HIGH RATE NITRIFICATION TREATMENT OF AMMONIUM NITROGEN POLLUTED GROUNDWATER USING A NOVEL ACRYL-RESIN FIBER FOR BIOMASS ATTACHMENT

NGUYEN THI HAI DUONG [1], LEU THO BACH [1], KENJI FURUKAWA [2*], TAKAO FUJII [3] AND TRAN HIEU NHUE [4]

Department of Environmental Science, Graduate School of Science and Technology, Kumamoto University[1], Department of Civil Engineering and Architecture, Faculty of Engineering, Kumamoto University, 2-39-1, Kurokami, Kumamoto 860-8555, Japan[2], Department of Applied Life Science, Sojo University, 4-22-1 Ikeda, Kumamoto, 860-0082, Japan [3], and Center for Environmental Engineering of Towns and Industrial Areas, Hanoi University of Civil Engineering, 55 Giai Phong Road, Hanoi, Vietnam [4]

Abstract This study demonstrates the use of Biofill (BL), an inexpensive and durable acryl-resin fiber material, as a biomass retainer for the nitrification of ammonium polluted groundwater. 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 experiments consistently demonstrated a high rate of nitrification using the BL material. The reactor achieved 95~99% 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 efficiency of the reactor. 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. The net 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 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, respectively, in the nitrifying sludge of present treatment system.

Keywords Groundwater, ammonium, nitrification, biomass carrier, acryl-resin

Introduction

Groundwater is used as the 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)]. Over the last ten years, the population of Hanoi has increased rapidly to more than 4 million, thus straining the capacity of the system. In addition, 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. Analyses conducted by the Center for Environmental Engineering of Towns and Industrial Areas (CEETIA) in 2000 showed that efficiencies of ammonium removal are very low at the WTPs where the source groundwater has high concentrations of ammonium and iron [6]. Thus for effective treatment, it would be technically and economically favorable to develop a purification system including biological nitrification treatment.

Ammonium can be removed by nitrification involving bacteria (nitrifiers) which use ammonium as an electron donor to provide energy for growth. The ammonium is oxidized ultimately to nitrate which can than be biologically denitrified, i.e. reduced to dinitrogen gas. Biological treatment is relatively inexpensive and produces no unwanted by-products when taken to completion. However, nitrifies are slowly growing organisms and will thus be washed out of continuous-flow reactors unless they are effectively immobilized. A high cell concentration is possible with immobilized biomass, which enhances volumetric efficiency. This allows for relatively small reactors and often offers protection from toxic shocks and adverse temperatures which would help maintain stable treatment.

This study focuses on biological treatment of groundwater contaminated with ammonium and presents the results of nitrification assays using a novel acryl-resin fiber material called Biofill (BL) as a biomass carrier. The objectives of this study were:

- To determine the maximum capacity of the BL material for retaining nitrifying activated sludge,
- To determine the maximum acceptable ammonium nitrogen loading capacity of a BL reactor,
- To determine the optimal influent iron concentration and evaluate the effects of pH and alkalinity on ammonium removal efficiency,
- To investigate the diversity of protozoa and bacterial community present in nitrifying sludge, and
- To evaluate the applicability of a biological nitrification process using acryl resin for ammonium removal from polluted groundwater in Hanoi.
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Materials and Methods

Hanoi-groundwater survey and synthetic groundwater

In order to assess the quality of Hanoi-groundwater, the field survey was conducted to collect the water samples from the wells of some water treatment plants as well as personal wells in Hanoi areas. Various chemical parameters were quantified in the field and samples were transported to Kumamoto University for further detailed analyses.

Based on the analytical results of groundwater in Hanoi areas, the synthetic groundwater used in this study was prepared with the similar composition as the polluted groundwater of Hanoi and its composition was shown in

Table 2.1.

Cultivation of nitrifying activated sludge

5 l of activated sludge, donated by the Northern Kumamoto Prefecture Wastewater Treatment Company, was used as seed sludge and cultivated in 15 l bucket by fill and draw under total oxidation conditions for more than one year. This activated sludge has a high nitrification activity (nitrifying activated sludge). A synthetic organic substrate containing peptone and meat extract was used as the carbon, nitrogen and phosphorous sources for cultivation of nitrifying activated sludge. The concentrated substrate 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. This concentrated synthetic organic substrate was kept in the refrigerator and was diluted to the proper concentration with tap water prior to use. In addition, the following inorganic salts were added to the substrate with

Table 2.1 Composition of the synthetic groundwater (mixed in tap water).

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 $CaCO_3$)	$NaHCO_3$

concentrations of (mg/l) NaCl 2, KCl 2.8, $CaCl_2$ 2.8, $MgSO_4 \cdot 7H_2O$ 4. The quantity of nitrifying biomass was estimated using mixed-liquor suspended solids (MLSS).

· Biomass carrier

In order to immobilize nitrifying activated sludge, a novel acryl-resin fiber material called Biofill (BL) donated by N. E. T. Co. Ltd. was used. This kind of biomass carrier material is light in weight, inexpensive and durable. Two strips of the BL material (Fig. 2.1)

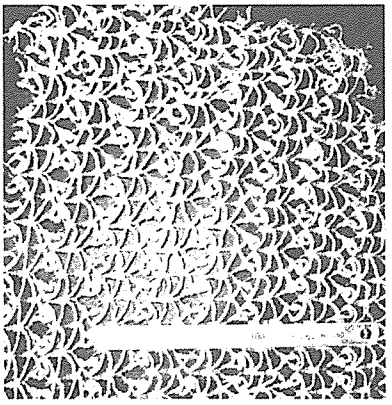


Fig. 2.1 Photograph of a novel acryl-resin fiber material (BL).

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. 2.2).

