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Wheat straw, sawdust, and biodegradable plastics as potential carbon sources for synthetic nitrate-polluted groundwater column denitrification

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ABSTRACT

Biological denitrification of nitrate-polluted groundwater involves the selection of an appropriate organic carbon source. This paper aimed to evaluate the effects of wheat straw, sawdust, and biodegradable plastics as carbon sources on denitrification through column experiments. Results showed that the biodegradable plastics group released less nitrogen compounds than the wheat straw and sawdust groups. In the column denitrification experiments, the nitrate concentrations in the effluents obtained from the sawdust and wheat straw columns were below 2.50 and 3.00 mg NO_3^-–N L^{-1} at 25°C ± 2°C and steady-state conditions, while the nitrate removal efficiency was about 95% and 94%, respectively. For biodegradable plastics, the nitrate removal efficiency was about 99%. Moreover, the nitrite concentrations were lower than 0.05 mg NO_2^-–N L^{-1} or undetected at steady-state conditions for all columns. These findings indicated that wheat straw, sawdust, and biodegradable plastics can be used as solid carbon sources for denitrification, and biodegradable plastics had the best performance for denitrification stimulation. Notably, nitrate breakthrough and nitrite accumulation occurred when the temperature was reduced to 16°C ± 2°C, indicating that the temperature influenced denitrification.

Keywords: Groundwater; Nitrate; Biological denitrification; Solid carbon source

1. Introduction

Nitrate contamination of groundwater has become a serious concern in many countries. This type of contamination is caused by the extensive use of nitrogen fertilizers and irrigation with domestic wastewater [1–6]. Consuming nitrate-contaminated water poses risks to human health, causing several diseases such as methemoglobinemia in infants (blue baby syndrome) and cancers [7–9]. To minimize these health risks, the World Health Organization (WHO) set the threshold of nitrate concentration in drinking water to 11.3 mg NO_3^-–N L^{-1} [10].

Denitrification is the most preferred process for removing nitrates from groundwater. This process employs various denitrifying microorganisms in the presence of an organic carbon source, which serves as an electron donor under a given set of conditions [11–13]. However, denitrification in a natural system is an extremely slow process and influenced by several factors, such as carbon supply, temperature, dissolved oxygen (DO), and phosphorus [14–18]. Moreover, the carbon concentration in groundwater is sometimes insufficient to promote denitrification. Therefore, using supplemental carbon sources is necessary. Previous studies have focused on the use of liquid carbon sources, and the use of ethanol, molasses, and methanol as carbon sources has been reported to stimulate microbial denitrification [19–22]. However, these carbon sources are expensive and require

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sophisticated processes, especially at fluctuating nitrate concentrations, and have the risk of overdosing with the resultant deterioration of effluent water quality [23]. Solid carbon sources, such as waste newspaper, wood chips, and synthesized biodegradable polymers, have recently attracted considerable attention, because they can support slow carbon release [12,24,25]. In addition, these sources are widely available from industries and agricultural sectors, and thus greatly reduce the handling costs. These advantages indicate their potential use in nitrate removal from groundwater.

Several factors, such as cost, denitrification rate, and content of unfavorable compounds, should be considered during carbon source selection. Cellulose is an abundant renewable and natural polymer that is widely available, inexpensive, and biodegradable. The feasibility of using cellulose-rich materials, such as woodchips, wheat straw, and pine bark, to enhance denitrification rate has been reported [26–30]. Meanwhile, biodegradable plastics are commonly used worldwide. Such plastics are easily degraded by microorganisms and absorbed by the natural environment. Studies have shown that the degradation of biodegradable plastics creates reducing conditions that can develop and sustain an anaerobic-reductive environment conducive to denitrification [23,31]. Moreover, while being degraded, biodegradable plastics produce acid intermediates that can be used as electron donors by denitrifying bacteria [32]. Thus, when biodegradable plastics are used as carbon sources in groundwater remediation, denitrifying bacteria simultaneously use biodegradable plastics, as biofilm carriers, and water-insoluble carbon sources for denitrification.

Previous studies have focused on the denitrification rates of certain types of solid carbon sources, and their results have shown that using wheat straw, synthesized biodegradable polymers, and sawdust as carbon sources can stimulate microbial denitrification [26,33]. However, less attention has been directed toward the comparison among different carbon sources for biological denitrification, even though such comparisons are necessary for the identification of economic, effective, and environment-friendly alternative carbon sources that are conducive to denitrification. Therefore, in the present study, dynamic column experiments were performed to evaluate the releasing law of nitrogen compounds and organic carbon in wheat straw, sawdust, and biodegradable plastics. The behaviors of these carbon sources during biological denitrification were also evaluated. Accordingly, a suitable organic substrate was selected as a potential carbon source for nitrate-polluted groundwater remediation.

The objectives of this study are: (a) to test the leaching laws of nitrate, nitrite, ammonium, and chemical oxygen demand (COD) of the selected solid carbon sources in column experiments; (b) to assess the ability of these carbon sources to promote denitrification in column experiments and select the optimal solid carbon source for nitrate-polluted groundwater remediation systems; and (c) to investigate the effects of temperature on potential denitrification.

