Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2020.24956

178 (2020) 136–142
February

Key influencing factors of nitrate removal from pumped groundwater by using liquor as the carbon source

Y. Jiang, Y. Xia, Q. Tong, Y. Chen*

College of Environmental Science and Engineering, Guilin University of Technology, Jiangan Road 12#, Guilin, China, emails: cyd0056@vip.sina.com (Y. Chen), 2648616097@qq.com (Y. Jiang), Xiayuan82@gmail.com (Y. Xia), 416807579@qq.com (Q. Tong)

Received 1 March 2019; Accepted 25 September 2019

ABSTRACT

In vast agricultural areas, nitrate-contaminated groundwater poses a threat to the drinking water security of residents. This research aims to develop a convenient approach to remove nitrate from pumped groundwater. A batch experiment was conducted to study the influencing factors including the dissolved oxygen (DO) content, carbon–nitrogen (C/N) mass ratio, water temperature, and water–sand (W/S) volume ratio under anaerobic and aerobic conditions. Liquor was innovatively used as the carbon source to enhance denitrification. The results demonstrated that liquor is a very useful carbon source for nitrate removal without the artificial cultivation of bacteria. The aerobic conditions not only left the nitrate removal efficiency unaffected but also restrained the generation of nitrite and ammonia nitrogen. The C/N ratio required for complete nitrate removal under aerobic conditions is 1.8–2.0, and a water temperature of 15°C–25°C was suitable for promoting the removal effects. The W/S volume ratio was recommended to be lower than 4:1 to provide suitable adhesion conditions for bacteria. The C/N ratio, water temperature and W/S ratio were the key influencing factors, which could be easily adjusted. This research shows that rural residents can remove nitrate from pumped groundwater by using liquor.

Keywords: Nitrate removal; Carbon source; Pump-and-treat; Influencing factor; Groundwater

1. Introduction

Nitrate is one of the most common groundwater pollutants in farm areas [1] because of the excessive use of nitrogenous fertilizers and the improper disposal of solid waste and domestic sewage in rural areas [2–5]. For instance, the nitrate concentration in groundwater can be as high as 300 mg/L in North China [6,7]. Because groundwater is the main source of drinking water, nitrate pollution in groundwater is directly related to public health. According to previous studies, drinking groundwater with a high nitrate concentration not only possibly causes methemoglobinemia [8] but also leads to diabetes, high blood pressure, and hyperthyroidism [9,10]. To avoid the risk of nitrate pollution in drinking water, the maximum concentration limit of nitrate-N by the World Health Organization and China is 10 mg/L. Therefore, excessive nitrate in groundwater should be removed before consumption [11].

In recent decades, many previous studies have suggested that biological denitrification technology is one of the most economical and effective methods to remove nitrate from groundwater [1,12–18]. Denitrification is the process in which organic carbon acts as an electron donor, and nitrate acts as an electron acceptor at the end of the denitrifying bacterial respiratory chain and is restored to nitrite, nitric oxide, nitrous oxide, and gaseous nitrogen. The main factors affecting denitrification include the organic carbon source, nitrate concentration, oxygen concentration, pH, and temperature [13]. Organic carbon sources have been widely studied and adopted, which include solid materials

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* Corresponding author.

such as newspapers, cotton, straw, and rice bran [17–21] and liquid materials such as sucrose, ethanol, acetate, methanol and liquor [1,22–24]. The different carbon sources result in diverse responses and operation techniques, and the treatment effects vary considerably, also depending on the suitable carbon–nitrogen (C/N) ratio [1,16,17,24–26]. When carbon source, oxygen, nitrate coexist in a system, oxygen maybe inhibits denitrification onset because oxygen is a preferred electron acceptor during bioprocesses. For instance, some studies have indicated that denitrification can begin just when oxygen concentration is less than 0.5 mg/L in aquifers [27,28]. Nevertheless, Gómez et al. [22] and Chen et al. [24] indicate that biological denitrification with ethanol acted as a carbon source is less affected by dissolved oxygen (DO) in the laboratory experiments. Temperature and pH also have range values preferred by denitrifiers. For example, Rust et al. [29] suggest that the pH range preferred by heterotrophic denitrifiers is generally between 5.5 and 8.0. Weil and Brady [30] suggest that the preferred temperature for denitrification processes is between 25°C and 35°C. In a word, the existing studies have provided lots of scientific basis for the removal of nitrate by denitrification. However, nitrate pollution in groundwater usually results from nonpoint source contamination. The current in-situ studies often focus on permeable reactive barrier [17], denitrification wall [16] or well inject-treat [1,26] technologies, but with a limited treatment scope. In-situ groundwater remediation technology by using denitrification is slowly being developed at present. Therefore, the technology's real water security potential remains unclear. Families in rural areas generally pump groundwater for use as drinking water; thus, it is very significant to develop a user-friendly practical pump-and-treat technology, which can be accepted by the public, including the option for adding carbon sources and key influencing factors.

