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ENHANCEMENT OF AMMONIA REMOVAL IN PEAT BIOFILTER SEEDED WITH ENRICHED NITRIFYING BACTERIA

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ABSTRACT

Night soil sludge was enriched to foster nitrifying bacteria by the addition of ammonium sulfate, and the enriched mixed culture of nitrifying bacteria obtained was inoculated to peat. Ammonia gas was introduced to the peat biofilter by gradually increasing from 48 to 265 ppm, at a space velocity from 87 to 437 h^{-1}, with an ammonia load from 0.5 to 5.8 g N kg^{-1} dry peat d^{-1} for 103 days. The ammonia removal characteristics observed over a 103 day experiment were as follows: an average ammonia removal ratio of 97 %, complete removal capacity of 4.5 g N kg^{-1} dry peat d^{-1}, maximum removal capacity of 5.6 g N kg^{-1} dry peat d^{-1}, maximum removal rate of ammonia, V_m of 46.1 g N kg^{-1} dry peat d^{-1}. The ammonia removal capacity of this peat biofilter was enhanced significantly and the maximum removal capacity and maximum removal rate were the highest among the data reported to date.

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INTRODUCTION

The high concentration of ammonia emitted from composting plants, night soil or waste water treatment, and various industries still causes environmental problems. Although ammonia has a detectable odor threshold concentration of approximately 17 ppm [1], the concentrations of ammonia from night soil treatment plants were reported to be between 0.2 and 50 ppm, between 20 and 200 ppm from fishmeal plants, and greater than 35 ppm in composting facilities [1-3]. In general, for the removal of odorous compounds in contaminated air, biological treatments have some potential advantages over other chemical and physical treatments [4,5], for example, that the pollutants do not enter into another phase, they are converted to harmless oxidation products and biological methods are relatively cheap because of the low investment and operational costs. A good operational stability over a long period can be achieved if careful maintenance is carried out.

Recently, some studies of microbial ammonia removal by a biofilter using peat [6-9] or wood bark [10] as an organic packing material, and activated carbon fiber (ACF) as an inorganic packing material [11] have been conducted. The biofilters were commonly seeded with uncharacterized sludge. In order to enhance the microbial removal rate, several methods have been proposed [5]: seeding of useful bacteria, immobilization of microorganisms, selection of carriers, and application of new microorganisms to processes. In previous studies we used night soil sludge as a seed for peat and ACF biofilters [6,9,11]. Although they showed a good removal rate of ammonia, significant biological removal started at around the 20th day and complete removal of ammonia was observed after 28 days, which is defined as the acclimation period. In order to shorten the acclimation period and enhance the ammonia removal rate, enrichment of the night soil sludge to foster a mixed culture of nitrifying bacteria will be useful. However, such a trial has never been conducted, mainly because the growth of chemoautotrophic nitrifying bacteria is extremely slow and their isolation is difficult [12,13]. Here, we conduct the enrichment of sludge to increase the population of nitrifying bacteria, and the characteristics of ammonia removal by a peat biofilter seeded with an enriched mixed culture of nitrifying bacteria are investigated.

MATERIALS AND METHODS

Enrichment culture

Twenty ml of night soil sludge was inoculated to 180 ml AL medium in a 300 ml bottle. AL medium contained: 2.5 g $(NH_4)_2SO_4$, 0.5 g KH_2PO_4, 50 mg $MgSO_4 \cdot 7H_2O$, 4 mg $MgSO_4 \cdot 7H_2O$, and 0.1 mg Fe-EDTA per liter of distilled water, at pH 8.0-8.2 [9,12]; 0.5 mg phenol red was added as an indicator. The bottle was aerated at 1 l min^{-1} at a temperature of 25 - 28 °C. During the enrichment period, pH was maintained at 6-8 by adding 0.1N NaOH. The concentration of NH_4^+ was also maintained at 300 - 700 ppm by adding 10-times-concentrated AL medium. The pH and concentrations of NH_4^+, NO_2^- and NO_3^- of the culture were measured once or twice a day.

