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Removal of NH_4^+–N ion in drinking water treatment using locally isolated heterotrophic nitrifier

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ABSTRACT

Batch removal treatment of NH_4^+–N from drinking water was carried using locally isolated heterotrophic nitrifier of *Bacillus cereus* I6. This work investigates the effect of initial NH_4^+–N concentration (5–25 mg/L), initial pH (pH 3–10) and inoculum size (0.5–3.0% v/v) on the removal of NH_4^+–N. From the isolation, screening and identification, heterotrophic *B. cereus* I6 (98% similarity) was the most potential degrading strain in NH_4^+–N removal. The removal was high at initial NH_4^+–N concentration of 5 mg/L (62%), initial pH of 5 (69.5%) and inoculum size of 3.0% v/v (85%). The removal decreased from 62 to 24.8% when the initial NH_4^+–N concentration was increased, while it had increased from 29 to 85% when the inoculum size was being increased. The most suitable conditions for NH_4^+–N removal by heterotrophic of *B. cereus* I6 was observed at an initial pH of 5.

Keywords: *Bacillus cereus*; Drinking water treatment; Heterotrophic nitrifier; NH_4^+–N removal; Biological aerated filter

1. Introduction

Ammonia is a compound of nitrogen and hydrogen with the formula of NH_3. It forms ammonium (NH_4^+) ion when dissolving in water. High level of NH_4^+ in drinking water will cause deleterious impact on drinking water treatment plants via complications in the chlorination process due to the creation of carcinogenic chloramines [1]. Drinking water containing more than 0.2 mg/L of ammonia would decrease the disinfection efficiency [2,3]. Moreover, NH_4^+ may interfere with the manganese filtration process because too much oxygen is being consumed by nitrification which consequently results in mouldy, earthy-tasting water [3,4].

The available treatment for removal of NH_4^+–N consists of physical-chemical (by activated carbon adsorption, ion exchange and chemical oxidation) or biological processes (via sequencing batch reactor and activated sludge system). However, physical–chemical process often provides long-term disadvantage including high operation and maintenance cost due to the requirement of extra chemical addition, regeneration of activated carbon or exchange of new membrane. The formation of carcinogenic or non-valuable by-product through physical–chemical treatment has promoted world wide the study on biological process as an alternative. Tekerlekopoulou and Vayenas [5] noted that NH_4^+–N removal through biological process is preferable due to low operating cost, low energy input and also appreciably small volume of sludge

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Table 1
Ingredients of media for screening and shaking test

Chemicals	Brand	Medium	
		A	B
Glucose, $C_6H_{12}O_6$ (g)	Systerm (Malaysia)	–	0.2
Ammonium sulphate, $(NH_4)_2SO_4$ (g)	Systerm (Malaysia)	0.5	0.037
Agar (g)	Sigma-Aldrich (USA)	15.0	–
Sodium chloride, $NaCl_2$ (g)	Systerm (Malaysia)	0.5	–
Potassium chloride, KCl_2 $2H_2O$ (g)	Systerm (Malaysia)	0.02	–
Red phenol (g)	Merck (Germany)	0.0075	–
Potassium dihydrogen phosphate, KH_2PO_4 (g)	Merck (Germany)	0.2	0.14
$MgSO_4{\cdot}7H_2O$ (g)	R&M Chemicals (United Kingdom)	–	0.2
$FeSO_4{\cdot}7H_2O$ (g)	R&M Chemicals (United Kingdom)	–	0.03
$MnCl_2{\cdot}4H_2O$ (g)	R&M Chemicals (United Kingdom)	–	0.015
Sterilized distilled water (mL)	–	1,000	1,000

generated. These biological advantages consequently provide an easy process handling for drinking water treatment.

Removal of NH_4^+–N from drinking water using mixed or pure cultures of bacteria is an economical and more environmental friendly process. Conventionally, biological NH_4^+–N removal through nitrification process involves two genera of autotrophic bacteria such as *Nitrosomonas* sp. for removal of NH_4^+ to form NO_2^- and *Nitrobacter* sp. for conversion of NO_2^- to NO_3^-. The problems using the conventional nitrification are often observed in which the nitrification process is extremely slow due to the low autotrophic growth, the autotrophic nitrifiers are vulnerable to high loads of organic matter and the process could only be performed well in aerobic conditions [6].

