Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1127780

57 (2016) 21990–21999
October

Biological denitrification using rice washing drainage (RWD) as carbon source for removing nitrate from groundwater

Qiaochong He[a,b], Chuanping Feng[a,b,*], Qili Hu[a], Rui Li[c], Nan Chen[a,b]

[a]*School of Water Resources and Environment, China University of Geosciences (Beijing), Beijing 100083, China, Tel. +86 13121918944; email: cong2005130079@163.com (Q. He), Tel. +86 10 82322281; Fax: +86 10 82321081; email: fengcp@cugb.edu.cn (C. Feng); Tel. +86 13121918964, +86 18813181663; emails: 13121918964@163.com, hustyle1234@163.com (Q. Hu), Tel. +86 13621327736; email: chennan@cugb.edu.cn (N. Chen)*
[b]*Key Laboratory of Groundwater Cycle and Environment Evolution (China University of Geosciences (Beijing)), Ministry of Education, Beijing 100083, China*
[c]*State Environmental Protection Key Laboratory of Simulation and Control of Groundwater Pollution, Chinese Research Academy of Environmental Sciences, Beijing 100012, China, Tel. +86 15652359313; email: 518lirui@163.com*

Received 23 July 2015; Accepted 28 November 2015

ABSTRACT

To investigate the feasibility of rice washing drainage (RWD) as carbon source for biological denitrification, the denitrification performance using RWD, maize stalks, poplar leaves, and sawdust as carbon sources was evaluated by batch experiments. Results showed that nitrate in synthetic groundwater could be removed effectively using RWD, maize stalks, and sawdust as carbon sources, and the nitrate removal efficiencies were 96, 98, and 96%, respectively, while using poplar leaves was 73%. Furthermore, RWD-based denitrification resulted in a favorable nitrate removal rate constant (2.649 d^{-1}), higher than others (2.412 d^{-1} for maize stalk, 0.427 d^{-1} for poplar leaf, 0.363 d^{-1} for sawdust). The optimum ratio of RWD to synthetic groundwater was obtained to be 50/350 (v/v), at which the nitrate removal efficiency reached 100% with no nitrite accumulation and the COD removal efficiency reached 90%, indicated that the denitrification with RWD could not only efficiently remediate the nitrate contaminated groundwater but also effectively treat the RWD.

Keywords: Denitrification; Rice washing drainage; Carbon source; Nitrate removal; Groundwater

1. Introduction

Groundwater is the main drinking water source in many countries and regions, especially in rural communities [1]. Nitrogen-based fertilizer used in agriculture, wastewater uncontrolled discharge [2], and landfill leachate [3] are primarily responsible for the increase in nitrogenous compounds in groundwater. In China, the pollution of groundwater with nitrate is severe, with nitrate concentration in groundwater exceeding 130 mg of NO_3^--N/L in some rural areas [4].

Elevated nitrate concentration in drinking water could have adverse effects on human health [2], such as leading to methemoglobinemia [5] and gastric cancer [6]. Concerning about this, the World Health Organization (WHO) has set a maximum contaminant

---

*Corresponding author.

level of 50 mg of NO_3^-/L (11.29 mg of NO_3^--N/L) [7], while 10 mg of NO_3^--N/L has been proposed by China [8].

In consideration of the hazards to human health, various technologies for nitrate removal have been developed, such as ion exchange [9], reverse osmosis [10], and catalytic reduction [11]. However, ion exchange requires disposal of waste brine and renewal of costly ion-exchange resin. Reverse osmosis needs sophisticated manipulation and the remainder treatment. Catalytic reduction would produce some hazardous by-products such as nitrite and ammonium [11]. Heterotrophic denitrification using organic carbon source as electron donor has been widely adopted due to low consumption and high efficiency [12,13]. However, the deficiency of organic matters in groundwater is an inevitable problem that heterotrophic denitrification process has to confront with [4,14]. Various liquid carbon sources, such as sucrose, ethanol [12], and methanol [13] have been used in nitrate contaminated groundwater remediation. Nevertheless, these liquid carbon sources are apt to overdose, resulting in secondary pollution and high cost. To avoid these problems, various solid carbon sources, such as wheat straw, sawdust, biodegradable plastic (BP) [14], and cassava distiller's dried grains [15] have been utilized as carbon sources to remove nitrate from groundwater. Although these solid carbon sources could make denitrification process stable operation, residual materials might bring about secondary pollution and reactor clogging.

On the other hand, rice, feeding more than 60% of the population and contributing nearly 40% of total calorie intake, is a staple food in China, and China is the largest rice producer and consumer in the world [16]. An enormous amount of wastewater is generated from the processes for cooking rice, which is called "rice washing drainage (RWD)" in this study. RWD contains a relatively high amount of solid particles including starch and some proteins, so the biochemical demand value (BOD) in this drainage are high and require expensive wastewater treatment [17]. However, domestic sewage of approximately 96% villages is directly discharged into the receiving waters without any treatments [18]. Therefore, untreated RWD is directly discharged into water body, which would exert serious risks to environment. From the viewpoint of environmental protection and resource conservation, it is necessary to effectively utilize RWD as a resource. This study will support a data basis for developing an individual reactor supplied to each house in rural villages.

In this study, to investigate the feasibility of RWD using as carbon source, the leaching behavior and denitrification performance of RWD were compared with those of maize stalks, poplar leaves, and sawdust by batch experiments. Furthermore, the denitrification experiments were performed at different ratios of RWD to synthetic groundwater to find out an optimal RWD dosage.

