Denitrification of nitrate-contaminated groundwater in columns packed with PHBV and ceramsites for application as a permeable reactive barrier
Liangtao Ye, Ge Yu, Shoubiao Zhou, Shengpeng Zuo and Caixia Fang

ABSTRACT
Groundwater contamination via nitrate leaching has been observed in many countries. The denitrification performance of a poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV)–ceramsite-based reactive barrier system was evaluated in continuous upflow bioreactors. Artificial groundwater spiked with nitrate (44.75–57.25 mg NO₃⁻-N/L) was tested under different hydraulic retention times (HRT; 5.2 h and 2.6 h). The continuous experiment for 35 days showed that more than 95% NO₃⁻ was removed. The maximum denitrification rate was determined to be 241 mg NO₃⁻-N/(L·d), and NO₂⁻ concentration was below 0.1 mg/L in the effluent at the substrate:ceramsite mass ratio of 1:40. Rapid biodegradation of PHBV granules caused an initial excess release of dissolved organic compound (DOC), and shortening HRT from 5.2 h to 2.6 h can result in a sharp decrease of DOC. The minimum DOC concentrations were determined to be 20–33 mg/L in the effluent when the HRT was set at 2.6 h. So the PHBV granules may be a suitable solid-phase carbon source for enhancing denitrification in nitrate-contaminated groundwater.

Key words | biodegradable polymers, denitrification, groundwater, permeable reactive barrier, solid carbon source

INTRODUCTION
The use of nitrogen fertilizers and irrigation with domestic wastewater is the main origin of nitrate pollution in groundwater, which is threatening potable water sources in China and elsewhere (Soares 2000; Shrimali & Singh 2001; Su & Puls 2004). In China, more than 50% of the population relies on groundwater as potable water (Jin et al. 2004). Nitrate is a priority pollutant of groundwater in many countries due to its toxicity related to methemoglobinemia in infants and tumor of the alimentary canal (Gómez et al. 2000; Vosoughifar et al. 2005). Both the European Union and World Health Organization have set the standard for nitrate in potable water at 11.3 mg NO₃⁻-N per litre (WHO 2004). Currently, China has set the surface water standard for nitrate at 20 mg NO₃⁻-N per litre (AQSIQ 1995).

The widespread occurrence of nitrate pollution in groundwater has attracted interest in feasible technologies for the treatment of nitrate. Conventional means for NO₃⁻-N removal, including a variety of physical–chemical approaches, such as ion exchange (Bae et al. 2002), reverse osmosis (Kim et al. 2007) and electrodialysis (Rittmann & McCarty 2001), have been proven effective. However, disadvantages such as poor selectivity, costly operation and maintenance, and sludge disposal have driven the search for alternative NO₃⁻-N removal technologies (Kapoor & Viraragahvan 1997; Qian et al. 2011). As an alternative, heterotrophic denitrification combined with a permeable reactive barrier system (PRBS) is likely to be feasible as an in situ groundwater remediation. Heterotrophic denitrification is an anoxic microbial process during which denitrifiers utilize organic carbon substances as electron donors and nitrate as terminal electron acceptors (Sierra-Alvarez et al. 2007; Qian et al. 2011). Therefore, organic carbon is the critical
element to achieve successful denitrification. Since carbon substances are usually inadequate for complete denitrification in nitrate-contaminated groundwater, an external carbon source addition should be required. Traditionally, a variety of dissolved carbon sources, such as ethanol, methanol or acetic acid have been effectively applied for nitrate removal (Mohseni-Bandpi & Elliott 1996; Mohseni-Bandpi et al. 1999; Lee et al. 2001). However, there are disadvantages such as sophisticated and costly process control and the risk of overdosing. To avoid these problems, solid biodegradable polymer (BDP) materials, such as polycaprolactone (PCL), poly(1,4-butandiol succinate), poly(3-hydroxybutyrate) and poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV), have been applied as carbon source and biofilm carrier for enhancing denitrification in recent years (Boley et al. 2000; Boley et al. 2005; Hiraishi & Khan 2003; Boley & Müller 2005; Wang & Wang 2009; Wang & Wang 2012; Wu et al. 2013). As we all know, the completion of high denitrification efficiency at least meets two basic conditions: enough contact time between carbon sources and nitrate, and the specific amount of carbon sources. However, a conventionally single BDP packed approach may bring low denitrification performance owing to inadequate BDPs in spatial distribution or high dissolved organic compounds (DOC) of the effluent owing to overdosing. Denitrification columns packed with PHBV–ceramsite at appropriate ratios could extend the spatial distribution of carbon sources and prolong the contact time between the substrate and nitrate, which may cut down the usage of carbon sources and result in high denitrification performance without over-release of DOC in the effluent. Therefore, it seems to be promising to implement the columns packed with PHBV–ceramsite into a PRBS to treat nitrate-contaminated groundwater. The long-term success of a PRBS is evaluated by its ability to maintain the reactivity of the materials with a sufficient hydraulic residence time (HRT) and low DOC in the effluent (Moon et al. 2008; Qian et al. 2011).

