Nitrogen reduction using bioreactive thin-layer capping (BTC) with biozeolite: A field experiment in a eutrophic river

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ABSTRACT

Bioreactive thin-layer capping (BTC) with biozeolite provides a potential remediation design that can sustainably treat N contamination from sediment and overlying water in eutrophic water bodies. Nitrogen (N) reduction using BTC with biozeolite was examined in a field incubation experiment in a eutrophic river in Yangzhou, Jiangsu Province, China. The biozeolite was zeolite with attached bacteria, including two isolated heterotrophic nitrifiers (*Bacillus* spp.) and two isolated aerobic denitrifiers (*Acinetobacter* spp.). The results showed that the total nitrogen (TN) reduction efficiency of the overlying water by BTC with biozeolite (with thickness of about 2 mm) reached a maximum (56.69%) at day 34, and simultaneous heterotrophic nitrification and aerobic denitrification occurred in the BTC system until day 34. There was a significant difference in the TN concentrations of the overlying water between biozeolite capping and control (t-test; $p < 0.05$). The biozeolite had very strong *in situ* bioregeneration ability. Carbon was the main source of nitrifier growth. However, both dissolved oxygen (DO) and carbon concentrations affected denitrifier growth. In particular, DO concentrations greater than 3 mg/L inhibited denitrifier growth. Therefore, BTC with biozeolite was found to be a feasible technique to reduce N in a eutrophic river. However, it is necessary to further strengthen the adaptability of aerobic denitrifiers through changing domestication methods or conditions.

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Introduction

Eutrophication is a critical problem that impairs the water quality of urban water bodies. With the rapid development of industrialization and urbanization, eutrophication of urban water bodies is increasingly serious in China. Both nitrogen (N) and phosphorus (P) concentrations are the main limiting factors for the eutrophication of water bodies (Lewis et al., 2011). Sediments play an important role in eutrophication, because they are regarded as a source or sink for N and P in water bodies (Nilsson and Jansson, 2002; Pan et al., 2012). Therefore, when external sources of N and P are strictly controlled, the subsequent reduction of N and P released from sediments will effectively control the eutrophication of water bodies.

At present, dredging and capping are the main approaches for decreasing N and P released from sediments and are widely used (Förstner and Apitz, 2007). However, dredging has the following disadvantages: (1) high cost; (2) sediment resuspension; (3) secondary pollution owing to transport and disposal of sediment; and (4) destruction of the benthic ecological environment. Capping has become a focus of current research and has developed rapidly from inactive, thick-layer capping (Kim and Jung, 2010) to active/bioreactive

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thin-layer capping (BTC) (Jacobs and Förstner, 1999; Berg et al., 2004; Lin et al., 2011; Özkundakci et al., 2011; Huang et al., 2011, 2012, 2013; Meis et al., 2012; Pan et al., 2012). Active capping materials used for the reduction of N and P in eutrophic water bodies are as follows: (1) calcite (Berg et al., 2004; Lin et al., 2011); (2) natural zeolite and its modified products (Jacobs and Förstner, 1999; Lin et al., 2011; Sun et al., 2011; Huang et al., 2011, 2012); and (3) different types of modified P inactivation agents (Oliveria et al., 2011; Xiong and Peng, 2011; Mailapalli and Thompson, 2011; Özkundakci et al., 2011; Meis et al., 2012; Pan et al., 2012). However, active capping materials may have the following issues in their actual application: (1) inability to be regenerated *in situ*; (2) declining effectiveness with time; and (3) limited utilization of active adsorbents.

Zeolite is commonly used as a biological carrier because of its porous properties (Jung et al., 2004). Nitrifiers attached to biozeolite can regenerate the zeolite ion exchange capacity for ammonium during the biological nitrification period (Lahav and Green, 1998; Jung et al., 2004). In addition, denitrifiers attached to biozeolite can remove ammonium *via* the biological denitrification reaction.