Therefore, it has led to extensive studies on the investigation of heterotrophic bacteria as potential nitrifiers that can be used to overcome the inherent problems. Recently, the heterotrophic nitrifiers that have been identified as potential bacteria in removal of NH_4^+ are *Thiosphaera pantotropha*, *Pseudomonas stutzeri*, *Pseudomonas putida*, *Bacillus* sp., *Comamonas* sp., *Alcaligenes faecalis*, *Diaphorobacter* sp. and *Acinetobacter calcoaceticus* [7–13]. Joo et al. [6] reported that heterotrophic nitrifier of *A. faecalis* No. 4 demonstrated high efficiencies of NH_4^+–N removal under aerobic conditions. Another study has been reported that *Pseudonocardia ammonioxydans* $H9^T$ could degrade NH_4^+–N to high removal percentage under high organic carbon to nitrogen ratios (C_{org}/N) [14].

Briefly, a biological aerated filter (BAF) system was used to study the simultaneous removal of ammonia and manganese from drinking water treatment. The BAF system is suggested to operate as an additional system to the current conventional water treatment [15–17]. The system is well known in wastewater treatment but not in drinking water treatment. The system was designed using transparent polyvinyl chloride column with a height of 1.5 m and diameter of 0.16 m and was operated in up-flow configuration. It was partially supported with a plastic medium for degrading biofilm growth and attachment. Mixed culture of sewage activated sludge (SAS) was used as inoculum seeds to the BAF system [15–17]. In this study, a local heterotrophic nitrifier was isolated from the mixed culture and the removal of NH_4^+–N using the locally isolated heterotrophic nitrifier was investigated in a batch experiment to further determine the effect of pH, initial NH_4^+–N concentration and inoculum sizes on the removal process.

2. Materials and method

2.1. Media

A standard medium of nutrient broth and nutrient agar were prepared for the bacteria enrichment and isolation. Media for screening test of the most NH_4^+–N removal strain (Medium A) and shake flasks experiments (Medium B) were listed in Table 1. The medium compositions in the screening test followed the method described by Sarathchandra [18]. The pH of medium A was adjusted to pH 8.0 using 0.5 M NaOH and sterilized at 121 ℃ for 15 min. For shake flasks experiment, medium B included: 0.2 g $C_6H_{12}O_6$, 0.37 g $(NH_4)_2SO_4$, 0.14 g KH_2PO_4, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.03 g $FeSO_4{\cdot}7H_2O$ and 0.015 g $MnCl_2{\cdot}4H_2O$ dissolving in 1,000 mL sterile distilled water and the initial pH was

adjusted to pH 6.5. Different amounts of NH_4^+–N was added to medium B for different initial concentrations of 5–25 mg/L.

2.2. Bacterial isolation and screening

The step taken for NH_4^+–N removal study consists of bacterial isolation, screening, identification, shake flask experiment and finally analysing. A local SAS was taken from a sewage treatment plant located in Putrajaya. The SAS is used as a mixed culture in a BAF system for the simultaneous removal of NH_4^+–N and manganese (Mn^{2+}) in drinking water treatment had demonstrated high removal efficiencies [15]. About 50 mL of mixed culture from BAF system was sampled and agitated to obtain homogeneous suspensions. The mixed culture (10 mL) was transferred into nutrient broth (100 mL) and then was shaken and incubated at 150 rpm and 37˚C for 24 h. After the incubation, the culture was serially diluted from 10^{-1} to 10^{-5} in sterile saline water. About 0.1 mL of each dilution samples was spread on nutrient agar plates and incubated in a growth chamber (GC 1050, Protech, Malaysia) at 37˚C for 48 h. The appeared cell colonies after the incubation were repeated streaking with plastic loop on fresh agar plates to obtain pure isolates.

Screening process for the most potential NH_4^+–N degrading strain was performed through chemical test by cultivating the obtained isolated bacteria in 5 mL of medium A and incubating at 37˚C. Colour changes of the incubation were observed daily. The most potential strain for NH_4^+–N removal was selected according to the more change of red to yellowish colour of medium A [18].

