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ORIGINAL PAPER

Denitrification of nitrate-contaminated groundwater using biodegradable snack ware as carbon source under low-temperature condition

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Abstract A considerable increase in nitrate concentration in groundwater has been observed in many countries. This research focuses on nitrate removal using biodegradable snack ware (BSW) as both carbon source and biofilm support for denitrifiers. The denitrification efficiency of a laboratory-scale denitrification reactor packed with BSW was examined in a low-temperature condition. The nitrate removal efficiency supported by BSW decreased to approximately 40% at 12°C from nearly 100% at 25°C with 50 mg/L of nitrate-nitrogen in the influent and 2 h of hydraulic retention time (HRT). The complete nitrate removal was obtained when nitrate-nitrogen concentration was no more than 15 mg/L at 2 h of HRT and at 12°C. If the initial concentration of nitrate-nitrogen was 50 mg/L, 5 h of HRT was needed for the complete nitrate removal. Nitrite concentration in the treated water decreased evidently as HRT was increased from 2 to 5 h, or as nitrate-nitrogen concentration in the influent decreased to 15 mg/L from 50 mg/L. It was observed that varying HRT and nitrate concentration in the influent had no noticeable effect on dissolved organic carbon content in the effluent under the experimental conditions. This study indicated that the complete nitrate removal could be achieved readily even at 12°C using BSW as carbon source by changing HRT or the initial concentration of nitrate in the influent, which has some useful implications in environmental engineering practice.

Keywords Biodenitrification · Biofilm support · Packed bed reactor · Solid carbon source

Introduction

Groundwater is one of main water sources for human consumption in most countries of the world. In some countries, especially those located in droughty area, groundwater is used as the only water resource, such as Saudi Arabia (Alabdula'aly 1997). In China, more than 50% of population uses groundwater as drinking water (Jin et al. 2004). Recently, a considerable increase in nitrate concentration in groundwater has been observed in many countries including China (Ovez 2006; Rocca et al. 2007; Sierra-Alvarez et al. 2007; Wang and Wang 2009). Concern over nitrate contamination is due to health problems related to methemoglobinemia in infants (blue baby syndrome) and cancer of the alimentary canal and there is no other group of carcinogens that can produce such a wide variety of tumors by consuming nitrate-contaminated water (Vosoughifar et al. 2005; Wang and Wang 2009). Nitrate pollution of groundwater is caused mostly by intensive use of nitrogen-based fertilizers in agriculture and uncontrolled land discharges of raw and treated wastewater (Shrimali and Singh 2001; Mahvi et al. 2005; Ghafari et al. 2008).

In comparison to physical and chemical methods (such as reverse osmosis, ion exchange and electrodialysis) to remove nitrate from groundwater, biological denitrification stands out for being the most economical and feasible on a large scale, as well as for being the only selective method

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for removing nitrate by transforming it into harmless nitrogen gas (Volokita et al. 1996; Moreno et al. 2005; Bidhendi et al. 2006). The majority of microbial denitrification treatment relies on heterotrophic bacteria which require an organic carbon source.

Groundwater has low carbon content; therefore, external carbon-containing substrates have to be added. Usually, dissolved carbon source such as ethanol, methanol or acetate is used as electron donors for nitrate reduction (Bandpi et al. 1999). The disadvantage of this treatment process is the need of a close, rather sophisticated process control, and has the risk of overdosing with the resultant deterioration of effluent water quality (Boley et al. 2000). To avoid above-mentioned problems, insoluble substrates (solid carbon sources) including wheat straw, cotton, polycaprolactone (PCL) and polyhydroxyalkanoates (PHAs) as an alternative to the liquid carbon sources have been successfully employed in denitrification process by some researchers in recent years (Soares and Abeliovich 1998; Boley et al. 2003; Hiraishi and Khan 2003; Wang and Wang 2009). Solid substrates used in the denitrification process serve as both constant sources of reducing power for denitrification and matrices favorable for development of microbial films (Hiraishi and Khan 2003).

Most of the denitrification processes are carried out under mesophilic conditions. However, in some regions in the northern countries, groundwater temperatures (from 10 to15°C) are below the mesophilic range (Kim et al. 2002; Nakajima-Kambe et al. 2005). As a result, the investigation on psychrophilic denitrification process could be useful in optimizing the design and operation of such treatment in low-temperature regions. As far as it is known, however, there is less information about denitrification of groundwater using solid carbon sources under low-temperature conditions.

