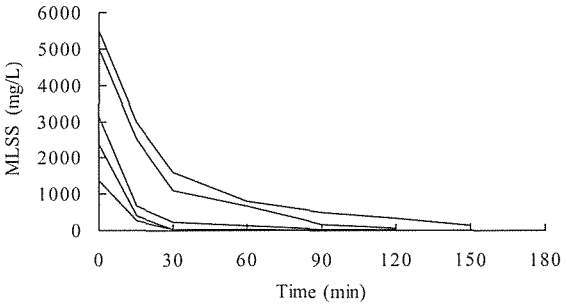


Fig. 4 Time courses of sludge retention on BL material

because of the low influent alkalinity (100 mg/l, see Fig.5 (a)). The effluent iron concentration was close to 0 mg/l (data not shown) and the reactor was colored due to the precipitation of oxidized iron on the BL materials. Subsequently, iron was eliminated from the influent and the influent alkalinity was increased for elevating buffering capacity. After 2 weeks of operation, more than 99% of nitrification efficiencies were obtained and effluent NO_2-N concentrations were close to 0 mg/l (Fig. 5(c)). Thereafter, the volumetric nitrogen loading rate (VNLR) was increased stepwise by decreasing the HRT to 0.5 hour. The influent alkalinity was also increased stepwise and then maintained at approximately 200~230 mg/l. As shown in Fig. 5(a, c and d), more than 95% of nitrification efficiencies were obtained at each HRT. NO_2-N concentrations of about 5~11 mg/l were observed in the effluent at the HRTs of 1 and 0.5 hour, which may have been due to the high loading rate associated with the short HRTs.

The reactor was initially aerated at an air flow rate of 0.5 l/min until the HRT was lowered to 3 hours corresponding to VNLR of 0.25 g-N/l.d. As shown in Fig. 5(b), further increases in VNLR were associated with a decrease in DO, which caused the unstable nitrifying activities. At a HRT of 1 hour (VNLR of 0.75 g-N/l.d), the DO concentration was decreased to 0.5 mg/L and the nitrification efficiency was dropped to 88%. At this point, the airflow rate was increased to 1.5 l /min(on day 162), after which the DO concentration and nitrification efficiency recovered to 1 mg/l and 95%, respectively. From day 200, the reactor was operated with a HRT of 0.5 hour (VNLR of 1.5 g-N/l.d). High NO_2-N concentrations of 10~11 mg/l were observed in the effluent and the nitrification efficiency was reduced to about 60% at this HRT. Subsequently, VNLR was reduced to 0.5 g-N/l.d by the decrease in influent NH_4-N concentration to 10 mg/l and then it was increased again to 30 mg/l (VNLR of 1.5 g-N/l.d) on day 232 (Fig. 3.7 (b)). As shown in Fig. 5 (d), the nitrification efficiencies were about 95% at VNLR of 0.5 g-N/l.d and 80% at VNLR of 1.5 g-N/l.d. As shown in Fig. 5(c), the effluent from the reactor was clear with SS less than 2.5 mg/l even under high precipitation of oxidized iron in the reactor and high air flow rate. Effluent with low SS is one of the unique features of reactor packed with BL material.