2.3. DNA extraction, PCR amplification and 16S rRNA sequencing

Bacterial DNA was extracted from a bacterial suspension in nutrient broth that was cultivated at 37°C for 24 h. The extraction was conducted using Wizard® Genomic DNA Purification Kit (Promega, USA) protocol for isolation of genomic DNA from Gram positive and negative bacteria. Universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTA CCTTGTTACGACTT) were used to amplify 16S RNA gene according to the polymerase chain reaction (PCR) amplification protocol provided by Promega Manufacture (USA). The PCR was performed using Mastercycler (Epgradient S, Eppendorf, Version 3.608). PCR-amplified product was purified by Wizard® Plus SV Minipreps DNA Purification System (Promega, USA). The PCR product was sent to First BASE Laboratories Sdn. Bhd (Kuala Lumpur, Malaysia) for the 16S RNA sequencing. Finally, the result of 16S rRNA sequence of the isolate was compared with those of other microorganisms by way of BLAST through National Centre for Biotechnology Information homepage (http://www.ncbi.nlm.nih.gov).

2.4. Phylogenetic analysis

The 16S rRNA gene sequence of the isolate strain was aligned with all the sequences available from the GenBank by BLAST. All sequences were retrieved from GenBank individually and aligned using ClustalW packaging in MEGA software (MEGA version 5, USA). The phylogenetic analysis was later performed by MEGA version 5 software using neighbour-joining tree method, which was tested by Bootstrap method (1,000 repetitions).

2.5. Shake flask experiments

After screening and identification, the selected heterotrophic strain was grown in 250 mL conical flasks containing 150 mL of nutrient broth, and it was cultivated in a shaking incubator (S1-600R, Japan) with a speed of 150 rpm and 37˚C for 24 h. The heterotrophic strain was harvested using a centrifuge (Kubota 5220, Japan) at 350 rpm for 15 min. After two rinses with sterile distilled water, the heterotrophic biomass was re-suspended in another sterile distilled water to prepare the stock solution. The dry weight of the heterotrophic biomass was determined after drying at 105˚C overnight. Batch experiment was conducted using 250 mL conical flasks containing 100 mL of medium B. The range of effects of initial NH_4^+–N concentration, initial pH and inoculum size were 5–25 mg/L, pH 3–10, and 0.5–3.0% v/v, respectively. During the study on the effect of initial NH_4^+–N concentration, 1 mL cell suspension (1% v/v) was suspended in the medium B (pH 6.5) and incubated at 150 rpm at 37˚C for 24 h, while initial NH_4^+–N concentration of 10 mg/L and 1% v/v inoculum were used to investigate the effect of pH. For the effect of inoculum size, initial pH and NH_4^+–N concentration were set as pH 6.5 and 10 mg/L, respectively. At the end of incubation, samples were centrifuged (Eppendorf 5804, Germany) at 5,000 rpm for 10 min. The supernatant was collected for analysis purposes.

2.6. Analytical method

The samples for chemical oxygen demand (COD) measurement were digested via Reactor Digestion Method (Method 8000) and determined using HACH spectrophotometer (HACH DR/2010, USA). Levels of

ammonium nitrogen (NH_4^+–N) were determined spectrophotometrically through the Nesslerization method at an absorbance of 425 nm using a HACH DR/2010 (Method 8038). Removal efficiency of the response was calculated using the following equation:

$$NH_4^+ - N(\%) = \frac{N_i - N_f}{N_i} \times 100 \quad (1)$$

where N_i and N_f are the initial and final concentrations (mg/L) of NH_4^+–N, respectively.

2.7. Statistical evaluation

The results were analysed using a one-way analysis of variance with a significant difference of $p < 0.05$. Statistical calculations were executed with SPSS software for Windows, version 16.0 (SPSS Inc. USA).

