Structure and function of slow release organic carbon source in groundwater in-situ denitrification

D.Y. Zhang, G.H. Li, Y. Wang and G.Z. Zhou
Department of Environment Science and Engineering, Tsinghua University, Beijing, China, 100084
(E-mail: zhangdayi@tsinghua.org.cn)

Abstract Many nitrate pollution cases exceed the threshold as recommended by the World Health Organization (50 mg NO₃/L) and by the USA (10 mg N/L) for drinking water. In-situ denitrification was regarded as a good method to decrease nitrate contamination but is restricted by carbon absent in groundwater. Considering the disadvantages of known carbon sources, this paper provided slow-release organic carbon-source (SOC) technique to solve the problem and the results showed that SOC materials showed good performance during simulated groundwater denitrification. Structure analysis suggested that hydroxy chemical bond existed between PVA and starch in SOC and surface configuration changed with materials dissolving into water. After seven days of domestication, with 40–50 mg/L initial NO₃-N, denitrification efficiency increased from 80.6% to 90.7% and the real COD consumption per N-NO₃ reduction was 1.82–3.73 with 2.79 as average. Denitrification process followed the law of zero order kinetics and the parameter of denitrification dynamics, K, was from 0.1366 to 0.1873. It was suggested that SOC was a potential carbon source material (electron donor) suitable for in-situ groundwater denitrification.

Keywords Denitrification; groundwater; in-situ; SOC

Introduction
Water is a fundamental material for life on earth. The whole mechanism of metabolism, synthesis and structure of colloidal cellular constituents and transport of nutrients inside cells and interactions with the environment are closely related to characteristics of water. About 2.66% of the total global water resources (groundwater, lakes and rivers, polar ice and glaciers) are fresh water, but only a small fraction (0.6%) is available as drinking water (Falbe et al., 1992). Therefore, it is necessary that the water reserves be looked after carefully and wastewater treatment be done efficiently.

Nitrate is found in most of the natural waters in moderate concentrations but is often enriched to the contaminant levels in the groundwater resources mainly from the excessive use of fertilizers and uncontrolled onland discharges of raw and treated wastewater. This leads to an increasingly important problem, limiting the direct use of the groundwater resources for human consumption in several parts of the world including India, China, Japan, USA and UK. Several parts of Europe also have similar problems. In the rural communities of California, there is no centralized water supply. The local groundwater is the major source of drinking water and is generally produced from private wells. About 40% of the water wells in the Sierra Pelona basin in Los Angeles county, California, produce water that exceeds the maximum permissible level of NO₃-N (Williams et al., 1998). The American Water Works Association reported that more than 20% of 5,826 groundwater samples from the domestic and irrigation wells in the state of Nebraska have shown nitrate concentrations in excess of the United States Health Standard.

Biological denitrification is an important alternative which actually removes nitrate from the drinking water sources. This process reduces nitrate to innocuous nitrogen gas rather than to ammonia which exerts an adverse impact on drinking water quality by
combining with chlorine to form chloramine and nitrogen trichloride. Under the anoxic conditions, denitrifying bacteria are capable of using the chemically bound oxygen in nitrate as a terminal electron acceptor. Utilising complex organic compounds are termed as the heterotrophic microorganisms and the denitrification processes are termed as autotrophic and heterotrophic, respectively. Biological denitrification is the only process that directly targets nitrate and does not shift the concentration of other ions. For these reasons, biological treatment represents a cost-effective technique for removing the nitrate ion from the contaminated water. Mateju et al. (1992) have published a comprehensive account of different methods of biological denitrification of the drinking water.

Several types of organic compounds have been used as the substrates in water denitrification processes. Most of the published research on water denitrification process uses methanol, ethanol or acetic acid as the organic carbon source. Although methanol assures the highest denitrification rate, it can constitute certain risk if the treated water is used for drinking purpose. A high efficiency of NO₃ removal was achieved. Sugar or glucose syrup was also used as the carbon source for heterotrophic denitrification where the rate of NO₃-N removal was found higher than 80% at the influent concentration of 400 mg NO₃/L. (Nurizzo and Mezzanatte, 1992). Dalmacija et al. (1991) used ethanol as the source of carbon for denitrifying bacteria to remove nitrate from river water which contained about 117 mg NO₃/L. Hamazah and Ghararah (1996) investigated the influence of the type of substrate used and nitrate loading on the denitrification process of the contaminated drinking water. The system consisted of an anoxic static bed column operated at various nitrate loadings in the range 240–1,300 mg NO₃-N/(L·day). About three weeks of continuous system operation were needed to establish steady-state conditions with respect to the effluent nitrate removal were found in the range of 95–97% with methanol and 88–92% with ethanol as substrate. The results showed that the system should be operated at a maximum nitrate loading of 450 mg NO₃-N/L day to treat waters having a high nitrate concentration of 500 mg/L to the allowable effluent nitrate and nitrite concentrations. Based on the stoichiometric relationships, a methanol to nitrate ratio of 0.56, ethanol to nitrate ratio of 0.374, and acetic acid to nitrate ratio of 1.27 were used to compare the efficiencies of these substrates. The corresponding stoichiometric C/N ratios for methanol, ethanol and acetic acid are 0.93, 0.87 and 2.24, respectively. Thus, ethanol gave better results when compared with methanol and acetic acid.

