Enhanced bacterial quorum aggregation on a zeolite capping layer for sustainable inhibition of ammonium release from contaminated sediment

Jinlan Xu, Haiyang Zhang, Rong Zhao and Fanxing Kong

ABSTRACT

The main objective of this study was to investigate how signal molecules enhance bacterial quorum aggregation on a zeolite capping layer for sustainable inhibition of ammonium release from contaminated sediment. Sediment remediation experiments were carried out by using nitrifying bacteria (WGX10, WGX18), denitrifying bacteria (HF3, HF7) and two kinds of signal molecules (OHHL, C8-HSL). The results showed that nitrifying bacteria and denitrifying bacteria could significantly aggregate on zeolite after adding 1.0 μM OHHL at a C/N ratio of 7. The maximum ammonium removal of five times the amount of ammonium adsorbed was achieved when 1.0 μM OHHL was added at the C/N ratio of 7 (the bio-regeneration rate was up to 88.32%), which was 1.24–2.02 times the ammonium removal amount at C/N ratios of 3, 5, 9. The concentration of total nitrogen in the overlying water was no more than 0.8 mg/L during four rounds of sediment remediation experiments. In addition, the bio-regeneration rate was up to 71.20%, which achieved sustainable inhibition of ammonium release from contaminated sediment.

Key words | ammonium release, contaminated sediment, inhibition, signal molecules, zeolite capping layer

INTRODUCTION

In-situ capping technology consists of capping sediments with sand, gravel, clay or other materials and this technology is currently one of the most promising technologies for water purification because it can control the release of pollutants from sediments (Kim & Jung 2010). In-situ capping technology involves the formation of a thin-layer capping of bio-zeolite (Huang et al. 2011; Lin et al. 2011) instead of traditional, physical, thick-layer capping methods (Berg et al. 2004), which mainly rely on passive physical barrier effects. Microbes on the zeolite degrade adsorbed ammonium and other pollutants in thin-layer capping systems of bio-zeolite, and bio-regeneration of bio-zeolite at the bottom of river is realized. Thus, this process leads to the sustainable adsorption of ammonium and other pollutants from sediments and improves the quality of the overlying water (Xu et al. 2011). However, problems have been encountered in the research of this technology. In particular, it is very difficult for denitrifying bacteria to attach to the surface of zeolite, which limits the bio-regeneration ability of the bio-zeolite and decreases its service cycle. Consequently, it is important for improving bio-regeneration of bio-zeolite and prolonging its life cycle to increase the amount of denitrifying bacteria attached on zeolite surfaces. By changing the structure of zeolite and the physiological behavior of bacteria, it is possible to enhance the rate of bio-regeneration of zeolite (Lin et al. 2015).

Studies have shown that bacterial quorum sensing (QS) plays an important role in the formation of biofilms and signal molecules can modulate the physiological behaviors of bacteria to promote their attachment and aggregation (Pérez-Montaño et al. 2015; Reen et al. 2015). There are some papers demonstrating the positive effects of N-acylhomoserine lactones (AHLs) in aerobic sludge granulation (Ren et al. 2010). AHLs add-back studies by Tan et al. (Tan et al. 2014) explained the positive role of AHLs in extracellular polymeric substances (EPS) synthesis and bacteria
aggregation. These studies showed the influences of signal molecules on the quorum aggregation of nitrifying bacteria. However, no related studies of denitrifying bacteria have been conducted. In addition, there were some previous studies about the additive concentration and types of signal molecules on bacterial quorum aggregation. However, the influences of the C/N ratio on bacterial quorum aggregation after adding the signal molecules are not clear.

As shown in Figure 1, a O= bond exists in the signal molecule N-(3-oxohexanoyl)-L-homoserine lactone (OHHL), C₁₀H₁₅NO₄. However, there is no bond in another signal molecule N-octanoyl-DL-homoserine lactone (C₈-HSL, C₁₂H₂₁NO₃). It is clear that there is a significantly different structure between the two signal molecules. And it causes different modes of attachment by nitrifying bacteria and denitrifying bacteria in the bio-zeolite. It is not clear whether the presence of the O= bond in the signal molecule enhances the quorum aggregation of denitrifying bacteria on zeolite.