Studies on simultaneous nitrification and denitrification (SND), which is an effective methods to convert ammonium nitrogen (NH_4^+-N) to N gas *via* simultaneous heterotrophic nitrification and aerobic denitrification, have drawn increased attention in wastewater treatment, because SND has advantages over conventionally separated nitrification and denitrification processes (such as short reaction times, small systems, and low cost) (Du et al., 2003; Nakano et al., 2004; Walters et al., 2009; Chen et al., 2012). Hence, with the advancement of research, increased numbers of heterotrophic nitrification and aerobic denitrification strains have been isolated from different environments and investigated for SND, including *Paracoccus* spp. (Patureau et al., 2000), *Pseudomonas* spp. (Kim et al., 2008), *Alcaligenes* spp. (Zhao et al., 2012), *Bacillus* spp. (Yang et al., 2011; Zhang et al., 2012), *Acinetobacter* spp. (Zhao et al., 2010), *Delftia* spp. (Wang et al., 2007), *Agrobacterium* spp. (Chen and Ni, 2012), *Comamonas* spp. (Chen and Ni, 2011), and *Serratia* spp. (Sakai et al., 1996).

Current research on simultaneous heterotrophic nitrification and aerobic denitrification has primarily focused on N removal from wastewater *via* laboratory experiments. However, few studies have investigated N reduction in eutrophic water bodies *via* simultaneous heterotrophic nitrification and aerobic denitrification under field conditions.

In order to solve the above problems of the current active capping materials, BTC with biozeolite (*i.e.*, zeolite with two isolated heterotrophic nitrifiers and two isolated aerobic denitrifiers attached) was proposed to reduce N in a eutrophic river by our group (Huang et al., 2011, 2012, 2013). On one hand, the rate of nitrification was improved by high-efficiency heterotrophic nitrifiers attached to biozeolite so that the *in situ* bioregeneration rate of the zeolite ion exchange capacity was increased. On the other hand, N pollutants [*e.g.*, NH_4^+-N, nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and organic nitrogen (Org-N)] were removed by heterotrophic nitrifiers and aerobic denitrifiers *via* SND and biological assimilation. Previous research results indicated that BTC not only completely inhibited NH_4^+-N release from sediments but also

reduced N from overlying water, sediment-interstitial water, and surface sediments. Moreover, *in situ* bioregeneration of the zeolite ion exchange capacity was feasible (Huang et al., 2011, 2012, 2013).

In the present study, a biozeolite were selected as a capping material. On the basis of previous research, the feasibility of N reduction in eutrophic water bodies using BTC with biozeolite at a dose rate of 2 kg/m^{-2} (about 2 mm thickness) was further examined through a field incubation experiment in a eutrophic river in Yangzhou, Jiangsu Province, China. The *in situ* bioregeneration of the zeolite ion exchange capacity was also investigated. Changes in nitrifier and denitrifier number attached to the biozeolite were conducted by plate counting. The mechanisms of BTC N reduction were further analyzed.

1. Material and methods

1.1. Study site

The study site was a eutrophic river in Yangzhou City, named "the Yangzhou Ancient Canal". The Yangzhou Ancient Canal is the oldest section of the Beijing-Hangzhou Canal, and starts from Wantou and enters the Yangtze River at Guazhou. Its length and basin area are respectively 30 km and 720,000 m^2, and the depth of the water is 3–5 m.

Urban sewerage, industries and agriculture are the main sources of pollution in the Yangzhou Ancient Canal. Its eutrophication is a serious problem. Despite efforts to restore the river by reducing external nutrient loading, large cyanobacterial biomasses of low diversity have appeared every summer. It has the following hydraulic characteristics: (1) cargo ships are banned in the Yangzhou Ancient Canal, and (2) with the exception of changing the water of the canal from the Beijing-Hangzhou Canal to improve water quality, the flow velocity of the canal is very slow at most times.

During our field investigations, the concentrations of TN, NH_4^+-N, NO_2^--N, NO_3^--N, total phosphorus (TP), and orthophosphate (PO_4^{3-}-P) of the canal section studied ranged from 2 to 19 mg/L, 1 to 7 mg/L, 0 to 0.6 mg/L, 0 to 1.5 mg/L, 0.3 to 2.0 mg/L and 0.05 to 1.95 mg/L respectively (mean 7.32 mg/L, 6.23 mg/L, 0.11 mg/L, 0.33 mg/L, 0.79 mg/L, and 0.54 mg/L respectively).

1.2. Experimental materials

1.2.1. Natural zeolite
Physical and chemical properties of the natural zeolite used in this study were as follows: grain size 1–2 mm, specific surface area 42.51 m^2/g, porosity 36.55%, average aperture 6.75 nm, bulk density 1.01 g/cm^3, true density 2.29 g/cm^3, maximum saturated adsorption capacity of the zeolite toward NH_4^+-N 13.35 mg NH_4^+-N/g zeolite.

