

# In-situ biological denitrification using pretreated maize stalks as carbon source for nitrate-contaminated groundwater remediation

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## ABSTRACT

A simulation apparatus of in-situ groundwater remediation (SAIR) that used maize stalks pretreated with sodium hydroxide (MSSH) as a carbon source was designed for nitrate-contaminated groundwater treatment. Two experiments, RA and RB, were constructed in this SAIR. The removal performance of SAIR fed with real nitrate contaminated water was investigated under static and dynamic conditions. In the static remediation experiment, good removal efficiency (>95% for nitrate, 89% for total nitrogen) was observed in both experiments. However, nitrate removal efficiency did not differ greatly between RA and RB at a hydraulic retention time (HRT) of 15 d. Overall, these results indicate that MSSH has potential for use as an alternative carbon source for denitrification.

**Key words** | denitrification, groundwater, maize stalks, nitrate-contaminated, permeable reactive barrier, pretreatment

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## INTRODUCTION

In recent years, nitrate contamination in shallow groundwater, a major drinking water source, has become an increasingly serious problem, resulting from the discharge of untreated domestic and industrial wastewater, excessive use of nitrogen fertilizers, and landfill leachate (Thomas *et al.* 2008). Elevated nitrate concentrations in drinking water not only lead to methemoglobinemia in infants (blue baby syndrome) (Mousavi *et al.* 2012), but could also cause gastric cancer owing to nitrate reduction to nitrite in the intestine (Mohseni-Bandpi *et al.* 2013). Consequently, the World Health Organization has stipulated a maximum contaminant level of 50 mg NO<sub>3</sub><sup>-</sup>/L (i.e., a nitrate-nitrogen value of 11.3 mg/L). In China, the maximum permissible concentration for nitrate in drinking water is 10 mg NO<sub>3</sub><sup>-</sup>-N/L.

The conventional remediation technology for contaminated groundwater has been the pump-and-treat (P&T) systems (Obiri-Nyarko *et al.* 2014). However, P&T systems

are not a realistic treatment solution due to the high costs of management and operation. A permeable reactive barrier (PRB) is considered to be the best option for in-situ remediation technology because of its increased cost effectiveness, environmentally sound nature, and lower maintenance requirements during long-term operation (Obiri-Nyarko *et al.* 2014). Gibert *et al.* (2008) simulated an aquifer and a denitrification wall in a laboratory setting, using softwood as the carbon source for column experiments. The denitrification wall successfully removed almost all of the nitrate (>96%) when the flow rate was 0.3 cm<sup>3</sup> min<sup>-1</sup>.

However, the lack of organic materials in contaminated groundwater has limited the application of biological remediation. Although liquid carbon sources have commonly been used for heterotrophic denitrification, these materials are expensive, uncontrollable, and lead to secondary pollution. Solid carbon sources are not only useful as electron

donors for biological denitrification, but also as carriers of microorganisms. Indeed, many agricultural wastes have been used for heterotrophic denitrification, including maize cobs (Cameron & Schipper 2010), wheat straw (Saliling *et al.* 2007), and rice stalks (Qian *et al.* 2011).

In this study, a fiber-reinforced plastic-based reaction tank was developed to simulate the in-situ biological denitrification process. In this process, heterotrophic denitrification bacteria used maize stalks that had been pretreated with sodium hydroxide (MSSH) as a solid carbon source and bio-film carrier. The goal of the present study was to investigate the denitrification performance of MSSH as a carbon source, and to solve the problem of the total plate count being too high.

## METHODS

### Preparation of materials

#### Carbon source

Maize stalks from mature plants were collected from the cropland of Jianxian (Tianjin, China). Following collection, the stalks were cut into small pieces (2–3 cm length) and then soaked for 24 h in 3% sodium hydroxide solution. Subsequently, the soaked maize stalks were flushed with running water repeatedly until the pH of the water was approximately 7.0. Maize stalks pretreated with sodium hydroxide are hereafter referred to as MSSH. The elemental composition of maize stalks and MSSH are given in Table 1.