2. Materials and methods

2.1. Carbon sources

In this study, RWD, maize stalks, poplar leaves, and sawdust were used as carbon sources. Rice was produced in northeast China. Seven hundred grams of rice was mixed with 700 mL of deionized water, and then washed by hand for 10 s, and the washed water was separated into a container. Afterward, 700 mL of deionized water was added into the washed rice and the procedure was repeated as mentioned above. Rice was washed in triplicate and all of the washed water was mixed. Finally, 1,920 mL of washed water was obtained and employed to simulate RWD generated from our daily life, and then kept in a refrigerator at 4℃. RWD was turbid and gelatinous, and also contained some solid particles that would settle under static condition. Two hundred milliliters of RWD was filtrated by 0.45-μm cellulose acetate membrane filters, filtrate was taken for COD detection, and solid particles were dried at 28℃ for 24 h, and then ground using a mortar for elemental analysis. Maize stalks were obtained from the suburbs of Daxing district (Beijing, China), sawdust from Kaibiyuan Company (Beijing, China), and poplar leaves from the schoolyard of China University of Geosciences (Beijing, China). Maize stalks, poplar leaves, and sawdust were ground to powder using a pulverizer (ZN-04, KINGSLH, China), then dried at 28℃ for 24 h.

2.2. Synthetic nitrate contaminated groundwater

Synthetic groundwater (per liter of deionized water) contained 0.304 g of $NaNO_3$, 0.022 g of KH_2PO_4, i.e. the concentration of NO_3^--N in the synthetic groundwater was 50 mg/L and the ratio of N/P was 10.

2.3. Activated sludge acclimation

Activated sludge for inoculation of denitrifying bacteria was collected from the Qinghe Wastewater Treatment Plant (Beijing, China) and acclimated in culture solution at 26℃ for one month. The culture solution was prepared by adding 0.1562 g of glucose, 0.304 g of $NaNO_3$, and 0.022 g of KH_2PO_4 in 1 L tap water. The culture solution was replaced every two days. After acclimation, the mixed liquid suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and sludge volume index (SVI) were 2,715, 1,792, and 99 mL/g, respectively.

2.4. Leaching experiments

Leaching experiments were conducted to investigate COD leaching behavior of each selected carbon source. The three equivalent carbon sources (1 g of maize stalks, 1 g of poplar leaves, and 1 g of sawdust) were added into three 250-mL conical flasks containing 250 mL of deionized water, respectively. Meanwhile, 250 mL of RWD was directly added into a conical flask. All conical flasks were sealed and shaken in a thermostatic shaker (DDHZ-300, Peiying, China) (26 ± 0.5˚C) at 150 rpm for 72 h. Three milliliters of supernatant was periodically taken from each flask to determine COD concentration.

2.5. Denitrification performance using different carbon sources

Denitrification experiments using different carbon sources were carried out to investigate the denitrification performance of each carbon source. 2.428 g of maize stalks, 1.746 g of poplar leaves, and 11.653 g of sawdust were added into 500-mL conical flasks containing 400 mL of synthetic groundwater, respectively. In addition, 400 mL of RWD was added into a 500-mL conical flask with 0.121 g of $NaNO_3$ and 0.009 g of KH_2PO_4 (50 mg of NO_3^--N/L and N/P ratio of 10). Five milliliters of domesticated sludge was added into each conical flask, and then purged nitrogen gas (N_2) for 5 min. These conical flasks were sealed using rubber stopper, and then placed in a thermostatic shaker (26 ± 0.5˚C) and shaken at 150 rpm.

2.6. Denitrification performance with different RWD dosages

To avoid the secondary pollution caused by excessive RWD, this batch experiment was carried out to find out an optimal dosage. The total liquid volume was kept at 400 mL in 500-mL conical flask, the volume ratios of RWD to synthetic groundwater of 10/390, 20/380, 50/350, 100/300, and 200/200 (v/v) were prepared by changing the dosages of RWD and synthetic groundwater. In addition, to investigate the effect of solid particles contained in RWD on denitrification performance, 50 mL of RWD filtrated by cellulose acetate membrane filters (0.45 μm) was added into a 500-mL conical flask containing 350 mL of synthetic groundwater, represented by F50/350. Similar to the procedure mentioned in Section 2.5, 5 mL of domesticated sludge was added into each conical flask and purged nitrogen gas for 5 min, and then all conical flasks were sealed and shaken in a thermostatic shaker (26 ± 0.5˚C) at 150 rpm.

2.7. Analytical methods

Six milliliters of supernatant was taken periodically from each flask for analysis of NO_3^--N, NO_2^--N, NH_4^+-N, COD, and pH. Prior to sampling, conical flasks were placed on standing for 30 min to settle the sludge. The samples were filtered with 0.45-μm cellulose acetate membrane filters before analysis.

According to the Water and Wastewater Monitoring Analysis Method [19], NO_3^--N, NO_2^--N, NH_4^+-N, and TN were measured with an ultraviolet spectrophotometer (DR6000, HACH, USA) and the detection limits were 0.08 mg/L for NO_3^--N, 0.003 mg/L for NO_2^--N, 0.025 mg/L for NH_4^+-N, and 0.05 mg/L for TN. COD was digested by potassium dichromate using a Speed Digester (5B-1F, Lianhua Tech Co., China) at 165˚C for 10 min and measured with an ultraviolet spectrophotometer (DR6000, HACH, USA). The pH was determined by pH meter (Seven Multi S40, Mettler Toledo, Switzerland). *C* and *N* contained in RWD (solid particles), maize stalks, poplar leaves, and sawdust were analyzed by an elemental analyzer (EA3000, EURO, Italy).

3. Results and discussion

3.1. Leaching experiments

As shown in Table 1, *C* contents were all above 40%, and no significant difference in *C* contents was observed among solid particles in RWD, maize stalks, and poplar leaves, while sawdust had a higher *C* content (46.863 ± 0.004%). On the other hand, solid particles contained in RWD had a higher N content (3.345 ± 0.032%).