2. Materials and methods

2.1. Chemicals

Chemicals used in this study were of analytical reagent grade. Zeolite (2.0–4.0 mm) and quartz sand were used as inert materials to fill the columns. Wheat straw was obtained from the suburbs of Beijing (Daxing) and shredded to ribbons (5 mm width). Sawdust (powder) was obtained from Kaibiyuan Company (Beijing). Biodegradable plastics (5 mm × 2 mm) composed of 60% starch and 30% polypropylene were obtained from Zhaohe Ecological Technology Company, Hebei, China.

Solid samples were washed with distilled water, air dried, and ground to a homogeneous fine powder, after which they were analyzed for elemental composition using an elemental analyzer (Elementar Vario MACRO cube, Elementar Analysensysteme GmbH, Germany). The elemental composition of the selected solid carbon sources is given in Table 1.

2.2. Groundwater sources

Groundwater used in the column experiments was pumped from China University of Geosciences (Beijing). The composition of the groundwater is given in Table 2. Before using, groundwater was amended with an appropriate mass of $NaNO_3$ to achieve a nitrate concentration of 51.30 ± 0.98 mg NO_3^-–N L^{-1}. The pH of the prepared synthetic groundwater was 7.0–8.0. Anaerobic sludge was obtained from the Qinghe wastewater treatment plant, Beijing, China, in which the suspended solids and volatile suspended solids were 2,560 and 1,869 mg L^{-1}, respectively.

2.3. Leaching column experiments

Secondary pollution of groundwater is often caused by pollutants released from solid carbon sources. Thus,

Table 1
Elemental composition of the selected solid carbon sources

	C (%)	N (%)	H (%)
Wheat straw	42.54	0.52	5.66
Sawdust	48.78	0.21	5.86
Biodegradable plastics	43.91	0.08	6.62

Table 2
Composition of raw groundwater used for the column study (mg L^{-1})

NO_3^-–N	2.50
NO_2^-–N	0.00
NH_4^+–N	0.16
TP	0.03
HCO_3^-	39.6
SO_4^{2-}	50.8
Cl^-	31.5
Ca^{2+}	38.6
Mg^{2+}	15.9
K^+	2.75
Na^+	23.5

the release of pollutants should be considered when these solid carbon sources are used for nitrate-polluted groundwater remediation. Moreover, dissolved organic carbon released from a solid carbon source should be sufficient and stable to meet the requirements of denitrifying bacteria. Consequently, leaching column experiments were established to simulate the migration and leaching laws of nitrate, nitrite, ammonium, and COD concentrations of each selected carbon source.

Three PVC columns (internal diameter: 10.0 cm; length: 50.0 cm) were used as reactors (Fig. 1). The inert material contained zeolite and quartz sand (1:1 by weight). Before the experiments, each carbon source and inert material were washed with distilled water, then allowed to dry naturally, and finally sterilized in a pressure cooker for 15 min. The columns were then disinfected with ethyl alcohol. The following were then added separately to the sterilized columns: 158.3 g of wheat straw and 1,583 g of inert material; 272.1 g of sawdust and 2,721 g of inert material; and 371.6 g of biodegradable plastic and 3,716 g of inert material. Powdered quartz sand was placed at both ends of the columns to hold the packing materials, and then the columns were sealed to create anaerobic conditions.

A polyethylene tank (25 L) served as the groundwater storage tank. Concentrations of NO_3^-–N, NO_2^-–N, NH_4^+–N, and COD in the groundwater were too low to be considered. The groundwater was pumped upward into the columns using a four-channel peristaltic pump (LEAD-2, Baoding Longer Precision Pump Co., Ltd., China) at a flow rate of 2.0 mL min^{-1}. The column experiments were operated at 20°C ± 2°C for 80 d, after which samples were collected from the effluent line and analyzed for NO_3^-–N, NO_2^-–N, NH_4^+–N, and COD.

2.4. Denitrification column experiments

Denitrification column experiments were conducted to evaluate the capacity of the solid carbon sources to enhance biological denitrification. Three PVC columns identical to those described in the leaching experiments were utilized with wheat straw, sawdust, and biodegradable plastics as carbon sources. The inert material contained zeolite and quartz sand (with a weight ratio of 1:1). Sterilization was performed using the same method as that described in the leaching column experiments. The packing materials in each column consisted of a carbon source and inert material, and the amounts of carbon and inert material were the same as those described in the leaching column experiments. Packing materials were immersed in 1,000 mL of sludge for 24 h, then mixed homogeneously, and packed into each column. Quartz sand was placed at both ends of the column, after which they were sealed and covered with an opaque material to prevent light exposure.

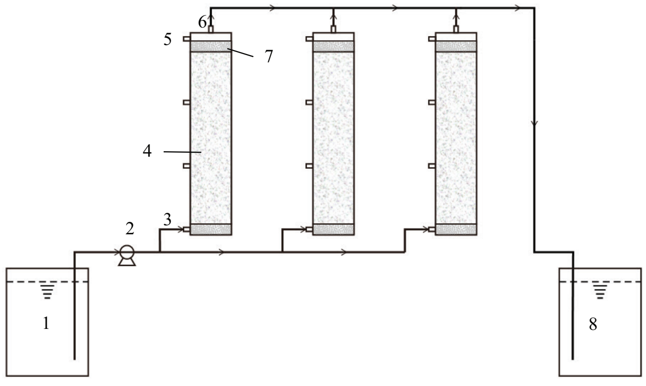

Fig. 1. Schematic of the column experiments: 1 – storage tank; 2 – peristaltic pump; 3 – inlet; 4 – packing materials; 5 – sampling port; 6 – effluent; 7 – quartz sand; and 8 – collecting tank.