Thus, two types of AHL signal molecules (OHHL and C₈-HSL) were selected for this study to investigate the effect of signal molecules on the quorum aggregation of denitrifying bacteria, the bio-regeneration of zeolite and the influence of sediment remediation after adjusting the ratio of C/N. The results of this research will provide a theoretical basis for zeolite capping on sediment remediation methods.

MATERIALS AND METHODS

Materials

In the experiment, nitrifying bacteria WGX10 and WGX18 Bacillus sp. and the denitrifying bacteria HF3 and HF7 were Acinetobacter sp. were used (Huang et al. 2012). The Haiyu zeolite, collected from Gongyi in Henan Province, China, is 1–2 mm in diameter. The chemical composition of the zeolite is shown in Table 1. SiO₂ and Al₂O₃ dominated (67.0–68.0% and 13.0–14.0%, respectively). The sediment was obtained from the Grand Canal in Yangzhou (China) and contained 3.14% organic matter and total nitrogen (1.24 mg/kg) (Table 2) (Huang et al. 2011). Two types of AHL QS signal molecules C₈-HSL was purchased from Green Herbs.

<table>
<thead>
<tr>
<th>Chemical composition of the zeolite</th>
<th>Chemical compounds (%)</th>
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<tr>
<td>Zeolite type</td>
<td>SiO₂</td>
</tr>
<tr>
<td>Haiyu zeolite</td>
<td>67.0–68.0</td>
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Figure 1 | There is a significant different structure between OHHL (with a O= bond) and C₈-HSL (without a O= bond); see Figures 3 and 7.
methods

Effects of signal molecules on bacterial quorum sensing of nitrifying and denitrifying bacteria in zeolite

To investigate the effect of signal molecules on bacteria attachment, an experimental study of bacteria attachment on 96-well plates and zeolite were performed.

Bacteria attachment on 96-well plates were as follows (Sabaeifard et al. 2014): 96-well plates (NEST, Shanghai, China) were used in this experiment to explore the improvement of attachment of denitrifying bacteria HF3 and HF7 and nitrifying bacteria WGX10 and WGX18 by signal molecules. HF3, HF7, WGX10, WGX18 and the mixed bacterial suspension were prepared, the pH value of the bacteria suspensions was 7.0 ± 0.1. 280 μL of the bacterial suspensions was placed in each well, the initial C/N ratio of the bacterial suspension was 3 without adding a carbon source. In addition, different concentrations of sodium acetate were added to change the C/N ratios of the bacterial suspensions to 5, 7, and 9 before the signal molecules were added at different concentrations. During the whole experimental period, the plates were kept at 30 ± 3 °C under aerobic conditions for 5 days before using the crystal violet staining method (Djordjevic et al. 2002) to measure the OD600 (the absorbance at the wavelength of 600 nm) of the attached biomass using a microplate reader (Stepanovic et al. 2000).

Bacterial attachment on zeolite was conducted in 250 mL of conical flasks in the same conditions. After 5 days, suspensions were poured from each of the conical flasks slowly, and then the zeolite was washed two times with sterile deionized water and weighed, the weight of the zeolite without biofilm was subtracted from the weight of the bio-zeolite to obtain the biomass aggregated on the surface of the zeolite (Alonso-Herrada et al. 2016).

5 g bio-zeolite was added into 100 mL of 0.3% sterile NaCl solution and the flasks were shaken at 120 rpm for 48 hours at 30 °C in order to make the attached biofilm separate from the zeolite. Then 8 mL suspensions were taken out to centrifuge at 10,000 rpm for 5 min at 4 °C and the supernatant solution was poured out. Next, 1 mL PBS (phosphate-buffered saline) was added to resuspend the biofilm; this was repeated three times. Then probe EUB 338 (GCTGCCCTCCCGTAGGAGT) (Shenggong, Shanghai, China) was used to dye the samples (Amann 1995). The dyed samples were placed on microscope slides and air dried for 2 hours. Finally, the samples were scanned (objective amplification is 100) using a laser scanning confocal microscope (Leica SP8, Germany). The above-mentioned operations were conducted in darkness. The attachment of bacteria on zeolite was analyzed using a scanning electron microscope (SEM, JSM-6510LV, Japan JEOL) operating at an acceleration voltage of 15 kV.

