



Nitrate removal from groundwater by cooperating heterotrophic with autotrophic denitrification in a biofilm–electrode reactor

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ABSTRACT

An intensified biofilm–electrode reactor (IBER) combining heterotrophic and autotrophic denitrification was developed for treatment of nitrate contaminated groundwater. The reactor was evaluated with synthetic groundwater (NO_3^- –N50 mg L^{−1}) under different hydraulic retention times (HRTs), carbon to nitrogen ratios (C/N) and electric currents (*I*). The experimental results demonstrate that high nitrate and nitrite removal efficiency (100%) were achieved at C/N = 1, HRT = 8 h, and *I* = 10 mA. C/N ratios were reduced from 1 to 0.5 and the applied electric current was changed from 10 to 100 mA, showing that the optimum running condition was C/N = 0.75 and *I* = 40 mA, under which over 97% of NO_3^- –N was removed and organic carbon (methanol) was completely consumed in treated water. Simultaneously, the denitrification mechanism in this system was analyzed through pH variation in effluent. The CO_2 produced from the anode acted as a good pH buffer, automatically controlling pH in the reaction zone. The intensified biofilm–electrode reactor developed in the study was effective for the treatment of groundwater polluted by nitrate.

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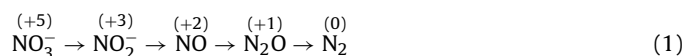
1. Introduction

Groundwater is widely used for drinking water in most countries. Nitrate is simply transported to groundwater through uncontrolled discharge of nitrate containing sources such as chemical fertilizers, industrial or domestic wastes, and landfill leachate [1]. The reduction of nitrate to nitrite will cause methemoglobinemia in the fetus, known as “blue baby syndrome” [2]. Also, during consumption, nitrate is reduced to nitrite in the intestine, which has been linked to several cancers. In view of these problems, many countries promulgate specific regulations to set the maximum contaminating levels of nitrate in groundwater. The value of nitrate nitrogen proposed by China is 10 mg L^{−1} [3], which is in accordance with that proposed by the World Health Organization [4].

The traditional physicochemical methods used to eliminate nitrate from water are ion exchange, reverse osmosis and electro-dialysis [5–7]. However, these approaches have been found to be cost-ineffective due to high installation or maintenance costs and also the concentrated waste brines require further treatment or disposal [4].

Biological denitrification is widely applied in nitrate removal from groundwater attributing to its high energy efficiency. In den-

itrification process, the complete reduction of nitrate (NO_3^-) into nitrogen gas (N_2) is achieved as the following approach [8]:



Biological denitrification can be achieved in an autotrophic or a heterotrophic manner. The heterotrophic denitrification process is applied most extensively because of its high efficiency and the simplicity of the reactors required. However, effluent turbidity increasing due to bacterial growth [9], and excessive organic carbon resulting in secondary pollution [10] make it unfavorable. A number of researchers developed natural materials (wheat straw, plant prunings etc.) as organic carbon sources for use in heterotrophic denitrification [11,12]. The method was cost-effective but the pre-treatment process was complicated and lengthy [13]. Furthermore, the CO_2 produced during heterotrophic denitrification was wasted in all of the studies mentioned above.

Autotrophic denitrification has the advantage of low microbial output and activated sludge, and no secondary pollution. Many researchers have studied autotrophic denitrification using sulfur as an electron donor, as it is cheaper and easier to handle than heterotrophic denitrification using organic chemicals [14,15]. However, a high content of sulfur would be created in the treated water. According to the literature, hydrogen bacteria is one of the autotrophic bacteria which grows fastest, its life cycle is 24 times to sulfur bacteria [16]. Therefore, hydrogen sparged into water to be treated hydrogenotrophically has been described [17,18]. How-

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ever, H_2 is so sparingly solubility in water (1.6 mg L^{-1} at 20°C) that sparging H_2 will create waste, and the requirement for an additional hydrogen source has disconcerting security issues, low utilization and high costs.

Biofilm–electrode reactors (BER) were developed on the basis of internal production of H_2 performed by the electrolysis of water. Sakakibara [19] and Feleke [20] immobilized denitrifying microorganisms on the cathode and hydrogen was simultaneously produced by the cathode. Ghafari [21] used an upflow bio-electrochemical reactor (UBER) to accommodate hydrogenotrophic denitrifying bacteria employing palm shell granular activated carbon (GAC) as the biocarrier and cathode material, and nitrate can be entirely reduced within application of a wide operational range of electric current ($10\text{--}16 \text{ mA}$) as well as HRT ($13.5\text{--}30 \text{ h}$). Though BERs mentioned above could be used for better hydrogen exploitation, cathode not only provided hydrogen, but also was used as carriers, and nitrate removal capacity was comparatively limited.