To develop an effective approach to remove nitrate from groundwater for rural families, Chen et al. [24] propose a user-friendly pump-and-treat technology through a water-bucket experiment, in which liquor was particularly recommended as a highly effective and low-cost carbon source. As estimated, a bottle of 500 mL liquor that costs approximately 10 Yuan renminbi, that is, less than 2 US dollars, can be used for 2 months by a family. Liquor is a household drink in many areas, especially in China. Therefore, the method is very likely to be accepted and used by the public, especially families in rural areas. To perfect the pump-and-treat technology, a batch experimental study was carried out under both anaerobic and aerobic conditions. The aim of this paper is to reveal several key factors that can influence the performance of the pump-and-treat technology, including the DO content, C/N ratio, water temperature, and water–sand (W/S) volume ratio. Because liquor is easily accessible by residents, this research can make the pump-and-treat technology for nitrate removal more practicable.

2. Materials and methodology

2.1. Experimental design

A batch experiment consisting of 42 microcosms was designed. The materials in each microcosm included groundwater, a carbon source, and sand particles, which were stored in a 1,000 ml screw-neck transparent glass bottle. Groundwater was pumped from a local aquifer and stored for microcosm formation within 2 d. On average, the chemical background of the groundwater is mainly characterized by a nitrate content of 3.2 mg/L, acetate content of 0.21 mg/L, electric conductivity of 206 us/cm, DO level of 7.6 mg/L, pH of 8.1, total organic carbon (TOC) content of 2.1 mg/L and water temperature of 24.5°C; in addition, no nitrite is detected. Liquor (20%, v/v ethanol) was used as the carbon source, in which ethanol acted as the electron donor at a concentration of 1.56×10^5 mg/L. Clear quartz sand collected from rivers was used as porous media with diameters of 2–10 mm but without special treatment. To simulate the shallow groundwater polluted by nitrate, $NaNO_3$ (analytical reagent) was added to prepare a target nitrate concentration of 100 mg/L. As denitrifying bacteria are ubiquitous in the environment [13,31], the groundwater and sand particles act as microbial inoculation media. This experiment disregarded the artificial cultivation of bacteria.

All microcosms were divided into three types (as shown in Table 1). Control type M0 was aimed at surveying nitrate removal under sterilized conditions and was compared with the other microcosm types under unsterilized conditions. The other microcosm types, including M1 and M2, were aimed at surveying nitrate removal under anaerobic and aerobic conditions, respectively. Among these microcosms, type M1 consisted of three groups (M1-1 to M1-3) with different C/N ratios. Type M2 consisted of ten groups (M2-1 to M2-10) to distinguish the effectiveness of nitrate removal, including different C/N ratios (M2-1, M2-2, and M2-3), different water temperatures (M2-1, M2-4, and M2-5), and different W/S ratios (M2-6 to M2-10). Overall, the batch experiment included 14 groups, and each group was performed in triplicate.

The sterile control group (M0-1) was sterilized with a high-temperature and high-pressure treatment (in a sterilization pot for 3 d; 60 min each day), and then 5 mL of mercuric chloride solution with a concentration of 500 mg/L was added to enhance the sterilization treatment. Nitrogen gas was blown into the anaerobic (M1) microcosms to decrease the initial DO concentration below 1.0 mg/L and then sealed and placed in a strictly anaerobic glove box. The aerobic (M2) microcosms with an initial DO concentration higher than 7.2 mg/L remained open and were placed in an aerobic environment. Due to the aerobic conditions being more representative of the actual operating conditions of rural families, the aerobic microcosms were of greater interest in the experiment.