Preparation of the zeolite adsorbing ammonium, preparation of bio-zeolite and the experiment of bio-regeneration of the zeolite

To investigate the removal efficiency of ammonium in bio-zeolite, bio-zeolite was prepared for the bio-regeneration experiments.

First, preparation of the zeolite adsorbing ammonium was as follows: samples of 5 g zeolite were placed in 250 mL conical flasks, each of which contained 200 mL of solution of ammonium chloride at ammonium chloride concentrations of 20 mg/L, 40 mg/L, 60 mg/L, 80 mg/L, or 100 mg/L. The mixtures were kept at constant temperature (30 ± 3 °C). After 5 days of adsorption, the residual ammonium concentration in the solution was quantified in order to determine the adsorption by the zeolite at different concentrations of ammonium chloride. Then, the solution was taken out slowly and the samples were washed twice with sterile deionized water. Using this method, zeolite with five amounts of ammonium adsorption (0.76 mg/g, 1.53 mg/g, 2.14 mg/g, 2.82 mg/g and 3.50 mg/g) were prepared, which simulated different ammonium release amounts in sediments.
Second, the preparation of bio-zeolite was as follows: 200 mL of mixed suspensions of denitrifying (WGX10 and WGX18) and nitrifying bacteria (HF3 and HF7) was added to conical flasks with 5 g zeolite of adsorbing ammonium. The preparation of bacteria suspensions has previously been described (Huang et al. 2011). The concentration of the signal molecules and the C/N ratios were as above. After 5 days, the suspensions were taken out slowly from each of the conical flasks, and the zeolite was washed twice with sterile deionized water, and then the bio-zeolite was prepared.

Third, the experiments of bio-regeneration were as follows: 200 mL of distilled water and 5 g zeolite adsorbing ammonium was added to each of the conical flasks. The flasks were kept at 30 ± 3 °C under aerobic conditions for 25 days before conducting physical and chemical analyses to determine the ammonium amounts of bio-regeneration.

The pilot of sustainable bio-zeolite capping layer of decontaminating sediments

To investigate the efficiency of bio-zeolite capping layer on sustainable inhibition, following experiments were prepared.

The preparation of bio-zeolite was as follows: bio-zeolite 1 (without signal molecules), bio-zeolite 2 and 4 (with signal molecules) and bio-zeolite 3 and 5 (with signal molecules and appropriate C/N ratio) were prepared as above. 3,000 g natural zeolite was placed in a plastic bucket to form biofilm. The plastic bucket was inoculated with 6,000 mL denitrifying bacteria suspension, 6,000 mL nitrifying bacteria suspension under aerobic condition. Signal molecules were added to the bacteria suspension and sodium acetate was added to the suspension to adjust its ratio. After 5 days, 12,000 mL suspension was taken out slowly from each of the three plastic buckets and then the three types of bio-zeolite were gently washed by sterile deionized water and dried naturally.

The pilot of sustainable bio-zeolite capping layer of decontaminating sediments was as follows: prepared bio-zeolite was placed in a 5 L wide-mouth jar (inside diameter of 15 cm and a height of 24 cm) in the sediment repair experiments. First, 500 g of sediment was added before adding 45.44 g of bio-zeolite. Next, 5 L of water was added to serve as the overlying water, the pH and the dissolved oxygen (DO) content of which was 6.79 ± 0.1 and approximately 3 mg/L, respectively. The experiments of controlling nitrogen release from the sediment was subjected to natural oxygen conditions. Due to the concentrations of ammonium release from the sediments being low after 50 days, the bio-zeolite and the sediments were taken out when the capping experiments had lasted for 30 days. The first round of experiments was finished. Then the fresh sediments were placed in the wide-mouth jars, next the bio-zeolite were reused for capping the fresh sediments after they were gently washed with sterile deionized water and dried naturally. The second round of capping experiments was started. All the experiments lasted for four rounds (120 days). The total nitrogen, ammonium, nitrate and nitrite concentrations in the overlying water were measured periodically. All of the above experiments were conducted at room temperature (26–30 °C). After four rounds of capping experiments, three groups of bio-zeolite were taken out to conduct physical and chemical analyses to determine bio-regeneration of bio-zeolite.