3. Results and discussion

3.1. Bacterial isolation and screening of potential isolated strain for removal of NH_4^+–N

A serial spreading of mixed culture on nutrient agar plates resulted with six colonies of strains. Five of the isolated bacteria were Gram positive strains with three rods (I2, I3 and I6) and two cocci (I1 and I5) and 1 g negative with rod shape (I4). The result shows that isolated bacteria from mixed culture of SAS consisted of a wide variety of species which provided potential strains for pollutant removal. *Pseudomonas*, *Bacillus* and *Aeromonas* species are commonly potential bacteria found in activated sludge that can be used for removal of pollutants in wastewater treatment.

It is impossible to directly test all of the isolated strains in shake flasks experiment due to more requirement of time for experiment set-up and analysis. The other way is firstly, screening the most potential strain for removal of NH_4^+–N using rapid test based on colour change. Then the most potential strain in NH_4^+–N removal was selected for species identification and shake flask experimental study. Fig. 1 shows the rapid removal of NH_4^+–N by the isolated strains.

After 2 days, it was found that the test tube containing mixture of medium A and isolated I6 shows the most obvious colour changing from red to yellow compared with others, followed by test tube I4, I2, I1, I3 and I5. Control without any strain was used in the screening test in which there was no conversion of colour in the medium A until the end of incubation period. The observation presented that I6 was the most potential as NH_4^+–N degrading bacteria. The colour change by phenol indicator in medium A was based on acidity levels. The more acidic condition of medium A would change the origin colour of red to yellow in which the initial pH of medium was pH 8. More removal of NH_4^+–N by I6 produced more hydrogen ion that caused the level of acidity in medium A increased in which consequently turned the red colour of medium A to yellow.

3.2. Identification of potential NH_4^+–N removal strain

After the rapid screening of the potential strains in removal of NH_4^+–N, I6 was selected for species identification. Strain I6 is a Gram-positive type. The cells of strain I6 was observed by SEM (Leo1450VPSEM, UK) and appeared as short rods with approximately 2.526 μm length and 0.810 μm width (Fig. 2).

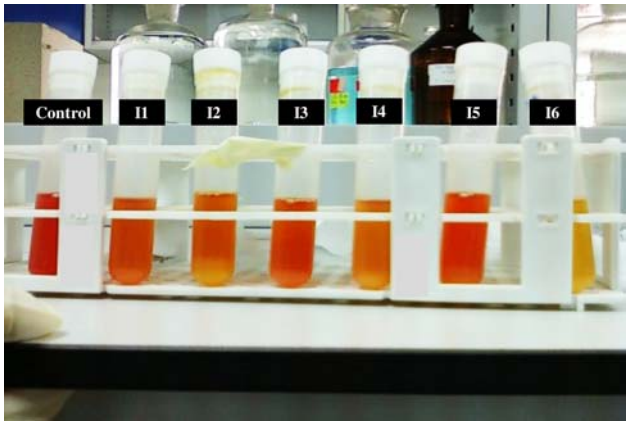

Fig. 1. Screening of isolated strains in removal of NH_4^+–N.

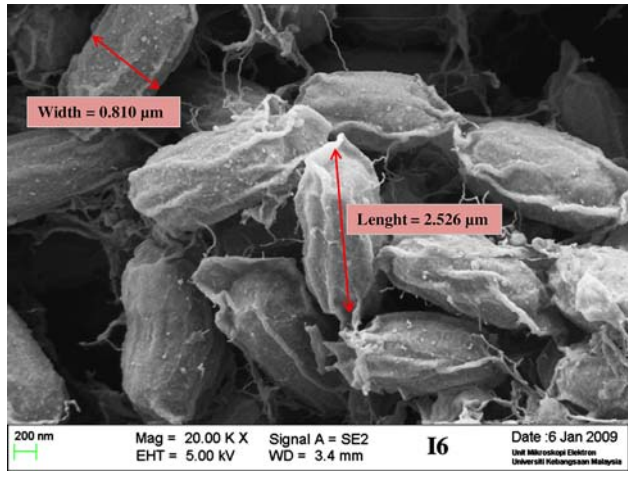

Fig. 2. Scanning electron micrograph of strain I6.