When NO_3^- concentration reached about 8 g l^{-1}, the culture was transferred to fresh medium and the next enrichment was carried out by the same method. This procedure was repeated for several months. At the end of enrichment, a sample was taken for the determination of the concentration of surviving microorganisms by counting on nutrient agar (NA) (Eiken Kagaku Co., Ltd., Japan) and P medium plus gelrite (10 g l^{-1}) (PG). P medium contained: 2.5 g $(NH_4)_2SO_4$, 34 g $Na_2HPO_4 \cdot 12H_2O$, 0.7 g KH_2PO_4, 0.5g $NaHCO_3$, 100 mg $MgSO_4 \cdot 7H_2O$, 5 mg $CaCl_2 \cdot 2H_2O$, and 1 mg Fe-EDTA per liter (pH 8.0 - 8.2) [13,14]; 0.5 mg phenol red was added as an indicator. Gelrite, a trade name of gellan gum (Kelco Division, Merck Co., USA), was used to make a solid medium.

Confirmation of nitrifying bacteria

At the end of enrichment, several kinds of bacteria grew on PG plates. Six of about 50 colonies appearing on PG plates were tested for ammonium oxidizing ability. Each isolate was inoculated to 5 ml AL medium and incubated at 30 °C at 120 strokes per minute (spm) for 2 weeks. At the end of the incubation period, the amounts of NO_2^- and NO_3^- production were tested using a Merckoquant test strip. The highest producer of NO_2^- and NO_3^-, B1123, was selected. Then, the isolate was inoculated to 100 ml AL medium and incubated at 30 °C at 120 spm for 8 days. The cells collected were mixed with 50 % glycerol solution which was stored at -20 °C as a stock culture.

The growth characteristics of B1123 in AL medium were investigated. One loop of a B1123 colony was grown on a PG plate, inoculated to 5 ml AL medium, then incubated at 30 °C at 120 spm for 8 days. This culture was transferred to 100 ml AL medium, then incubated again at 30°C at 120 spm. During incubation, pH, optical density at 660 nm (OD_{660}), and the concentrations of NO_2^- and NO_3^- were monitored.

Experimental apparatus

As the carrier in the biofilter, peat moss A (Takahashi Peat Moss Co., Ltd., Hokkaido, Japan) was packed in glass columns (5 cm inner diameter, 50 cm height) in a laboratory-scale biofilter, as previously reported [9,11]. The characteristics of the peat were described previously [6, 9]. After the measurement of moisture content, about 60 g dry peat plus 2.2 g $Ca(OH)_2$ were sterilized at 120 °C for 20 minutes. This peat was sprayed with cell suspension of B1123 grown in AL medium, mixed and packed into columns to a height of 18 cm. The height of the peat bed was compacted to 14 cm within two weeks. Detailed experimental conditions are summarized in Table 1. The 0.05 - 1.5 N aqueous ammonia solution in a 3 l bottle was fed, using a peristaltic pump, to the top of an ammonia generator column of PVC (5 cm outer diameter, 52 cm height) which was packed with glass beads, through which air was passed in the countercurrent flow. Air containing a specific concentration of ammonia was then supplied to the biofilter columns. The ammonia load into the biofilter was changed by controlling the inlet concentration and/or space velocity. Forty ml of sterilized distilled water was sprayed aseptically and manually on the peat bed every 2 days to maintain the moisture content of the bed at about 70 %. All experiments were conducted at room temperatures of 24 - 28 °C. During the operation, the pH of the biofilter was maintained at 6 - 8 by adding 40 ml of 1 % Na_2CO_3. When nitrite and nitrate concentrations reached about 10 g l^{-1}, 100 ml of sterilized distilled water was sprayed to wash the peat bed.

Bacterial count

The cell number counted by the most probable number (MPN) method [5, 6, 9,11] was considered to be nitrifying bacteria mainly because enriched nitrifying bacteria are a

Table 1. Experimental condition of peat biofilter seeded with enriched nitrifying bacteria and the cell number of nitrifying bacteria.

Characteristics	
Packing dry weight (g)	56.7
Packed volume (l)	0.275
Packing density (g dry peat l^{-1})	206
Packing height (cm)	14
Initial moisture content (%)	64
Initial pH	7.46
Supplied ammonia	
Inlet concentration (ppm)	50 - 265
Flow rate (l min^{-1})	0.4 - 2.0
Space velocity (h^{-1})	87 - 438
Load (g N kg^{-1} dry peat d^{-1})	0.50 - 5.8
Cell number measured by MPN method (cells g^{-1} dry peat)	
Initial	6.0×10^7
After 68 days	2.2×10^8

mixture of autotrophic and heterotrophic bacteria. The homogenized peat sample was also serially diluted in sterilized distilled water, then spread on NA and PG plates, and colonies appearing on plates were counted.