Test and analysis methods

Ammonium, nitrate, and nitrite were measured using a UV spectrophotometer (DR5000, Hach). Nessler’s reagent and N-(1-naphthyl) ethylenediamine spectrophotometry method were used to analyze the ammonium and nitrite (Walter 1961), respectively. Nitrate analysis was carried out using UV spectrophotometry (Walter 1961). DO was determined using a DO meter (HQ500d, Hach), and pH was measured using a pHS-3c pH meter (Shanghai Precision and Scientific Instrument Co., Ltd).

After the capping experiment, the bio-zeolite was taken out and placed in 800 mL Erlenmeyer flask, and then washed with ethanol. The supernatant solution was discarded after centrifuging after which the physical and chemical desorption experiments were carried out to measure the capacity of bio-regeneration of zeolite. For the physical desorption experiment, 450 mL of 2 mol/L CaCl$_2$ solution was added to each of 800 mL Erlenmeyer flask with 45.44 mL bio-zeolite. Next, the flasks were shaken at 160 rpm for 24 hours at 25 °C and then centrifuged to determine the ammonium concentration in the supernatant solutions, which represented the quantity of ammonium physically desorbed by from the bio-zeolite. For the chemical desorption experiment, K$^+$ was added to replace ammonium. After the physical desorption experiment, 2 mol/L KCl solution was added, and the flasks were shaken for 24 hours at 25 °C before centrifuging to determine the ammonium contents in the supernatant solutions, which represented the quantity of ammonium chemically desorbed from the bio-zeolite. The sum of ammonium...
desorbed through physical and chemical methods represented residual ammonium in the zeolite. The ammonium of bio-regeneration on the zeolite was obtained by subtracting the amounts of residual ammonium in the zeolite from the total amount of ammonium removed.

**Data processing**

The ammonium of bio-regeneration of the zeolite was calculated using the following formula (1):

\[ m_0 = m - m_1 \]  

where \( m_0 \) was the ammonium of bio-regeneration per unit mass of the bio-zeolite (mg/g), \( m \) was the total amount of ammonium removed per unit mass of the bio-zeolite (mg/g), and \( m_1 \) was the residual amount of ammonium in the zeolite.

The amounts of total nitrogen or ammonium removed in the sediment were calculated using two formulas (2, 3) given below. The amount of total nitrogen (or ammonium) reduction in the sediment was calculated using formula (2) when the total nitrogen (or ammonium) concentration in the overlying water was higher than the initial total nitrogen (or ammonium) concentration in the overlying water.

\[ R_n = V(C_n^0 - C_n) \]  

However, when the total nitrogen (or ammonium) concentration in the overlying water was less than the initial total nitrogen (or ammonium) concentration in the overlying water, the decrease in the amount of total nitrogen (or ammonium) in the sediment was calculated using formula (3).

\[ R_n = V(C_n^0 - C_0) \]  

The amount of total nitrogen (or ammonium) removed in the overlying water was calculated using formula (4) as follows:

\[ W_n = V(C_0 - C_n) \]

where \( R_n \) was the amount of total nitrogen (or ammonium) removed (mg/g) from the sediment in the experiment during the \( n \)th round of the experiment, \( W_n \) was the amount of total nitrogen (or ammonium) removed (mg/g) in the overlying water in the \( n \)th round of the experiment, \( V \) was the volume (L) of the overlying water in the reactor, \( C_n^0 \) was the total nitrogen (or ammonium) concentration (mg/L) of the overlying water in the zeolite capping systems when the experiment lasted until the final day in the \( n \)th round of the experiment, \( C_n \) was the total nitrogen (or ammonium) concentration (mg/L) in the blank control system when the experiment lasted until the final day in the \( n \)th round of the experiment, and \( C_0 \) was the initial total nitrogen (or ammonium) concentration (mg/L) in the overlying water.

The total amount of total nitrogen (or ammonium) reduction in four rounds of experiments can be calculated using formula (5):

\[ X = \sum_{n=1}^{4} V(C_n^0 - C_n) \]  

where \( C_n^0 \) was the total nitrogen (or ammonium) concentration (mg/L) in the blank control system when the experiment lasted until the final day in the \( n \)th round of the experiment; and \( X \) was the total amount of total nitrogen (or ammonium) removed from the zeolite systems during the four rounds of the remediation experiments.