Several attempts have also been made to make use of solid substrates. Mori (1996) attempted to denitrify contaminated water (125 mg NO₃/L) by passing it with ethanol (100 mg/L) through a column containing charcoal as the contact material for about 15 days. The use of polybutyric acid as a solid substrate for the microbial nitrate elimination in drinking water was examined (Muller et al., 1992). The feasibility of the co-immobilization of bacteria and polybutyric acid makes this process useful for denitrification of water during treatment processes. Volokita et al. (1996) studied the microbial removal of NO₃ from the drinking water in laboratory columns separately packed with unprocessed cotton and shredded newspapers. The unprocessed cotton and the newspaper served as the sole chemical and physical substrates for the microbial population in the laboratory columns. The unprocessed cotton was entirely consumed in this process. For both the substrates, the removal of nitrate was rapidly achieved without formation of nitrite. However, the cellulose dependent denitrification was affected by changes in the temperature. Further, pretreatment of the newsprint with diluted sodium hydroxide or diluted hydrochloric acid or autoclaving did not improve the efficiency of the process. Use of hydrogen as the electron donor in biological denitrification offers substantial cost savings as compared to the use of organic substrates due to less sludge production and easier post-treatment.
Considering the disadvantages of known carbon sources, slow-release organic carbon-source (SOC) has the potential to solve the problem. With SOC in the groundwater, carbon materials (electron donor) would dissolve into the environment on control to support denitrification in different situations. In this paper, several kinds of SOC were manufactured and evaluated in a simulated groundwater system.

**Methods**

**Experimental materials**

Four kinds of SOC materials were used in this investigation. Frame material was PVA while starch was used as the carbon source (electron donor). Starch was mixed with water at inverse proportion of 1:10 (m/m) and heated at 60°C for 1 hour under whisking. PVA was added into the solution and calefacted to 90°C rapidly and whisked for 30 minutes. Molding was at 4°C for 2 hours and raw and processed materials were incised into lumpish solid with cubage of 1 cm³. According to different starch contents, they were named GEPVAS-20, GEPVAS-40, GEPVAS-60 and GEPVAS-80, as shown in Table 1.

**Experimental methods**

To evaluate the use of SOC, a simulated underground circumstance was fabricated using the equipment developed as shown in Figure 1. With N₂ import and export, the anaerobic environment could sustain stable denitrification.

The experiment was divided into two sections. In Section I, no SOC was in the system during denitrification in order to evaluate the carbon usage, C/N and parameters of denitrification. In Section II, measurable SOC was in the system during denitrification in order to evaluate the real effect of SOC in in-situ denitrification.

**Analytical methods**

To discuss the performance and effect of SOC, inner structures and surface characters were studied. In the denitrification section, COD and nitrate were detected using different methods.

SOC structure was analyzed by Raman Spectrometry to express the correlated function of starch and PVA (David et al., 2001). Micro-Raman measurements of the deposited materials were performed using a REM2000 system with 632.8 nm radiation from a He-Ne laser using a back-scattering geometry. Microscope objectives × 100 or × 50 were used to focus the laser beam onto a spot of approximately 1–5 μm in diameter, and to collect the scattered light, which then passed through the spectrometer onto a CCD detector. Furthermore, a confocal hole with a diameter of 200 μm, a spectrograph entrance slit of 150 μm, and 1,800 grooves/mm diffraction grating were employed.

Surface characters were analyzed by scanning electron microscope (SEM, Philips 525M). The system provided manipulation of the SOC sample with respect to the tungsten tip with steps of 200 nm in the x, y and z direction. The piezo elements could be activated during SEM operation, although some interference with the SEM signal was visible. The set-up enabled manipulation of objects while watching with a resolution of

| Table 1 Starch and PVA contents in serials of SOC |
|---------------------|-----|-----|------|------|-----|
| Section              | Starch | PVA | Starch/PVA | pH | H₂O content (%) |
| GEPVAS-20            | 20    | 80  | 1/4         | 5–7 | 85–90 |
| GEPVAS-40            | 40    | 60  | 2/3         | 5–7 | 85–90 |
| GEPVAS-60            | 60    | 40  | 3/2         | 5–7 | 85–90 |
| GEPVAS-80            | 80    | 20  | 4/1         | 5–7 | 85–90 |
10 nm at video frequency and an electron beam energy of 30 keV. In addition, a voltage difference could be applied between the tip and the sample and the current could be measured (de Jonge and van Druten, 2003).