Analysis

The moisture content of peat was determined by drying for more than 8 hours at 80 °C in an oven. Inlet and outlet ammonia concentrations in the biofilter were measured using ammonia gas detection tubes (Gastec, Co., Ltd., Japan). The lower detection limit of the tubes was 0.25 ppm. To test the presence of nitrate and/or nitrite in the drained water, a Merckoquant test strip for nitrate and nitrite (Merck KGaA, Germany) was used.

RESULTS AND DISCUSSION

Characterization of enriched ammonia-oxidizing bacteria

After 6 months of enrichment, nitrifying bacteria, B1123, were obtained. By detailed analysis, B1123 was found to contain a predominantly autotrophic nitrifying bacteria and a minority of heterotrophic bacteria, the separation of which was extremely difficult. Therefore, B1123 was used for further experiments. The growth curve of the mixed culture of nitrifying bacteria of B1123 on AL medium is shown in Figure 1 a. The optical density (OD_{660}) of the culture was below 0.05 after 10 days and the cell numbers counted on PG plates were on the order of 10^6 - 10^7. The pH was maintained at 6 - 8 by adding 0.1 N NaOH. The total concentration of NO_2^- and NO_3^- reached 3 g l^{-1} after 8 days (Figure 1 b), indicating that more than 90 % of the ammonium was oxidized to NO_2^-.

When B1123 was cultivated in NB medium for heterotrophic bacteria, OD_{660} increased, and two days were required to reach the stationary phase; no production of NO_2^- and NO_3^- was observed. It is assumed that the heterotrophic bacteria which coexisted with nitrifying bacteria grew rapidly in organic NB medium, but had no ability to oxidize nitrogen compounds to NO_2^- and NO_3^-. To confirm the survival of nitrifying bacteria even in NB medium, the cells of B1123 grown in NB medium were washed, suspended in AL medium, and then incubated at 30°C. After 10 days, pH decreased to about 7 and productions of NO_2^- and NO_3^- were confirmed; then the concentration of the two substances increased very rapidly (data not shown).

Ammonia removal characteristics

Ammonia removal by the peat biofilter involved two processes [9, 15]. First, ammonia was removed by physical and chemical adsorption by peat. After peat was saturated with ammonia, ammonia was oxidized by nitrifying bacteria. Therefore, the time required to reach a steady state of ammonia oxidation by nitrifying bacteria is the acclimation

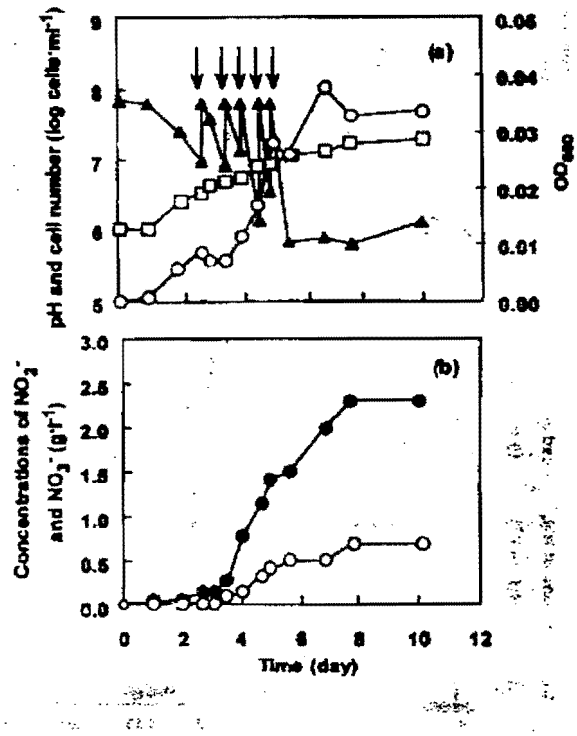

Figure 1. Growth characteristics of enriched nitrifying bacteria, B1123, in AL medium: (a) optical density at 660 nm (OD_{660} ○, cell number □, and pH (▲), and (b) production of NO_2^- (○), and NO_3^- (●). Addition of 0.1 N NaOH (↓).