Its source was a mine in Bayannaoer City, Inner Mongolia Autonomous Region of China. Its main mineral composition as determined by X-ray diffraction (XRD) was clinoptilolite, orthoclase and quartz. Its elemental composition determined by X-ray fluorescence (XRF) was as follows, in mass %: Si = 73.98, Al = 8.99, Ca = 5.07, K = 4.68, Fe = 3.11, Na = 2.13, Mg = 1.51, and other trace elements (such as Mn, Ti, Zn, Cu, Ni, Co, Sr, Zr, and Rb) added up to 0.53. The Si/Al ratio was 8.23.

1.2.2. Biofilm formation on zeolite

Biofilm formation on the zeolite was cultivated by a mixed culture containing two isolated heterotrophic nitrifiers (WGX10 and WGX18) and two isolated aerobic denitrifiers (HF3 and HF7) through the method of artificial aeration. WGX10 and WGX18 were identified as *Bacillus subtilis* and *Bacillus amyloliquefaciens*, respectively, by the Auto-Microbic system (AMS) and 16S rRNA gene sequence analysis. HF3 and HF7 were identified as *Acinetobacter junii* and *Acinetobacter calcoaceticus*, respectively. The four strains were isolated from sediments. The origins, physiological and biochemical characteristics, and detailed procedures regarding biofilm formation on the zeolite were previously illustrated by Huang et al. (2012).

1.3. Incubation experiments

The incubation experiments were set up in two PVC-U double-walled corrugated pipes with a height of 5 m and a diameter of 1 m. With the help of a 40-ton crane, the two pipes were laid on the surface of the sediments lightly, and inserted vertically and slowly into the sediments of the Sanwan section of the Yangzhou Ancient Canal, respectively. The thickness of the sediments was about 2.5 m and the height of the overlying water was about 2 m in the two pipes. A schematic of the experimental set-up is shown in Fig. 1.

The first pipe served as the "control system", which was maintained without any capping material as a reference. The
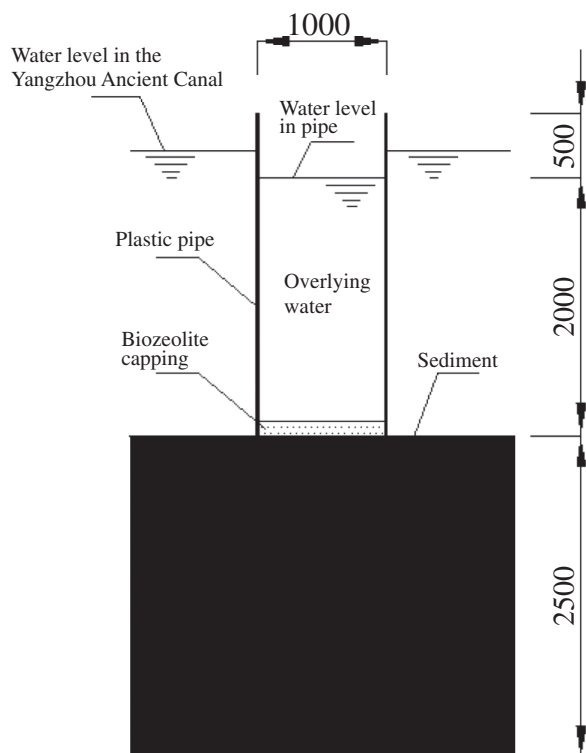

Fig. 1 – Schematic of the experimental set-up. (1) Overlying water, (2) biozeolite capping, (3) sediment, (4) water level in the pipe, (5) water level in the Yangzhou Ancient Canal, (6) plastic pipe.

second pipe served as the "biozeolite capping system", which contained the biozeolite capping layer of 2 mm (i.e., the biozeolite of dose rate of 2 kg/m^2 was spread evenly onto the sediment in the biozeolite capping system).

The incubation experiments were initiated on April 22, 2011 and lasted 168 days. During July 10 to July 13, the two pipes were submerged due to heavy rains, and water exchange occurred between the pipes and the Yangzhou Ancient Canal, which lead to a halt in investigating the reduction efficiency of TN of the overlying water. However, the water level in the Yangzhou Ancient Canal dropped quickly on July 14, and the incubation experiments were carried on to analyze the changes in nitrifier and denitrifier number and the *in situ* bioregeneration of the zeolite ion exchange capacity.