#### Groundwater

The influent for the in-situ groundwater remediation experiments was prepared using groundwater pumped from

Jianxian (Tianjin, China). The water was amended with potassium nitrate to give a final nitrate concentration of 80 mg/L.

### Activated sludge acclimation

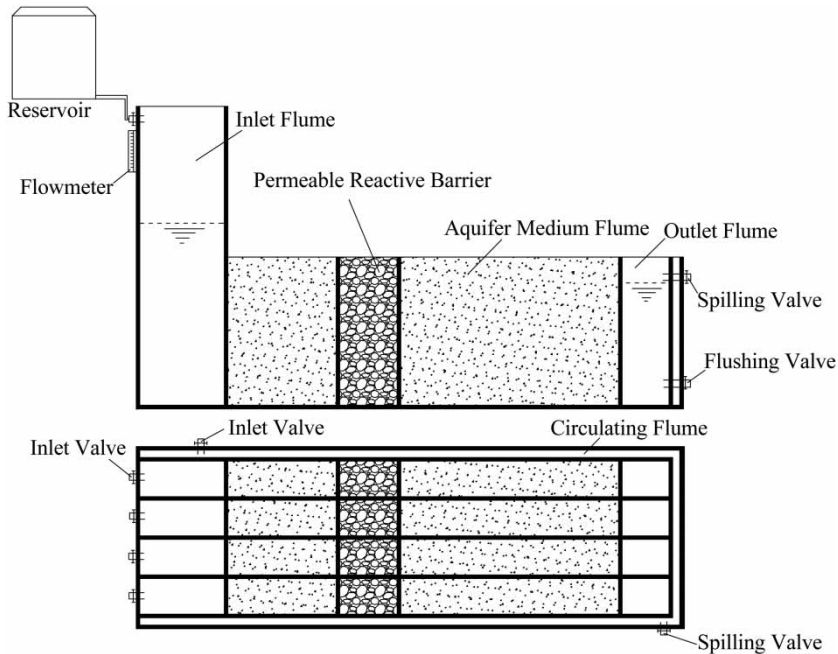
Anaerobic sludge was collected from the Qinghe wastewater treatment plant in Beijing and cultivated in a liquid nutrient medium at 25 °C for 1 month. The nutrient medium was prepared by adding KNO<sub>3</sub> (0.370 g/L); C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> (0.156 g/L); CH<sub>3</sub>OH (320 μL/L); and KH<sub>2</sub>PO<sub>4</sub> (0.044 g/L). During acclimation, the nutrient medium was replaced every 3 days. The mixed liquid suspended solids and sludge volume index were 2,820 mg/L and 110 mL/g, respectively.

### Experimental apparatus

As shown in Figure 1, the simulation apparatus of in-situ groundwater remediation (SAIR) experiments consisted of a reservoir (334 L), inlet flume (15 cm width), glass tube float flowmeter, aquifer medium flume, PRB (15 cm width), outlet flume (10 cm width), and a circulating flume (5 cm width). A SAIR of 160 cm × 40 cm × 50 cm was made from fiber-reinforced plastic. Valves were installed to allow the groundwater to spill out from the outflow and circulating flumes. In addition, a flushing valve was installed at the bottom of the outlet flume to wash the reactors. The reservoir was raised to 1 m higher than the SAIR, after which groundwater flowed into the inlet flume using the potential energy of water. A glass tube float flowmeter was installed in the inlet flume to adjust the flow rate, replacing the peristaltic pump and saving energy. The PRB was placed at two-thirds of the aquifer medium flume with a width of 15 cm. Silver sand (0.5–1.0 mm) was placed in the aquifer medium flume. The porosity and hydraulic conductivity of the silver sand, which were determined from the volume of groundwater that was added to the solids, were calculated to be 35% and 4 × 10<sup>-3</sup> cm/s. Before the experiment, the SAIR was flushed with groundwater for 5 h to avoid the influence of the silver sand. The temperature of the simulation experiment was consistent with that of the groundwater because of the circulating flume surrounding the reactor. Multi-group experiments were started simultaneously, which reduced the time required for the