**RESULTS AND ANALYSIS**

**Effects of signal molecules on bacterial quorum aggregation on zeolite**

The OD_{600} values of nitrifying bacteria (WGX10 and WGX18) were up to 1.77 and 1.51 after adding 1.0 \( \mu \)M OHHL, 1.94, 2.25 and 1.92, 1.76 times higher than with no signal molecule. After adding C8-HSL the OD_{600} values were 0.91 and 0.67, 0.92 and 0.86, respectively (Figure 2(a)). However, the OD_{600} values of denitrifying bacteria HF3 and HF7 were 1.00 and 0.91, which was 1.69, 1.57 and 1.89, 1.63 times higher than with no signal molecules. After adding C8-HSL the OD_{600} values were 0.59 and 0.58, 0.53 and 0.56 respectively. Therefore, attachment of the nitrifying bacteria on the pore surface was easier than denitrifying bacteria when OHHL was added. This result could be due to the initial attachment capacity of nitrifying bacteria (OD_{600} was 0.91 and 0.67) better than denitrifying bacteria (OD_{600} was 0.59, 0.58), indicating that the attachment capacity of nitrifying bacteria (WGX10 and WGX18) could be improved significantly by adding 1.0 \( \mu \)M OHHL. An O=C-bond was found in OHHL after comparing the structure
of OHHL and C8-HSL, which might be one of the reasons that OHHL could enhance the quorum aggregation of bacteria. Furthermore, the attachment capacity of bacteria was improved only in 1.0 μM OHHL concentration, suggesting that the concentration of OHHL had a strict limitation for nitrifying bacteria (WGX10 and WGX18). J. Hrenovic et al. (Hrenovic et al. 2008) used HDTMA bromide to modify the natural zeolite surface, which enhanced the adsorption capacity of phosphate-accumulating bacteria (the amount of immobilised cells increased from $3.3 \pm 0.27 \times 10^9$ to $5.28 \pm 0.16 \times 10^9$ CFU/g). In this study signal molecules also enhanced the adsorption capacity of nitrifying bacteria and denitrifying bacteria on zeolite by modifying the bacteria surface.

Denitrifying bacteria (HF3 and HF7) aggregated on the pore surface increased significantly (OD600 values of HF3 and HF7 increased from 0.94, 0.92 to 1.59, 1.64, respectively) (Figure 2(b)) after adding 1.0 μM OHHL at a C/N ratio of 7, 69% and 78% higher than the bacteria under low C/N conditions (C/N is 3). Besides, the OD600 values of nitrifying bacteria WGX10 and WGX18 only increased by 19% and 35% (from 1.77, 1.51 to 2.10, 2.04) in the same situation. The results mentioned above showed that great improvement of adsorption capacity on the pore surface was found in denitrifying bacteria.

Significant improvement of mixed bacteria attached on the pore surface was obtained after adding 1.0 μM OHHL at a C/N of 7 (Figure 3(a1) and 3(a2)), the corresponding OD600 values of the mixed bacteria increased from 1.76 to 2.23, 27% higher than the mixed bacteria under low C/N conditions (C/N is 3). It showed that high a C/N would promote aggregation of mixed bacteria on the pore surface. A similar pattern was also observed on zeolite when bacteria attached to the zeolite increased by 41% (from 0.2150 g to 0.3032 g, Figure 3(b1) and 3(b2)) with the increase of the C/N ratio from 3 to 7. However, no significant change of adsorption (0.0835 g, C/N = 3; 0.0836 g, C/N = 7) was found in bacteria on zeolite after adding C8-HSL. It is obvious that the number of bacteria attached on the zeolites was significantly increased when adding OHHL as shown in Figure 3(c1) and 3(d1)) compared with the conditions without signal molecules (Figure 3(c2) and 3(d2)). This result indicated that the nitrifying bacteria and denitrifying bacteria could greatly aggregate on zeolite by adding 1.0 μM OHHL at the C/N ratio of 7.
Figure 3 | Effects of signal molecules on mixed bacteria aggregation. (a1–a2) Effects of OHHL, C8-HSL (C/N = 3) and C/N ratio (with OHHL) on the attachment of mixed bacteria on the hole board. (b1–b3) Effects of OHHL, C8-HSL (C/N = 3) and C/N ratio (with OHHL) on the attachment of mixed bacteria on the zeolite. (c1–c2) SEM images of bacteria (scanning factor: 10 × 100); (d1–d2) SEM images of bacteria on zeolite. (Initial bacteria concentration OD$_{600}$ and pH of bacteria suspension is 0.2 and 7.0 ± 0.1, respectively.)
Effects of bacterial quorum aggregation on bio-regeneration of zeolite