To reduce HRTs and efficiently improve the denitrification rate, multi-electrode and three-dimensional electrode systems were proposed [22–24]. These types of systems achieved excellent performance mainly attributed to the enlarged surface area of their electrodes. Simultaneously, the pH was maintained at around neutral and a favorable anoxic environment was distinct advantages. The denitrification rate could reach more than $0.20 \text{ mg NO}_3^- \text{--N cm}^{-2} \text{ d}^{-1}$ while the current efficiency could reach as high as 200%, indicating excellent potential in applications for groundwater remediation [4,24].

Though hydrogenotrophic BERs has made substantial development, long acclimatization processes of autotrophic bacteria, biofilm detachment and low capacity of bacteria are still problems. Moreover, all autotrophic denitrification are limited by inorganic carbon (CO_2 , carbonate etc.) availability [25].

To the best of our knowledge, few researchers have referred to nitrate removal using cooperation between heterotrophic and hydrogenotrophic denitrification. Rocca [26] coupled heterotrophic–autotrophic denitrification (HAD) processes, supported by cotton and zero valent iron, and HAD of drinking water was shown to have reasonable application in permeable reactive barriers (PRB). However, by-products such as total organic carbon (TOC) increased and bacterial release became limiting factors. In any case, no publications have advocated cooperating heterotrophic and autotrophic denitrification and the effective utilization of the product (CO_2) formed by heterotrophic denitrification.

In this study, an intensified biofilm–electrode reactor (IBER) combining heterotrophic and hydrogenotrophic denitrification was developed, in which bacteria use fiber threads as independent carrier and form a biofilm, carbon rods are used as the anode, with stainless steel wire embedded in fiber threads acting as the cathode to provide H_2 for autotrophic bacteria. The bacteria then used CO_2 , which was the product of heterotrophic denitrification, as part of inorganic carbon source. The objective of this work was to investigate the effect of HRT, carbon to nitrogen ratios (C/Ns), and electric current (I) on nitrate remediation and to optimize the operating parameters of the reactor for cooperative heterotrophic denitrification and autotrophic denitrification.

2. Materials and methods

2.1. Experimental apparatus

A set of closed plexiglass cylinders (diameter 300 mm, height 300 mm), as illustrated in Fig. 1, was used in this study. The main reactor compartments consisted of an influent plastic tank (25 L), a water collector (diameter 80 mm, height 200 mm), carriers (fiber threads), eight carbon rods (diameter 10 mm, height 250 mm) as

anodes and stainless steel wire (diameter 1 mm, length 5000 mm) as cathodes. The water collector was installed in the center of the cylinder. 108 holes (diameter 5 mm, distance between hole centers 20 mm) were drilled symmetrically in the collector wall. Each hole was filled with 7 or 8 fiber threads (thickness 1 mm, length 100 mm). All threads bound on the collector made up about 1/10 of the effective total reactor volume. Stainless steel wire was spirally inserted in the fiber threads as the cathode to produce hydrogen by electrolyzing water. Carbon rods encircled the collector, equi-spaced along the cylinder wall to provide the inorganic carbon source (CO_2) through carbon oxygenation. A peristaltic pump with two channels (LEAD-2, Lange Company, Baoding, China) was used to control the flow rate of influent and effluent. The water inside the reactor was recirculated using a magnetic pump (MP-15RN, Xishan Company, Wenzhou, China). A DC regulated power supply (HY1792-5S, 0–50 V, 0–5 A) was used to provide the current.

Synthetic groundwater was pumped from the influent tank into the IBER through the bottom inlet of the reactor and was circulated by a magnetic pump. Influent flowed through the biofilm and nitrate was denitrified by heterotrophic and autotrophic bacteria. Effluent was drawn from the water collector.

As mentioned in our previous study, the advantages of this process design are strong adhesion of microorganisms, low cost and easy management [27]. The biofilm was not only generated rapidly and efficiently but also worked as a filter, preventing suspended solids (SS) and other impurities getting into the treated water, therefore saving a sedimentation tank or membrane filtration [23]. Simultaneously, the inorganic carbon source and hydrogen gas could be utilized more effectively in this reactor design.

2.2. Microorganisms and synthetic water

Denitrifying bacteria which formed the biofilm on the surface of fiber threads was collected from the Qinghe wastewater treatment plant (Beijing, China).