Previous studies conducted by authors demonstrated that biodegradable snack ware (BSW) is a kind of perfect carbon source for nitrate removal from groundwater through batch experiments, with a relatively high denitrification rate and low cost (Wang and Wang 2009). The main objective of this study was to investigate the denitrification performance of a continuous-flow reactor packed with BSW as carbon source under a low-temperature condition (12°C), with a particular emphasis on effects of initial nitrate concentration and hydraulic retention time (HRT) on denitrification performance, in order to optimize the running parameters of the reactor to treat nitrate-contaminated groundwater under the low-temperature condition. This study was carried out between November 2008 and December 2009 in Beijing Agro-Biotechnology Research Center and Tsinghua University, China.

Materials and methods

Materials

BSW is made from reed and sugar cane, manufactured by Zhejiang Shuangyu Plastic Ltd., China. Prior to use, BSW was cut into pieces with size of 1.5 × 1.5 cm.

A synthetic influent water medium was used to simulate the composition of nitrate-contaminated groundwater, which consisted of the groundwater from Changping campus, Tsinghua University, China, containing about 50 mg/L of nitrogen and 10 mg/L of phosphorus by adding $NaNO_3$ and KH_2PO_4, unless other stated. Any supplementary nutrients were not added into the groundwater treated in this study, except for nitrate and phosphate. The characteristics of groundwater used in the experiments are as follows: nitrate-nitrogen (NO_3-N), 6.5–9.6 mg/L; dissolved oxygen (DO), 3.0–4.5 mg/L; nitrite-nitrogen (NO_2-N), 0.0 mg/L; dissolved organic carbon (DOC), 0.0–1.3 mg/L; and pH 7.4–7.9.

Activated sludge, obtained from Tsinghe Sewage Plant, Beijing, was used as the seed source for denitrification.

Experimental set-up

The continuous experimental set-up consisted of a cylindrical plexiglass biodenitrification unit of 4.5 cm inner diameter and 45 cm height, completely submerged and operating with an upward flow mode. The flow rate was regulated by a peristaltic pump (Fig. 1). The reactor was packed with 60 g of BSW as carbon source and biofilm support. Considering the working volume of the reactor and the volume of BSW packed, HRT with various flow rates was calculated and listed in Table 1.

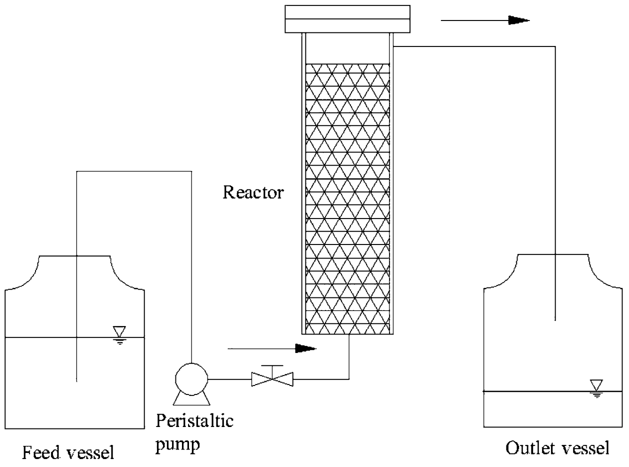

Fig. 1 A schematic presentation of the experimental set-up

Table 1 Flow rate and corresponding HRT and initial NO_3-N concentration adopted in the continuous-flow experiment at 12°C

Running sequence	Flow rate (mL/h)	HRT (h)	Initial NO_3-N concentration (mg/L)
1	230	2	50
2	230	2	40
3	230	2	30
4	230	2	20
5	230	2	15
6	184	2.5	50
7	153	3.0	50
8	132	3.5	50
9	115	4.0	50
10	102	4.5	50
11	92	5	50

Experimental procedure

30 mL of activated sludge, mixed with 3 L of influent containing 50 mg/L of NO_3-N, was pumped into reactor. At the first 3 days, the influent containing activated sludge was recirculated with daily replenishment of nitrate and 3 h of HRT at around 25°C, in order to inoculate BSW with denitrifying microorganisms. After this inoculation period, the reactor was started up (day 1) with 50 mg/L of NO_3-N in the influent and 2 h of HRT at 25°C. When stable denitrification rate was achieved, temperature was lowered down to 12°C, and then the system run continuously. To optimize reactor operation under the low-temperature condition, different initial NO_3-N concentration and HRT were set (Table 1). The system was kept running under each condition listed in Table 1 for 3 days.