A polyethylene tank (25.0 L) served as the synthetic groundwater storage tank. Synthetic groundwater containing 51.30 ± 0.98 mg NO_3^-–N L^{-1} was pumped upward into the columns using a four-channel peristaltic pump. The column experiments were divided into two periods according to the different experimental conditions. In the first period, the column experiments were conducted at 25°C ± 2°C for 66 d at an influent flow rate of 2.0 mL min^{-1}. In the second period, the temperature decreased to 16°C ± 2°C, the influent flow rate was maintained at 2.0 mL min^{-1}, and the experiments were conducted for 49 d.

2.5. Analytical techniques

Electrodes were used to measure the pH (UB-7, Denver Instrument, USA), oxidation-reduction potential (ORP) (ORPTestr 10, Oakton, USA), and DO (DO 110, Eutech Instruments, Singapore). NO_3^-–N, NO_2^-–N, NH_4^+–N, and COD were measured according to the procedures described by the Water and Wastewater Monitoring Analysis Method [34]. Before analysis, all samples were filtered using 0.45 μm membrane filters. Triplicate analyses were carried out for each sample. The results of the parallel experiments were within the allowable range, and the arithmetic mean was used as the final result.

3. Results and discussion

3.1. Leaching column experiments

3.1.1. Release of nitrogen compounds

As shown in Fig. 2(a), large amounts of nitrate were released from the wheat straw, sawdust, and biodegradable plastics at the beginning of the experiment, and their maximum nitrate concentrations were 4.26, 17.09, and 4.84 mg NO_3^-–N L^{-1}, respectively, at day 1. The nitrate concentrations of the biodegradable plastics rapidly decreased to values below 0.50 mg NO_3^-–N L^{-1} at day 5 and then remained nearly constant until the end of the experiment. Similarly, the nitrate concentrations of the sawdust rapidly decreased to values below 2.00 mg NO_3^-–N L^{-1} at day 14. By contrast, the nitrate concentrations in the effluent from wheat straw column stayed quite stable, ranging from 0.89 to 2.50 mg NO_3^-–N L^{-1} after 5 d.

As shown in Fig. 2(b), minimal amounts of nitrite were released from the three solid carbon sources. The nitrite concentrations, except those released during the first few days, were <0.02 mg NO_2^-–N L^{-1} or were undetected throughout the experiment. Therefore, secondary pollution caused by nitrite released from the solid carbon source can be disregarded.

As shown in Fig. 2(c), significant amounts of ammonium were released from wheat straw during the experiment

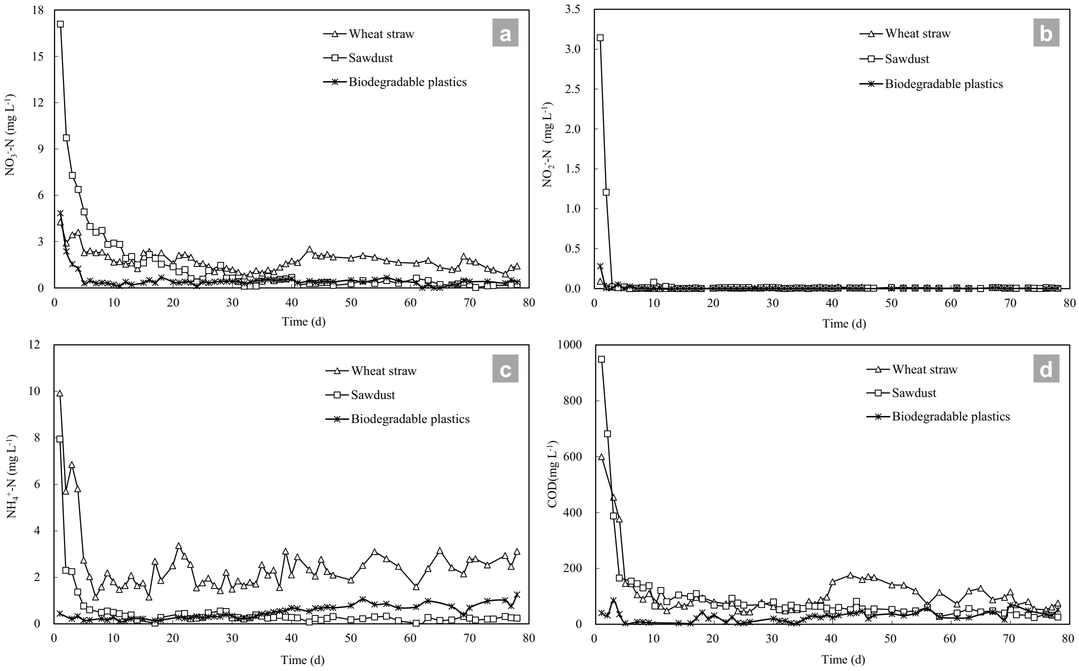

Fig. 2. Concentrations of nitrate (NO_3^-–N), nitrite (NO_2^-–N), ammonium (NH_4^+–N), and COD in column effluents over time at a flow rate of 2.0 mL min^{-1} during the leaching column experiments. Groundwater pumped from China University of Geosciences (Beijing) was used as feed water.

and the concentration in this column was higher than in the other two columns, with the maximum levels of 9.90 mg NH_4^+–N L^{-1} being observed on the first day. The ammonium concentrations then rapidly decreased to 2.03 mg NH_4^+–N L^{-1} on the fifth day, after which they remained at 1.15–3.36 mg NH_4^+–N L^{-1} until the end of the experiment. The ammonium concentration in the sawdust column was high initially but decreased to values lower than 1.00 mg NH_4^+–N L^{-1} on the fifth day. The ammonium concentration in the column filled with biodegradable plastics remained nearly constant at 0.10–1.30 mg NH_4^+–N L^{-1}.