· Reactor description and operation

Fig. 2.2 shows the schematic diagram of the experimental system. The 5-l reactor was made of PVC and the influent was fed by using a variable speed peristaltic pump. BL strips were set symmetrically in the reactor. Aeration at the base of the reactor kept the contents well mixed and oxygenated.

In order to investigate the maximum capacity of the BL material for retaining biomass, the reactor was seeded with nitrifying activated sludge at various initial MLSS concentrations ranging from 1,500 to 5,000 mg/l. The remaining, unattached MLSS was measured following 15, 30, 60, 90, 120 and 150 min and the decrease in MLSS

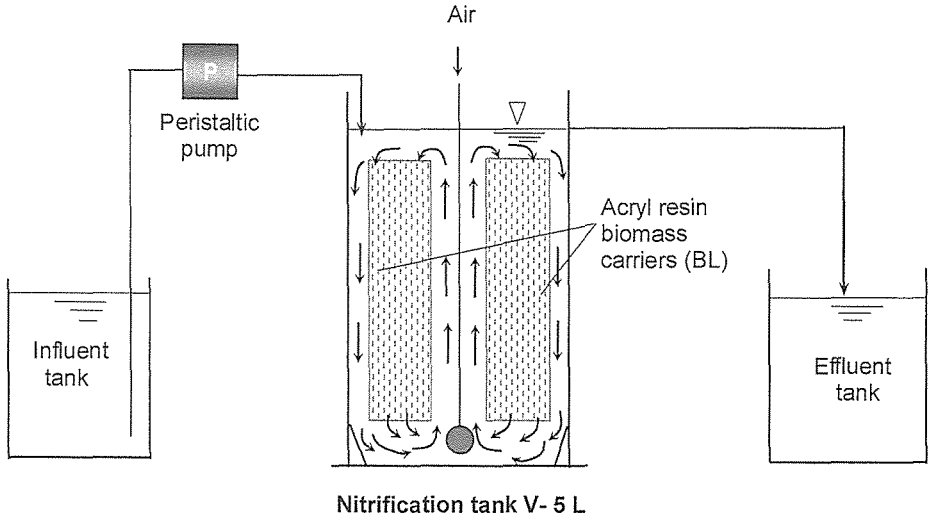


Fig. 2.2 Schematic diagram of the experimental system used in this research.

concentration was used to estimate the extent of sludge attachment to the BL material.

To determine the maximum acceptable ammonium loading capacity, the reactor was initially inoculated with 15 g of nitrifying activated sludge. This seed sludge completely attached to the BL materials within 2 hours 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 adjusting the influent flow rate.

The reactor was operated in the dark at room temperature. Influent alkalinity and the pH of the reactor contents were regulated by addition of a $NaHCO_3$ solution. The flow rate of the air supply was 0.5~ 1.5 L air/ min.

Analytical methods

Flow rate, pH, DO and alkalinity were monitored every 2 days and SS, NH_4-N, NO_3-N and NO_2-N levels were determined in the effluent. The pH and DO were measured by using a Mettler Toledo-320 pH meter (Switzerland) and UC-12 Digital DO/ O_2/ 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]. The analyses of microorganisms presented in reactor were conducted by the observation using stereoscopic microscope Nikon eclipse E-600, Nikon Co. Ltd, Japan.

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 were taken from the reactor at the operational periods with the influent ammonium concentrations of 10 and 30 mg/l, which then mentioned as samples A and B, respectively.

· DNA extraction and PCR amplification of 16S rDNA

Total genomic DNA was extracted from the samples using the Isoplant kit (Nippongene, Tokyo) following the manufacturer's instruction.

All PCRs were conducted with an equimolar mixture of two forward primers (CTO189fM (5'- GGAGRAAAGCAGGGGATCG - 3'), CTO189fC (5'- GGAGGAAAGTAGGGGATCG - 3'); R=A or G; Y= C or T) 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 $MgSO_47H_2O$, 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°C for 2 min followed by 40 cycles of denaturation at 94°C for 15 sec with primer annealing at 60°C for 2 sec and elongation at 68°C 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 *Taq*I 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 *Taq*I, 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

Groundwater contamination and abatement measures adopted in Hanoi city

In order to assess the quality of Hanoi-groundwater, the field survey was conducted. The locations of sampling points are shown in Fig. 3.1 and Table 3.1 shows the analytical results of surface water and groundwater in Hanoi areas.

According to the analytical results, the pollution of groundwater in Hanoi urban area is especially evidenced by the presence of ammonium. High ammonium levels of about 15~28 mg/l were detected in the groundwater taken from the wells (depth,100m) of Ha Dinh and Phap Van areas, which located in the southern part of Hanoi.

There are different explanations concerning to why the ammonium contamination of groundwater is occurring. One proposal considers the transfer of polluted water from surface sources into upper aquifer zones, which then passes to deeper zones through geological windows or discontinuities in subsurface impervious strata. In this scenario, pollution from anthropogenic origins is responsible for the nitrogenous compounds that ultimately enter the deep aquifer zones and contaminate these potable water sources. However, natural origins of the contamination are also considered. Since Hanoi is located on the Bac Bo plain delta, and the geology of which is composed of quaternary sediments resting on the tertiary deposit of Neogene age. The unconsolidated quaternary sediment consisted of sand and gravel interbedded with clay and silt, often contain organic matter, which contaminates the groundwater sources. Regardless of the debated origins of the nitrogenous compounds, the pollution problem is a reality that must be dealt with.