In order to develop a PRBS for nitrate-contaminated groundwater, the denitrification performance of continuous packed-bed reactors, using PHBV as an electron donor and inertial shale ceramsites as a biofilm carrier, was tested. The objectives of this study were: (1) to determine the nitrate removal efficiency (NRE) at different substrate: ceramsite mass ratios; (2) to compare the effects of different HRTs on denitrification performance; and (3) to obtain an idea of the appropriate barrier thickness to attain acceptable denitrification efficiency in a PRBS.

**MATERIALS AND METHODS**

**Materials**

The PHBV used in this study has a molecular weight of 4.6 × 10^5 g/mol (4–6.5 mm averaged particle size in diameter). The shale ceramsites have an average particle size of 5–8 mm in diameter.

**Reactor set-up**

In this study, continuous experiments were carried out in an upflow packed-bed reactor using 10.5 cm inner diameter by 42 cm height polyvinyl chloride (Figure 1). The four sampling ports of the column including the effluent port were evenly distributed at intervals of 10 cm from the bottom. Due to the high nitrate-nitrogen in groundwater around cities, for instance, 61.07 mg NO3-N/L was detected from groundwater around Chaohu, China (Qian et al. 2011), the four reactors were fed with artificial groundwater containing 325–413 mg/L KNO3 (44.75–57.25 mg NO3-N/L), 180 mg/L KH2PO4, 180 mg/L KH2PO4 and 17 mg/L MgSO4. The three reactors were packed with mixtures of PHBV granules and shale ceramsites at three different mass ratios, and one reactor was packed with single shale ceramsites as a control. The shale ceramsites were pre-washed with deionized water and then dried for 4 h at 105 °C in the oven. All the packed materials were piled up to a height of 40 cm in the four columns. The reactors were completely submerged and operated in continuous upflow mode (Figure 1). At the initial stage of the experiment, the artificial groundwater was mixed with activated sludge from a local municipal wastewater treatment plant (sewage treatment plant of South Wuhu, Wuhu, China).

**Experimental procedures**

PHBV and ceramsites were added into the reactors at three mass ratios (PHBV:ceramsites (m/m) = 1:21, 1:28, 1:40). In
order to determine the optimum PHBV:ceramsites ratio with maximum nitrate removal, four sets of experiments were performed under ambient temperatures of 20–25 °C. The continuous upflow reactor was operated for 35 days, and the artificial groundwater was pumped into the reactors at a flow rate of 5 mL/min (HRT = 5.2 h) and 10 mL/min (HRT = 2.6 h).

Analytical methods

Nitrate was determined by a UV-spectrophotometer (Hach DR-5000) at 220 nm and 275 nm, and nitrite was assayed by the hydrochloric acid naphthyl ethylenediamine spectrophotometry method (China SEPA 2002). DOC concentrations were measured by following the standard method of China NIS (1989). The pH value was measured with a pH meter.

Data analysis

The NRE was defined by the following equations:

\[
NRE(\%) = \left( \frac{1 - C_o}{C_i} \right) \times 100,
\]

where \( C_o \) and \( C_i \) are nitrate concentrations in influent and effluent (mg/L), respectively.

The nitrate removal rate (NRR) was defined by the following equations:

\[
NRR = \frac{\Delta m}{VT} \text{ (mg NO}_3^-\text{-N/(L·d))},
\]

where \( \Delta m \) is the concentration difference of NO\(_3\) -N in influent and effluent (mg) per unit time; \( V \) denotes the effective volume of the column (L); and \( T \) denotes the reaction time (d).