COD and nitrate analyses were performed as APHA Standard Methods. COD measurement was using Hach COD Reactor (45600-02), and nitrate was detected by UV spectrophotometer (SHIMADZU, UV2501PC).

Results and discussion

SOC structure

Methylene peaks in GEPVAS were 1,435 cm\(^{-1}\) and 1,105 cm\(^{-1}\), which were 1,441 cm\(^{-1}\) and 1,155 cm\(^{-1}\) in PVA and 1,459 cm\(^{-1}\) and 1,131 cm\(^{-1}\) separately in starch. This suggested that Einstein shift occurred in SOC materials because of the action of different hydroxy by PVA and starch. OH-peak of H\(_2\)O was separated into two parts, which were 3,390 cm\(^{-1}\) (combined hydroxy of H\(_2\)O) and 3,250 cm\(^{-1}\) (unrestricted hydroxy of H\(_2\)O), as shown in Figure 2.

Peak 1,250 cm\(^{-1}\) was observed in GEL SOC material and did not exist in either starch or PVA. It was suggested that hydroxy chemical bond existed between PVA and starch and this kind of hydroxy bond impacted the phenomenon of SOC in denitrification.

Figure 1 Experimental equipment

Figure 2 Raman Spectrum characters
Surface characters

Analyzed by SEM, the surface of materials changed significantly during the denitrification process. The surfaces of raw SOC were coarse with starch grain and the organism dissolved into the water to act in denitrification, which caused a smoother surface and the starch grain to disappear. Differences of surface were shown in Figure 3 and it was obvious that the configuration of each kind of SOC was quite different. In GEPVAS-20, reticulate structure was observable. This kind of reticulate structure was imperfect with the increase of content of starch and could be explained as the decrement of PVA organism and the superstratum function of starch.

Denitrification simulation

In order to determine the electron donor and SOC performance in denitrification, two sections were designed to differentiate carbon sources and affirm parameter of denitrification dynamics. In section I, denitrification occurred with SOC in simulated system, while in section II, it occurred without SOC.

As shown in Figures 4 and 5, in Section I (denitrification with SOC in system), electron donor was partly from dissolved organisms and partly from the SOC itself. The mechanics was therefore more complex and uncalculated. In the initial term, the values of COD and nitrate were 100–140 mg/L and 40–50 mg/L. During the first seven days, COD and nitrate remained as initial level, which suggested that the bacteria needed a period of domestication. In 8–17 days, nitrate reduced significantly together with COD decreasing. Nitrate reduced to 5–9 mg/L, below WHO level. In CK treatment, COD and nitrate remained at the initial level during the whole period of operation. It was suggested that in simulation system without carbon source, denitrification was limited and could not occur. However, the superfluous of COD was unrestricted and maintained above 70 mg/L, which was caused by slow release carbon of SOC. Although the released domino effect could be proved in such phenomena, the excessive COD must be controlled to appropriate level.

As shown in Figures 6 and 7, in Section II (denitrification without SOC in system), the electron donor consisted of the organism dissolved by SOC without any other carbon source. In initial term, the values of COD and nitrate were also 100–140 mg/L and 40–50 mg/L, respectively. As for Section I, there were a period of domestication in the first seven days and nitrate decreased with COD after 8–17 days. COD also decreased. Nitrate

Figure 3 Surface characters: (a) GEPVAS-20; (b) GEPVAS-40; (c) GEPVAS-60; (d) GEPVAS-80.
Note: *: before denitrification; **: after denitrification

Downloaded from https://iwaponline.com/ws/article-pdf/6/3/105/418199/105.pdf by guest
Figure 4  Trends of nitrate in Section I. *•* GEPVAS-20; ■ GEPVAS-40; ▲ GEPVAS-60; ▲ GEPVAS-80; ○ CK1; △ CK2

Figure 5  Trends of COD in Section I. *•* GEPVAS-20; ■ GEPVAS-40; ▲ GEPVAS-60; ▲ GEPVAS-80; ○ CK1; △ CK2

Figure 6  Trends of nitrate in Section II. *•* GEPVAS-20; ■ GEPVAS-40; ▲ GEPVAS-60; ▲ GEPVAS-80; ○ CK1; △ CK2
also reduced to 4–8 mg/L, but it was quite different with COD expression. In latter period of operation, COD were below 30 mg/L and down to 5 mg/L in separate treatment, which improved that 80–96% of COD could be used by denitrifying bacteria during denitrification. Another result of rapid COD’s consumption, denitrification was limited and slower than in Section I.