2.2. Sample analysis

Water samples were collected during the experimental duration of 480 h. The samples were used as follows. A water sample of 10 mL was poured into a brown borosilicate glass bottle of 30 mL, which was used to detect the DO level, water temperature, and pH value by using a German WTW handheld multiparameter test instrument (multi 3420). A water sample of 1 mL was used to measure the nitrate and nitrite concentrations using ion chromatography (Dionex ICS 2100). The minimum detection limit of nitrate and nitrite was 0.1 mg/L, and the minimum detection

Table 1
Experimental settings of the different microcosm types

Microcosm type	Group no.	Solids (g)	Water (ml)	Initial nitrate level (mg/L)	Initial C/N ratio	Water temperature (°C)	W/S ratio
Control type, M0	M0-1	400	800	125.9–134.3	1.6–1.7	26.1–29.1	5:1
Anaerobic type, M1	M1-1	400	800	109.3–109.4	1.9–2.0	27.7–29.5	5:1
	M1-2	400	800	109.4–109.9	1.4–1.5	27.0–29.2	5:1
	M1-3	400	800	99.9–102.1	1.1–1.2	26.4–28.4	5:1
Aerobic type, M2	M2-1	400	800	110.4–110.6	1.8–2.0	27.3–30.4	5:1
	M2-2	400	800	111.0–111.9	1.5–1.7	27.0–30.2	5:1
	M2-3	400	800	103.8–104.8	1.1–1.3	26.8–28.8	5:1
	M2-4	400	800	112.0–112.4	1.8–1.9	12.8–22.7	5:1
	M2-5	400	800	113.9–114.3	2.0–2.1	5.8–10.7	5:1
	M2-6	100	800	103.4–103.7	2.6–3.0	28.5–30.9	20:1
	M2-7	200	800	92.3–95.8	2.8–2.9	29.0–31.2	10:1
	M2-8	400	800	96.6–98.1	2.5–2.9	29.0–31.0	5:1
	M2-9	500	800	105.0–105.5	2.6–2.9	28.5–31.1	4:1
	M2-10	0	800	99.1–100.7	2.5–2.7	28.1–30.8	–

Notes: C/N ratio is the mass ratio of the carbon in the liquor to the nitrogen in the groundwater. The W/S ratio is the volume ratio of the water solution and sand particles in the microcosm. The water temperatures are actual values.

limit of acetate was 0.5 mg/L. A water sample of 5 ml was filled into a headspace bottle of 10 ml with a Teflon gasket lining to obtain the ethanol concentration by using a gas chromatograph (American Agilent 6890N) equipped with a 30 m × 0.53 mm × 1 μm DB-WAXetr capillary column and a flame ionization detector. The minimum detection limit of ethanol was 0.1 mg/L.

3. Results and discussion

The analysis results were used to generate plots of the average values and standard errors. Overall, the results reflect the nitrate concentration changes under the various conditions and the influences of the DO level, C/N ratio, water temperature, and W/S ratio on nitrate removal. The results are described and discussed as follows.

3.1. Changes in the nitrate concentration and nitrate removal

3.1.1. Control type

The control-type microcosms (M0-1) indicated the concentration changes of nitrate and ethanol as well as DO under sterilized conditions. As shown in Fig. 1, it is clear that these concentrations experienced no significant change during the experiment. The nitrate and DO concentrations ranged from 133.2 to 129.2 mg/L and from 6.7 to 5.5 mg/L, respectively, on average. The ethanol concentration ranged from 91.1 to 87.3 mg/L. Usually, ethanol acted as the available carbon source can be oxidized by oxygen and/or nitrate in the catalysis of microorganisms following biochemical reactions (1) and (2) [23,24]. Nevertheless, ethanol was not significantly consumed by either aerobic metabolism or denitrification in this experiment because of the absence of microorganisms. Therefore, the performances of M0-1 can be compared with those of the other microcosm types under unsterilized conditions.