Low amounts of nitrogen compounds were released from the biodegradable plastics in the leaching column experiments. Thus, using biodegradable plastics as carbon sources can prevent secondary pollution and is feasible for future applications. Similarly, the sawdust column released high levels of nitrate although only in the early stages of the experiment. It also released low amounts of nitrite and ammonium, and thus, secondary pollution caused by the nitrogen compounds released from sawdust can be disregarded. By contrast, wheat straw released the largest amount of nitrogen compounds, indicating that its use has the potential for secondary pollution even though it is often used as the carbon source in groundwater remediation systems [35]. These findings coincided with the results of elemental analysis of these carbon sources, which revealed that the total nitrogen contents of wheat straw were highest among the selected carbon sources.

3.1.2. Release of COD

Ensuring that the denitrifying microorganisms have sufficient levels of organic carbon is important during denitrification. Fig. 2(d) shows the changes in the COD of the effluent over time. Within 5 d, large amounts of COD were released from the wheat straw and sawdust, with the maximum concentrations of 599.6 and 948.5 mg L^{-1}, respectively, being observed on the first day. These results indicated that the COD release rates of the wheat straw and sawdust were high during the initial period of the leaching experiments, demonstrating that the available organic carbon was sufficient to support denitrification. It has been reported that <95% of nitrates were effectively removed from domestic effluent using sawdust in 5.5 d [36,37]. However, these carbon sources have the potential to cause the COD to be too high, thereby exceeding the requirement for microbial growth and causing secondary pollution of groundwater. Dhamole et al. [38] found that the application of wheat straw in denitrification resulted in high levels of dissolved organic carbon and color. In future applications, it may be beneficial to pretreat these carbon sources prior to use to avoid secondary pollution of the groundwater.

The COD concentrations rapidly decreased from the first day, with the concentrations in the wheat straw experiments falling below 150.0 mg L^{-1} by day 5, after which they fluctuated between 52.0 and 176.0 mg L^{-1} until the end of the experiment. For sawdust, the COD concentrations rapidly decreased to 154.0 mg L^{-1} in 6 d, after which they continued to decrease gradually until reaching 25.6 mg L^{-1} at the end of the experiment. These findings demonstrated that the amount of COD released by wheat straw and sawdust decreased with time until it can no longer meet the requirements for microbial growth. Previous studies revealed that the efficiency of the wheat straw decreased with time, and that replenishment of the system with fresh carbon source can enhance the nitrate removal efficiency [35]. Meanwhile, the COD released from the biodegradable plastics remained low, ranging from 3.25 to 52.0 mg L^{-1}, and its COD release rate remained constant throughout the leaching experiments.

These findings coincided with the results of batch leaching experiments, which indicated that the amount of COD released from the wheat straw and sawdust was much higher than those released by the biodegradable plastics in the absence of microorganisms [39]. However, the longevity of solid carbon sources in the remediation systems is important to their actual application. In most cases, only slowing the decomposition rates of the solid carbon sources is sufficient to support nitrate removal, because the amount of carbon is larger than those of the nitrate inputs. Meanwhile, although the amount of COD released by the biodegradable plastics was minimal, denitrifying bacteria obtained carbon by continuously degrading biodegradable plastics [40]. Shen et al. [41] suggested that biodegradable plastics were first hydrolyzed by extracellular enzymes to their monomer, dimer, trimer, and tetramer forms, after which they were further biodegraded or used directly as electron donors to reduce the nitrate and carbon source required for living cells in the solid denitrification system. Consequently, the slow and prolonged release of available carbon from biodegradable plastics can be obtained in future applications.

3.2. Denitrification column experiments

3.2.1. Environmental parameters in the columns

The pH value plays a vital role in the growth and reproduction of microorganisms. Denitrifying bacteria have an optimum growth pH range of 6.0–8.5 [17]. Throughout the experiment, the pH of the three columns remained in the range of 6.0–7.5, which was favorable for the growth and reproduction of the denitrifying bacteria. The ORP was measured as an overall redox parameter to indicate the reduction conditions of the columns. Efficient denitrification depends on sound redox conditions. At the beginning of the experiment, a reducing environment was formed inside the columns, and the ORP value remained from –260 to –293 mV after 1 week, indicating that the system was in a completely anaerobic state during the experiment.

In a reaction system, denitrifying bacteria prefer O_2 to nitrate, and thus the reduction of nitrogen is disrupted. In addition, the DO in the system facilitates the accumulation of N_2O. Thus, the influence of DO should be eliminated during denitrification. Vaishali and Subrata [13] suggested that denitrification generally occurs at a DO content of <2 mg L^{-1}. In this experiment, DO entered the column at concentrations of around 7.0 mg L^{-1}, but was rapidly consumed and decreased along the column to below 1.2 mg L^{-1} in all three columns after 7 d. These results indicated that after the feed water flowed into the columns, the DO was consumed rapidly. Thus, low DO contents do not affect denitrification.