Denitrification efficiency and C/N (COD consumption per N-NO\textsubscript{3} reduction) in different sections is shown in Table 2. From the results, the denitrification efficiency in Section I was 83.2–89.7% and in Section II was 80.6–90.7%. It was a high efficiency for in situ denitrification and suitable for further research and development. Because of carbon release from SOC, the value of C/N in Section I was not real C/N, which was from 1.04 to 2.22 with 1.68 as average. The real C/N was calculated in Section II and from 1.82 to 3.73 with 2.79 as average.

According to the trends of nitrate in Section II, parameters of denitrification dynamics were analogised. Correlation analysis showed that denitrification process followed the law of zero order kinetics:

\[
\frac{dN}{dt} = -K
\]

\[N = -Kt + N_0\]

where \(K\) is the denitrification rate (mg/L h) and \(N_0\) is the nitrate concentration (mg/L).

**Table 2** Denitrification efficiency and C/N in different sections

<table>
<thead>
<tr>
<th>Material</th>
<th>Initial COD</th>
<th>Initial N-NO\textsubscript{3}</th>
<th>Final COD</th>
<th>Final N-NO\textsubscript{3}</th>
<th>Denitrification efficiency (%)</th>
<th>C/N</th>
<th>C/N (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEPVAS-20*</td>
<td>139.60</td>
<td>47.43</td>
<td>71.20</td>
<td>8.51</td>
<td>82.1</td>
<td>1.76</td>
<td>1.68</td>
</tr>
<tr>
<td>GEPVAS-40*</td>
<td>179.60</td>
<td>47.43</td>
<td>88.00</td>
<td>6.14</td>
<td>87.1</td>
<td>2.22</td>
<td></td>
</tr>
<tr>
<td>GEPVAS-60*</td>
<td>134.00</td>
<td>48.22</td>
<td>98.80</td>
<td>4.96</td>
<td>89.7</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>GEPVAS-80**</td>
<td>145.20</td>
<td>49.41</td>
<td>18.40</td>
<td>9.30</td>
<td>81.2</td>
<td>3.16</td>
<td></td>
</tr>
<tr>
<td>GEPVAS-20**</td>
<td>145.60</td>
<td>45.46</td>
<td>28.80</td>
<td>8.71</td>
<td>80.8</td>
<td>1.82</td>
<td></td>
</tr>
<tr>
<td>GEPVAS-40**</td>
<td>145.60</td>
<td>42.89</td>
<td>18.40</td>
<td>3.97</td>
<td>80.6</td>
<td>3.73</td>
<td></td>
</tr>
<tr>
<td>GEPVAS-60**</td>
<td>113.20</td>
<td>42.49</td>
<td>18.40</td>
<td>9.30</td>
<td>90.7</td>
<td>2.46</td>
<td></td>
</tr>
<tr>
<td>GEPVAS-80**</td>
<td>113.20</td>
<td>42.49</td>
<td>18.40</td>
<td>3.97</td>
<td>90.7</td>
<td>2.46</td>
<td></td>
</tr>
<tr>
<td>CK</td>
<td>395.60</td>
<td>43.48</td>
<td>377.60</td>
<td>41.31</td>
<td>5.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CK</td>
<td>427.20</td>
<td>40.12</td>
<td>445.60</td>
<td>40.91</td>
<td>-2.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

C/N: COD consumed per N-NO\textsubscript{3} reduction

*: Section I; **: Section II
Parameters of denitrification dynamics of different kinds of SOC were calculated and shown in Table 3. Scale of $K$ ranged from 0.1366–0.1873 and GEPVAS-20 showed the best performance.

Conclusions
This laboratory study successfully demonstrated that SOC materials could effectively reduce NO$_3^-$ in a simulated groundwater environment. A synthetic groundwater containing NO$_2^-$-N concentrations of 45 mg/L and NO$_3^-$ was reduced below the standards set by the USEPA (10 mg NO$_3^-$-N/L) when SOC was put into the system. Structure analysis showed that a hydroxy chemical bond existed between PVA and starch in SOC. Surface configuration changed with materials dissolving into the solution. The majority of the NO$_3^-$ removal occurred in 10 days after seven days domestication of bacteria. Under these conditions, denitrification efficiency was 80.6–90.7% and the real COD consumed per N-NO$_3^-$ reduction was 1.82–3.73 with 2.79 as average. The denitrification process followed the law of zero order kinetics and $K$ (parameter of denitrification dynamics) of different kinds of SOC was from 0.1366 to 0.1873. It was suggested that SOC was a potential carbon source material (electron donor) suitable for in-situ groundwater denitrification. SOC superfluous of COD was found in this investigation, especially in Section I, which could cause risk of high organic pollution. Further research is being undertaken to solve this problem and has reached its preliminary achievement objectives.

Acknowledgements
The authors would like to thank NFSC Project 40372107 and MOE Project 20030003096 for their financial support.

References