The variation trend of COD concentration with time was of prime significance for investigating the leaching behavior of each carbon source. As shown in Fig. 1, the COD concentration in RWD reactor increased in 7 h, whereas in maize stalks, poplar leaves, and sawdust reactors increased in 22 h. In addition, the COD release rates of RWD, maize stalks, and poplar leaves were higher than that of sawdust. Furthermore, the initial COD concentration (1,131 mg/L) in RWD reactor was the highest, and a sharp increase was observed during 5–7 h, COD concentration reached approximately

Table 1
C and N contents in solid particles in RWD, maize stalks, poplar leaves, and sawdust

Carbon source	*N* content (%, dry weight)	*C* content (%, dry weight)
Solid particles in RWD	3.345 ± 0.032	42.092 ± 0.203
Maize stalks	1.027 ± 0.009	41.180 ± 0.058
Poplar leaves	1.560 ± 0.050	41.235 ± 0.071
Sawdust	0.790 ± 0.016	46.863 ± 0.004

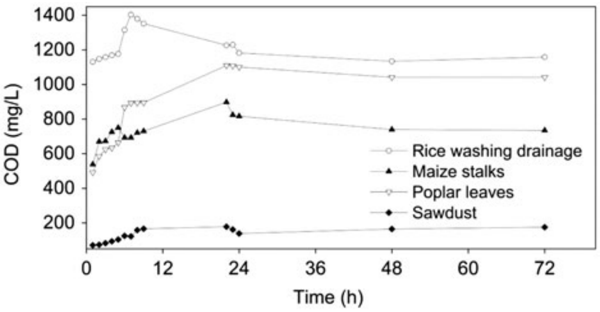

Fig. 1. COD change in RWD, maize stalks, poplar leaves, and sawdust.

1,400 mg/L at 7 h, the COD increase might be attributed to organic matters released from solid particles contained in RWD. However, the COD concentration in RWD reactor began decreasing from 7 h and reached approximately 1,200 mg/L at 24 h, and then remained almost constant. The COD decrease was probably resulted from the microbial growth because RWD was easy for breeding bacteria [17]. In maize stalks and poplar leaves reactors, the COD concentrations increased significantly during the first 5 h, and then gradually increased to 898.12 and 1,109.91 mg/L at 22 h, respectively. In sawdust reactor, the COD concentration increased slowly and reached 178.1 mg/L at 22 h. In contrast to RWD, COD concentrations decreased slightly in maize stalks, poplar leaves, and sawdust reactors, and then remained almost constant from 22 h. Zhang et al. [14] also found that the COD release rates of solid carbon sources were higher at the beginning of leaching experiments.

In the leaching experiments, from 24 to 72 h, average COD concentration was 1,158 mg/L in 250 mL of RWD, 763 mg/L in 1 g of maize stalks in 250 mL of deionized water, 1,061 mg/L in 1 g of poplar leaves, and 159 mg/L in 1 g of sawdust. Therefore, 2.428 g of maize stalks, 1.746 g of poplar leaves, and 11.653 g of sawdust should be added in 500-mL conical flask containing 400 mL of deionized water to make the total COD value be approximately equal to that in 400 mL of RWD in the next experiments for denitrification performance using different carbon sources.

3.2. Denitrification performance

3.2.1. Nitrate removal

As shown in Fig. 2(A), the nitrate concentrations decreased with time in all carbon sources reactors, while nitrate concentration in the blank reactor (no addition of carbon source) remained almost unchanged. Nitrate concentrations using maize stalks and sawdust as carbon sources decreased significantly during the first 1 d, and the nitrate removal efficiency reached almost 98% on Day 3 using maize stalks, and 96% on Day 6 using sawdust, indicating that maize stalks had a higher nitrate removal rate than sawdust. This result was in accordance with that reported by Greenan et al. [20]. For poplar leaves, the nitrate removal efficiency was the lowest and reached 73% at the experiment end. This was due to the fact that poplar leaves contained high amounts of lignin, which had an adverse effect on its degradability and microbial activity [21], lack of available carbon source for denitrification would lead to inadequate nitrate removal [22]. However, for RWD, the nitrate removal efficiency reached almost 40% on Day 0.25, and then increased to 96% on Day 3.

If fitted the experimental data with first-order kinetics, the rate constant was 2.649 d^{-1} ($R^2 = 0.925$) for RWD, which was higher than other carbon sources (2.412 d^{-1} for maize stalks, $R^2 = 0.916$; 0.427 d^{-1} for poplar leaves, $R^2 = 0.887$; 0.363 d^{-1} for sawdust, $R^2 = 0.935$). The higher nitrate removal rate in RWD reactor might be attributed to that: (1) the RWD could produce biodegradable substances that could be more easily used by bacteria, such as organic acids and alcohols [23]; (2) it was speculated that RWD might contain vitamin C because rice contained vitamin C [24]. Furthermore, addition of vitamin C would accelerate the micro-organism growth, and then speed up the transformation of nitrate to nitrite [25]. Nevertheless, further study should be carried out to analyze the effect of vitamins on denitrification; (3) the RWD contains various trace metals, such as Fe of 992.50 μg/L and Mn of 89.30 μg/L, which would cause an increase in metabolic activity [26].