The maximum ammonium removal was achieved by adding 1.0 μM OHHL when the amounts of adsorbed ammonium in the zeolite were 0.76 mg/g, 1.53 mg/g, 2.14 mg/g, 2.82 mg/g, and 3.50 mg/g (Figure 4). This was noticeably higher than the corresponding values of the control group and the C8-HSL group, possibly due to the bacteria adsorption amount being 0.215 g (5 g zeolite) by adding 1.0 μM OHHL while there was only 0.083 g (5 g zeolite) of bacteria aggregation by adding C8-HSL. Hedström and Amofah (Hedström & Amofah 2008) found that the ammonium adsorption capacity increased with the decrease of grain size, and the highest adsorption capacity achieved was 2.7 mg NH₄⁺-N/g, while in this study, the ammonium adsorption capacity could be up to 3.50 mg/g. In addition, the highest removal of ammonium was observed, which were 0.54 mg/g (71.05%), 0.73 mg/g (47.71%), 0.95 mg/g (44.39%) and 1.22 mg/g (43.26%), respectively with the application of 1.0 μM OHHL in four ammonium adsorption amount (0.76, 1.53, 2.14, 2.82 mg/g). Rožič et al. (Rožič et al. 2000) investigated the removal of ammonium from aqueous solutions using a Croatian clinoptilolite and...
bentonite. The highest removal efficiency for NH$_4^+$-N (61.1%) was achieved on natural zeolite at the lowest initial concentration, i.e. 100 mg N-NH$_4^+$/L. With the increase of the initial concentration of ammonium, the removal efficiency rapidly decreased, consistent with the result of this study. This result suggested that ammonium removal increased with the increase of ammonium adsorption capacity at lower ammonium adsorption capacity. However, ammonium removal was significantly reduced to 0.81 mg/g (the bio-regeneration rate was just 13.71%) when ammonium adsorption amount in the zeolite reached 3.50 mg/g, indicating that the bio-regeneration of zeolite would be restrained by excessive ammonium adsorption in the zeolite. Wang and Peng (Wang & Peng 2010) investigated that biofilm covered on the zeolite did not affect the ion exchange for smaller particle size but decreased the ion exchange capacity by around 22% for larger particle sizes; bio-regeneration could recover about 78.0% and 65.9% of the ion-exchange capacity respectively for smaller and larger particle sizes of zeolite, respectively (Wen et al. 2006).

Figure 5 shows that the maximum removal of ammonium (0.67 mg/g, 1.25 mg/g, 1.89 mg/g, 2.25 mg/g, 1.17 mg/g) was observed with the application of 1.0 μM OHHL at a C/N ratio of 7, which was 1.24–2.02 times the removal amount of the condition when adding 1.0 μM

![Figure 5](https://iwaponline.com/wst/article-pdf/76/12/3428/242262/wst076123428.pdf)