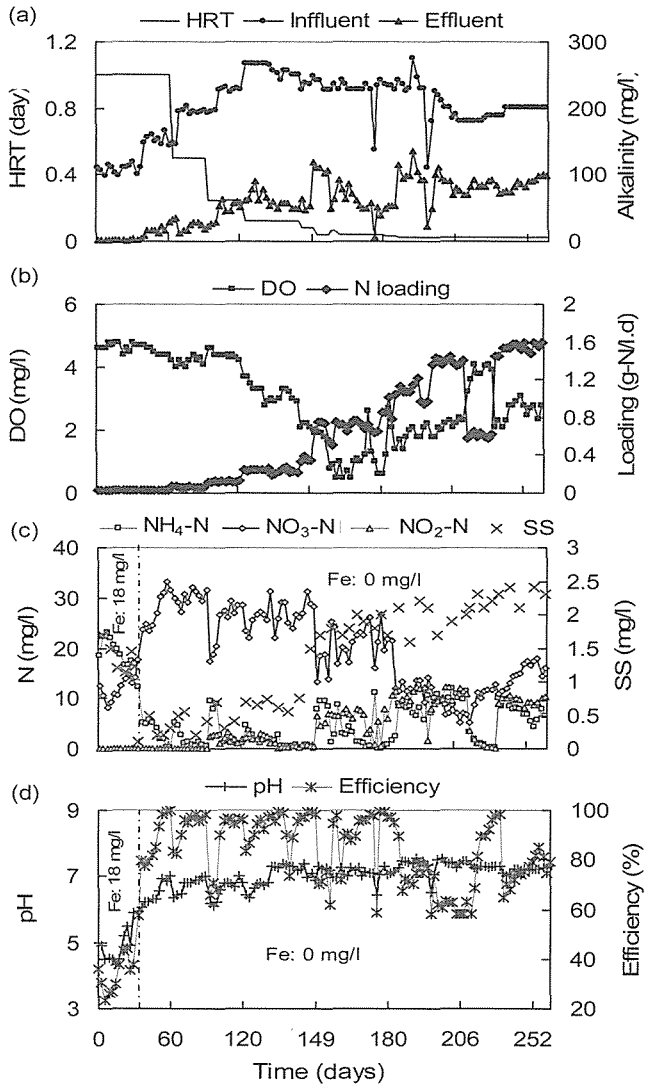


Fig. 5 Time course of nitrification treatment
(a) HRT, influent, effluent alkalinity
(b) DO, nitrogen loading rate
(c) Effluent NH_4-N, NO_3-N, NO_2-N, SS
(d) pH and nitrification efficiency

Effect of HRT on nitrification efficiency

Fig. 6 shows the effect of HRT on nitrification efficiencies. The reactor was able to nitrify over 95% of influent NH_4-N even under I hr of HRT. These results show that attached immobilization using BL material enables the complete retention of slow growing nitrifiers even under short HRT operation.

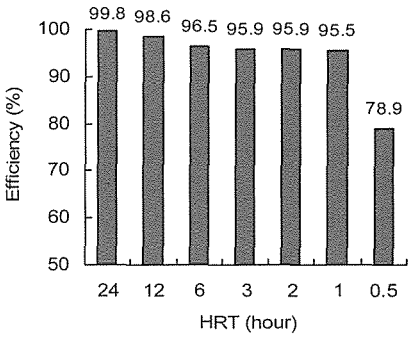


Fig. 6 Effect of HRT on nitrification efficiency

Effect of nitrogen loading rate

Fig.7 shows the relationship between VNLRs and the volumetric NH_4-N removal rates. For the achievement of nitrification efficiencies of more than 95%, VNLR must be kept under 0.75 g-NH_4-N/l.d. This is a fairly high value in terms of practical purposes and can warrant the application of BL material for the biological nitrification treatment of ammonium contaminated groundwater.

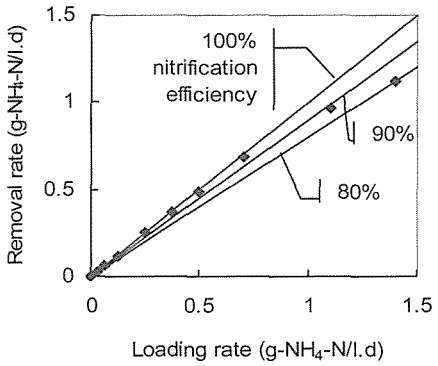


Fig. 7 Relationship between VNLRs and volumetric NH_4-N removal rates

Sludge yield

The observed sludge yield (Y_{obs}) was calculated from the following equation using experimental data.

$$Y_{obs} = \frac{(X_t - X_0) + \sum_{i=1}^{t} (Q \times C)_i}{\sum_{i=1}^{t} S_i}$$

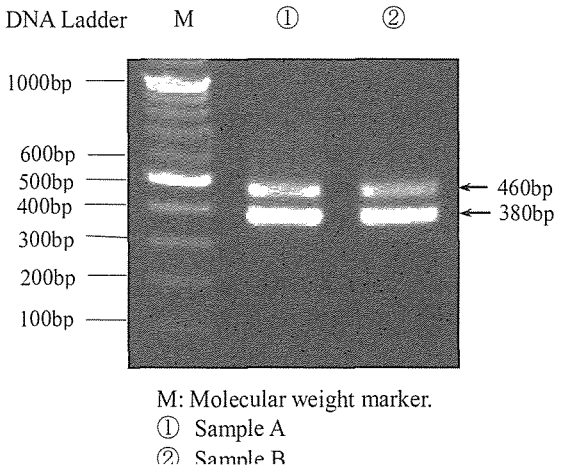
where Y_{obs} refers the observed sludge yield coefficient (g-VSS/g-NH_4-N removed), X_t and X_0 refer the amount of biomass in the reactor at time t (g-VSS) and the amount of biomass in the reactor at time t = 0 (g-VSS), respectively. Q, C, ΣS and t refer the flow rate (l/d),the effluent VSS concentration(g-VSS/l), total amount of NH_4-N removed (g-NH_4-N) and operation time (day), respectively.

After 266 days of continuous operation, the reactor operation was stopped and the biomass was completely detached from the BL material. 18.6 g-VSS was recovered indicating a net increase of 16.65 g (including effluent VSS and withdrawn sludge). Removal amount of NH_4-N during 266 days of continuous operation was 250.1 g. The observed sludge yield (Y_{obs}) was calculated to be 0.067 g-VSS/g-NH_4-N removed.