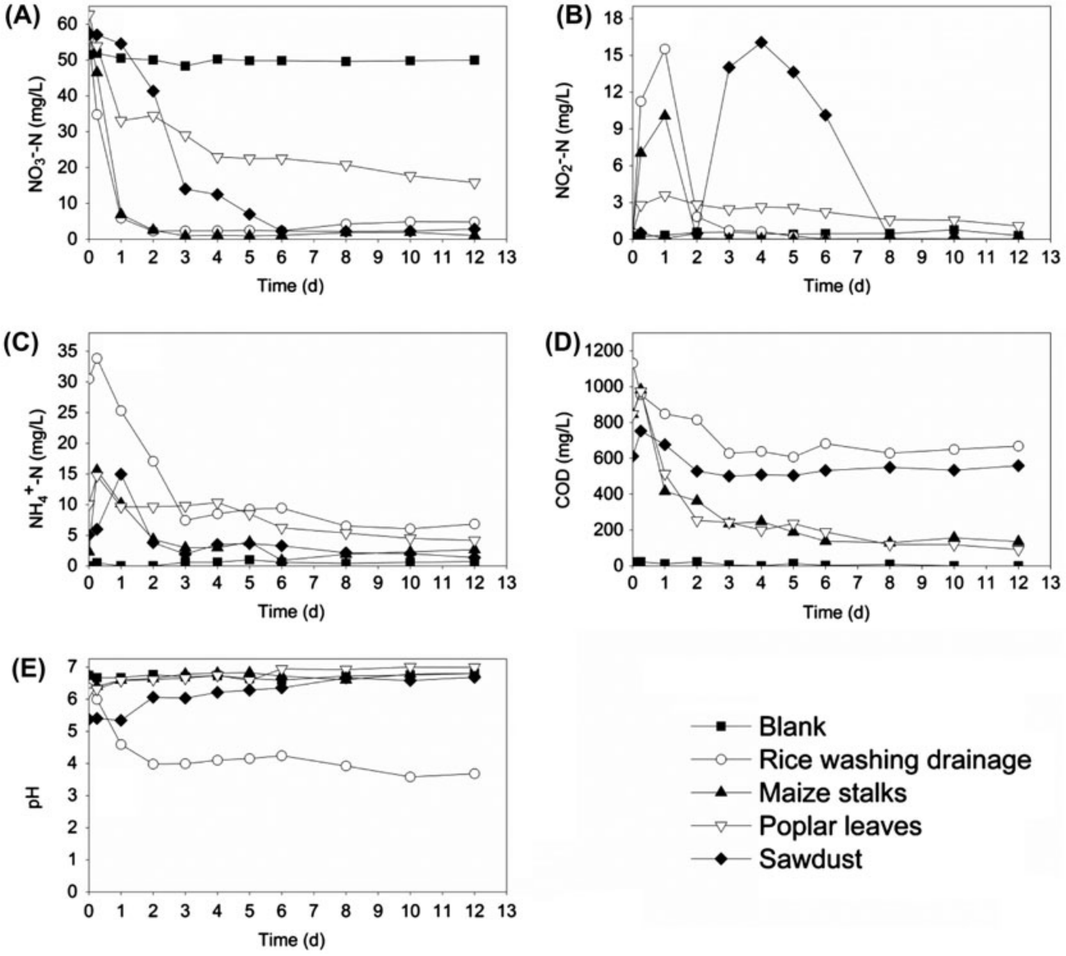

Fig. 2. Changes in nitrate, nitrite, ammonium, COD, and pH when using RWD, maize stalks, poplar leaves, and sawdust as carbon sources during the batch experiments.

However, the nitrate concentration in RWD reactor increased gradually from Day 6, the phenomenon might be attributed to nitrogenous compounds released from substances in the RWD. On the other hand, owning to the fermentation of RWD, pH in RWD reactor decreased significantly and reached about 4.0 on Day 2, and then remained almost constant (Fig. 2(E)). Low pH could inhibit the microbial activity because optimum pH value for microbial denitrification ranged from 7.0 to 9.0 [27]. Therefore, decomposition of substances in the RWD and low pH might lead to the increase in nitrate concentration in RWD reactor. In contrast, the pH in maize stalks, poplar leaves, and sawdust reactors firstly decreased slightly and then increased gradually, ranging from 6.0 to 7.0 after Day 2. The increase in pH was resulted from the occurrence of alkalinity produced in denitrification process [22]. In the batch experiments, although COD concentration in each carbon source was designed to be same according to the results of above leaching experiments, COD concentration in each carbon source was different (Fig. 2(D)). This phenomenon was attributed to that the release behavior of each carbon source was different, and RWD had a higher carbon release rate than solid carbon sources due to that solid particles in RWD mainly consisted of starch and protein.

3.2.2. Nitrite accumulation

As shown in Fig. 2(B), higher nitrite concentrations were detected in RWD, maize stalks, and sawdust reactors. The nitrite concentration increased with time and reached 15.51 mg of NO_2^--N/L in RWD reactor and 10.06 mg of NO_2^--N/L in maize stalks reactor on Day 1, then sharp declines in nitrite concentrations were observed after Day 1, reached 0.7 mg of NO_2^--N/L and 0.06 mg of NO_2^--N/L, respectively, and then remained almost constant. In sawdust reactor, the nitrite concentration increased drastically after Day 2 and reached 16.05 mg of NO_2^--N/L on Day 4, and then decreased slowly to less than 0.04 mg of NO_2^--N/L on Day 8, and then was kept at 0.03 mg of NO_2^--N/L at the experiment end. In poplar leaves reactor, the nitrite concentration increased slightly and reached 3.59 mg of NO_2^--N/L on Day 0.25, and then nitrite concentration was kept at a relatively lower level (1.09 mg of NO_2^--N/L). On the other hand, the nitrite concentration in blank reactor was below 0.5 mg of NO_2^--N/L throughout the experiment due to no denitrification. At the end of the experiment, nitrite concentration was 0.7 mg of NO_2^--N/L for RWD, 0.06 mg of NO_2^--N/L for maize stalks, 0.03 mg of NO_2^--N/L for sawdust, and 1.09 mg of NO_2^--N/L for poplar leaves.

Compared with Fig. 2(A), it could be seen that the peak in nitrite almost corresponded with the minimum value of nitrate. The phenomenon was due to the fact that nitrite was an intermediate product of denitrification [13]. Furthermore, relatively high concentration of residual nitrate at the beginning of the experiment could inhibit the synthesis and activity of nitrite reductase [28]. Therefore, nitrite concentration increased with the decrease in nitrate concentration at the initial stage. Subsequently, low concentration of residual nitrate lessened the inhibition on nitrite reductase, and then nitrite concentration decreased with time.