3.2.2. Performance of the columns

Because a certain concentration of nitrogen compounds can be released from wheat straw and sawdust, the influence of their adsorption on concentration in the denitrification column experiments can be ignored. Similarly, the influences of the nitrate, nitrite, and ammonium absorbed by the zeolite and biodegradable plastics can be neglected during the long column experiments [18]. Consequently, denitrification mainly led to nitrate removal and accumulation of the by-products.

As shown in Fig. 3(a), the nitrate concentrations in the effluent decreased from 51.72 to 5.01 mg NO_3^-–N L^{-1} (wheat straw), to 5.59 mg NO_3^-–N L^{-1} (sawdust), and to 0.37 mg NO_3^-–N L^{-1} (biodegradable plastics) on the first day, with removal efficiencies of 90.3%, 89.2%, and 99.3%, respectively. Thereafter, the nitrate concentrations in the effluent from the sawdust and wheat straw columns continued to decrease gradually, with the concentrations falling below 2.50 mg NO_3^-–N L^{-1} by day 6 (sawdust) and below 3.00 mg NO_3^-–N L^{-1} by day 24 (wheat straw), resulting in nitrate removal efficiencies of over 95% and 94%, respectively, after which the concentrations remained nearly constant. For biodegradable plastics, the nitrate concentrations in the effluent remained constant, ranging from 0.08 to 0.95 mg NO_3^-–N L^{-1} by day 67, indicating that the biodegradable plastics can support complete denitrification (>99%). Thus, biodegradable plastics were the best carbon sources that promote denitrification. Although the nitrate concentrations in the other two columns were much higher, their nitrate concentrations were lower than the standard set by WHO. Thus, biodegradable plastics, wheat straw, and sawdust can be used as carbon sources for denitrification, and biodegradable plastics are the best among these sources. Previous studies also showed that these carbon sources can be used to remove nutrients from drinking water and domestic effluent [36,37,41,42].

The denitrification efficiency was divided by the mass of the solid carbon source for normalization to compare it with those obtained in other studies. The mass of the wheat straw was 158.3 g; sawdust, 272.1 g; and biodegradable plastics, 371.6 g. Nitrite was not used in the calculation of the denitrification rate, because it was produced in small amounts during denitrification. Meanwhile, the ammonium accumulation was high and thus adjusted during the calculation. Within 66 d, the average denitrification rates of the wheat straw, sawdust, and biodegradable plastics were 0.23, 0.11, and 0.14 mg NO_3^-–N L^{-1} d^{-1} g^{-1}, respectively. Gibert et al. [43] used cork as the carbon source in a denitrification column experiment with a flow rate of 0.3 and 1.1 mL min^{-1}, and the denitrification efficiency was 0.034 and 0.022 mg NO_3^-–N L^{-1} d^{-1} g^{-1}, respectively.

As an intermediate product of nitrate reduction, nitrite is undesirable, because it is more toxic to humans than nitrate. Certain amounts of accumulated nitrite were observed simultaneously with nitrate reduction on the first day of the experiments, with concentrations of 0.654, 0.034, and 0.008 mg NO_2^-–N L^{-1} being obtained for the wheat straw, sawdust, and biodegradable plastics, respectively (Fig. 3(b)). Thereafter, the concentration of the accumulated nitrite in the effluent was <0.050 mg NO_2^-–N L^{-1} or undetected under steady-state conditions. These concentrations were far lower than the values reported in previous studies [43]. Shen et al. [41] also observed nitrite accumulation when poly(butanediol succinate) was used as the carbon source in a packed-bed bioreactor, where the average nitrite concentration was 0.20 mg NO_2^-–N L^{-1} throughout the operating period. This concentration was higher than the nitrite accumulation observed in the present study.