**Table 1** | Elemental composition of the carbon source

Carbon source	C (%)	N (%)	H (%)	S (%)
Maize stalks	46.93 ± 0.32	2.09 ± 0.20	5.66 ± 0.36	0.67 ± 0.28
MSSH	45.79 ± 0.39	1.18 ± 0.18	6.17 ± 0.25	0.39 ± 0.42



**Figure 1** | Schematic of the in-situ remediation system.

experiment because the device contained four reactors side by side.

## Experimental procedure

### Elemental analysis of carbon sources

Maize stalks and MSSH were dried and ground to a homogeneous fine powder using a grinder, then analyzed for elemental composition. The contents of C, H, N, and S were determined by an elemental analyzer. The elemental composition is given in Table 1.

### Leaching experiments

The leaching solution composition of added carbon sources was analyzed. Identical amounts of maize stalks and MSSH (5 g, dry weight) were placed in two airtight flasks with 500 mL deionized water and soaked at 20 °C for 3 days. In addition, the liquid used to pretreat the maize stalks was also analyzed (hereafter referred to as PMSSH). The mixed liquor was then filtered and analyzed to determine the leaching solution composition (Table 2).

**Table 2** | Concentration of elements in the leaching solutions of the carbon sources

Composition/(mg/L)	Leaching solution		
	Maize stalks	MSSH	PMSSH
Mg	330	170	210
Al	0.26	0.02	0.2
Mn	0.007	0.002	0.004
Ca	35	15	27
Fe	0.32	0.03	0.08
Ni	1.85	0.7	0.8
K	41	12	1.5
Cu	1.35	0.34	1.2
Cr <sup>6+</sup>	0.33	0.18	0.04
NO <sub>3</sub> <sup>-</sup> -N	4.63	3.95	1.5
NO <sub>2</sub> <sup>-</sup> -N	0.8	0.012	38.2
NH <sub>4</sub> <sup>+</sup> -N	104.75	13.6	0.012

### In-situ groundwater remediation simulation experiments

In this study, static and dynamic in-situ groundwater remediation experiments were conducted, as the flow rate of groundwater is a significant factor. The in-situ static and

dynamic groundwater remediation experiments were conducted in two different reactors (RA and RB) containing different filling media. Specifically, RA was packed with MSSH to investigate the effects of bacteria generated from MSSH on removal performance. RB was packed with MSSH and activated sludge to evaluate the performance of SAIR.

In the static experiment, groundwater was used to fill the four reactors separately, then kept in a static state. In the dynamic experiment, the flow rate of influent was adjusted by a glass tube float flowmeter installed in the inlet flume. The water flow rates of groundwater into the filter were 15.6 mL/min, 7.8 mL/min and 5.2 mL/min, respectively. Denitrification performance was also investigated under different hydraulic retention times (HRTs). Samples were filtered through a membrane filter (pore diameter 20  $\mu\text{m}$ ) and then subjected to analysis as described below.

### Analytical methods

According to *Water and Wastewater Monitoring Analysis Method* (SEPA 2002),  $\text{NO}_3^-$ -N,  $\text{NO}_2^-$ -N,  $\text{NH}_4^+$ -N, total nitrogen (TN), and total phosphorus (TP) were determined by ultraviolet spectrophotometry (DR5000, HACH, USA). The pH and dissolved oxygen (DO) were determined using a multi-parameter portable analyzer (HQ30d, HACH, USA). Elemental C, N, H, and S in the maize stalks were measured using an elemental analyzer (EA3000, EURO, Italy). The standard deviations were analyzed at a confidence level of 90%, and all analyses were carried out in Origin 8.0 (OriginLab, trial version).