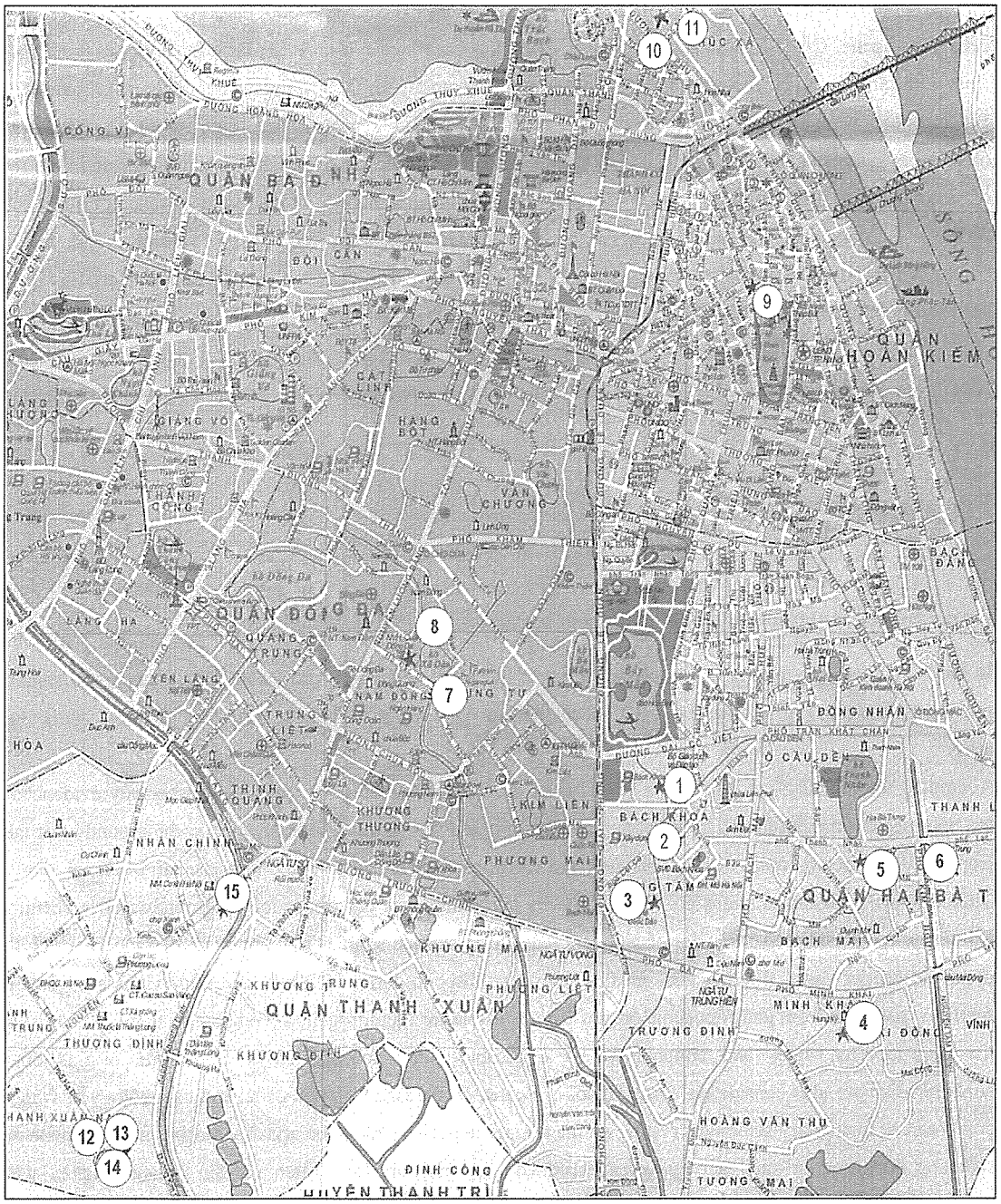


Fig. 3.1 Surface water and groundwater sampling points in Hanoi area.

Table 3.1 Analytical results of water quality in Hanoi area.

No	Name of well	Temp, °C	pH	NO_3-N, mg/l	NH_4-N, mg/l	SS, mg/l	SO_4^{2-}, mg/l	SiO_2, mg/l	S^{2-}, mg/l	IC, mg/l	TOC, mg/L	TC, mg/l	Fe, mg/l	Ca, mg/l	Mg, mg/l	Na, mg/l
1	Bach khoa	26.1	6.84	4.3	12.4		1.6	52.8	0.5	24.8	1.4	26.2	11.5	25.1	12.4	29.0
2	Dong Tam **	25.3	6.60	5.0	25.0		0.8	23.7	0.5	26.0	2.8	28.8	20.5	51.8	15.9	26.2
3	HUCE	25.3	6.69	7.8	10.0		5.2	53.8	0.0	26.6	1.6	28.2	31.6	42.8	18	44.0
4	Minh Khai **	34.3	6.70	9.7	7.6		14.4	42.6	0.9	25.5	1.7	27.2	24.0	45.9	20.8	72.4
5	Quynh Mai	25.3	6.80	0.9	3.6		4.2	35.0	1.3	13.6	0.5	14.1	4.3	15.2	10.9	9.1
6	Kim Nguu canal *	23.4	7.00	12.0	34.0	80	18.2	41.7	1.6	20.9	13.8	34.7	3.3	37.2	14.3	44.6
7	Lu River-out *	21.3	8.11	6.5	10.1	35	23.4	23.0	0.5	14.3	3.2	17.5	0.7	30.8	10.6	31.2
8	Lu River-in *	22.3	7.72	8.5	37	50	5.0	34.9	1.3	20.2	5.9	26.1	1.2	36.1	12.9	40.2
9	HoanKiem Lake *	19.9	10.30	4.3	0.3	175	1.6	21.8	1.4	1.9	8.3	10.2	1.0	13.7	1.4	8.3
10	Yen Phu 12	25.0	7.38	1.7	1.1		2.1	22.1	1.4	4.6	0.5	5.1	2.5	32.8	8.6	7.2
11	Yen Phu 20	25.2	7.10	3.6	4.6		2.6	45.1	2.2	6.3	0.6	6.9	9.1	40.5	13.8	18.2
12	Ha Dinh 5	26.0	6.82	3.3	15.3		2.2	41.3	0.1	23.5	2.5	26.0	15.6	25.5	14.8	27.4
13	Ha Dinh 6	26.3	6.88	3.2	18.5		2.6	37.9	0.9	22.3	1.3	23.6	12.1	23.8	12.7	30.8
14	Ha Dinh 8	26.0	6.86	3.7	18.8		2.1	44.6	0.1	17.8	1.7	19.5	15.0	23.4	13.5	28.7
15	Cau Moi *	22.0	7.43	7.2	31.0	25	11.7	90.9	0.8	18.1	5.4	23.5	1.0	33.1	16.6	44.4
16	Van Dien *	21.3	7.80	10.2	13.3	50	29.2	29.3	0.5	12.6	4.6	17.2	1.5	33.5	14	47.9
17	Phap Van No.1	27.0	6.90	3.2	28.0		2.8	30.9	0.0	20.7	3.2	23.9	9.5	25.3	13.2	32.6
18	Phap Van No.2	26.3	6.80	2.8	19.0		2.4	44.0	0.0	21.1	3.0	24.1	9.7	21.2	12	34.2
19	Phap Van No.3	26.3	6.80	2.9	15.4		2.1	41.4	1.2	18.7	2.8	21.5	9.5	20.9	11.8	36.2
20	Univ. tap	20.3	8.06	6.5	0.1		5.3	15.8	0.4	7.2	0.4	7.6	0.1	26.9	6.3	3.0