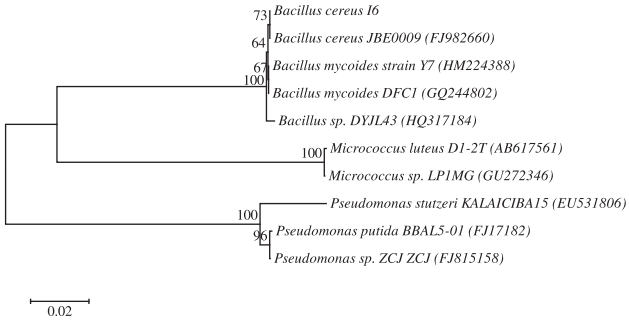

Fig. 3. Phylogenetic tree based on 16S rRNA sequences between strain *B. cereus* I6, other members of *Bacillus*, and heterotrophic nitrifiers from other groups. Bootstrap values (1,000 replications) are indicated at the interior branches. The scale bar represents 0.02 inferred substitutions/nucleotide positions.

A fragment of approximately 1,500 bp 16S RNA was obtained from PCR and sequencing. Homology searches of the 16S RNA gene sequence of strain I6 in GenBank by BLAST revealed that it had high similarity to sequences of the species *Bacillus cereus* JBE0009 (FJ982660) with the 99% identity. As obtained in the screening test, *B. cereus* I6 was classified as heterotrophic nitrifier, based on a previous study by Kim et al. [11]. A neighbour-joining phylogenetic tree was constructed based on 16S rRNA sequences as illustrated in Fig. 3 to show the phylogenetic relationships among *B. cereus* I6 strain, other members of *Bacillus* strains, and heterotrophic nitrifiers from other genera. The results suggested that strain *B. cereus* I6 and *B. cereus* JBE0009 were in the same group. This tree also showed a clear evolutionary divergence of *B. cereus* I6 from strain types belonging to other heterotrophic nitrifier groups as well as *Pseudomonas* and *Micrococcus*.

In Malaysian regulation for safe drinking water, there is no specific standard limit for *B. cereus*. For drinking water, the microbial contamination is controlled according to the standard limits for total coliform and *Escherichia coli*. World Health Organization (WHO) has also regulated that total coliform and *E. coli* should be absent after disinfection process [19]. Thus, the use of *B. cereus* in biological system can be reliable and safe since they will be finally disinfected in conventional drinking water treatment plant.

3.3. Profile of NH_4^+–N removal

Time profiles of NH_4^+–N removal by heterotrophic nitrifier of *B. cereus* I6 is shown in Fig. 4. Initial NH_4^+–N concentration decreased with time. The growth of the strain was slow during the first 6 h incubation, however the obviously increasing growth rate was observed afterwards. At that moment, the removal of NH_4^+–N significantly increased from 7.7% (6 h) to 78.5% (30 h) ($p < 0.05$). The maximum removal of NH_4^+–N was observed at 30 h contact time with 78.5% removal and NH_4^+–N concentration was measured as 1.4 mg/L which was below maximum concentration limit (< 1.5 mg/L) for drinking water regulated by WHO [19]. After 30 h incubation, the strain death rate exceeds the production of new cells until the end of 48 h incubation. Thus, it caused the removal of NH_4^+–N to stop after 30 h. During incubation, initial pH of 6.5 decreased to 5.7 at the end of incubation. A decrease in pH value had confirmed that the locally heterotrophic *B. cereus* I6 removed the NH_4^+–N producing more H^+ ions. The ability of locally isolated heterotrophic nitrifier of *B. cereus* I6 in biological NH_4^+–N removal had negated that the removal can only be performed by autotrophic strain such as *Nitrosomonas*. Brycki et al. [20] found that

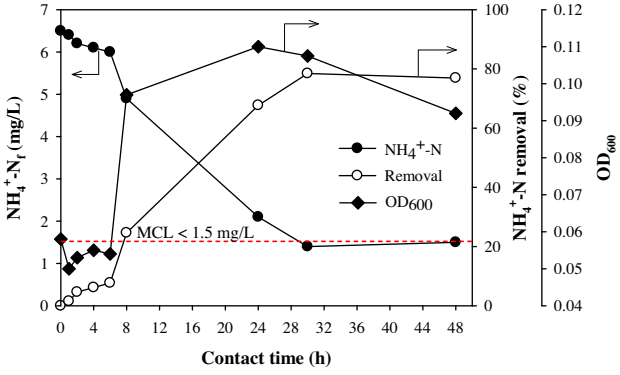

Fig. 4. Time profiles of NH_4^+–N removal by heterotrophic nitrifier of *B. cereus* I6.