## RESULTS AND DISCUSSION

### Leaching experiments

Table 2 summarizes the elements in the leaching solutions after 3 days of soaking. All three leaching solutions contained some metallic elements. It is well known that K and Mg are necessary for the activity of several enzymes and that Ca can contribute to the stabilization of microbial cell walls (Madigan *et al.* 2006). Some trace metallic elements, such as Fe, Cu, Mn, and Ni, typically play a significant role as cofactors for enzymes (Labbé *et al.* 2003). In

addition, some unessential elements such as Al and Cr were found; however, they were present at levels well below the inhibitory concentration (Stasinakis *et al.* 2003). When the leaching solutions of the maize stalks were compared, the concentrations of nitrogen in the MSSH leaching solution were reduced. Therefore, using MSSH as a carbon source can reduce nitrogen pollution of aquatic systems.

### Basic environmental parameters of SAIR experiments

The temperature of in-situ groundwater remediation experiments remained at 10–11.5 °C throughout the experiment. This temperature was kept close to the actual temperature of groundwater to ensure reliability of the simulation. The pH remained neutral over time and along the length of reactors, with values fluctuating between 7.1 and 8.2 (data not shown). The DO levels in the influent were around 8.5–10.0 mg/L, and rapidly decreased to approximately  $1.3 \pm 0.4$  mg/L in the effluent (data not shown). The  $\text{COD}_{\text{Mn}}$  concentrations in the PRB and effluent were  $7.5 \pm 1.5$  mg/L and  $3.0 \pm 0.3$  mg/L, respectively, indicating that the availability of organic carbon and DO were not limited by the denitrification process.

In theory, the chemical oxygen demand (COD) consumption is 2.86 for 1 mg  $\text{NO}_3^-$ -N reduction under anoxic conditions. However, in practice, COD/N requirements range from 5 to 10, with a minimum ratio of 3.5–4.0 being essential (Bernat *et al.* 2008). In this study, total organic carbon (TOC) accounted for 55.7% of the  $\text{COD}_{\text{Mn}}$  in MSSH leachate, and the release rate was 39.86 mg TOC ( $\text{g}^{-1}$  MSSH). The amount of MSSH packed in the PRB was 1,300 g; therefore, the TOC/N was approximately 6.0, which is in the range of the optimum C/N.

### In-situ static groundwater remediation experiments

#### Nitrate and TN removal

The denitrification performance of the static experiments is shown in Figure 2. Nitrate removal was observed in RA and RB. Interestingly, nitrate could be effectively removed in RA without inoculation with domesticated denitrifying bacteria. Nitrate decreased from  $80.0 \pm 1.5$  mg  $\text{NO}_3^-$ -N/L to  $35.5 \pm 0.3$  mg  $\text{NO}_3^-$ -N/L (RA) and  $7.5 \pm 0.2$  mg  $\text{NO}_3^-$ -N/L (RB) in



**Figure 2** | Evolution of nitrate ( $\text{NO}_3^-$ -N), total nitrogen (TN), nitrite ( $\text{NO}_2^-$ -N), and ammonium ( $\text{NH}_4^+$ -N) (mg/L), and removal efficiency (%) for in-situ static groundwater remediation experiments.

the PRB in the first 12 days, then decreased slowly to  $5.0 \pm 0.8$  mg  $\text{NO}_3^-$ -N/L in both RA and RB. The effluent nitrate concentration gradually decreased over time in RA and RB, with levels decreasing to  $5.0 \pm 1.5$  mg  $\text{NO}_3^-$ -N/L after day 70, then remaining almost constant. In other similar studies, polycaprolactone, used as a carbon source and biofilm carrier, formed biofilm in 16 days (Shen *et al.* 2013). The removal of TN was consistent with that of nitrate. The nitrate removal efficiency was 92.14% and 95.57% in RA and RB, respectively, while the TN removal efficiency was