Identification of bacterial community

Two samples A and B of nitrifying sludge were taken from reactor at the operational periods with influent ammonium concentrations of 10 and 30 mg/l, respectively. The bacterial 16S rDNA-specific primer pair (CTO189fMC and CTO654r) was used for the amplification of 16S rDNA fragments in a non-conserved region among a β-subdivision ammonia oxidizer. Fig. 8 shows the results of electrophoresis analyses of amplified 16S rDNA from samples A and B. The intense bands of 460 and 380 bp were detected for both samples. The bands of 460 bp indicate the presence of β-subdivision ammonia oxidizer. This result confirms the present of ammonium-oxidizing bacteria in tested sludge samples

RFLP analyses were conducted using the restriction enzyme *Taq*I for digestion of amplified PCR products. Fig. 9 shows the results of RELP analyses for 13 clones (clone No 1~13) obtained from sludge sample A and 10 clones (clone No 14~23) of sample B. Based on RELP analyses, these 23 clones were categorized into 3 groups as shown in Table 2. Clones 1, 2, 6, 8, 3, 15, 20, 14 were chosen as the representative clones of groups for further sequent analyses. The comparative results of selected clones sequences with currently recognized ammonium-oxidizing bacteria in the DDBJ database indicated that groups I-a, II-a and III had 94~96% similarity with *Nitrosomonas oligotropha*; group I-b had 94% similarity with *Nitrosomonas oligotropha* and 96% with *Nitrosomonas cryotolerans*; group II-b had 94% similarity with *Nitrosomonas communis*. These results revealed that there were two different kinds of ammonium-oxidizing bacteria in the tested sludge samples. A probable explanation for this observation is that these two sludge samples were fed with different ammonium loading rates (0.5 and 1.5 g-NH_4-N/l.d for sludge samples A and B, respectively). However, from the results illustrated in Table 3.2, a lineage of *Nitrosomonas oligotropha* could be identifying as the predominant bacterium in the tested nitrifying sludge.



M: Molecular weight marker.
① Sample A
② Sample B

Fig. 8 Electrophoresis analysis of amplified 16S rDNA using the extracted DNA from sludge samples as templates

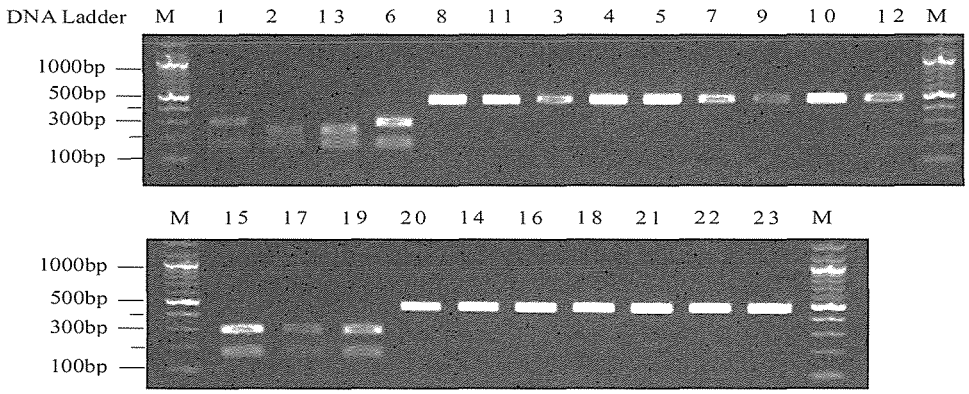


Fig. 9 RFLP analyses of restriction fragments obtained from clones 1-13 of sample A and clones 14-23 of sample B using the restriction enzyme *Taq*I

Table 2 Grouping of amplified DNA fragments obtained from the sludge samples

Group	Clone number		Bacterium with highest homology
	Sample A	Sample B	
I-a			*Nitrosomonas oligotropha* (96%)
I-b	2, 13		*Nitrosomonas oligotropha* (94%) *Nitrosomonas cryotolerans* (96%)
II-a	6	15, 17, 19	*Nitrosomonas oligotropha* (96%)
II-b	8, 11	20	*Nitrosomonas communis* (94%)
III	3, 4, 5, 7, 9, 10, 12	14, 16, 18, 21, 22, 23	*Nitrosomonas oligotropha* (96%)

CONCLUSION

The following conclusions have been drawn from the experimental results of nitrification treatment for NH_4-N polluted groundwater using BL material as a biomass carrier.

1) The biomass retention capacity of BL material was determined to be 6,200 g-MLSS/m^3 .

2) Results of continuous-flow experiments consistently demonstrated a high rate of nitrification. The reactor achieved 95~99% nitrification efficiencies at ammonium loading rates up to 0.75 g-N/l.d.

3) The influent iron, alkalinity concentrations, pH and HRT were all correlated with the nitrification efficiencies. For effective nitrification efficiencies, these operational parameters must keep as free of influent iron, influent alkalinity of 200~230 mg/l, pH of 6.8~7.2 and an HRT of greater than 1 hr.

4) The net observed sludge yield (Y_{obs}) was determined to be 0.067 g-VSS/g-NH_4-N removed.

5) A lineage of *Nitrosomonas oligotropha* was proved to be the predominant nitrifier in the nitrifying sludge.

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