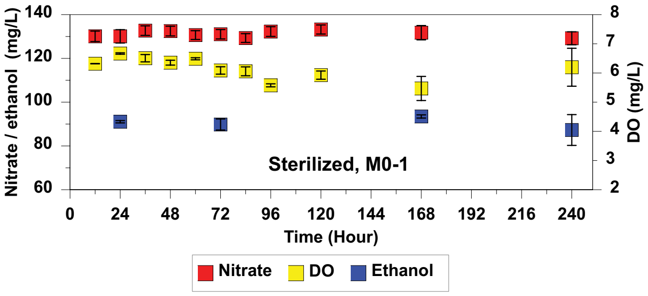

Fig. 1. Concentration changes of nitrate, ethanol and DO in control-type microcosm M0-1.

$$5CH_3CH_2OH + 12NO_3^- \rightarrow 10HCO_3^- + 6N_2 + 2OH^- + 9H_2O \quad (1)$$

$$CH_3CH_2OH + 3O_2 \rightarrow 2CO_2 + 3H_2O \quad (2)$$

3.1.2. Anaerobic type

The anaerobic-type microcosms, including the M1-1, M1-2, and M1-3 groups, were subjected to anaerobic conditions, in which the DO concentrations were lower than 1.0 mg/L. As designed, these microcosms had different C/N ratios. According to the concentration changes of nitrate and ethanol (Fig. 2), nitrate attenuation was notable. Although M1-1, M1-2, and M1-3 had different C/N ratios of 1.9–2.0, 1.4–1.5 and 1.1–1.2, respectively, these ratios were higher than the theoretical C/N ratio (0.71) derived from biochemical reaction (1), indicating the sufficiency of carbon source. As all other prerequisites were similar, including nitrate concentration, water temperature and W/S ratio (Table 1), their nitrate concentrations decreased almost at the same rate under the conditions of sufficient carbon source. Before

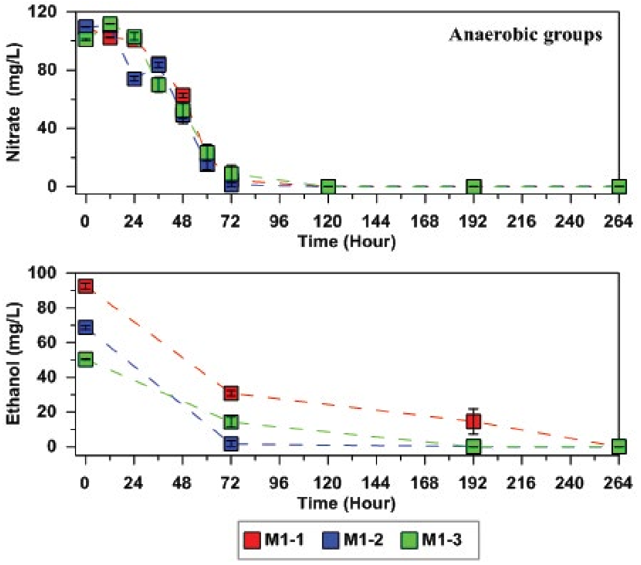

Fig. 2. Changes in the nitrate and ethanol concentrations in the anaerobic groups (M1-1, M1-2, and M1-3).

the 120th hour, the nitrate removal rates of M1-1, M1-2, and M1-3 were 0.9, 0.8 and 0.8 mg/L/h, respectively. Nitrate was completely removed at the 120th hour in each group, accompanied by a distinct decrease in ethanol concentration.

3.1.3. Aerobic type

The aerobic-type microcosms included the ten groups of M2-1 to M2-10, which were subjected to aerobic conditions. In contrast to the anaerobic-type microcosms, the aerobic-type microcosms had a high initial DO concentration, and oxygen was allowed to enter the microcosms. The DO level played an important role in using the added ethanol as an electron donor for microbial aerobic metabolism, which likely affected nitrate removal. In addition, the changes in the C/N ratio, water temperature and W/S ratio were considered key influencing factors.

Under aerobic conditions, in comparison, the nitrate concentrations in M2-1 to M2-10 were completely centralized, as shown in Fig. 3. When the above factors changed, the nitrate concentration showed different features. For example, the nitrate in M2-1 was completely removed at the 264th hour but nitrate removal was poor in M2-2 and M2-3. M2-5 had more nitrate residue than M2-4, and M2-10 had more nitrate residue than M2-6 to M2-9. The underlying reason for these differences is further discussed below.