89.63% and 95% in RA and RB, respectively. The denitrification performance of RB was slightly better than that of RA, indicating that the inoculation of domesticated denitrifying bacteria was not the limiting factor for denitrification because MSSH itself could readily breed denitrifying bacteria under the condition of a slow flow rate. The values of bacterial counts were  $10^2$  CFU/mL and  $10^3$  CFU/mL in the RA and RB effluents, respectively. Because the bacterial counts in groundwater were far lower than in activated sludge, this sort of denitrifier acclimation could require a

longer time than inoculation with activated sludge. However, this method could prevent the deterioration of the effluent water quality and potential for contamination with pathogenic bacteria in activated sludge contaminating the water being treated (Wang & Wang 2013).

In the static experiments, nitrate and TN concentrations rapidly decreased and remained almost constant in the PRB; however, nitrate and TN concentrations gradually decreased over time in the effluent. Nitrate and TN were removed in the PRB, but not in the outlet flume, resulting in a large concentration difference between the effluent and the PRB. Therefore, nitrate and TN were moved from the outlet flume to the PRB until the concentration was balanced.

#### Nitrite and ammonium accumulation

During the first 36 days of the experiment, nitrite as the intermediate product of nitrate reduction accumulated significantly in the PRB of RA and RB, reaching  $2.13 \pm 0.1$  mg  $\text{NO}_2^-$ -N/L and  $2.6 \pm 0.1$  mg  $\text{NO}_2^-$ -N/L, respectively. There was then an abrupt decrease in nitrite accumulation from the initial levels to  $0.4 \pm 0.1$  mg  $\text{NO}_2^-$ -N/L and  $0.5 \pm 0.1$  mg  $\text{NO}_2^-$ -N/L in the PRB of RA and RB on day 57, after which these concentrations remained constant (below  $0.5 \pm 0.1$  mg  $\text{NO}_2^-$ -N/L). Similarly, the nitrite concentration of the effluent also increased and then remained stable in RA and RB. Nitrite accumulation was probably caused by the incomplete reduction of nitrate, after which  $\text{NO}_2^-$  was not further reduced to  $\text{N}_2$ . Several factors have been shown to cause this phenomenon, such as organic carbon supply, pH, oxygen content, and phosphate concentration (Gómez *et al.* 2002).

Significant amounts of ammonium accumulated in the PRB were observed throughout the experiments in RA and RB, reaching a maximum of  $2.3 \pm 0.3$  mg  $\text{NH}_4^+$ -N/L and  $7.5 \pm 0.3$  mg  $\text{NH}_4^+$ -N/L, respectively. However, the effluent ammonium concentrations were below  $1.0 \pm 0.1$  mg  $\text{NH}_4^+$ -N/L in RA and RB throughout the experiments. The ammonium accumulation was probably due to the occurrence of dissimilatory nitrate reduction to ammonium (DNRA). DNRA is a further anaerobic reduction reaction that converts nitrate to ammonium rather than  $\text{N}_2$  (Shen *et al.* 2013).