RESULTS AND DISCUSSION

Denitrification performance

Results of nitrate removal in four biological PRBS are shown in Figure 2. It can be seen that NRE was almost independent of PHBV:ceramsite ratios in three treatment reactors. During phase 1 (start-up period; HRT = 5.2 h), the NRE at three mass ratios increased significantly. Nitrate was mostly depleted (>89%) during the initial 9 days, and it reached the peak with the maximum removal efficiency of 99% at 11 d when the PHBV:ceramsite ratio was 1:40. The acclimation time (the lag time) was 16 days and 3 days when using PCL and starch/polyacaprolactone (SPCL 11) as carbon sources, respectively (Boley et al. 2000; Shen & Wang 2011). The
acclimation time in this study was 9 days, suggesting moderate acclimation time was needed using PHBV as carbon sources. During phase 2, HRT was adjusted to 2.6 h, and the NRE decreased sharply to 78%–85% in three treatment reactors at 14 d. This is obviously ascribed to the overloaded nitrate suddenly in the reactors and the acclimatization failure of denitrifying microorganisms to the new system in a short time. However, the NRE recovered to 92%–96% at 21 d without any adjustment of influent nitrate load. It kept stable at 24–35 d, and near-complete nitrate removal was achieved at 35 d in three treatment reactors (Figure 2).

Since three treatment reactors displayed similar NRE, the minimum DOC in effluent could be a representative indicator to determine the optimal substrate:ceramsite mass ratio in these cases. As indicated in Table 1, the concentrations of excess DOC in effluent decreased (from 88–133 mg/L to 20–33 mg/L at 28–35 d when the reactor functioning was stable) as the PHBV:ceramsite mass ratio was declining in the reactor, indicating that a substrate:ceramsite ratio of 1:40 may be an appropriate dosage for nitrate removal from groundwater. Therefore, the substrate:ceramsite ratio of 1:40 was selected for evaluating its feasibility for treating nitrate-contaminated groundwater.

Denitrification performance in a PRBS packed with PHBV–ceramsite at the mass ratio of 1:40 is depicted in Figure 3. During phase 1 (HRT = 5.2 h), high NRE (>82%) was observed within 20 cm from the bottom in 6 days and almost complete removal was achieved within 20 cm at 8–11 d (Figure 3(a)). The denitrification rate was observed to be 6–97 mg NO$_3$–N/(L·d) and it reached its peak at 8–11 d except at 10 cm from the bottom (Figure 3(b)). During phase 2 (HRT = 2.6 h), there was a decrease of nitrate removal in the effluent when HRT was shortened to 2.6 h at 14 d, and then the NRE increased significantly and kept stable within 20 cm from the bottom. The denitrification rate was observed to be 151–241 mg NO$_3$–N/(L·d) within 10–40 cm in the reactor at 14–35 d. It is reported that the highest denitrification rates were 530 and 31.9 mg N/(L·d) when two natural cellulose materials (wheat straw and reed stalk) were used as the substrate in an upflow bio-reactor with HRTs of 9.16 h and 45 h, respectively (Soares & Abeliovich 1998; Qian et al. 2011). For single BDP materials, the highest denitrification rate was determined to be 504–3,984 mg N/(L·d) using PCL as solid carbon source when HRT was set at 0.875 h (Boley et al. 2000). For mixed BDPs, the highest denitrification rates were 686 mg N/(L·d) and 349 mg N/(L·d) in continuous upflow reactors using cross-linked starch/PCL blends and PHBV/PLA blends as carbon sources when HRT was set at 1 h (Shen & Wang 2011; Wu et al. 2012). The comparison of the aforementioned denitrification performance indicates that the denitrification rates are affected by the change of HRT.

### Spatial–temporal variation of DOC, nitrite and pH

Spatial–temporal variations of DOC, nitrite and pH in the bioreactor packed with PHBV–ceramsite at the mass ratio of 1:40 are indicated in Figure 4.

A continuous increase of DOC was observed during phase 1 (HRT = 5.2 h), and DOC reached its highest value of 667–1,253 mg/L within 10–40 cm of the bottom at 11 d (Figure 4(a)). Concurrently, NRE seemed to increase synchronously with the rapid increase of DOC. At 14 d, HRT was adjusted to 2.6 h, and DOC within 10–40 cm of the bottom decreased significantly. Then constant DOC concentrations were observed to be 20–33 mg/L in the effluent at

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**Table 1** The DOC in effluent at three PHBV:ceramsite ratios for 28–35 d

<table>
<thead>
<tr>
<th>DOC (mg/L)</th>
<th>Mass ratio (m/m)</th>
<th>Effluent (28–35 d)</th>
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<tbody>
<tr>
<td>1:21</td>
<td>88–133</td>
<td></td>
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<tr>
<td>1:28</td>
<td>53–56</td>
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<tr>
<td>1:40</td>
<td>20–33</td>
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</table>
Figure 3 | Change of NRE and removal rates relative to time and distance at the mass ratio of 1:40.