3.2. Key factors influencing on nitrate removal

3.2.1. Dissolved oxygen

In this experiment, the anaerobic and aerobic microcosm types were designed to reveal the influences of the DO content. The concentrations of DO were <1 mg/L and >7.2 mg/L under anaerobic and aerobic conditions, respectively. Compared with the anaerobic groups (M1-1 to M1-3), nitrate removal in the aerobic groups (M2-1–M2-3) is slower. Nitrate could not be completely removed before the 120th hour. Therefore, it can be confirmed that the presence of higher concentrations of DO can affect the action of denitrification.

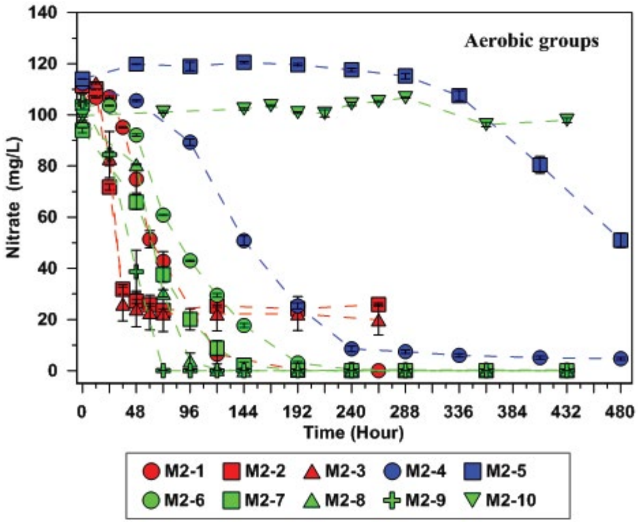

Fig. 3. Changes in the nitrate concentration in the aerobic groups (M2-1 to M2-10).

A high DO content may decrease the nitrate removal rate. Before the 120th hour, the nitrate removal rates of M2-1, M2-2, and M2-3 were 0.9, 0.7 and 0.7 mg/L/h, respectively, which were slightly lower than those under anaerobic conditions. Fig. 4 also shows that the DO concentration in M2-1 preferentially decreased, which was accompanied by decreases in nitrate concentration. Moreover, ethanol was consumed. DO, which is a stronger electron acceptor than nitrate, has attracted considerable attention in the study of the denitrification mechanism and can compete with nitrate to capture available electron donors (organic carbon source) in the environment, thereby inhibiting denitrification [13,22]. Therefore, it is very possible that DO inhibited nitrate removal through the gradual depletion of ethanol. When ethanol was completely consumed, the DO concentration increased again under aerobic conditions.

Previous studies have suggested that there is a DO concentration threshold for the onset of denitrification, which is considered to be approximately 0.5 mg/L in aquifers [27,28]. However, this phenomenon was not observed in the experiment. When the nitrate in M2-1 to M2-3 began to be removed, the DO concentrations were usually higher than 3.0 mg/L. Nitrate began to be removed quickly in M2-1 to M2-3 after the 24th hour (Fig. 4). The onset of denitrification was not significantly delayed under aerobic conditions. The reasons causing the different DO concentrations for denitrification onset from the previous studies maybe because of the facultative nature of denitrifying bacteria [32–34] and the artificial supply of liquor in the experiment. Facultative denitrifying bacteria can metabolize nitrate under aerobic and anaerobic conditions. When more sufficient ethanol is provided, DO even with high concentration isn’t easy to hinder the onset of denitrification [1], but decrease the rate of nitrate removal [22,24], such as in M2-2 and M2-3 of the experiment. Because available carbon source usually is lack in aquifers [1,13,26],

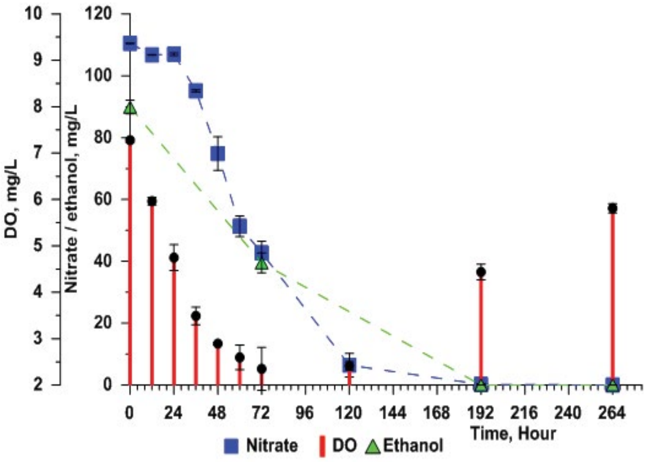

Fig. 4. Influences of the DO content on nitrate removal in M2-1 under aerobic conditions.

aerobic bacteria can preferentially use limited carbon source and make denitrification difficult to begin. As an example, Carter et al. [35] have isolated some species of denitrifying bacteria from soils that we're able to use oxygen and nitrate as electron acceptors simultaneously under the condition of up to 80% air saturation.