time, which is usually about one month. Figure 2 shows the time course of ammonia removal by a peat biofilter seeded with enriched nitrifying B1123 bacteria. The inlet ammonia concentration was introduced at about 100 ppm, at SV 87 h^{-1}, and at load of about 0.5 g N kg^{-1} dry peat d^{-1}. In a previous study, in the peat biofilter seeded with original night soil sludge, total ammonia adsorption during the acclimation period was 2.6 g N kg^{-1} dry peat for 20 days of operation [9]; after that, ammonia was detected at the outlet. Total removal of 2.7 g N kg^{-1} dry peat of ammonia by this peat with enriched nitrifying bacteria was attained in about 7 days, at which time no ammonia was detected at the outlet. After that, when the load was increased to about 0.6 g N kg^{-1} dry peat d^{-1} at 21 days, the removal ratio decreased for 3 days due to unknown reasons, but complete removal was observed again at 25 days.

During the first 47 days, the inlet concentration of ammonia was maintained at an average of 72 ppm (Figure 2a), and the load was increased gradually from 0.5 to 2 g N kg^{-1} dry peat d^{-1} (Figure 2b) by changing the space velocity from 87 to 390 h^{-1} (Figure 2a). After that, the inlet concentration was increased gradually to 265 ppm at an average SV of about 320 h^{-1} where the load was as high as 5.8 g N kg^{-1} dry peat d^{-1}. The kinetic data were taken on the 55th day. The load was further increased to 5.78 g N kg^{-1} dry

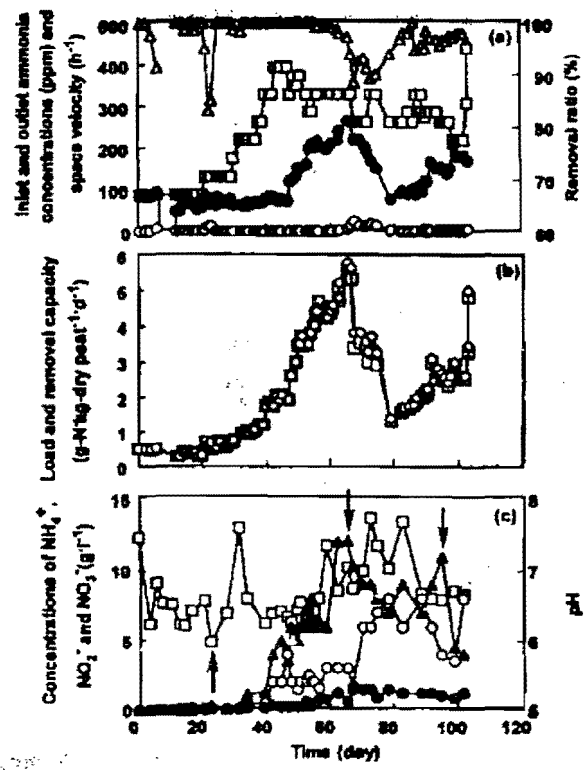

Figure 2. Time course of ammonia removal by peat biofilter seeded with enriched nitrifying bacteria: (a) inlet (●) and outlet (○) ammonia concentrations, removal ratio (Δ) and space velocity (□); (b) ammonia load (□) and ammonia removal capacity (○) and (c) pH (□), NH_4^+ (●), NO_2^- (○) and NO_3^- (▲) concentrations. Addition of 40 ml of 1 % Na_2CO_3 (↑) and washing of column by 100 ml distilled water (↓).

peat d^{-1} (Figure 2b) and a removal ratio of more than 98% was attained (Figure 2a). However, after samples were taken for cell number counting at 68 days, the removal ratio decreased even when the load was reduced to about 1.5 g N kg^{-1} dry peat d^{-1}, indicating that there were drastic disturbances on peat. Then, the removal of ammonia improved again to a load of 3.2 g N kg^{-1} dry peat d^{-1} at 92 days and then increased to 5.0 g N kg^{-1} dry peat d^{-1} at 103 days (Figure 2b). The average removal ratio of ammonia was 97.3% for a 103-day experiment. From the relationship between load and removal capacity of ammonia, the complete removal was at 4.5 g N kg^{-1} dry peat d^{-1} and the maximum removal capacity was 5.6 g N kg^{-1} dry peat d^{-1} (Figure 3). The data obtained in this experiment, as well as previous data, are shown in Table 2. Ammonia removal by enriched nitrifying bacteria is the most efficient method.