Sampling and analysis

Every 24 h, water samples (50 mL) were collected from the inlet and the outlet of the reactor, obtaining three replicates for each run assayed. Water samples were filtered through 0.45 μm-pore membrane and were tested within 1 h of collecting. The filtrate was subjected to analyses of concentrations of NO_3-N, NO_2-N, ammonia and DOC according to standard methods (SEPAC 2002). DOC concentration was determined by a TOC analyzer (TOC-V wp, Shimadzu Corp., Kyoto, Japan). Each of the measurements conducted during this research was done in duplicate to provide greater confidence in the results.

Results and discussion

Reactor start-up

After 3-day recirculation of nitrate-contaminated groundwater containing activated sludge for inoculation microorganisms, the influent was continuously pumped into the reactor. At the first day for system start-up, 34% of removal efficiency was obtained from around 50 mg/L of NO_3-N in the influent (Fig. 2). From then on, removal efficiency increased gradually with running time. This suggests that the system supported by BSW could be started up readily, and the colonization of the substrate by bacteria was the rate-limiting factor for nitrate removal during the start-up period of the reactor (Volokita et al. 1996). After 7 days of running, almost complete nitrate removal was achieved, and NO_2-N concentration in the effluent was below 0.01 mg/L.

When temperature was decreased to 12°C from day 11 onwards, the breakthrough of nitrate was observed in the effluent; NO_3-N concentration exceeded 30 mg/L (Fig. 2), and removal efficiency reduced to approximately 40% from nearly 100% at 25°C. At the same time, the relatively high NO_2-N concentration was observed, ranging between 0.07 and 0.09 mg/L. This could be attributed to metabolic activities of microorganisms being inhibited due to a low-temperature condition, resulting in the accumulation of intermediates of denitrification process.

It has been reported in the literature that the low-temperature conditions affected seriously denitrification rates supported by solid carbon sources. Aslan and Turkman (2004) used wheat straw as substrate to remove nitrate from drinking water. When temperature was below 20°C, removal efficiency of nitrate decreased to about 10% from almost 100% at over 20°C. Volokita et al. (1996) reported denitrification rate at 14°C was approximately 40% of the

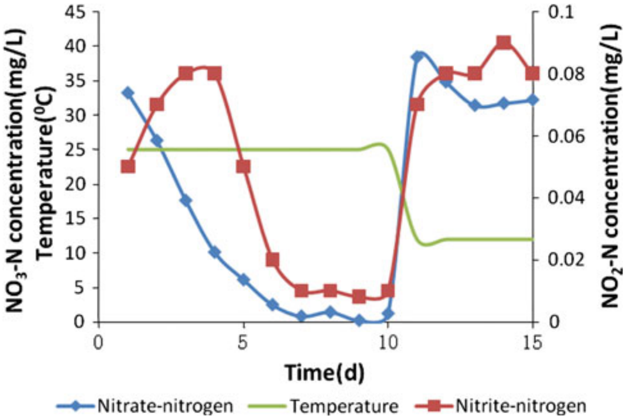

Fig. 2 Concentrations of NO_3-N and NO_2-N in the effluent of a reactor packed with 60 g of BSW and operated at the temperatures indicated and 2 h of HRT

rate observed at 25°C used cotton as carbon source to denitrification of groundwater. The obtained experimental results were similar with above-mentioned ones; however, the tolerance to the low temperature with BSW as carbon source was superior to wheat straw and cotton.

Effect of the initial NO_3-N concentration on denitrification at 12°C

To investigate the denitrification performance using BSW as carbon source under the low-temperature condition, NO_3-N concentration in the influent was changed between 15 and 50 mg/L with 2 h of HRT. Figure 3 illustrates the effect of NO_3-N concentration in the influent on denitrification at 12°C. Complete nitrate removal was observed as initial NO_3-N concentration was 15 mg/L.

When NO_3-N concentration in the influent was increased from 15 to 20 mg/L, denitrification rate was observed to raise significantly (Fig. 3). Denitrification rate had a negligible change, and was maintained in the range of 9–10 mg/(L h) with 20–50 mg/L of initial concentration of NO_3-N, indicating a zero-order kinetic model can be used to described the denitrification process under this condition (Foglar and Briski 2003). Several studies have shown that denitrification rate increased consistently with an increase in the initial NO_3-N concentration using soluble carbon sources (Elefsiniotis and Li 2006), which conflicts with obtained results. This may be due to two different types of carbon sources employed, and to different concentrations of nitrate and DOC in the influent, because denitrification reaction usually follows zero-order kinetic model when both the carbon source and nitrate or nitrite are in enough concentration not to limit growth (Glass and Silverstein 1998).