* Surface water; ** Personal well

At present, all WTPs in Hanoi are using a physico-chemical treatment process for the purification of groundwater (see **Fig. 3.2**). This treatment process effects for only iron removal. As shown in **Fig. 3.2,** with use of such process, ammonium can not be removed from groundwater. The countermeasures adopted to reduce levels of nitrogenous compounds in groundwater of Hanoi have included treatment by strong oxidation such as chlorination. While this has the drawback of high treatment costs, it includes the beneficial formation of chloramines with long lasting disinfection potential. However, the formation of harmful chlorinated organic compounds, while reduced by the formation of chloramines, is still a possibility. Associated health concerns have restricted the use of this method. Biological treatment methods are also being considered because of their potential of converting nitrogenous compound to non-toxic, environmentally safe forms; thus biological methods are worth further pursuing.

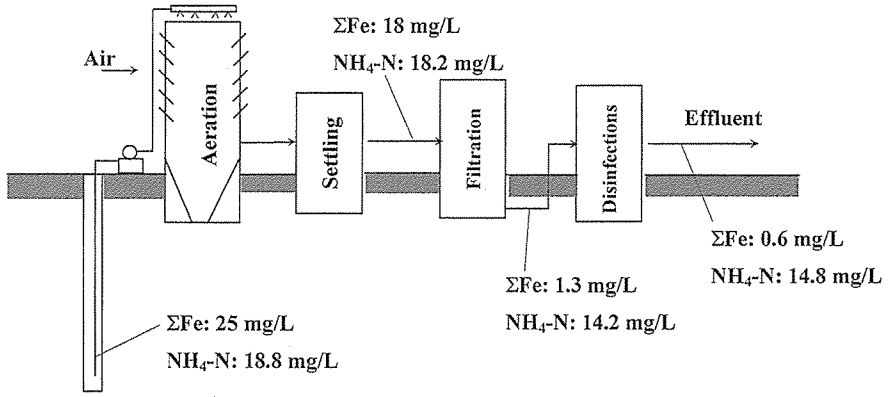


Fig. 3.2 Schematic view of a conventional water treatment process using in Hanoi WTPs [6].

Biological nitrification of groundwater contaminated with ammonium

· Cultivation of nitrifying activated sludge

5 l of activated sludge was used as seed sludge and cultivated in 15 l bucket by fill and draw under total oxidation conditions using a synthetic organic substrate containing peptone and meat extract. As shown in **Fig. 3.3** the concentration of sludge increased with increasing COD loading rate. From day 168, the MLSS concentration was reduced to about 4000 mg/l and sludge was cultivated with stable COD loading rate of 250 mg/l.d. A activated sludge with high nitrifying activities was obtained after long period of cultivation with high sludge retention time. In order to assess the nitrifying activities of the cultivated sludge, samples of mixed liquor were separated from biomass by centrifugation (10 min at 3,000 rpm). TOC and NO_3-N concentrations were determined hourly. **Fig. 3.4** shows the time courses of TOC and NO_3-N concentrations in mixed liquor. The TOC concentration decreased with increasing of NO_3-N concentration. This indicates the nitrifying activity of cultivated sludge. It is clear that the hourly NO_3-N concentration increased linearly with a slope of 6.92. Thus the specific nitrification rate can be estimated as 1.73 mg-N/g-MLSS/h.

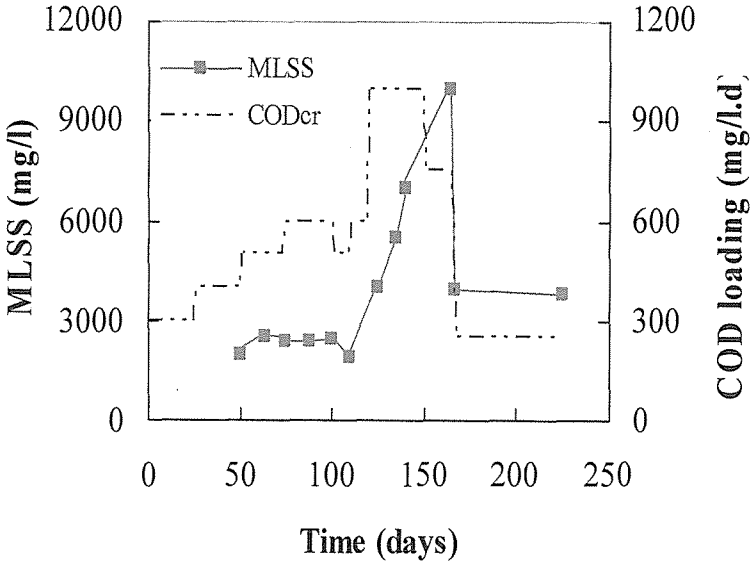


Fig. 3.3 Time courses of sludge concentration and COD loading rate.