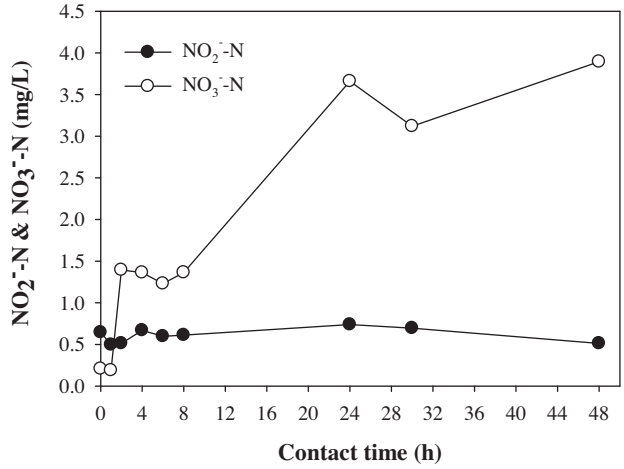

Fig. 5. Time profiles of NO_2^-–N and NO_3^-–N concentrations.

heterotrophic strain of *Bacillus licheniformis* could also execute NH_4^+–N removal to nitrogen gas (N_2). The high growth rate of heterotrophic strains compared with autotrophic ones has allowed the removal of NH_4^+–N to perform.

Fig. 5 shows the time profiles of nitrite (NO_2^-–N) and nitrate (NO_3^-–N) during the removal of NH_4^+–N. As can be seen, the production of NO_2^-–N was not as much as NO_3^-–N along the 48 h incubation. The maximum production of NO_2^-–N was achieved at 24 h with concentration of 0.51 mg/L and it decreased to 0.38 mg/L at the end of incubation times. Furthermore, the NO_3^-–N slowly increased at the first 8 h incubation but reached a higher production rate between 8 and 48 h with a rate of 0.06 mg/L h. The production of NO_2^-–N and NO_3^-–N gave evidence that the heterotrophic nitrifier of *B. cereus* I6 had removed NH_4^+–N which was later converted to these both components. The most promising of heterotrophic nitrifier, not only can remove the NH_4^+–N, but also act as aerobic denitrifier for biological NO_3^-–N conversion to N_2 [13,21]. Moreover, it has been reported that aerobic denitrification performed by heterotrophic strains possessed less complex metabolic pathways than that of autotrophs [11].

3.4. Effect of initial NH_4^+–N concentration

Fig. 6 represents the effect of initial NH_4^+–N concentration on the removal. As can be seen, the final concentration of NH_4^+–N is directly proportional to the initial concentration with coefficient determination (R^2) of 0.9976. However, by increasing the NH_4^+–N concentration from 5 to 25 mg/L, the removal of NH_4^+–N dropped from 62 to 24.5% and the COD/NH_4^+–N ratios decreased from 40 to 8. A high specific growth rate of heterotrophic nitrifiers leads to the requirement of sufficient organic carbon supplements for cells synthesis. As the NH_4^+–N increased, the COD supplement of 200 mg/L at the initial incubation was not sufficient for heterotrophic bacteria and finally limited the removal process. In Malaysian drinking water treatment, COD is not a major problem, but the existence in high concentration can lead to the depletion of natural oxygen resources and to the development of septic conditions [15]. The contaminant is easily removed through a conventional system of aeration and filtration compared with NH_4^+–N. The COD removal in this study was in the range 62.5–84.5% with effluent concentration of 31–75 mg/L. The COD concentrations of the effluents are within the normal conditions in Malaysian raw water which is used for production of safe drinking water.