In addition, aerobic conditions can inhibit the accumulation of denitrification intermediate products because of nitrification process as below.

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 4H^+ + 2H_2O \tag{3}$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \tag{4}$$

The experimental results showed that the different DO concentrations exert different effects on the accumulation of denitrification intermediate products, such as nitrite and ammonia nitrogen. The maximum nitrite and ammonia nitrogen concentrations in aerobic microcosm M2-1 were 2.2 and 0.41 mg/L, respectively, whereas the maximum nitrite and ammonia nitrogen concentrations in anaerobic microcosm M1-1 were 13.3 and 1.7 mg/L, respectively. These results might indicate that the pump-and-treat method under aerobic conditions increases the DO content in water and is also conducive to inhibiting the accumulation of denitrification intermediate products.

3.2.2. C/N ratio

The organic carbon source is the electron donor for nitrate removal through denitrification. The C/N ratio usually characterizes the mass ratio of the carbon source to the nitrogen source. In the process of denitrification, the nitrate removal effect can be affected by the adequacy of the carbon content. Pure denitrification exhibits a higher demand for carbon sources under aerobic conditions than under anaerobic conditions because of the presence of DO. This research compared the nitrate removal effects of the different C/N ratios under aerobic and anaerobic conditions.

Under anaerobic conditions, the C/N ratios of M1-1, M1-2, and M1-3 were 1.9–2.0, 1.4–1.5, and 1.1–1.2, respectively, which greater than the theoretical C/N ratio (0.71) derived from biochemical reaction (1). No nitrate concentration was detected at the 120th hour, and the removal rate was approximately 100%. The results indicate that sufficient carbon source (liquor) guaranteed the complete nitrate removal without the presence of excessive DO concentration.

Under aerobic conditions, the microcosms of M2-1, M2-2, M2-3, and M2-8 reflected the nitrate removal effects at the different C/N ratios. No nitrate was detected in M2-1 and M2-8 at the 200th hour, and the corresponding designed C/N ratios were 1.8–2.0 and 2.5–2.9, respectively. However, the nitrate removal rate was lower than 85% in M2-2 and M2-3, and the corresponding designed C/N ratios were 1.5–1.7 and 1.1–1.3, respectively. The low C/N ratio was associated with the poor removal effect. Compared with the cases under anaerobic conditions, complete nitrate removal required a high C/N ratio under aerobic conditions because of the reaction of DO and ethanol, as described in reaction (2).

This experiment shows that complete nitrate removal requires a C/N ratio above 1.8–2.0 when liquor is considered the carbon source under aerobic conditions. If the C/N ratio is lower, then this condition can cause the accumulation of acetate, an intermediate product. Therefore, selecting the appropriate C/N ratio is necessary.

3.2.3. Water temperature

The groundwater temperature differs according to the region. Groundwater can be affected by the air temperature after being extracted from the ground. Given that the temperature is a factor impacting microbial activity and growth, the temperature also affects the rate and efficiency of biochemical reactions. Based on local climate changes, this research considered three water temperature ranges: high, normal, and low temperatures. Under aerobic conditions with a sufficient carbon source and a W/S ratio of 5/1, the actual change ranges of the water temperature for microcosms M2-1, M2-4, and M2-5 were 27°C–30°C, 12°C–23°C, and 5°C–11°C, respectively, thereby potentially representing high, normal, and low temperatures, respectively.

The experimental results (Fig. 3) indicate that a low water temperature is associated with a low nitrate removal rate and low denitrification efficiency. At high temperatures, the nitrate removal rate of M2-1 reached 99.8% at the 192nd hour, and the remaining concentration was 0.2 mg/L. The nitrate removal rate of M2-4 reached 92% at normal temperature by the 240th hour. The nitrate removal rate of M2-5 was only 51% at a low temperature when the experiment had ended (at the 480th hour).