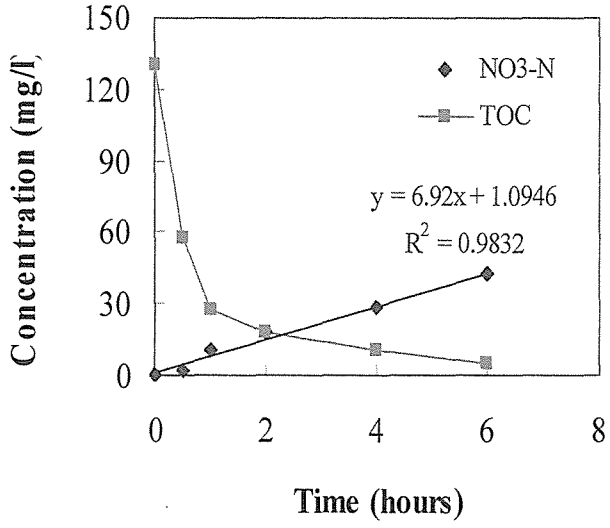


Fig. 3.4 Time courses of TOC and NO_3-N concentrations in mixed liquor.

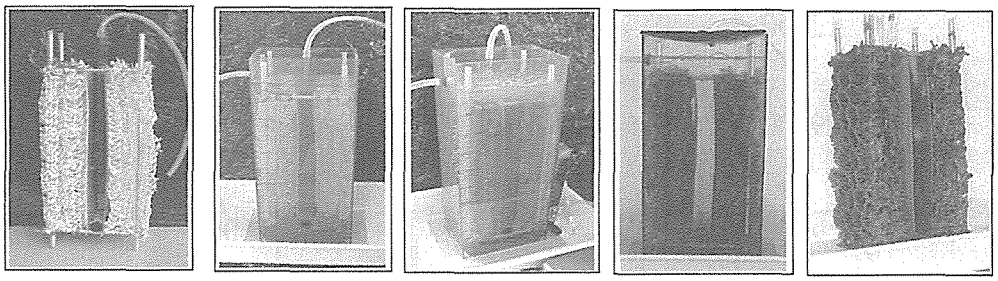


Fig. 3.5 Photographs of BL material and reactor during sludge attaching experiment.
(a) BL material.
(b) BL material dipped with nitrifying activated sludge.
(c) Starting of aeration.
(d) After 2 hours of aeration.
(e) BL material with attached nitrifying activated sludge.

· Sludge retention capacity

In order to investigate the maximum capacity of the BL material for retaining biomass, the BL strips 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 of the seeded sludge, when the initial concentration was below 5,000mg/l, was retained by BL within 2 hours of aeration (see Fig. 3.5). However, sludge seeded at initial concentrations above 5,000 mg/l did not attach completely within 2 hours. Time courses of sludge retention using 2 strips of BL material are shown in Fig. 3.6.These results indicate that the maximum sludge retention capacity of BL is about 6,200g-MLSS/m^3.

· Nitrification performance of reactor

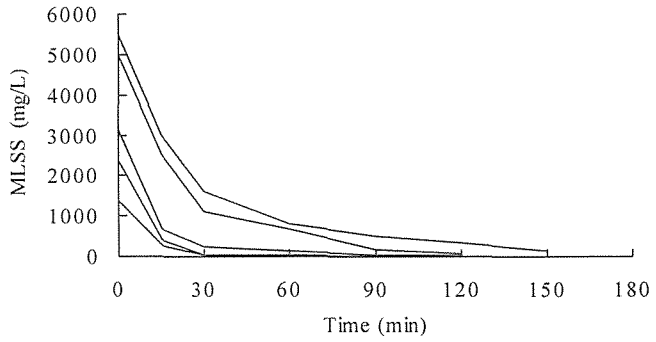


Fig. 3.6 Time courses of sludge retention on BL material.

After attachment of sludge on the BL material, operation 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. 3.7 (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 because of the low influent alkalinity (100 mg/l, see Fig.3.7 (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 alkalinity was increased to buffer the pH. After about 2 weeks more than 99% nitrification to NO_3-N was obtained and effluent NO_2-N concentrations were close to 0 mg/l (Fig. 3.7 (c)). Thereafter, the nitrogen volumetric loading rate (VLR) was increased stepwise by decreasing the HRT to 0.5 hour. The alkalinity of the influent was also increased stepwise and then maintained at approximately 200~230 mg/l to achieve a pH of 7± 0.2. As shown in Fig. 3.7 (a, c and d), more than 95% nitrification efficiency occurred 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.