3.2.3. Ammonium accumulation

As shown in Fig. 2(C), initial ammonium concentration was almost 0 mg of NH_4^+-N/L in blank reactor, 30.50 mg of NH_4^+-N/L in RWD reactor, 2.30 mg of NH_4^+-N/L in maize stalks reactor, 10.04 mg of NH_4^+-N/L in poplar leaves reactor, and 5.06 mg of NH_4^+-N/L in sawdust reactor. As shown in Table 1, RWD, maize stalks, poplar leaves, and sawdust contained a certain amount of nitrogen, resulting in the ammonium occurrence in the solution. Zhang et al. [14] also found that solid carbon sources (wheat straw, sawdust) released significant amounts of ammonium. Furthermore, the ammonium concentration increased with time and reached maximum value at 6 h (33.85 mg of NH_4^+-N/L) in RWD reactor, 6 h (15.59 mg of NH_4^+-N/L) in maize stalks reactor, 6 h (14.72 mg of NH_4^+-N/L) in poplar leaves reactor and 24 h (14.94 mg of NH_4^+-N/L) in sawdust reactor. The sharp increase in ammonium concentration might be due to the occurrence of dissimilatory nitrate reduction to ammonium (DNRA) that was considered as a counterproductive process in denitrification studies [14,29]. After that, the ammonium concentrations in RWD, maize stalks, poplar leaves, and sawdust reactors decreased with time, the phenomenon might be attributed to ammonia oxidizing bacteria (AOB) that could coexist with anaerobic heterotrophic bacteria [30], and the AOB could oxidize ammonium to nitrate [31] because sampling might cause negative pressure in the reactor and a small amount of oxygen was squeezed into the reactor, resulting in the increase in the dissolved oxygen in the solution. However, it is essential to carry out a further study on the existence of AOB. The ammonium concentration in blank reactor remained almost constant (around 0.5 mg of NH_4^+-N/L) throughout the experiments. At the end of the batch experiments, the ammonium concentration was around 7 mg of NH_4^+-N/L in RWD reactor, 2 mg of NH_4^+-N/L in maize stalks reactor, 4 mg of NH_4^+-N/L in poplar leaves reactor, and 1.5 mg of NH_4^+-N/L in sawdust reactor. Interestingly, although initial ammonium concentration was higher in RWD reactor, the ammonium concentration reached a lower level (7 mg of NH_4^+-N/L) at the experiment end, suggesting that the denitrification using RWD as carbon source could not only efficiently remove nitrate, but also could effectively treat RWD.

3.3. Denitrification performance with different RWD dosages

3.3.1. Nitrate removal

As shown in Fig. 3(A), it was observed that the nitrate concentration at each RWD addition dosage decreased during the first 1.5 d. In particular, nitrate concentration at 50/350, 100/300, and 200/200 decreased significantly and reached 0 mg of NO_3^--N/L at 50/350, 0.38 mg of NO_3^--N/L at 100/300, 0.97 mg of NO_3^--N/L at 200/200 on Day 1.5, while nitrate concentration at 10/390, 20/380, and F50/350 decreased slightly and reached 42.13 mg of NO_3^--N/L at 10/390, 36.42 mg of NO_3^--N/L at 20/380, and 20.66 mg of NO_3^--N/L at F50/350 on Day 1.5. The maximum nitrate removal efficiency was 16.70% at 10/390, 23.45% at 20/380, 57.02% at F50/350, 100% at 50/350, 100% at 100/300, and 96.62% at 200/200. The higher nitrate removal efficiency at 50/350, 100/300, and 200/200 was due to that the larger RWD dosage could guarantee the sufficient organic matters for denitrification. However, although the solution at 200/200 contained higher RWD, the nitrate efficiency at 200/200 was lower than that at 50/350, which might be owing to that low pH (around 4 as shown in Fig. 3(E)) inhibited the activity of denitrification bacteria whose optimum pH ranged from 7.0 to 9.0 [27]. On the other