**Figure 5** The effect of concentration of OHHL on the bio-regeneration of zeolite under different C/N ratio conditions. (a–e) Amount of ammonium adsorption is 0.76 mg/g, 1.53 mg/g, 2.14 mg/g, 2.82 mg/g, 3.50 mg/g, respectively. (Initial bacteria concentration OD$_{600}$ and pH of bacteria suspension is 0.2 and 7.0 ± 0.1, respectively.)
OHHL with different C/N ratios (3, 5, 9). The bio-regeneration rate was up to 88.32% under this condition (1.0 μM OHHL, C/N is 7), while 78.0% and 63.0% of bio-regeneration rate were obtained by Wang and Peng (Wang & Peng 2010), indicating better bio-regeneration in this study. The above-mentioned revealed that much ammonium in zeolite was removed to achieve bio-regeneration of zeolite after adding OHHL at a C/N ratio of 7, which could be due to substantial bacterial quorum aggregation in zeolite under this condition (adsorption amount was 0.3022 g per 5 g zeolite). Hrenovic et al. (Hrenovic et al. 2008) found that the maximum removal efficiency (72.45 ± 9.33%) of phosphorus was achieved when the highest amount (1.96 ± 0.24 × 10^8 CFU/g) of P-accumulating bacteria was achieved.

Compared with C/N ratios of 3, 5, 9, the removal efficiency of ammonium in zeolite was improved significantly by adding 1.0 μM OHHL at a C/N ratio of 7 when the ammonium absorption amount was 1.53, 2.14 and 2.82 mg/g, which might be due to the amount of bacteria quorum aggregation in zeolite increasing from 0.2150 g to 0.3022 g. The amount of ammonium removal increased from 0.70 mg/g to 1.25 mg/g, from 0.97 mg/g to 1.89 mg/g, and from 1.22 mg/g to 2.25 mg/g respectively with the ammonium adsorption capacity of 1.53, 2.14, and 2.82 mg/g. However, compared with C/N ratios of 3, 5, 9, the removal efficiency of ammonium (at a C/N ratio of 7) in zeolite just increased slightly under the condition of 0.76 mg/g and 3.50 mg/g of ammonium adsorption amount. This could be due to the decreased bioactivity of nitrifying bacteria and denitrifying bacteria under greater and lower ammonium concentrations.

The pilot of sustainable bio-zeolite capping layer of decontaminating sediments

Figure 6 shows five types of bio-zeolite capping system. The concentration of total nitrogen in the overlying water was no more than 0.8 mg/L in bio-zeolite 3 capping system during four rounds of sediment remediation experiments (120 days), satisfying class III water quality standard for surface water (TN < 1.0 mg/L) (SEPA 2002). Almost 95% (14.8 mg/L) of total nitrogen was inhibited, greatly much lower than total nitrogen release from sediments (15.6 mg/L) in blank, which is better than other four bio-zeolite capping systems. However, the total nitrogen release from sediments is up to 4.6 mg/L, 7.0 mg/L and 6.5 mg/L in bio-zeolite 2 (preparation by adding OHHL, C/N is 3), bio-zeolite 4 (preparation by adding C8-HSL, C/N is 3) and bio-zeolite 5 capping system (preparation by adding C8-HSL, C/N is 7), respectively, significantly higher than the class III water quality standard for surface water (TN < 1.0 mg/L) (SEPA 2002). This may be due to more bacteria attached on bio-zeolite 3 with ammonium adsorption in bio-zeolite from 1.53 mg/g to 2.14 mg/g, achieving the maximum bio-regeneration to remove 80% of ammonium in bio-zeolite. Therefore, the total nitrogen release from sediments could...
be effectively under control in bio-zeolite 3 capping system to remediate sediments sustainably in 120 days.

The balance of bio-zeolite capping layer of decontaminating sediments

Morin and Morse (Morin & Morse 1999) found that fast release of ammonium was a major factor in pollution of overlying water. In the present study, the fast release of ammonium in the sediments accounted for 75–100% of the total released ammonium, which was much higher than that report (11–80%). The sediments in this study were seriously polluted (ammonium was up to 11.45 mg/L in the blank control group) which would cause serious pollution to the overlying water. The release of ammonium was inhibited obviously by bio-zeolite 3 capping system during four rounds of sediment remediation experiments. The ammonium concentration in the overlying water decreased dramatically to 0.02–0.29 mg/L (Figure 7(a)), lower than the class III water quality standard for surface water (NH$_4^+$ < 1.0 mg/L) (SEPA 2002). It could be seen that bio-zeolite 3 capping system could effectively inhibit the fast release