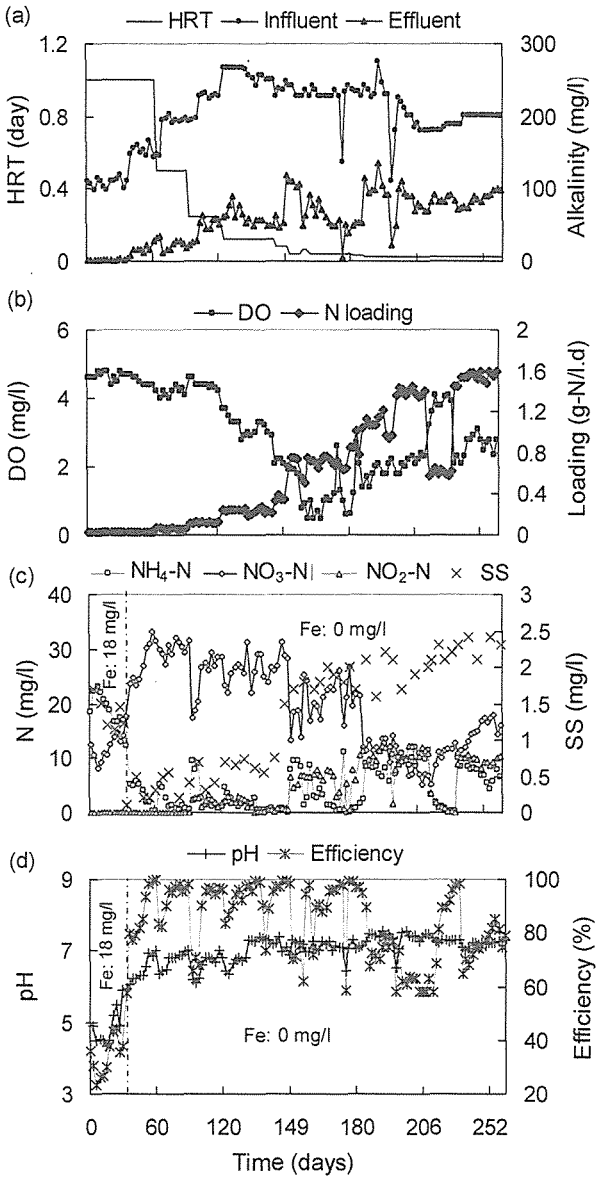


Fig. 3.7 Time course of:
(a) HRT, influent, effluent alkalinity;
(b) DO, nitrogen loading rate;
(c) Effluent NH_4-N, NO_3-N, NO_2-N, SS;
(d) pH and nitrification efficiency.

To support the activity of nitrifying bacteria attached on BL material, the reactor was initially aerated at an air flow rate of 0.5 l air/min, by which stable nitrification was maintained until the HRT was lowered to 3 hours corresponding to a nitrogenous VLR of 0.25 g-N/l.d. As shown in Fig. 3.7 (b), further increases in VLR were associated with a decrease in DO, which caused the nitrifying

activity to become unstable. At a HRT of 1 hour (VLR of 0.75 g-N/l.d), the DO concentration was decreased to 0.5 mg/L and the nitrification efficiency dropped to 88%. At this point, the airflow rate was increased to 1.5 l air/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 (VLR of 1.5 g-N/l.d). With this short HRT, high NO_2-N concentrations of 10~11 mg/l were observed in the effluent and the nitrification efficiency of reactor was reduced to about 60%. Subsequently, VLR was reduced to 0.5 g-N/l.d by decrease the influent NH_4-N concentration to 10 mg/l and then it was increased again to 30 mg/l (VLR of 1.5 g-N/l.d) on day 232 (Fig. 3.7 (b)). As shown in Fig. 3.7 (d), the nitrification efficiencies were about 95% at VLR of 0.5 g-N/l.d and 80% at VLR of 1.5 g-N/l.d.

As shown in Fig. 3.7 (c), the effluent from the reactor was exceptionally clear with SS less than 2.5 mg/l even when high precipitation of oxidized iron occurred in the reactor and when the airflow rate was increased. This aspect of low effluent SS is one of the main features of using acryl-resin fiber BL material. With such a system, use of a settling tank is not needed.

· Effect of pH on nitrification efficiency

Time courses of pH and nitrification efficiency are illustrated in Fig. 3.7 (d), which shows that a low influent alkalinity concentration induces drop in pH and the nitrification efficiency. It should be noted that pH of lower than 6 inhibits growth of nitrifiers and affects to the nitrification efficiency of reactor. Fig. 3.7 (d) shows that, for achievement of more than 90% of nitrification efficiencies, pH must be kept at 6.8~7.2.

· Effect of HRT on nitrification efficiency

The effect of HRT on the nitrification efficiency was investigated. The average values of nitrification efficiencies of reactor operated at each HRT are summarized in Fig. 3.8. At a HRT of 1 hour, the reactor was able to nitrify over 95% of 30 mg-N/l applied ammonium. These results revealed that with use of acryl-resin fiber BL material as a biomass attachment medium could retain a high concentration of nitrifiers; therefore, a decrease in HRT until 1 hour does not effected to nitrifying activity of the reactor.

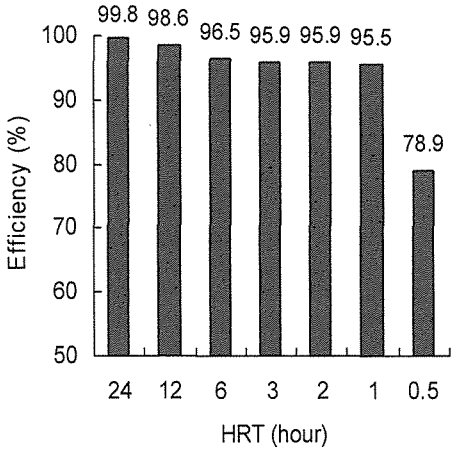


Fig. 3.8 Effect of HRT on nitrification efficiency.

• **Effect of nitrogen loading rate**

Capacity of the reactor for the nitrification treatment of 30 mg-N/l applied ammonium is illustrated in Fig. 3.9. In order to achieve a nitrification efficiency of more than 95%, a maximum VLR value of 0.75 g-NH_4-N/l.d could be applied. 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.