3.5. Effect of initial pH

Fig. 7 depicts that the final concentration and removal of NH_4^+–N were affected by initial pH during the removal. A high removal above 65% was achieved at initial pH of 3–5. The maximum removal of NH_4^+–N was observed at pH 5 with 69.5% removal and final concentration of 3.05 mg/L. The removal significantly decreased to 53% (pH 6), 35% (pH 7), 25% (pH 8), 22% (pH 9) and 32% (pH 10) as the initial pH was increased. Heterotrophic *B. cereus* I6 is a spore-forming strain which at low pH, the heat activation of *B. cereus* I6 was enhanced [22,23] and consequently increased the germination of spores. Therefore, low pH in the range 3–5 caused higher removal of NH_4^+ by heterotrophic *B. cereus* I6 at which the forming spores assisted in the removal. Instead, only slightly spores were activated at neutral pH [23].

3.6. Effect of inoculum sizes

For the higher inoculum size, the lag phase of the bacteria growth will be shorter compared to the smaller inoculum size, which consequently increased the

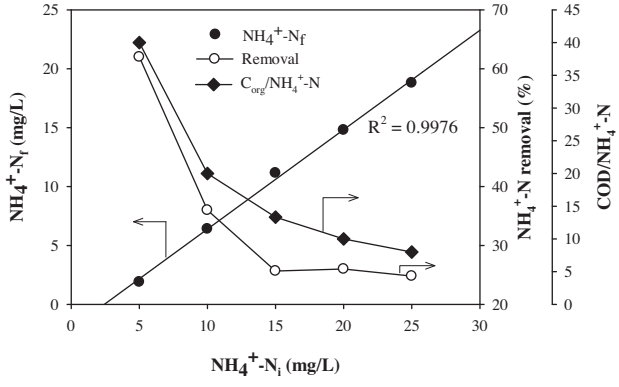

Fig. 6. Effect of initial concentration on NH_4^+–N removal.

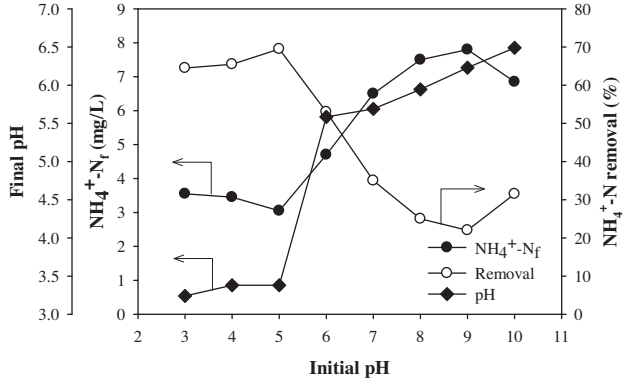

Fig. 7. Effect of initial pH on NH_4^+–N removal.

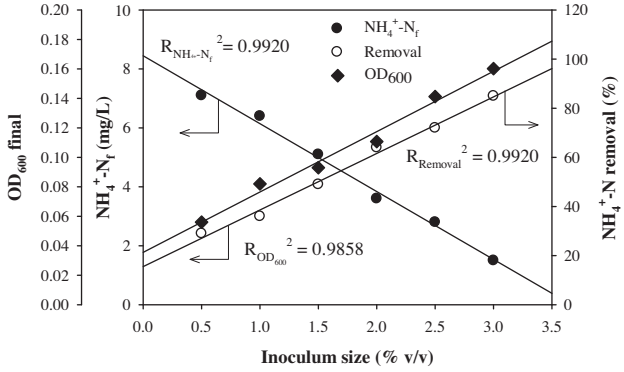

Fig. 8. Effect of inoculum sizes on removal performance.

removal of NH_4^+–N. Fig. 8 illustrates the performance of heterotrophic *B. cereus* I6 in the removal as the inoculum sizes were increased. The removal of NH_4^+–N was significantly increased ($p < 0.05$) as the inoculum sizes increased from 0.5 to 3.0% v/v with R^2 of 0.992. By using 0.5% v/v inoculum in the NH_4^+–N removal, the final optical density (OD) of strain growth was observed only 0.056. Meanwhile, at higher inoculum of 3.0% v/v, the final OD was 0.16 which was much higher than that of small inoculum sizes. High NH_4^+–N removal of 85% at 3.0% v/v inoculum was due to more cells involved in the removal compared with lesser inoculum sizes.