Figure 4 | Spatial–temporal variations of nitrite, DOC and pH at the mass ratio of 1:40.
28–35 d (Figure 4(a)). As shown in Figure 4(a), the DOC concentrations generally decreased with height from the bottom in this study. Previous studies showed that DOC concentrations decreased with the increase of the flow rates (or the decrease of HRT) in upflow denitrification reactors when using solid-phase carbon sources (e.g. poplar, hornbeam, pine shavings, wheat straw and starch/PCL blends) (Aslan & Türkman 2005; Shen & Wang 2011), which is consistent with this study. In addition, the phenomenon that effluent DOC increased quickly during the start-up period and then dropped strikingly during the stable period in this study was also reported (Aslan & Türkman 2005; Shen & Wang 2011; Wu et al. 2012). Wu et al. (2012) explained that during the start-up period, the biofilm is immature and falls off from the carriers easily, which leads to the increase of DOC in the effluent. In contrast, during the stable period, as the biofilm is mature and sticks to the carriers tightly, more DOC is fully consumed by denitrifying bacteria, which reduces the release of DOC in the effluent. So the high concentration of DOC released by the microbial decomposition of solid PHBV in the beginning further enhances rapid microbial growth, and abundant microbes boost the consumption of DOC in turn. Therefore, we believe that release of DOC is primarily affected by microbial activity and HRT during the denitrification process in this study.

Under anoxic conditions, nitrate is the most favorable terminal electron acceptor. However, during the denitrification process, it may release undesirable nitrite ions as intermediates (Gee & Kim 2004), which can also be electron acceptors and converted to \( N_2 \) in the presence of an excess of DOC. In this study, during the start-up period when the denitrification system was not stable, denitrifying microorganisms were not able to convert nitrite to \( N_2 \) in a timely way, which resulted in significant nitrite accumulation. During the stable period, even when HRT was shortened to 2.6 h, nitrite concentrations sharply decreased within 10–40 cm from the bottom, and \( \text{NO}_2\text{-N} \) did not exceed 0.1 mg/L in the effluent during the period of 20–35 d.

The variation of pH values within 10–40 cm throughout the experiment is also depicted (Figure 4(c)). pH values were observed to decrease from 5.66–8.20 (phase 1) down to 5.10–7.17 (phase 2) in the PHBV-fed reactor. One possible explanation is that \( CO_2 \) formation occurs following degradation of the organic matter, such as PHB (Müller et al. 1992), and \( CO_2 \) further acidifies the aquatic environment of the reactor, therefore reducing the pH (Hamlin et al. 2008). In addition, the variation of pH will exert its impact on bacterial activity and NRR. For instance, Shen et al. (2013) found that when pH was beyond the optimal range, increase of effluent nitrate and decrease of NRR were observed. In this study, at acidic pH 5.4 and 6.2, the denitrification rates in the effluent were 161 and 216 mg/(L·d), respectively, while the denitrification rate in the effluent was 235 ± 5 mg/(L·d) at the normal value (pH 6.7). Therefore, our study is consistent with previous studies on the optimal pH range for denitrification (Tang et al. 2011; Shen et al. 2013).

Finally, we give the concept model of nitrogen balance of the PRBS indicated in Figure 5. From the concept model, we can see that influent nitrate nitrogen is primarily transformed into \( N_2 \) and is partly assimilated into the microorganisms to support microbial growth and activity. The excess nitrogen is discharged into influent in the form of nitrate, nitrite and ammonia.

**CONCLUSIONS**

The PRBS, using PHBV as electron donor and ceramsite as biofilm carrier for nitrate-contaminated groundwater treatment, was successfully operated for 35 d. The results showed that influent nitrate (44.75–57.25 mg \( \text{NO}_3\text{-N} \)/L) in the PRBS was almost completely transformed to \( N_2 \) and over 95% \( \text{NO}_3\text{-N} \) was removed. The highest denitrification rate was observed to be 241 mg \( \text{NO}_3\text{-N} \)/(L·d) in the effluent.
when the substrate:ceramsite ratio was 1:40 and HRT was 2.6 h. During the operation, the hydrodynamics characteristics, such as HRT, were changed, but this change did not lead to a decline in the denitrification performance.

An appropriate thickness of the PRBS is obtained according to the spatial ion distribution data in this study. A minimum barrier thickness of 20 cm seemed to be required to ensure complete nitrate removal without the accumulation of nitrite under the PHBV:ceramsite ratio of 1:40 and HRT of 2.6 h.

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