The cell number of heterotrophic bacteria appearing on NA plates was significantly decreased after 68 days. During this experimental period, the number of nitrifying bacteria counted by the MPN method increased about fourfold (Table 1). We isolated some autotrophic nitrifying bacteria from enriched B1123 bacteria which included an ammonia-oxidizing bacterium, *Nitrosomonas eutropha*, and a nitrite-oxidizing bacterium, *Nitrobacter hamburgensis*. The characteristics of these bacteria will be described in a separate paper.

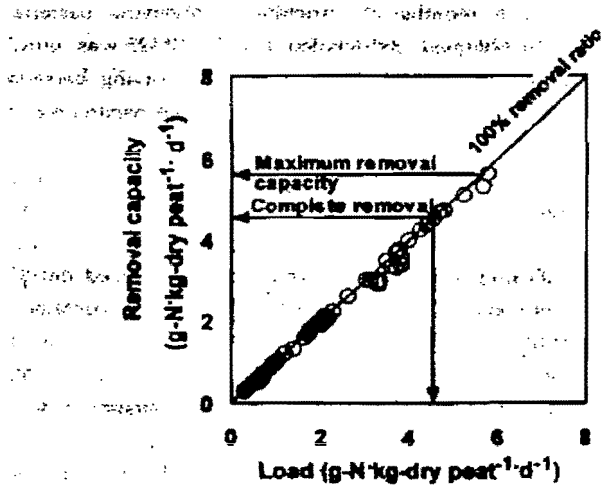

Figure 3. Relationship between ammonia load and ammonia removal capacity in peat biofilter seeded with enriched nitrifying bacteria.

Table 2. Ammonia removal characteristics by biofilters seeded with different mixed culture of nitrifying bacteria.

Ammonia removal characteristics	Peat seeded with night soil sludge [9]	ACF seeded with night soil sludge [11]	Peat seeded with B1123
Complete removal (g-N·kg^{-1} peat d^{-1})	1.6	1.5	4.5
Maximum removal capacity (g N·kg^{-1} peat d^{-1})	3.2	3.5	5.6
V_m (g N kg^{-1} peat d^{-1})	11.4	8.4	46.1
K_s (ppm)	226	26	350
Cell number by MPN method (cells g^{-1} dry peat) after kinetic data were taken	6.8×10^7	1.05×10^9	2.2×10^8
Specific ammonia oxidation rate (g N·$cell^{-1}$ h^{-1})	7.0×10^{-12}	3.34×10^{-13}	8.6×10^{-12}
Relative ammonia-oxidation rate	1	0.05	1.2

When the mixed culture of B1123 was applied to an activated carbon fiber (ACF) biofilter, the characteristics of which were described in a previous paper [11], the removal capacity was 1.2 g N kg^{-1} dry ACF d^{-1} at 20 days (data not shown), after which improvement was negligible. The cell number could not be determined by the MPN method at a dilution of 10^3, for unknown reasons. ACF was not an appropriate carrier for this nitrifying population.

Drain analysis

Results of the analysis of drain from a peat biofilter seeded with B1123 are shown in Figure 2c. When pH decreased to 6.0, the column was neutralized by adding 40 ml of 1 % Na_2CO_3 solution, and pH was maintained at around 7. During the experiments, the pH ranged from 6.0 to 7.6 with an average of 7.0. Almost no ammonium was detected for 30 days, and NO_2^- and NO_3^-, were detected from 20 days (Figure 2c). When the load of ammonia was increased from 20 to 67 days, there was a significant increase in NO_2^- followed by an increase in NO_3^- (Figure 2c), from the 30th - 67th days, confirming that ammonia was converted to NO_2^- and to NO_3^- in consecutive reactions. Complete removal of ammonia of 4.5 g N kg^{-1} dry peat d^{-1} was observed at 55 days, when the concentrations of NH_4^+, NO_2^- and NO_3^- were 0.4, 2.0, and 6.0 g l^{-1}, respectively. When the NO_3^- concentration reached 12 g l^{-1} at 67 days, the removal ratio decreased to 90%. It was assumed that nitrification was inhibited by such a level of NO_3^-. Therefore, the peat bed was washed by spraying 100 ml sterilized distilled water. The washing of peat with distilled water was repeated at the 97th day.