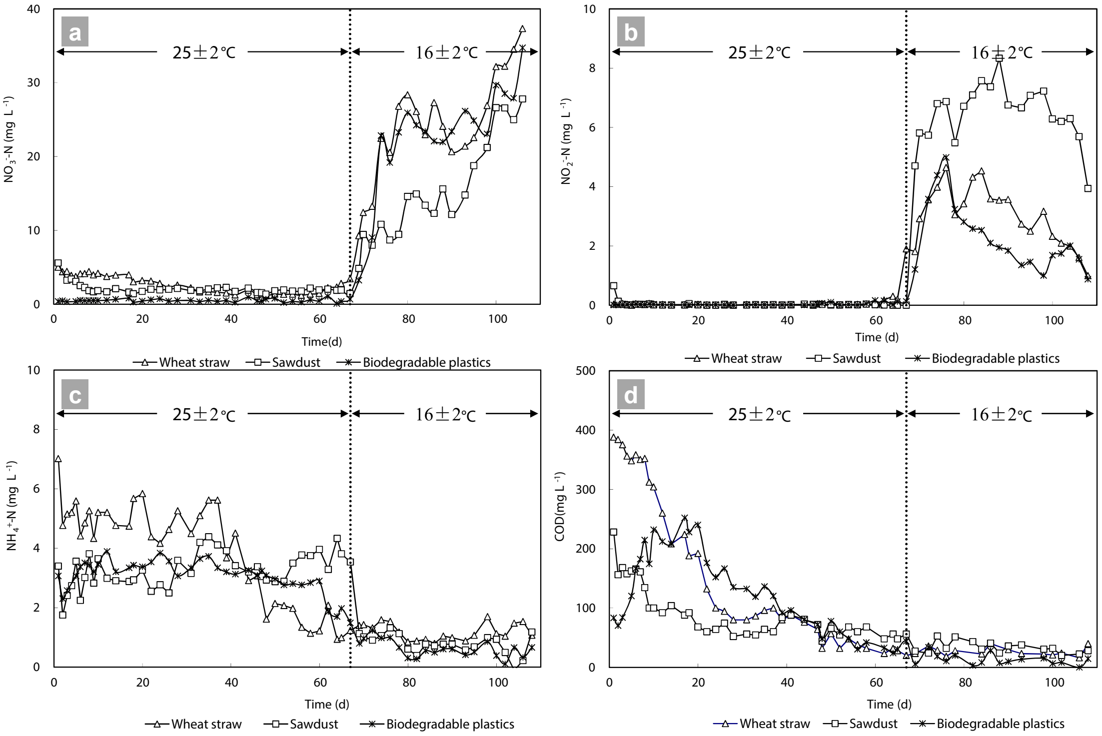

Fig. 3. Change in nitrate (NO_3^-–N), nitrite (NO_2^-–N), ammonium (NH_4^+–N), and COD in the column effluents over time at a flow rate of 2.0 mL min^{-1} (residence time of 13 h) when wheat straw, sawdust, and biodegradable plastics were used as carbon sources during the denitrification column experiments. Feed water for the column experiments was 51.30 ± 0.98 mg NO_3^-–N L^{-1}. After 67 d, the experimental temperature was reduced from 25°C ± 2°C to 16°C ± 2°C.

The nitrite accumulation observed in these studies was attributed to the incomplete reduction of nitrate caused by factors such as organic carbon supply and ambient conditions [14,43–45]. In the present study, the low nitrate concentration and nitrite accumulation reflected the favorable conditions and sufficient supply of organic carbon for complete denitrification.

As shown in Fig. 3(c), significant ammonium production was observed in the wheat straw column and had values of 7.01 mg NH_4^+–N L^{-1} on the first day of the experiment. Afterward, there was a decrease in ammonium accumulation from the initial levels to 4.77 mg NH_4^+–N L^{-1} for wheat straw on day 2, and the concentrations fluctuated between 4.50 and 8.50 mg NH_4^+–N L^{-1} by day 41. The concentration decreased to 1.60 mg NH_4^+–N L^{-1} at day 48, after which it remained almost constant until the end of the experiment. Moreover, significant amounts of accumulated ammonium were observed in columns of sawdust and biodegradable plastics within 67 d, during which the values fluctuated between 1.75 and 4.38 mg NH_4^+–N L^{-1} for sawdust and 1.48 and 3.89 mg NH_4^+–N L^{-1} for biodegradable plastics. As shown in Fig. 2(c), the ammonium concentration in the denitrification experiment was higher than that in the leaching experiment, except for the first few days, suggesting that the dissimilatory nitrate reduction to ammonia (DNRA) occurred. DNRA is another potentially significant component of the nitrogen cycle, and it competes with denitrification [46]. While the conditions promoting DNRA and denitrification are similar (e.g., anaerobic environment, nitrate availability, and organic substrates), denitrification represents a permanent nitrogen removal pathway, whereas DNRA is a nitrogen-conserving mechanism that transforms nitrate to ammonium rather than N_2 [47]. Several factors have been proposed to favor DNRA over denitrification as a pathway for nitrate reduction, including high temperature, high ratios of C/N, and low NO_3^- availability [47,48]. Tiedje [49] thought that the capacity of DNRA to accept electrons is eight, whereas that of denitrification is five. In strongly anaerobic habitats, the lack of electron acceptors is probably the factor that has the most limiting effect for growth. Thus, DNRA is preferred at a high available C/N ratio and low NO_3^- availability. As shown in Fig. 3(d), after 41 d the concentration of COD in the column with wheat straw became lower, which may hinder DNRA, so the accumulation of ammonium was weakened. Gibert et al. [43] also found that high levels of organic carbon could enhance the accumulation of ammonium by accelerating DNRA.

3.2.3. Evolution of COD

The supply of organic carbon from the solid carbon source was evaluated on the basis of the COD concentration in the effluent. The results of the comparison between Figs. 3(d) and 2(d) revealed that the COD concentration in the denitrification experiment was higher than that in the release experiment at most time points during the first 30 d of the experiment, indicating that a solid carbon source is prone to degradation in the presence of microorganisms to provide organic carbon.

As shown in Fig. 3(d), the COD concentration in the effluent showed a rapid decrease. There was minimal difference between the concentrations in the sawdust columns of the release experiments and those of the denitrification experiments. Both experiments showed a decreasing trend. It can be speculated that when wheat straw and sawdust are used as carbon sources, the release of organic carbon decreases gradually to levels that are far lower than the level required by the microorganisms. Conversely, the COD released from biodegradable plastics was low in the release experiments, while it was much higher in the denitrification experiments. Moreover, the COD increased from 83.2 to 252.0 mg L^{-1} in the first 17 d of the denitrification experiments. These results showed that a large amount of dissolvable carbon was produced after the biodegradable plastics were decomposed by the microorganisms, leading to complete denitrification. After 18 d, the COD concentration decreased, possibly because of microbial growth.