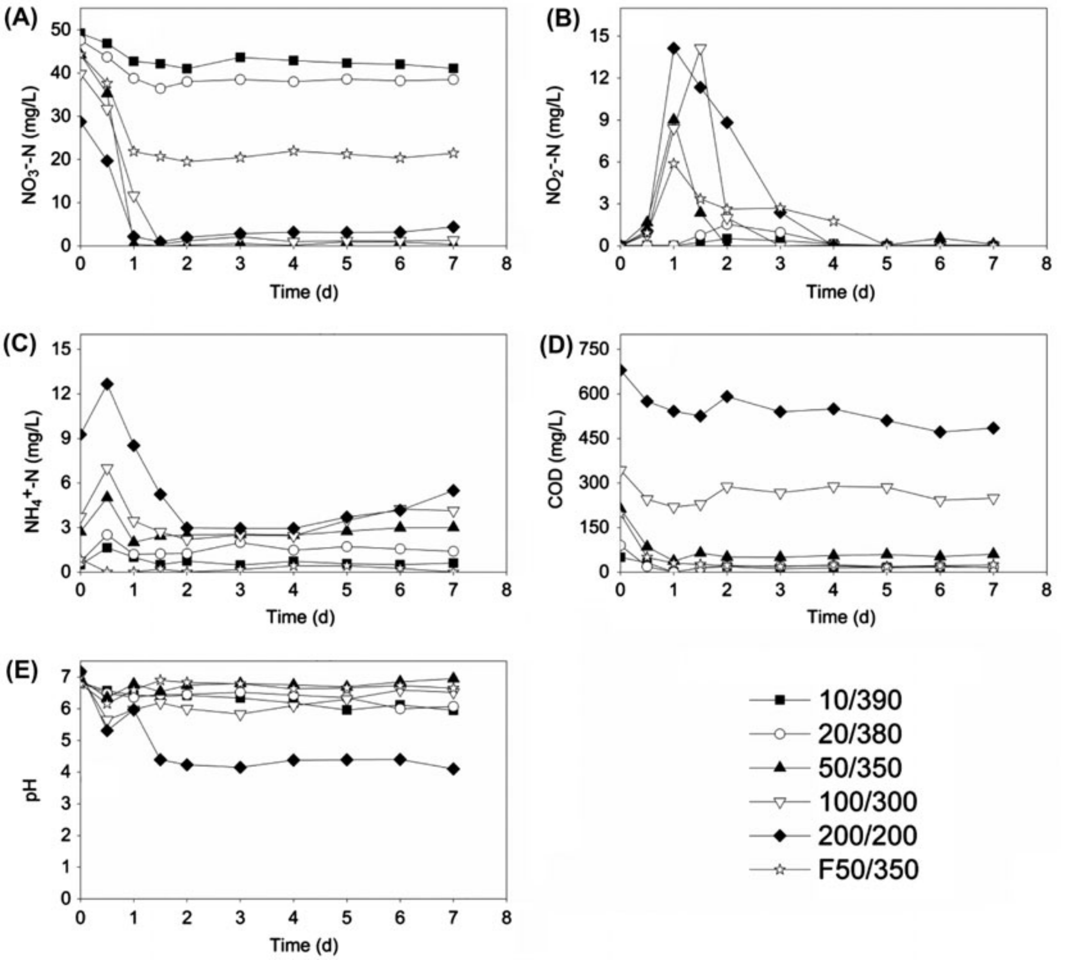

Fig. 3. Changes in nitrate, nitrite, ammonium, COD, and pH at different ratios of RWD to synthetic groundwater.

hand, there was the release of nitrogenous compounds from RWD along with the removal of nitrate. Furthermore, although initial solution COD concentration in the reactor at 50/350 was similar to that at F50/350 (Fig. 3(D)), the denitrification performance of the former was more excellent than that of the later, implying that solid particles contained in RWD played an important role in biological denitrification, namely, they might act as bacteria carriers and solid carbon sources beneficial for the denitrifying bacteria growth. The nitrate concentration at each ratio was kept at almost constant at 10/390 (around 42 mg of NO_3^--N/L), 20/380 (around 38 mg of NO_3^--N/L), 50/350 (around 0.5 mg of NO_3^--N/L), 100/300 (around 1 mg of NO_3^--N/L), 200/200 (around 3.5 mg of NO_3^--N/L), and F50/350 (around 21 mg of NO_3^--N/L). However, results showed that the nitrate concentration at 200/200 began increasing slightly 1.5 d later and was highest at the experiment end, which was attributed to a higher RWD dosage. Therefore, it was extremely necessary to control RWD addition dosage for a high-performance nitrate removal. The results indicated that RWD could be used as carbon source for denitrification.

3.3.2. *Accumulation of nitrite and ammonium*

As shown in Fig. 3(B), there was little nitrite accumulation at 10/390 and 20/380 throughout the experiments. However, nitrite concentration increased with time at 50/350, 100/300, 200/200, and F50/350, reached maximum on Day 1 (9.01 mg of NO_2^--N/L) at 50/350, on Day 1.5 (14.14 mg of NO_2^--N/L) at 100/300, on Day 1 (14.13 mg of NO_2^--N/L) at 200/200, and on Day 1 (5.88 mg of NO_2^--N/L) at F50/350. Furthermore, compared with Fig. 3(A), it could be seen that the peak in nitrite almost corresponded with the minimum value of nitrate, which was due to that nitrite was an intermediate product of denitrification [13]. Afterward, nitrite concentration at each ratio decreased with time and then remained almost constant (below 0.5 mg of NO_2^--N/L) at the end of experiments.

As shown in Fig. 3(C), little ammonium accumulation (below 1 mg of NH_4^+-N/L) was observed at F50/350 during the experiment period. This was probably due to few solid particles contained in the solution. However, ammonium concentrations at other ratios increased and reached 1.64 mg of NH_4^+-N/L at 10/390, 2.50 mg of NH_4^+-N/L at 20/380, 5.02 mg of NH_4^+-N/L at 50/350, 6.99 mg of NH_4^+-N/L at 100/300, and 12.66 mg of NH_4^+-N/L at 200/200 on Day 0.5, and then decreased gradually. However, ammonium concentration began increasing again from Day 2, and then reached almost constant and was around 0.7 mg of NH_4^+-N/L at 10/390, 1.5 mg of NH_4^+-N/L at 20/380, 2.5 mg of NH_4^+-N/L at 50/350, 4.0 mg of NH_4^+-N/L at 100/300, and 5.0 mg of NH_4^+-N/L at 200/200. The results indicated that the more the RWD contained, the higher the ammonium concentration reached. The first increase in ammonium concentration was speculated to be the occurrence of DNRA and ammonification of protein in the solution, and the decrease in ammonium concentration was mainly attributed to AOB. The second increase in ammonium concentration was ascribed to protein contained in RWD in the reactor.

3.3.3. Changes in COD and pH

Fig. 3(D) demonstrated that the COD concentration decreased significantly at each ratio during 1.5 d, showing that organic matters in the solution were utilized by denitrifying bacteria during this period. The COD concentration remained around 15 mg/L at 10/390 and 20/380, and below 60 mg/L at 50/350 from Day 1.5, suggesting that the organic matters were efficiently used by micro-organism. On the other hand, COD concentrations at 100/300 (around 250 mg/L) and 200/200 (around 500 mg/L) were high even at the end of experiments.