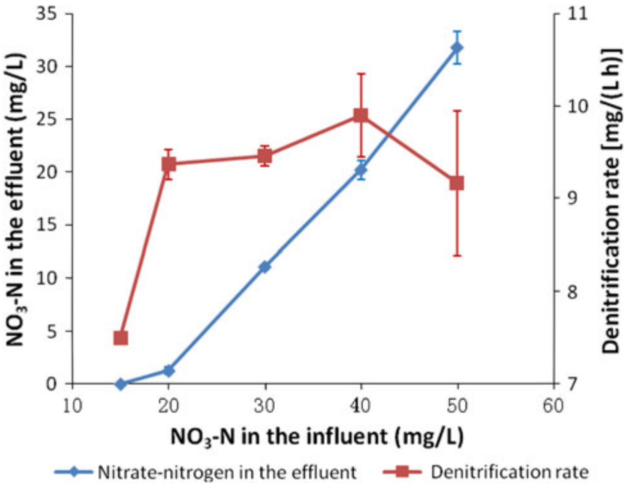

Fig. 3 Changes in concentration of NO_3-N in the effluent and denitrification rate with the varying NO_3-N concentrations in the influent at 12°C

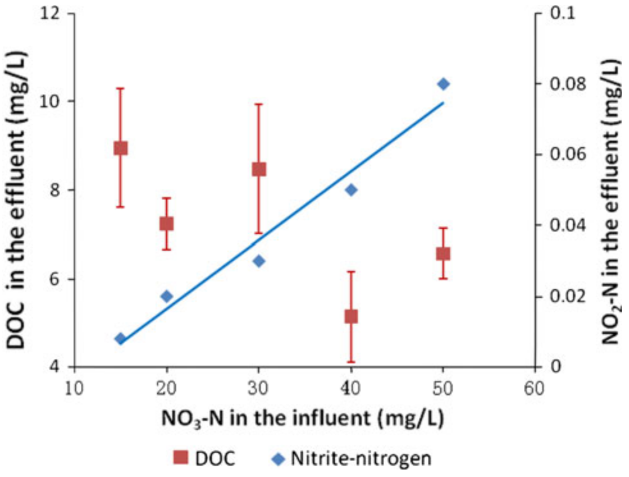

Fig. 4 Effects of the varying NO_3-N concentrations in the influent on NO_2-N and DOC concentrations in the effluent at 12°C

A linear correlation was observed ($Y = 0.00194X - 0.02259$, $R = 0.9835$) between NO_2-N concentration in the effluent and NO_3-N concentration in the influent (Fig. 4). Accumulation of nitrite, a highly toxic intermediate in the process of denitrification, has been reported to be influenced by several factors such as oxygen concentration, pH, the type of carbon source, and biofilm composition (Gómeza et al. 2000, 2002; Moreno et al. 2005). The experiments clearly indicate that nitrate concentration in the influent may affect the nitrite accumulation in the treated water using BSW as carbon source. As shown in Fig. 4, nitrate concentration in the influent had a negligible effect on DOC content in the effluent, which ranged between 5 and 10 mg/L.

Effect of HRT on denitrification at 12°C

The HRT of the biological reactor is a very important parameter for nitrate removal (Soares and Abeliovich 1998; Kesserű et al. 2002; Aslan and Turkman 2006; Behera et al. 2007). Since the majority of denitrifiers are mesophilic, their metabolic activity would slow down along with the fall of environment temperature. To extend the reaction time, therefore, is one of the necessary measures so that nitrate concentration in the effluent meets drinking water standard, when low-temperature groundwater is treated, or in cold winter (Welander and Mattiasson 2003).