To investigate the changes in nitrifier and denitrifier number and the *in situ* bioregeneration of the zeolite ion exchange capacity, twelve small bags with nylon nets having a pore size of 32 mesh (a diameter of 0.5 mm) were sewn, 5 g biozeolite was put into each bag, the mouth of each bag was sealed with silk thread, and the twelve bags were tied together with a light rope connected to a float and placed onto the sediment in the biozeolite capping system.

A DO probe and pH probe were placed at 20 mm above the top of the sediment or capping layer in each pipe to measure water temperature, DO concentration and pH of overlying water once every 2–7 days at a set time. About 500 mL water was slowly removed using a microwater pump with a diameter of 8 mm, 10 cm above the top of the sediment or capping layer in each pipe once every 2–7 days at a set time. Source water from the canal was then added to maintain a constant water volume. The concentrations of total nitrogen (TN), NH_4^+-N, NO_2^--N, and NO_3^--N in the water samples were analyzed within 2 hr according to the standard method (State Environmental Protection Administration (SEPA) of China, 2002) with a UV-visible spectrophotometer (UV759S, Shanghai Precision & Scientific Instrument Co., Ltd., China). Bags containing biozeolite were removed carefully at regular intervals, and the number of nitrifiers and denitrifiers attached to the biozeolite was measured by plate counting (Yu et al., 1990). NH_4^+-N adsorption by the biozeolite was analyzed using the following method: the NH_4^+-N adsorbed in the biozeolite was extracted by the simultaneous addition of 0.01 mol/L $CaCl_2$ and 2 mol/L KCl, and the quantities of physically and chemically adsorbed NH_4^+-N of the biozeolite were examined, respectively.

1.4. Data analysis

The reduction efficiency of TN of the overlying water was calculated using Eq. (1).

$$P = \frac{C_0 - C_i}{C_0} \times 100\% \tag{1}$$

where P is the percent reduction efficiency of TN of the overlying water, and C_i (mg/L) and C_0 (mg/L) are the TN concentrations of the overlying water from the biozeolite capping system and the control system, respectively.

A *t*-test was carried out to assess the significance of differences between the biozeolite capping system and the control system. Significance was assumed when *p* < 0.05.

2. Results and discussion

2.1. Reduction efficiency of N of the overlying water

From 0–73 days, the water temperature, DO concentration, and pH of the overlying water 10 cm above the top of the sediment or capping layer ranged from 18–28°C, 0.30–4.82 mg/L, and 7.4–8.0, respectively. The changes of TN, NH_4^+-N, NO_2^--N, and NO_3^--N concentrations of overlying water in the biozeolite capping system and control system during the incubation experiments are shown in Fig. 2, respectively.
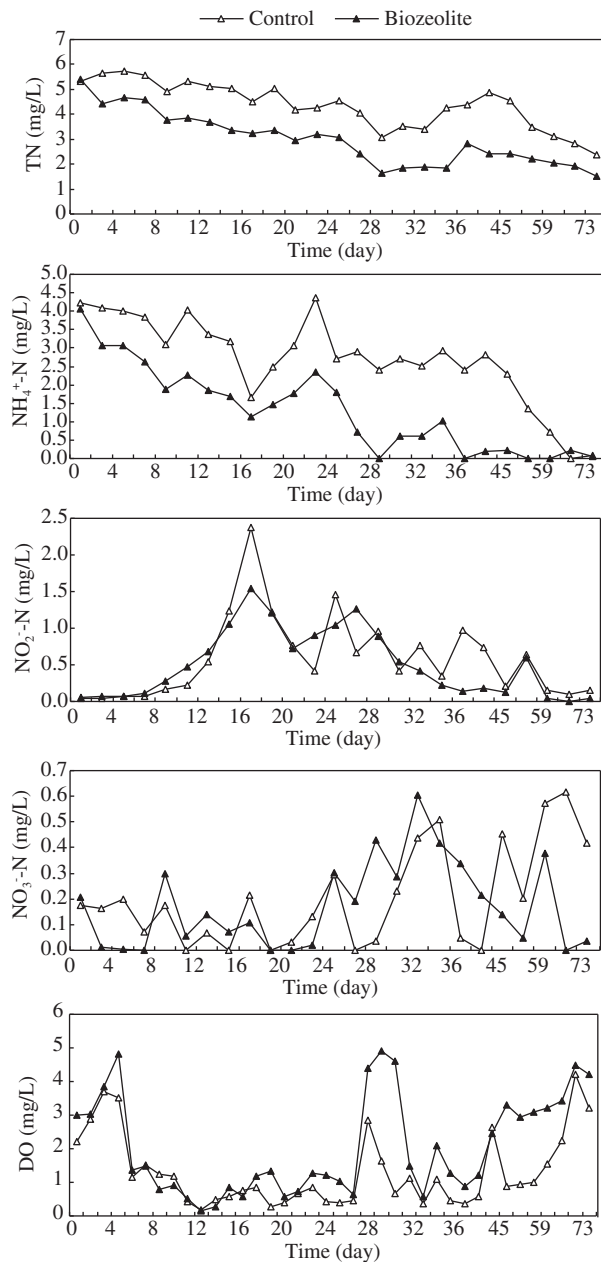