These experiments showed that a high removal capacity of the biofilter can be maintained by controlling the pH between 7.5 – 8.0 and low nitrite and nitrate concentrations in the peat. The leaching of accumulated NO_3^- helps the dilution of the product, and increases the nitrification capacity [7].

Kinetics analysis

Kinetic analysis of the biological removal of ammonia was conducted using a Michaelis-Menten type equation, and the removal efficiency was evaluated by assuming a plug air flow, as described previously [9,11]. The final equation is

$$\frac{C_{ln}}{R} = \frac{K_s}{V_m} + \frac{C_{ln}}{V_m}, \qquad (i)$$

where

V_m : maximum removal rate (g N kg^{-1} dry peat d^{-1})

K_s : saturation constant (ppm)

$C_{ln} = (C_o - C_e) \ln(C_o/C_e)^{-1}$

C_o : inlet concentration of ammonia (ppm)

C_e : outlet concentration of ammonia (ppm)

$R = SV\,(C_o - C_e) \cdot \alpha^{-1}$

SV : space velocity (d^{-1})

α : conversion coefficient (kg dry peat g N^{-1}).

Figure 4 shows the kinetic analysis of ammonia removal by the peat biofilter seeded with a mixed culture of B1123. The maximum removal rate, V_m, obtained was 46.1 g N kg^{-1} dry peat d^{-1} and the saturation constant, K_s, was 350 ppm. The V_m obtained was 4 times higher than that reported in a previous paper [9] (Table 2). The estimation of these parameters is useful for comparing the performance characteristics of various biofilters with different seeding sources, structures and carriers. The biological ammonia removal rate on peat was reported to be between 0.2 - 1.5 g N kg^{-1} dry peat d^{-1} [15]. In waste water treatment using a fluidized-bed bioreactor packed with granular activated carbon (GAC), V_m of the ammonia oxidizer was recalculated to be in the range from 0.12 to 2.1 g N kg^{-1} dry GAC d^{-1} and K_s varied from 0.1 to 598 ppm-NH_3 [16]. The ammonia removal rate obtained in this study was higher than these values.

By using the cell number 2.2 x 10^8 cells g dry peat obtained by the MPN method (Table 2), the specific ammonia oxidation rate was calculated to be 8.6 x 10^{-12} g N $cell^{-1}$ h^{-1} (Table 2). The ammonia oxidation rate of peat biofilters was significantly higher than that of the ACF biofilter, mainly because peat had a high adsorption capacity and significantly high buffering capacity [9]. The kinetic analysis indicated that the ammonia oxidation rate of *Nitrosomonas europaea* ranged from 1.56 x 10^{-14} to 1.81 x 10^{-13} g N $cell^{-1}$ h^{-1}, where the fluorescent antibody method or the MPN method was used for counting the cell number [17, 18]. The ammonia oxidation rate of enriched B1123 was significantly higher than those values.

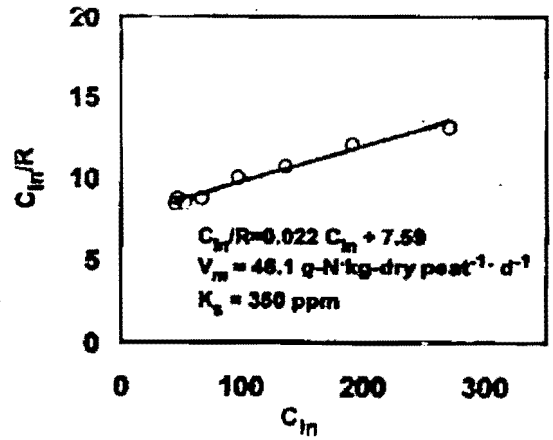

Figure 4. Results of kinetic analysis of peat biofilter seeded with enriched nitrifying bacteria.

Although the enriched nitrifying bacteria contained the contaminant of heterotrophic bacteria, application of this culture to the peat biofilter enhanced the removal characteristics of ammonia, and the value obtained in this experiment may be close to the maximum one inherent in nitrifying bacteria.

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