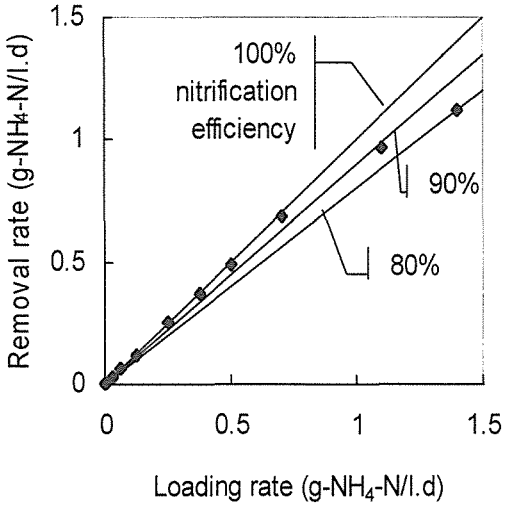


Fig. 3.9 Relationship between nitrogen VLR and NH_4-N removal rate.

• **Sludge yield**

The net observed sludge yield in the reactor was estimated by monitoring the NH_4-N removal as well as the VSS contents in both the reactor and the effluent. This value can be determined by the following equation.

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

Where:

Y_{obs}: Observed sludge yield coefficient (g-VSS/g-NH_4-N removed).

X_t: The amount of biomass in the reactor at operation time t (g-VSS).

X_0: The amount of biomass in the reactor at operation time t = 0 (g-VSS).

Q: Discharge flow rate (l/d).

C: The effluent VSS concentration (g-VSS/l).

$\sum S$: Total substrate removed (g-NH_4-N).

t: Operation time (day)

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

· Identification of microorganisms and bacterial community in nitrifying sludge

The biological component of the activated sludge system is comprised of microorganisms. Bacteria, fungi, protozoa, and rotifers constitute the biological component, or biological mass, of activated sludge. The efficiency of biological treatment system using activated sludge is supposed to relate to the bacterial population and protozoa presented in activated sludge. Different species of microorganisms can be found and have been listed by various authors [9-13]. Microorganisms are directly affected by their treatment environment. Changes in food, dissolved oxygen, temperature, pH, total dissolved solids, sludge age, presence of toxins, and other factors create a dynamic environment for the treatment organisms. Food (organic loading) regulates microorganism numbers, diversity, and species when other factors are not limiting [10]. The relative abundance and occurrence of organisms at different loadings can reveal why some organisms are present in large numbers while others are absent.

· Predominant microorganisms in nitrifying sludge

In this study, the analyses of microorganisms that dominate in sludge of the reactor were conducted by the observation using stereoscopic microscope. Fig. 3.10 shows photographs of biomass (as floc) attached on the yarns of BL material (left site) and predominant microorganisms (right site). The floc photographs show where, in relationship to the biomass, the treatment organisms can be found. Bacteria are a part of the floc or present as free cells around the floc. Swimming and gliding ciliates work the open water engulfing bacteria or other prey. Stalked ciliates attach to the biomass and vortex suspended bacteria into their gullets, while crawlers break bacteria loose from the floc surface. Three microorganic species of *Aspidisca sp.*, *Euglypha sp.*, and *Philodina sp.* were the most frequently observed and these protozoa were identified as the predominant microorganisms in the nitrifying sludge of present treatment system. Being strict aerobes, these microorganisms prove to be excellent indicators of an aerobic environment.

Aspidisca sp. is a member of the crawling ciliates, this protozoan is flat from top to bottom. The beating of the cilia gives the appearance of a protozoan that is crawling as it moves across the surface of floc particles. Crawling ciliates were found in large numbers when the nitrification efficiency of reactor was stable and high. These crawling ciliates indicate a stable and healthy sludge.

Euglypha sp. is a shelled (testate) amoeba. The shell of this *Euglypha sp.* consists of oval plates. Its spines project backward from the lower half of the shell. The shell of Euglypha is often transparent, allowing the hyaline (watery) body to be seen inside the shell. The pseudopodia extend outward in long, thin, rays when feeding or moving. Euglypha primarily eats bacteria and their numbers usually increase with increasing sludge age.

Philodina sp. is a member of rotifers. They have a very important role in nutrient cycling. And as they convert a good deal of their food into biomass, they play a key role in energy flow and nutrient cycling. When they consume nutrients, these nutrients are then passed on to the next trophic level. Rotifers are carbon transporters as they form a link between pico and nanoplankton carbon and macrozooplankton. Since they are sensitive to toxins and are thus great monitors of the health of water.