Fig. 2 – Changes of total nitrogen (TN) (a), ammonium nitrogen (NH_4^+-N) (b), nitrite nitrogen (NO_2^--N) (c), nitrate nitrogen (NO_3^--N) (d), dissolved oxygen (DO), and (e) concentrations of overlying water in biozeolite capping and control systems.

Fig. 2a shows that the TN reduction efficiency of the overlying water by BTC with biozeolite (the thickness of about 2 mm) reached a maximum (56.69%) at day 34. From 0–73 days, there were significant differences in the TN concentrations of the overlying water between the biozeolite capping system and the control system (t-test; $p < 0.05$), which indicated that BTC with biozeolite could reduce N effectively in the overlying water.

Fig. 2b demonstrates that from 0–28 days, the NH_4^+-N concentration changed from 4.05 mg/L to approximately 0 mg/L in the biozeolite capping system, and 4.23 to 2.41 mg/L in the control system, suggesting that BTC with biozeolite could effectively reduce NH_4^+-N. The NH_4^+-N concentration reached about 0 mg/L in the control system at day 66, which implied that the canal water body was able to convert NH_4^+-N into NO_3^--N via a biological nitrification reaction (Huang et al., 2012).

As shown in Fig. 2b–d, significant nitrification occurred in the biozeolite capping system. In particular, the control system had strong nitrification, which further indicated that the canal water body could transform NH_4^+-N into NO_3^--N (Huang et al., 2012). However, the NO_3^--N concentration in the control system was significantly greater than that in the biozeolite capping system at day 73, indicating that there was a weak biological denitrification in the control system. After day 6, the NO_2^--N concentration in the biozeolite capping system increased gradually, reaching a maximum at day 16, and then decreased to a low level (<0.2 mg/L) from days 17–34, which suggested that the accumulation of NO_2^--N occurred for about 30 days. The NO_3^--N concentration in the biozeolite capping system remained at a low level (<0.2 mg/L) until day 22. Afterwards, it increased and then decreased. The above results may be explained by the following reasons. Before day 34, SND occurred in the biozeolite capping system. The mechanism of SND is as follows: On one hand, NH_4^+-N oxidation by heterotrophic nitrification occurs via two different metabolic pathways (Ding et al., 2011; Zhao et al., 2012). One is heterotrophic nitrification via the oxidation of NH_4^+-N into NO_2^--N and NO_3^--N. The other is by the oxidation of NH_4^+-N into nitrogenous gas directly via hydroxylamine. On the other hand, aerobic denitrification is thought to occur by the following processes (Ding et al., 2011; Zhang et al., 2012): NO_3^--N is first converted into NO_2^--N by nitrate reductase; NO_2^--N is transformed into NO via nitrite reductase; NO is further converted into N_2O by nitric oxide reductase; and N_2O is transformed into N_2 via nitrous oxide reductase. NO_2^--N accumulation in the biozeolite capping system may result from the activity of nitrite reductase being less than that of nitrate reductase (Ding et al., 2011; Ge et al., 2012). First, no nitrate accumulation resulted from the conversion of NH_4^+-N into NO_2^--N, NO_3^-N, or nitrogenous gas by heterotrophic nitrification, and NO_2^--N and NO_3^--N were simultaneously transformed into nitrogenous gas via aerobic denitrification (Du et al., 2003; Ding et al., 2011). Nevertheless, after day 34, the increase in NO_3^--N concentration indicated that there was an imbalance between heterotrophic nitrification and aerobic denitrification in the biozeolite capping system (Nakano et al., 2004), resulting from the decrease in aerobic denitrification.