Figure 7 | Bio-zeolite capping systems for controlling the release of ammonium, nitrate, and nitrite in the overlying water. (The DO content and pH of the overlying water is respectively approximately 3 mg/L and 6.79 ± 0.1.)
of ammonium. The lowest nitrate nitrogen concentrations (0.02–0.03 mg/L) were obtained in the overlying water and the nitrite was even not observed (Figure 6). The above-mentioned results might be because the sediment nitrification rate of bio-zeolite 3 capping system was up to 0.606 gN/(m²·d), which was higher than the corresponding value (0.21–0.42 gN/(m²·d)) of the study by Pauer and Auer (Pauer & Auer 2000). In addition, 51.20 mg (5 g zeolite) of adsorbed ammonium was observed in bio-zeolite 3 system (Table 3) and the bio-regeneration rate was up to 71.20%, which lead to sustainable inhibition. However, the release of ammonium was clearly observed in bio-zeolite 1 capping system during the third round of sediment remediation experiment. The corresponding ammonium value in the overlying water reached up to 5.31 mg/L. High residual ammonium (up to 108.97 mg) was found in the zeolite after desorption leading to low bioregeneration capacity (bio-regeneration rate was only 19.43%) for inhibiting ammonium release sustainably in sediments. The bio-regeneration rate (45.11%) in bio-zeolite 2 was lower than that in bio-zeolite 3 but higher than that in bio-zeolite 1. Significant ammonium release was observed in bio-zeolite 2 during the third round of sediment remediation, and hence, sediments could not be remediated continuously. Therefore, the longer lifetime (120 d) of zeolite was achieved due to the greater bio-regeneration rate (71.20%) in bio-zeolite 3. The NH₄⁺ flux from sediment (146.61–594.20 mg/(m²·d)) in this study was 5.73–23.21 times the research (25.6 mg/(m²·d)) by Lin et al. (Lin et al. 2011). However, only 2.58 kg/m² of bio-zeolite 3 was employed to cover sediment surface area, less than the investigation (12.7 kg/m² of zeolite) by Lin et al. (Lin et al. 2011).

### CONCLUSIONS

The main results obtained in this study can be summarized as follows. (1) The quorum aggregation of nitrifying and denitrifying bacteria on zeolite improved significantly after adding 1.0 μM OHHL at a C/N ratio of 7. (2) This ammonium removal (0.54–1.22 mg/g) was improved with the increased amount of ammonium adsorbed under lower ammonium adsorption capacities (0.76–2.82 mg/g, 1.0 μM OHHL); however, the bio-regeneration of zeolite would be inhibited by excessive amounts of ammonium adsorbed. (3) The maximum removal of ammonium was observed with the application of 1.0 μM OHHL at a C/N ratio of 7, which was 1.24–2.02 times the removal amount when adding 1.0 μM OHHL at C/N ratios of 3, 5, 9; The bio-regeneration rate was up to 88.32% under this condition. (4) Bio-zeolite 3 capping system could effectively inhibit the fast release of ammonium and the longer lifetime (120 days) of zeolite was achieved due to the greater bio-regeneration rate (71.20%), which achieved sustainable inhibition of ammonium release from contaminated sediment.

### ACKNOWLEDGEMENTS

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### REFERENCES


### Table 3 | Balance of sustainable bio-zeolite capping layer of decontaminating sediments

<table>
<thead>
<tr>
<th>Capping system</th>
<th>The preparation condition of bio-zeolite</th>
<th>The total ammonium removal (mg)</th>
<th>Residual ammonium in the zeolites (mg)</th>
<th>Ammonium removal by biodegradation (mg)</th>
<th>Ammonium removal by biodegradation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-zeolite 1</td>
<td>No signal molecule</td>
<td>135.25</td>
<td>108.97</td>
<td>26.28</td>
<td>19.43</td>
</tr>
<tr>
<td>Bio-zeolite 2</td>
<td>Adding OHHL C/N is 3</td>
<td>156.94</td>
<td>86.14</td>
<td>70.8</td>
<td>45.11</td>
</tr>
<tr>
<td>Bio-zeolite 3</td>
<td>Adding OHHL C/N is 7</td>
<td>177.79</td>
<td>51.20</td>
<td>126.59</td>
<td>71.20</td>
</tr>
</tbody>
</table>
monocytogenes biofilm formation. Applied & Environmental Microbiology 68 (6), 2950–2958.


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