3.7. Comparison with other studies

Table 2 lists the comparison of NH_4^+–N removal using isolated heterotrophic and autotrophic nitrifiers. In this study, the maximum removal of NH_4^+–N by *B. cereus* I6 was found to be 85% with removal rate of 0.35 mg/L h at an optimum pH of 6.5 and inoculum size of 3.0% v/v. Zhao et al. [13] found that heterotrophic *A. calcoaceticus* HNR isolated from activated sludge could remove 92% of NH_4^+–N. Meanwhile, Kim et al. [11] found that *B. subtilis* PK15 and *B. cereus* PK5 removed about 90 and 4% of NH_4^+–N, respectively, when operated under 30% of dissolved oxygen (DO), C/N ratio of 8 and initial NH_4^+–N concentration of 200 mg/L. However, when the removal was performed under 5% of DO, C/N ratio of 8 and initial NH_4^+–N concentration of 100 mg/L, *B. cereus* PK5 showed a maximum removal of 28% with removal rate of 0.035 mg/L h. Moreover, Ahmad et al. [14] found that 82–88% of NH_4^+–N was removed using inoculation of *P. ammonioxydans* $H9^T$ in a laboratory scale airlift reactor operated under aerobic condition.

As can be seen in Table 2, the initial NH_4^+–N concentration studied by Zhao et al. [13] and Ahmad et al. [14] were both 120 mg/L which is 12 times higher than in this study. Nevertheless, *A. calcoaceticus* HNR showed a higher removal rate of 2.5 mg/L h compared with *B. cereus* I6 which represented a removal rate of 0.35 mg/L h. Instead, *B. cereus* I6 isolated from sewage-activated sludge presented a higher removal rate than *B. subtilis* PK15 and *B. cereus* PK5 as used by Kim et al. [11]. Furthermore, by using autotrophic *Nitrosomonas europaea* under oxygen limitation, the removal of NH_4^+–N was only in the range 26–62% [24]. In another study, 96% removal of NH_4^+–N with a rate of 0.013 mg/L h was obtained in a bioreactor

Table 2
Comparison of NH_4^+–N removal using pure culture of strains

Strains	Nitrifier groups	Strains source	Initial NH_4^+–N (mg/L)	pH	NH_4^+–N removal (%)	Removal rate (mg/L h)	References
B. cereus I6	Heterotrophic	SAS	10	6.5	85	0.35	This study
B. cereus PK5	Heterotrophic	Soil	100	7	28	0.035	[11]
B. subtilis PK15	Heterotrophic	Soil	200	7	90 ± 6	0.11 2	[11]
Acinetobacter calcoaceticus HNR	Heterotrophic	Activated sludge	120	8	92	2.5	[13]
Pseudonocardia ammonioxydans $H9^T$	Heterotrophic	Coastal sediment	120	7.8	82–88	–	[14]
Nitrosomonas europaea	Autotrophic	Activated sludge	1,000	7.9	26–62	5.46–12.92	[24]
Nitrosomonas eutropha, N. halophila and N. europaea	Autotrophic	Mixed liquor of municipal treatment	2,500	7.8	96	0.013	[25]

constructed with nitrifying biofilms of *Nitrosomonas eutropha*, *N. halophila and N.europaea* [25]. The difference of NH_4^+–N removal rate among the previous studies was due to the several factors such as strain types, treatment systems, initial NH_4^+–N concentrations and carbon source for strain growth. Thus, under a suitable condition, the heterotrophic nitrifiers may perform better removal than autotrophic ones in simultaneous nitrification-denitrification.

4. Conclusions

This study investigated the removal of NH_4^+–N using locally isolated heterotrophic nitrifier of *B. cereus* I6 from sewage-activated sludge that has been used in BAF for simultaneous removal of NH_4^+–N and manganese in drinking water treatment. It was found that some of the factors studied affected the removal, including initial NH_4^+–N concentration, pH and inoculum size. Increasing the initial NH_4^+–N concentration from 5 to 25 mg/L decreased the removal. The highest removal of NH_4^+–N was at the initial pH 5, while the lowest removal was observed at pH 9. The effect of inoculum sizes on removal of NH_4^+–N was also studied, where the removal increased as the inoculum size increased. The maximum removal was achieved at 3.0% v/v inoculum with 85% removal.

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