### In-situ dynamic groundwater remediation experiments

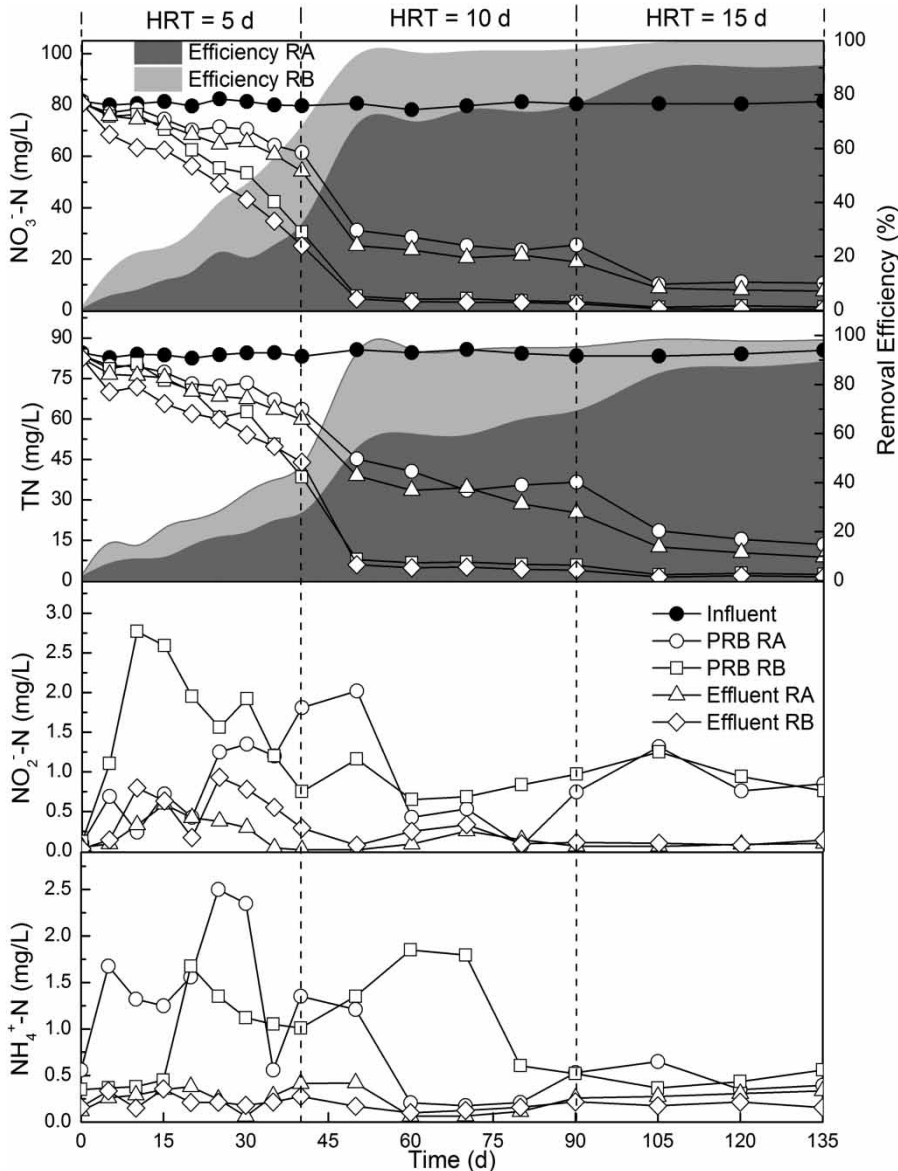
#### Nitrate and TN removal

The denitrification performance of dynamic experiments is given in Figure 3. The concentration of nitrate in the effluent decreased as the HRT increased. The effluent nitrate concentrations in RA and RB decreased to  $54.2 \pm 0.3$  mg  $\text{NO}_3^-$ -N/L and  $25.2 \pm 0.3$  mg  $\text{NO}_3^-$ -N/L with 24.12% and 54.5% nitrate removal respectively when the HRT was 5 d. The effluent nitrate concentrations in RA were  $18.9 \pm 0.3$  mg  $\text{NO}_3^-$ -N/L and  $7.6 \pm 0.2$  mg  $\text{NO}_3^-$ -N/L at HRTs of 10 and 15 d, respectively, with nitrate removal efficiencies of 76.49% and 90.71%, respectively. However, the nitrate removal efficiency in RB was above 96% and no significant differences were observed between operation at HRTs of 10 and 15 d. The removal of TN was consistent with that of nitrate; consequently, the performance of denitrification was positively associated with the HRT. This was possible because more substrate was released and consumed under the longer HRTs. These findings were similar to those of Yusoff *et al.* (2010), who also found that the biomass was washed out and denitrifying bacteria had a short contact time with the surfaces of carbon sources at lower HRTs.