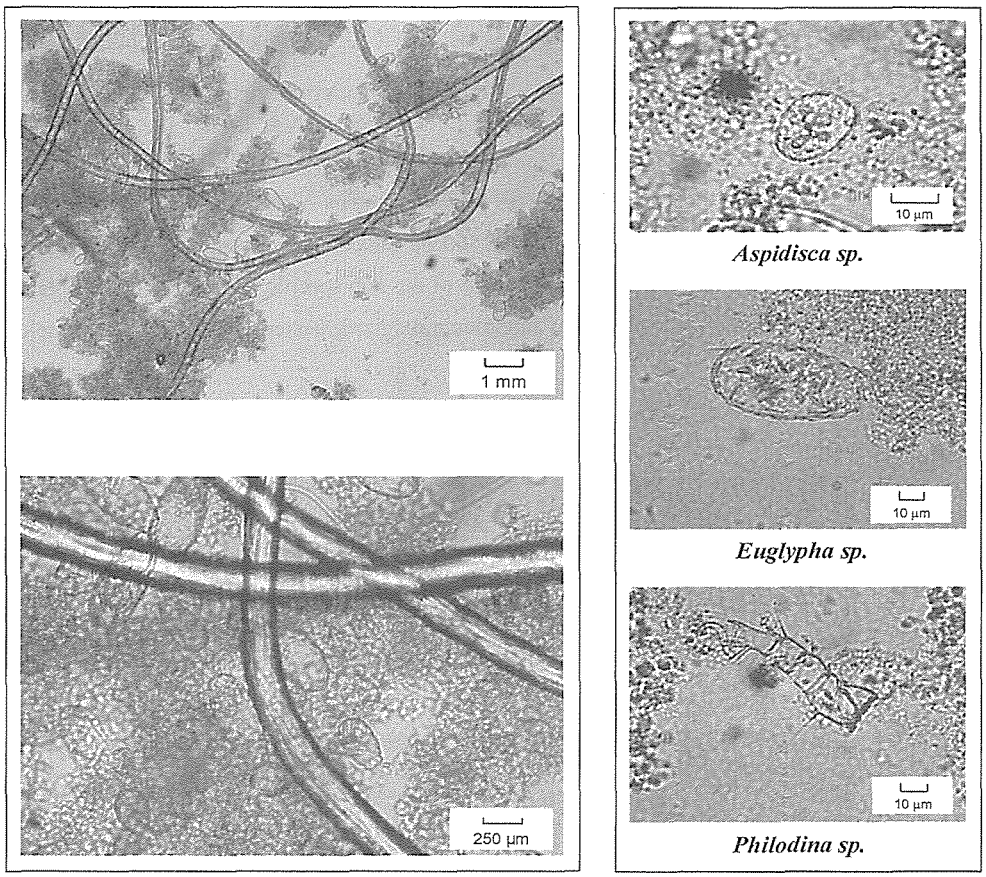
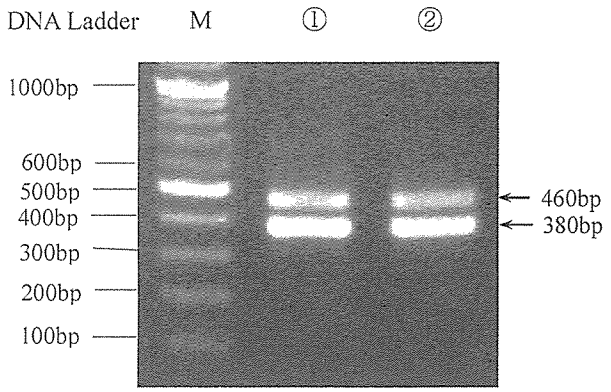


Fig.3.10 Photograph of biomass attached on the yarns of BL material(left) and predominant microorganisms(right)

• **Identification of bacterial community in nitrifying sludge**

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. Total genomic DNA was extracted and according to the electrophoresis analyses, almost the same DNA amounts were determined from these two samples (data not show). These results suggested that there were the same bacterial population in these two samples. In order to amplify 16S rDNA fragments in a non-conserved region among a β-subdivision ammonia oxidizer, the bacterial 16S rDNA-specific primer pair (CTO189fMC and CTO654r) was used. Fig. 3.11 shows the results of electrophoresis analyses of amplified 16S rDNA from samples A and B. For both samples, the intense bands of 460 and 380 bp were detected. The bands of 460 bp indicate the present of β-subdivision ammonia oxidizer while the bands of 380 bp were determined to be of the non-specific product by the previous research (data not show). This result confirms the present of ammonium-oxidizing bacteria in tested sludge samples.



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

Fig. 3.11 Electrophoresis analyses of amplified 16S rDNA using the extracted DNA from sludge samples as templates.

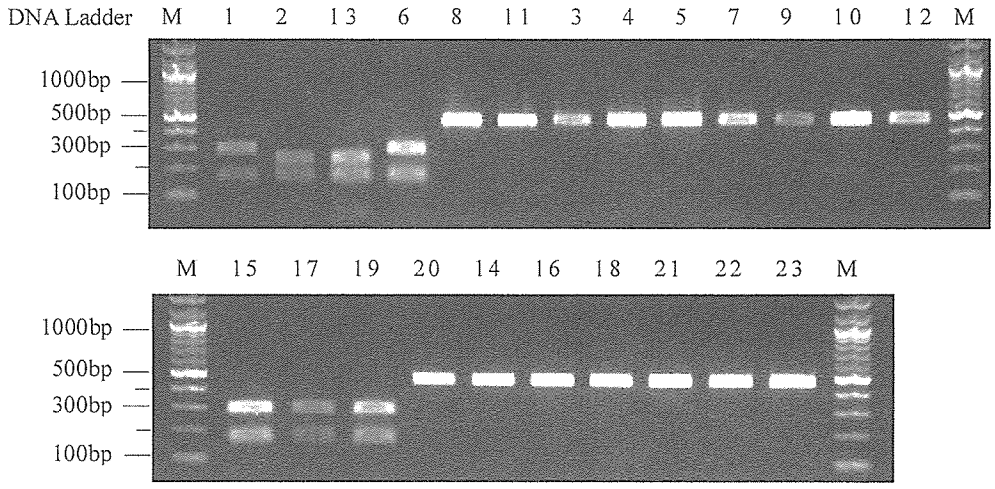


Fig. 3.12 RFLP analyses of restriction fragments obtained from clones 1~13 of sample A and clones 14~23 of sample B using the restriction enzyme *Taq*I.

Table 3.2 Grouping of amplified DNA fragments obtained from the sludge samples.

Group	Clone number		Bacterium with highest homology
	Sample A	Sample B	
I-a	1		*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%)

For specifically identification of ammonium oxidizing bacteria presented in sludge samples, the RFLP analyses were conducted using the restriction enzyme *Taq*I to digest the amplified PCR products, and categorize to groups with the same size of restriction fragments. Fig. 3.12 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 3.2. 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.

Conclusions

The following conclusions have been drawn from the experimental results obtained during the study of nitrification treatment of NH_4-N polluted groundwater using an acryl-resin fiber BL material as a biomass attachment medium.

1. Use of an inexpensive and durable acryl-resin fiber BL material for nitrification treatment of NH_4-N polluted groundwater was demonstrated. The biomass retention capacity of BL material for nitrifying activated sludge was determined to be 6,200 g-MLSS/m^3 and the effluent from the nitrifying reactor packed with BL