To determinate the optimum retention time for NO_3-N removal, the reactor was operated at different retention times varying from 2 to 5 h, with flow rate ranged from 230 to 92 mL/h at constant NO_3-N concentration of 50 mg/L. The corresponding hydraulic loading rate ranged from 3.47 to 1.39 $m^3/(m^2$ day). The effect of changing HRT on the

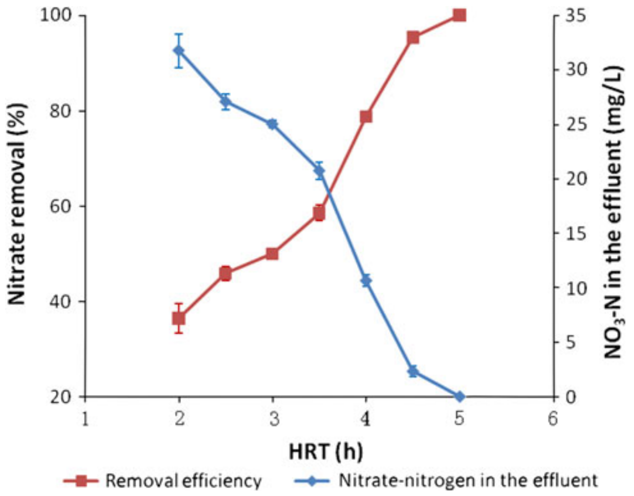

Fig. 5 Effects of HRT on nitrate removal at 12°C and 50 mg/L of NO_3-N in the influent

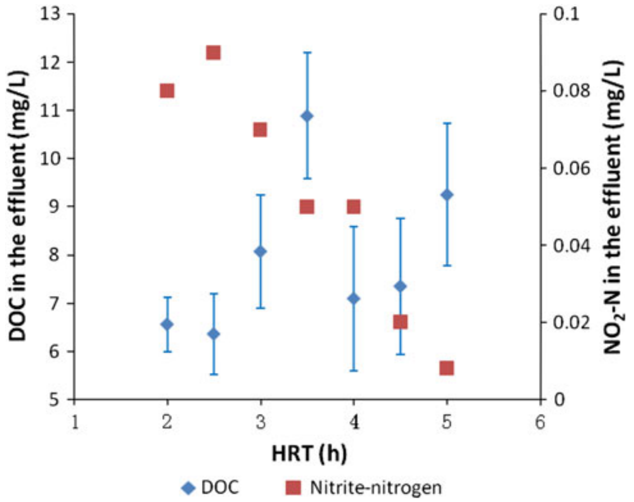

Fig. 6 Effects of HRT on NO_2-N and DOC concentrations in the effluent at 12°C and 50 mg/L of NO_3-N in the influent

removal efficiency of nitrate is shown in Fig. 5. The nitrate removal efficiency varied from about 40% at 2 h of HRT to 100% at 5 h of HRT.

The effect of HRT on nitrite accumulation is illustrated in Fig. 6. An evident increase in NO_2-N concentration in the effluent was observed as the HRT decreased. Thus, HRT plays an important role in the denitrification performance of the system support by BSW. The low HRT value resulted in a decrease in nitrate removal and an increase in nitrite accumulation because of the low contact time for microbial activity, wash-out of bacteria and extracellular enzymes (Soares and Abeliovich 1998; Aslan and Turkman 2006; Behera et al. 2007). Although Aslan (2005) suggested that effluent DOC increased by two times at 2-h residence time than that at 7-h residence time using ethanol as carbon source, significant differences in the DOC concentration in the treated water were not observed when HRT value was varied from 2 to 5 h in this study (Fig. 6), due to insoluble carbon source used.

The treated water never exhibited noticeable color and odor, and ammonia was never detected, as well as clogging of the reactor due to the entrapment of N_2 bubbles was not occurred during the whole experiment.

Although low temperature affected seriously denitrification rates supported by solid carbon sources, such as wheat straw and cotton, the optimum running parameters for the continuous-flow reactor have not been explored under the low-temperature conditions. This study indicated that the complete nitrate removal could be achieved readily by changing HRT or the initial concentration of nitrate in the influent. When the complete nitrate removal was achieved, the treated water contained the low concentrations of nitrite and DOC.

Conclusion

The nitrate removal efficiency of the reactor packed with BSW decreased to approximately 40% at 12°C from nearly 100% at 25°C under the conditions of 50 mg/L of NO_3-N in the influent and 2 h of HRT. If the initial concentration of NO_3-N was 50 mg/L, 5 h of HRT was needed for the complete nitrate removal at 12°C. The complete nitrate removal was also observed when initial NO_3-N concentration was no more than 15 mg/L at 2 h of HRT and at 12°C. Nitrite concentration in the treated water decreased markedly as HRT was increased from 2 to 5 h, or as NO_3-N concentration in the influent decreased to 15 mg/L from 50 mg/L. It was observed that varying HRT and nitrate concentration in the influent had no noticeable effect on DOC content in the effluent under the experimental conditions. As a final note, the observation that nitrate removal was complete even at 12°C could have some useful implications in environmental engineering practice.

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