In the dynamic experiments, the denitrification efficiency of RB was superior to that of RA. This was ascribed to the lower bacterial counts in groundwater than in activated sludge, which resulted in slow growth of the biofilm on the surface of the carbon source. Wang & Wang (2013) also reported that nitrate could be removed with a biofilm formation period of 23 days in a denitrification reactor without inoculation of external microorganisms when using biodegradable snacks as a carbon source.

#### Nitrite and ammonium accumulation

Significant nitrite accumulation was observed in the PRB of RA ( $1.8 \pm 0.4$  mg  $\text{NO}_2^-$ -N/L) and RB ( $2.0 \pm 0.5$  mg  $\text{NO}_2^-$ -N/L) when the HRTs were 5 and 10 d. However, nitrite concentrations decreased to  $0.9 \pm 0.3$  mg  $\text{NO}_2^-$ -N/L in the PRB of RA and RB when the HRT was 15 d. Furthermore, nitrite concentrations were kept at a low level in the effluent of RA ( $0.08 \pm 0.012$  mg  $\text{NO}_2^-$ -N/L) and RB ( $0.09 \pm 0.015$  mg



**Figure 3** | Evolution of nitrate ( $\text{NO}_3^-$ -N), total nitrogen (TN), nitrite ( $\text{NO}_2^-$ -N), ammonium ( $\text{NH}_4^+$ -N) (mg/L), and removal efficiency (%) for in-situ dynamic groundwater remediation experiments.

$\text{NO}_2^-$ -N/L). An evident decrease in nitrite concentration in the PRB was observed as the HRT increased. Therefore, the nitrite accumulation may be attributable to the short HRT which resulted in increased nitrite accumulation and decreased nitrate removal because of the low contact time for microbial activity and wash-out of bacteria (Damaraju *et al.* 2015).

High ammonium formation in the PRB of RA ( $2.0 \pm 0.2$  mg  $\text{NH}_4^+$ -N/L) and RB ( $1.4 \pm 0.2$  mg  $\text{NH}_4^+$ -N/L) was

observed when the HRT was 5 and 10 d. As shown in Figure 3, there was an abrupt decrease in ammonium accumulation from the initial levels to  $0.3 \pm 0.1$  mg  $\text{NH}_4^+$ -N/L when the HRT was 15 d. Conversely, the effluent ammonium concentrations were below  $0.38$  mg  $\text{NH}_4^+$ -N/L in RA and RB throughout the experiments. Further, ammonium was generated from DNRA. Overall, these findings indicate that DNRA occurred more easily at shorter HRTs than at longer HRTs.

## Surface morphology

At the end of the experimental period, MSSH samples were collected from the PRB of the reactor and analyzed by SEM, together with untreated maize stalks and MSSH from before the experiments. The surface structure of untreated maize stalks was porous and fibrous, with a flaky appearance of husk ash that was beneficial for the formation of biofilm. The surface structure of MSSH before the experiment was uneven, with a compact structure of protrusions and voids, which was more favorable to the multiplication of denitrifying bacteria and the formation of biofilm. These findings suggested that a part of the hemicellulose and lignin in the maize stalks was degraded by the sodium hydroxide solution. A large number of pore structures could be seen in the surface of MSSH after the experiment, which probably reflected fractures in the fibers due to microbial use. The increased pore structures provided more space for bacterial attachment and growth. Therefore, maize stalks can be used as biofilm carriers.

## CONCLUSIONS

The results demonstrated that nitrate can be effectively eliminated from nitrate-contaminated groundwater under static and dynamic conditions using an in-situ remediation reactor packed with MSSH as carbon source and biofilm support. In static remediation experiments, nitrate was effectively reduced in the conventional denitrification process, with a nitrate reduction efficiency of more than 92%, regardless of whether the system was inoculated with external microorganisms. This was because indigenous microorganisms in groundwater proliferated readily, resulting in stable biofilm formation. In dynamic remediation experiments, external microorganisms were necessary for high nitrate removal efficiency, and the performance of denitrification was positively associated with the HRT.

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