The nitrate removal efficiency of denitrification is closely related to the water temperature, even if the carbon source is adequate. A proper increase in water temperature is beneficial to promoting the growth and activity of microorganisms as well as increasing the reaction efficiency. Based on this research, a water temperature of 15°C–25°C is suitable for nitrate removal, and such a temperature is easily achieved through room temperature regulation.

3.2.4. W/S ratio

In a natural system, microorganisms are attached to solid particles for growth, and the solid particle content affects the number and activity of microorganisms. Previous studies indicate that 99% of the microorganisms are attached to the surface of particles in porous media [36–38]. In the present research, the W/S ratio indicates the particle content. The results show that the denitrification capacity differs significantly at the different W/S ratios.

In this study, five conditions were designed, namely, W/S ratios of 20:1, 10:1, 5:1, and 4:1 and no sand, corresponding to microcosms M2-6, M2-7, M2-8, M2-9, and M2-10, respectively. The five conditions provided adequate carbon sources and similar aerobic conditions and water temperatures; the results are illustrated in green lines in Fig. 3. The nitrate removal rate gradually increased with increasing solid particle content. No notable nitrate removal is observed in microcosm M2-10 under the no sand condition. The absence of solid particles is not advantageous to the growth and activity of bacteria. The nitrate removal percentages of M2-6, M2-7, M2-8, and M2-9 reached 100% after 432, 240, 144, and 96 h, respectively, thereby indicating that filling the device with the appropriate quantity of solid particles is necessary to achieve an enhanced nitrate removal effect. The determination of the W/S ratio requires combining the water demand and handling capacity of the device. According to this study, a W/S ratio lower than 4:1 should be adopted.

3.3. Outlook of the research

This research aims to reveal the key factors for developing nitrate removal technology from pumped groundwater in rural areas, as shown in the schematic diagram of Fig. 5. Through this microcosm study, the results indicate that the C/N ratio, water temperature, and W/S ratio are key factors influencing nitrate removal but not DO removal. When liquor used as the carbon source and with the presences of the appropriate water temperature and W/S ratio, the effect of DO can be ignored. The pump-and-treat method to remove nitrate from pumped groundwater by using liquor as the carbon source has a significant practical value.

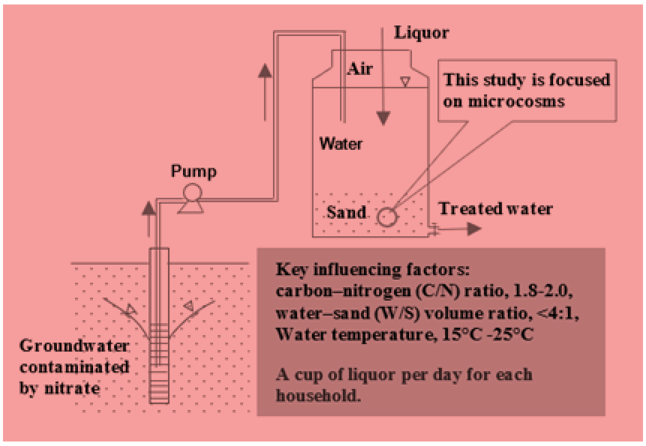

Fig. 5. Schematic diagram of the pump-and-treat method for nitrate removal from pumped groundwater.

4. Conclusions

Liquor can be used as a convenient carbon source to stimulate denitrification for effective nitrate removal from pumped groundwater. When enough liquor is added, denitrification can begin within approximately 24 h under aerobic conditions and without significant delay. To a certain extent, a high DO concentration is conducive to inhibiting the accumulation of intermediate products during denitrification.

The key factors that influence nitrate removal from pumped groundwater include the C/N ratio, water temperature and W/S ratio. When liquor is used as a carbon source, a C/N ratio of 1.8–2.0, a water temperature of 15°C–25°C and a W/S ratio lower than 4:1 are more suitable, and the complete removal of nitrate under aerobic conditions can be attained.

Acknowledgments

This research is funded by the National Natural Science Foundation of China and the National Science and Technology Support Project of China under Projects 41362012 and 2012BAC16B02, respectively.

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