3.2.4. Influence of temperature on denitrification

Researchers have found that temperature greatly affects denitrification rate [50]. In the present study, the temperature was reduced from 25°C ± 2°C to 16°C ± 2°C after 67 d to investigate the effects of temperature on denitrification. As shown in Fig. 3(a), complete nitrate reduction was achieved at 25°C ± 2°C, and the effluent nitrate concentrations of each column were lower than the drinking water threshold set by WHO. Furthermore, the nitrite accumulation in each column was minimal under these conditions. Thereafter, the temperature was reduced to 16°C ± 2°C, and nitrate breakthrough occurred, with the maximum nitrate concentration in the effluent being 37.30 mg NO_3^-–N L^{-1} (wheat straw), 34.70 mg NO_3–N/L (biodegradable plastics), and 27.80 mg NO_3^-–N L^{-1} (sawdust), giving corresponding nitrate removal efficiencies of 24.6%, 30.0%, and 43.8%, respectively. Furthermore, the nitrite accumulation became increasingly evident, reaching a maximum of 8.33 mg NO_2^-–N L^{-1} in the sawdust, 4.99 mg NO_2^-–N L^{-1} in the biodegradable plastics, and 3.24 mg NO_2^-–N L^{-1} in the wheat straw. These values were considerably higher than the maximum value set by WHO.

Nitrate breakthrough and nitrite accumulation when the temperature was reduced from 25°C ± 2°C to 16°C ± 2°C indicated that denitrification could not be completed at low temperature, so nitrate could not be completely removed, and toxic nitrite would accumulate. These findings are similar to those reported by Li et al. [16], who found that denitrification rates increased by 2.2- to 3.6-fold when temperature increased by 23°C–30°C. Vaishali and Subrata [13] also found that high temperature favored better denitrification efficiency than low temperature.

In contrast to the nitrate and nitrite concentrations, the ammonium concentration decreased rapidly. All the columns had values below 2.00 mg NH_4^+–N L^{-1} after 67 d (Fig. 3(c)). This primarily occurred because low temperature is adverse to DNRA, which resulted in decreased ammonium accumulation. These results are in agreement with those of Ogilvie et al. [51], who reported that nitrate-ammonifiers are effective competitors for nitrate at high temperatures. Dunn et al. [52] also reported that DNRA is more prevalent than denitrification during summer.

3.2.5. Morphological changes on the surface of the solid carbon source

The carbon source inside the column was collected, washed with distilled water, dried, and evaluated by scanning electron microscopy (SEM) at the end of the experiment to observe the damage caused to the solid carbon source surface by denitrifying bacteria.

Wheat straw is a complex mixture of cellulose, hemicelluloses (including xylan), pectins, and lignins. Fig. 4(a) shows a SEM photo of a fresh wheat straw surface, while Fig. 4(b) shows that of wheat straw at the end of the experiment. The fresh wheat straw was smooth and had no trace of damage, whereas the wheat straw obtained after treatment was rough and exhibited damages. These findings suggested that organic matter, such as cellulose on the surface, was decomposed by the bacteria. The SEM images of the sawdust are presented in Fig. 5. Before the sawdust was used, the cellulose and xylogen had an orderly structure. After the sawdust was used, the fiber bundle disappeared, and a large amount of cellulose was degraded or consumed. These results indicated that the cellulose and xylogen were degraded and consumed by the microorganisms during denitrification. Sawdust is mainly composed of lignin, cellulose, and semicellulose, which cannot be used directly by the denitrifying bacteria and thus should be hydrolyzed first into compounds with low molecular weights [53]. The images confirmed the feasibility of using sawdust as a solid carbon source for

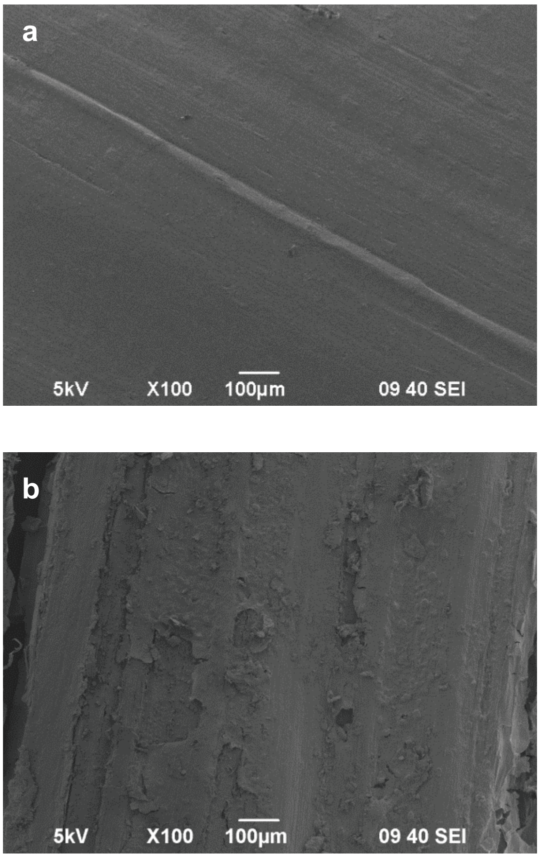

Fig. 4. SEM images of wheat straw: (a) unused wheat straw and (b) used wheat straw.