In biological denitrification, carbon source was electron donor and nitrate was electron accepter. Without consideration of intermediate products, it was assumed that carbon contained in RWD was transformed to CO_2 through offering four electrons and nitrate was transformed to N_2 via accepting five electrons. According to electron balances, the stoichiometric relationship could be described as follows:

$$5C \sim 4N \tag{1}$$

According to Eq. (1), the theoretical C/N ratio was 1.07 for complete nitrate removal in the denitrification process. Fernández-Nava et al. [32] also reported that the theoretical COD/N was 2.86 ($C/N = 1.07$) based on the electron balance. In the present study, MLSS in RWD was 897 mg/L and C content in RWD (solid particles) was 42.092%, so the carbon in solid part in RWD was 377.56 mg of C/L. On the other hand, COD in RWD filtrate was 1,131 mg/L, so the total C concentration in RWD filtrate was 424.03 mg of C/L. Thus, carbon concentration in RWD was 801.59 mg of C/L. The ratio of 50/350 was transformed C/N of 2.29 ((50 × 801.59 mg of C/L)/(350 × 50 mg of N/L)), and the C/N at F50/350 was 1.21 ((50 × 424.03 mg of C/L)/(350 × 50 mg of N/L)). Although the C/N (1.21) at F50/350 was higher than theoretical value (1.07) for denitrification, the nitrate removal efficiency was just 57.02%, whereas the nitrate removal efficiency was 100% at 50/350 with the C/N of 2.29, demonstrating that solid particles had a substantial effect on denitrification performance. Similarly, Fernández-Nava et al. [32] found that the optimum COD/N was 6.5 ($C/N = 2.43$) with wastewater from a sweet factory, 5.5 ($C/N = 2.06$) with a residue from a soft drinks factory and 4.6 ($C/N = 1.73$) with a residue from a dairy plant, while the optimum C/N was 1.08 with ethanol and 1.1 with methanol [12], indicating the optimum C/N for denitrification differed with different carbon sources, and the optimum C/N with pure carbon source was lower than that with wastewater as carbon sources. This phenomenon was probably because wastewater had lower carbon loading rate compared with methanol or ethanol, and nitrate reduction rate gradually decreased with the decrease in carbon loading rate [33]. Therefore, higher C/N using wastewater was required for complete denitrification. Furthermore, COD in the reactor is not only used for respiration but also for cell growth and maintenance [34], and RWD was easy for breeding bacteria [17], followed by more COD consumption. Hence, the optimum C/N (2.29) in this study was higher than theoretical value (1.07).

As shown in Fig. 3(E), although the pH in fresh RWD was 7.35, the pH values sharply decreased at each ratio during first 0.5 d due to the acids produced by the RWD fermentation [23]. Afterward, the pH increased due to the acid utilization by denitrifying bacteria, and then remained almost constant 1.5 d later. Furthermore, the pH value at 10/390 and 20/380 (pH around 6) was lower than that at 50/350, F50/350, and 100/300 (pH around 7) owning to the lower denitrification performance at 10/390 and 20/380. Interestingly, the tendency of pH change at 50/350 was similar to that at F50/350, kept at around 7 from Day 1.5. Compared with the solution at F50/350, more solid particles contained in the solution at 50/350, hence, more acids would be produced. However, the pH in the solution at 50/350 remained around 7 from Day 1.5, this phenomenon was due to that alkalinity generated by excellent denitrification performance (Fig. 3(A)) neutralized acids generated from RWD fermentation. On the other hand, pH at 200/200 gradually decreased and reached below 5 on Day 1.5, which was not suitable for denitrification because optimum pH for denitrifying bacteria ranged from 7.0 to 9.0 [27].

4. Conclusion

In this study, the denitrification performance was investigated using RWD, maize stalks, poplar leaves, and sawdust as carbon sources, followed by research on nitrate removal efficiency with different RWD dosages. The main conclusions were drawn as follows: (1) A higher nitrate removal rate was achieved when using RWD as carbon source; (2) The optimum ratio of RWD to synthetic groundwater (50 mg of NO_3^--N/L) was determined to be 50/350 (v/v); (3) Denitrification process could effectively treat nitrogenous compounds and acids produced from RWD. The study supplied a promising approach for cooperating groundwater nitrate-contaminated remediation with RWD treatment.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (NSFC) (Nos. 51578519, 21407129), the Foundation for the Advisor of Beijing Excellent Doctoral Dissertation (Nos. 20121141501, 20131141502) and the Fundamental Research Funds for the Central Universities (No. 2652015239).

References

[1] M.N. Almasri, J.J. Kaluarachchi, Implications of on-ground nitrogen loading and soil transformations on ground water quality management, J. Am. Water Resour. Assoc. 40 (2004) 165–186.

[2] S. Ghafari, M. Hasan, M.K. Aroua, Bio-electrochemical removal of nitrate from water and wastewater—A review, Bioresour. Technol. 99 (2008) 3965–3974.

[3] T.V. Nooten, L. Diels, L. Bastiaens, Design of a multifunctional permeable reactive barrier for the treatment of landfill leachate contamination: Laboratory column evaluation, Environ. Sci. Technol. 42 (2008) 8890–8895.

[4] D.J. Wan, H.J. Liu, J.H. Qu, P.J. Lei, S.H. Xiao, Y.N. Hou, Using the combined bioelectrochemical and sulfur autotrophic denitrification system for groundwater denitrification, Bioresour. Technol. 100 (2009) 142–148.

[5] S. Mousavi, S. Ibrahim, M.K. Aroua, S. Ghafari, Development of nitrate elimination by autohydrogenotrophic bacteria in bio-electrochemical reactors—A review, Biochem. Eng. J. 67 (2012) 251–264.

[6] S.S. Mirvish, N-nitroso compounds, nitrate, and nitrite: Possible implications for the causation of human cancer, Prog. Water Technol. 8 (1977) 195–207.

[7] WHO, Guidelines for Drinking-water Quality, Incorporating First and Second Addenda, vol. 1, Recommendations, third ed., World Health Organization, Geneva, 2008, p. 191.

[8] NHFPC, Standards for Drinking Water Quality, first ed., National Health and Family Planning Commission of the PRC, Beijing, 2006, p. 8.

[9] M. Alikhani, M.R. Moghbeli, Ion-exchange polyHIPE type membrane for removing nitrate ions: Preparation, characterization, kinetics and adsorption studies, Chem. Eng. J. 239 (2014) 93–104.