Compared to the control system, the biological actions of biozeolite capping over time are shown in Table 1.

Table 1 shows that the main N reduction pathways changed over time with regard to biological denitrification

Table 1 – Biological actions of biozeolite capping in different periods, compared with control.				
Experimental phase	Days 3–16	Days 17–34	Days 35–45	Days 46–73
Reduction of inorganic nitrogen (IOrg-N) concentration of control (mg/L, designated as A)	0.04	0.47	0.83	2.30
Reduction of IOrg-N concentration of biozeolite (mg/L, designated as B)	0.36	1.13	1.16	0.37
Reduction of TN concentration of control (mg/L, designated as C)	1.11	0.26	−0.29	2.19
Reduction of TN concentration of biozeolite (mg/L, designated as D)	1.21	1.37	−0.56	0.91
B–A (mg/L)	0.32	0.66	0.33	−1.93
D–C (mg/L)	0.10	1.11	−0.27	−1.29
Main pathway of nitrogen reduction	Biological denitrification (31%) and biological assimilation (69%)	Biological denitrification (IOrg-N of 59% and organic nitrogen (Org-N) of 41% converted to N$_2$)	Biological assimilation (IOrg-N converted to Org-N)	No significant biological action

(SND) and biological assimilation. The N reduction by biological denitrification occurs *via* two main pathways, which are the oxidation of NH$_4^+$-N into nitrogenous gas directly *via* heterotrophic nitrification and the conversion of NO$_3^-$-N into nitrogenous gas by aerobic denitrification. After day 34, the effect of aerobic denitrification is not significant. The conclusions are the same as discussed above.

2.2. The changes in the DO concentration of the overlying water in the two systems

The changes in the DO concentrations of the overlying water in the biozeolite capping system and control system during the incubation experiments are shown in Fig. 2e.

The DO concentration of overlying water in the two systems either increased or decreased due to the balance between the reaeration rate and the microbial consumption rate of DO. Fig. 2e showed that from 1–8 days, the DO concentration in the biozeolite capping system was greater than that in the control system due to sorption of organics by the zeolite, consistent with the results reported by Kim and Jung (2010). However, from days 9–35, there were no significant differences in DO concentrations between these two systems, and their DO concentrations were less than 1.5 mg/L, because the heterotrophic nitrifiers and aerobic denitrifiers had consumed DO due to increased degradation of the available organics. From 36–168 days, the DO concentration in the biozeolite capping system was greater than that in the control system again, which may result from the reduction in available organics in the biozeolite capping system.

2.3. Changes in nitrifier and denitrifier numbers attached to biozeolite

The changes in nitrifier and denitrifier numbers attached to biozeolite are shown in Fig. 3.

Fig. 3 demonstrates that the number of nitrifiers attached to biozeolite increased gradually until day 36, decreased slightly between days 37 and 73, and finally increased quickly from days 74–121. Changes in denitrifier numbers have the same tendency as nitrifier numbers before day 73. However, denitrifier numbers dropped slowly after day 73, in contrast to

nitrifier numbers. These results suggest that both nitrifiers and denitrifiers attached to biozeolite could grow well before day 36, and then died off gradually, which may be due to the absence of carbon in the biozeolite capping system from days 37–73, illustrating why aerobic denitrification decreased after day 34 (Du et al., 2003; Walters et al., 2009). However, there appears to be a contradiction between the significant nitrification occurring and the decrease in nitrifier numbers, such that there was no evidence of strong nitrification in the canal water body (Huang et al., 2012). After day 73, the increase in nitrifier numbers resulted from the addition of carbon after water exchange. Nevertheless, the decrease in denitrifier numbers indicated that, in addition to carbon, the DO concentration may be a main factor in aerobic denitrifier growth (Ding et al., 2011). The DO concentrations during days 59–73 and 110–168 were greater than 3 mg/L (Fig. 2e), suggesting that DO concentrations greater than 3 mg/L could inhibit the growth of aerobic denitrifiers. Previous studies from our group showed that no significant nitrification occurs under DO concentrations less than 1 mg/L (Huang et al., 2012). Hence, the optimal DO concentration of aerobic denitrifier growth may be 1–3 mg/L.