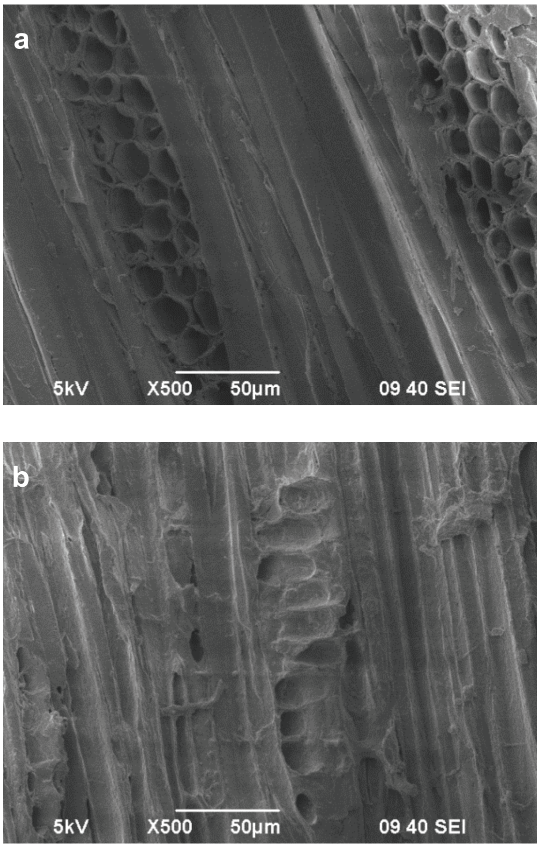

Fig. 5. SEM images of sawdust: (a) unused sawdust and (b) used sawdust.

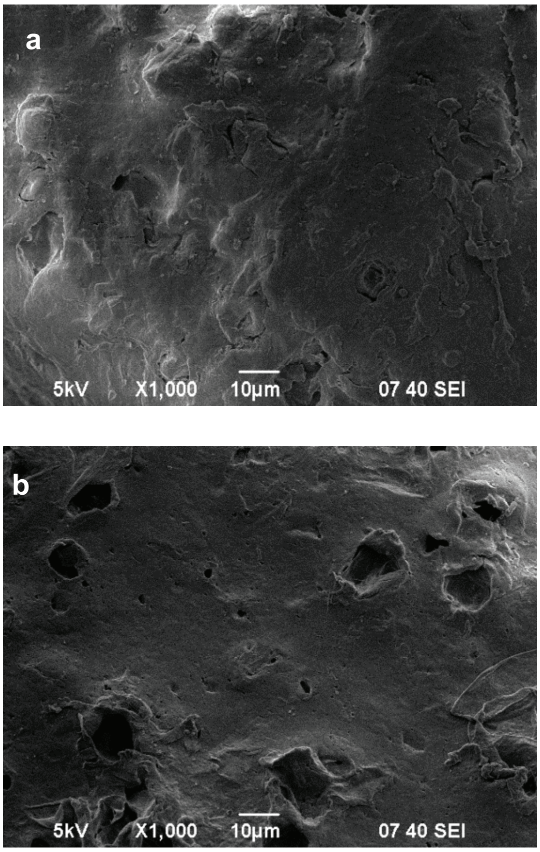

Fig. 6. SEM images of biodegradable plastics: (a) unused biodegradable plastics and (b) used biodegradable plastics.

denitrification. Meanwhile, the SEM images of the biodegradable plastics are shown in Fig. 6. The surfaces of these materials had no hole before the reaction. The holes appeared after the biodegradable plastics were used probably because of corrosion during the degradation process.

Overall, SEM revealed that different types of damages to wheat straw, sawdust, and biodegradable plastics appeared during denitrification. Additionally, the results presented herein confirm that they can be degraded and used by microorganisms and therefore have the potential for application as solid carbon sources for denitrifying microorganisms.

4. Conclusion

This study demonstrated that wheat straw, sawdust, and biodegradable plastics can enhance the performance of biological denitrification. Compared with wheat straw and sawdust, biodegradable plastics released less amounts of nitrogen compounds in the leaching column experiments. Therefore, secondary pollution was avoided when biodegradable plastics were used as carbon sources. When the temperature was 25°C ± 2°C, the nitrate removal efficiencies of the sawdust and wheat straw columns were over 95% and 94%, respectively, which was lower than the nitrate removal efficiencies of the columns containing biodegradable plastics (>99%). Furthermore, the nitrite concentrations in all the columns were lower than 0.050 mg NO_2^-–N L^{-1} or undetected under steady-state conditions. Nitrate degradation was accompanied by minimal ammonium production, indicating that DNRA occurred. When the temperature was reduced to 16°C ± 2°C, nitrate breakthrough occurred, and nitrite accumulated, indicating that high temperatures are more conducive to denitrification than low temperatures.

These results showed that wheat straw, sawdust, and biodegradable plastics can be used as solid carbon sources for denitrification in future applications, and that biodegradable plastics were the most appropriate carbon source for stimulation of denitrification activity owing to the lower amount of nitrogen compounds released, higher denitrification efficiency, and lower nitrite accumulation.

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