[10] L.A. Richards, M. Vuachère, A.I. Schäfer, Impact of pH on the removal of fluoride, nitrate and boron by nanofiltration/reverse osmosis, Desalination 261 (2010) 331–337.

[11] W.R. Zhao, X. Zhu, Y. Wang, Z.Y. Ai, D.Y. Zhao, Catalytic reduction of aqueous nitrates by metal supported catalysts on Al particles, Chem. Eng. J. 254 (2014) 410–417.

[12] M.A. Gómez, J. González-López, E. Hontoria-García, Influence of carbon source on nitrate removal of contaminated groundwater in a denitrifying submerged filter, J. Hazard. Mater. 80(2000) 69–80.

[13] Q.H. Wang, C.P. Feng, Y.X. Zhao, C.B. Hao, Denitrification of nitrate contaminated groundwater with a fiber-based biofilm reactor, Bioresour. Technol. 100 (2009) 2223–2227.

[14] J.M. Zhang, C.P. Feng, S.Q. Hong, H.L. Hao, Y.N. Yang, Behavior of solid carbon sources for biological denitrification in groundwater remediation, Water Sci. Technol. 65 (2012) 1696–1703.

[15] R. Wan, X. Zheng, Y.G. Chen, H.C. Wang, Using cassava distiller's dried grains as carbon and microbe sources to enhance denitrification of nitrate-contaminated groundwater, Appl. Microbiol. Biotechnol. 99 (2015) 2839–2847.

[16] L.Y. Cao, X.D. Zhan, S.G. Chen, Y. Feng, W.M. Wu, X.H. Shen, S.H. Cheng, Breeding methodology and practice of super rice in China, Rice Sci. 17 (2010) 87–93.

[17] M. Watanabe, K. Ichinose, K. Sasano, Y. Ozaki, T. Tsuiki, H. Hidaka, S. Kanemoto, Effect of enzymatic treatment on sedimentation and flocculation abilities of solid particles in rice washing drainage and its relationship with protein profiles, J. Biosci. Bioeng. 112 (2011) 67–70.

[18] J. Zou, X. Guo, Y. Han, J. Liu, H. Liang, Study of a novel vertical flow constructed wetland system with drop aeration for rural wastewater treatment, Water Air Soil Pollut. 223 (2012) 889–900.

[19] SEPA, Water and Wastewater Monitoring Analysis Method, fourth ed., China Environmental Science Press, Beijing, 2002, pp. 254–284.

[20] C.M. Greenan, T.B. Moorman, T.C. Kaspar, T.B. Parkin, D.B. Jaynes, Comparing carbon substrates for denitrification of subsurface drainage water, J. Environ. Qual. 35 (2006) 824–829.

[21] O. Gibert, J. Pablo, J.L. Cortina, C. Ayora, Chemical characterisation of natural organic substrates for biological mitigation of acid mine drainage, Water Res. 38 (2004) 4186–4196.

[22] S.J. Ge, Y.Z. Peng, S.Y. Wang, C.C. Lu, X. Cao, Y.P. Zhu, Nitrite accumulation under constant temperature in anoxic denitrification process: The effects of carbon sources and COD/NO_3-N, Bioresour. Technol. 114 (2012) 137–143.

[23] M. Watanabe, M. Makino, N. Kaku, M. Koyama, K. Nakamura, K. Sasano, Fermentative l-(+)-lactic acid production from non-sterilized rice washing drainage containing rice bran by a newly isolated lactic acid bacteria without any additions of nutrients, J. Biosci. Bioeng. 115 (2013) 449–452.

[24] K.A. Lisko, J.F. Hubstenberger, G.C. Phillips, H. Belefant-Miller, A. McClung, A. Lorence, Ontogenetic changes in vitamin C in selected rice varieties, Plant Physiol. Biochem. 66 (2013) 41–46.

[25] P.M. Yan, Q. Wang, Z. Li, X.Y. Yan, H.F. Zhang, Effects of vitamin C on the content of nitrite in fermented cabbage, Sci. Technol. Food Ind. 31 (2010) 232–235 (in Chinese).

[26] N. Labbé, S. Parent, R. Villemur, Addition of trace metals increases denitrification rate in closed marine systems, Water Res. 37 (2003) 914–920.

[27] Y.N. Tang, C. Zhou, M. Ziv-El, B.E. Rittmann, A pH-control model for heterotrophic and hydrogen-based autotrophic denitrification, Water Res. 45 (2011) 232–240.

[28] Y.Z. Peng, G.B. Zhu, Biological nitrogen removal with nitrification and denitrification via nitrite pathway, Appl. Microbiol. Biotechnol. 73 (2006) 15–26.

[29] Z.Q. Shen, Y.C. Zhou, J.L. Wang, Comparison of denitrification performance and microbial diversity using starch/polylactic acid blends and ethanol as electron donor for nitrate removal, Bioresour. Technol. 131 (2013) 33–39.

[30] X.C. Xu, Y. Xue, D. Wang, G.W. Wang, F.L. Yang, The development of a reverse anammox sequencing partial nitrification process for simultaneous nitrogen and COD removal from wastewater, Bioresour. Technol. 155 (2014) 427–431.

[31] L. Peng, B.J. Ni, D. Erler, L. Ye, Z.G. Yuan, The effect of dissolved oxygen on N_2O production by ammonium-oxidizing bacteria in an enriched nitrifying sludge, Water Res. 66 (2014) 12–21.

[32] Y. Fernández-Nava, E. Marañón, J. Soons, L. Castrillón, Denitrification of high nitrate concentration wastewater using alternative carbon sources, J. Hazard. Mater. 173 (2010) 682–688.

[33] Y.T. Pan, B.J. Ni, P.L. Bond, L. Ye, Z.G. Yuan, Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment, Water Res. 47 (2013) 3273–3281.

[34] H. Constantin, M. Fick, Influence of C-sources on the denitrification rate of a high-nitrate concentrated industrial wastewater, Water Res. 31 (1997) 538–589.