2.4. The *in situ* bioregeneration ability of biozeolite

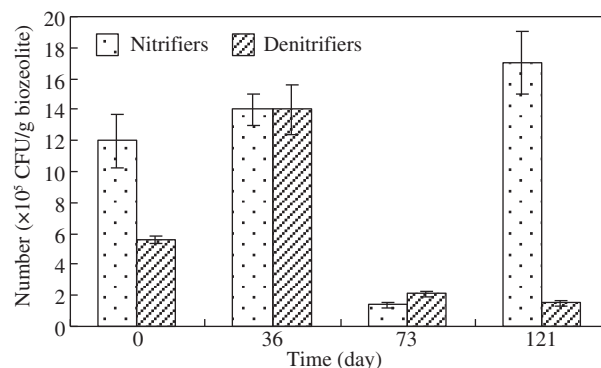The changes in ammonium adsorption by the biozeolite over time are shown in Fig. 4.

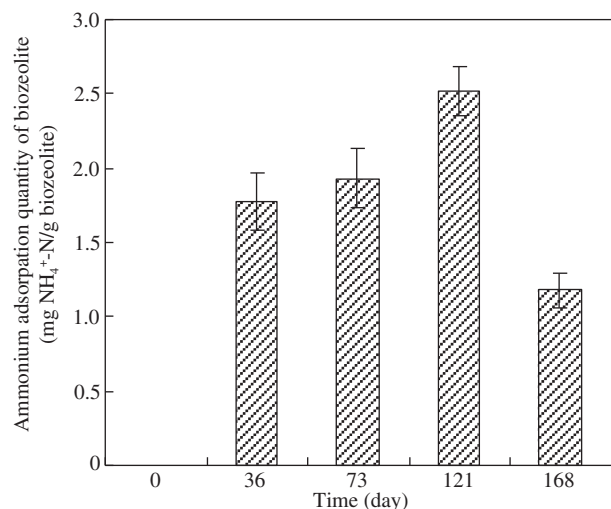Fig. 3 – Changes of nitrifier and denitrifier numbers on the biozeolite.

Fig. 4 – Change of ammonium adsorption quantity in the biozeolite *vs.* time.

Fig. 4 shows that ammonium adsorption by the biozeolite stabilized until day 73 due to the saturation of adsorption of ammonium by the biozeolite, increased during days 74–121, owing to the increase in ammonium concentration after water exchange, and finally decreased significantly from 2.52 to 1.18 mg NH_4^+-N/g biozeolite because of the significant *in situ* bioregeneration of the biozeolite during nitrification (Lahav and Green 1998; Jung et al., 2004). These results indicate that the biozeolite has very strong *in situ* bioregeneration ability. The extent of *in situ* bioregeneration of the biozeolite seemed to be related to the nitrifier number, which suggested that the increase in nitrifier numbers increased the *in situ* bioregeneration rate of the biozeolite.

2.5. The mechanism of BTC N reduction

BTC with biozeolite reduces N in eutrophic water bodies *via* a two-step process. First, biozeolite quickly removes ammonium from sediments and overlying water by physical adsorption and ion exchange during the first several days (*i.e.*, ammonium is stored in the biozeolite). Second, heterotrophic nitrifiers and aerobic denitrifiers reduce N pollutants (*e.g.*, NH_4^+-N, NO_2^--N, NO_3^--N, and Org-N) through SND and biological assimilation. Furthermore, significant *in situ* bioregeneration of the biozeolite occurs during nitrification.

Recently, anammox has been identified as an important pathway for removal of reactive N. Although anammox was not investigated in this study, the process may also contribute to the reduction of N from the aquatic ecosystem (Hou et al., 2013). However, further work is still required to confirm the hypothesis.

3. Conclusions

(1) N reduction in eutrophic water bodies using BTC with biozeolite *via* SND is feasible.
(2) The effects of *in situ* bioregeneration of biozeolite were significant during nitrification.

(3) Carbon was the main factor in heterotrophic nitrifier growth. However, both DO concentration and carbon were main factors in aerobic denitrifier growth.
(4) It is important to further strengthen the adaptability of aerobic denitrifiers through the optimization of biofilm formation *via* the control of DO concentrations between 1 and 3 mg/L.

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