The synthetic water was prepared by dissolving 0.304 g L^{-1} NaNO_3 , 0.044 g L^{-1} KH_2PO_4 , and CH_3OH according to different C/Ns in tap water. The original pH of this solution was normally about 7.5 and needed no further adjustment.

2.3. Experiment procedure

About 4 L of anaerobic sludge was placed in a refrigerator with nutritive material at 4°C . CH_3OH , NaNO_3 and KH_2PO_4 were added into the anaerobic sludge according to C/N/P = 3:1:0.2. After 7 days, anaerobic sludge was placed into the reactor and 8 L of tap water was added to a total of 12 L. CH_3OH , NaNO_3 and KH_2PO_4 were then added to the influent tank and the applied current was adjusted to 10 mA. The sludge–water was circulated by a magnetic pump for the first 3 days. 15 days later, influent and effluent could be run continuously because a dark grey color covered the threads, indicating that the biofilm had formed well. The concentration of $\text{NO}_3^- \text{--N}$ in influent was maintained around 50.00 mg L^{-1} . The system was started up when more than 80% of the nitrate was eliminated and the whole cultivated process took 25 days. Dissolved oxygen (DO) was kept below 0.5 ppm and pH was automatically maintained at 7.0–8.0 in the reactor.

Operational conditions (HRT, C/N, I and $\text{COD}_{(\text{cr})}$) for each run were maintained until steady state in the nitrate and nitrite removal. Each sample was taken for at least three successive HRTs.

2.4. Analytical methods

$\text{NO}_3^- \text{--N}$, $\text{NH}_3 \text{--N}$ and $\text{NO}_2^- \text{--N}$ were determined by ultraviolet spectrophotometer (HACH, DR 5000) according to standard

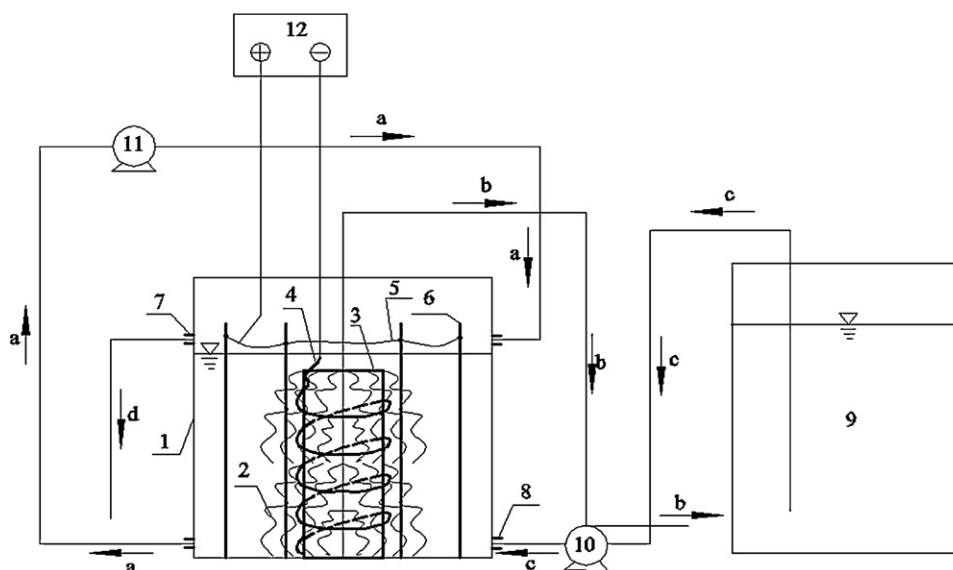


Fig. 1. Experimental apparatus. 1 reactor; 2 carriers (fiber thread); 3 water collector; 4 cathode (stainless wire); 5 conducting wire; 6 anode (carbon rods); 7 overflow pipe; 8 inlet; 9 influent tank; 10 peristaltic pump; 11 magnetic pump; 12 power supply; a backflow pipe; b effluent pipe; c influent pipe.

methods. $\text{COD}_{(\text{cr})}$ was also measured by standard methods. pH in influent and effluent was determined by pH meter (LIDA pH 3C). A thermometer was placed into the reactor to monitor the real-time temperature during the experiments.

3. Results and discussion

3.1. Effect of HRT on nitrate and nitrite removal

It is apparent from Fig. 2 that the concentration of NO_3^- -N in effluent remained consistently below 2.00 mg L^{-1} at different HRTs. NO_3^- -N out of biofilm (the solution between cylinder interior wall and the outer part of the fiber threads biofilm, and there are bacteria in suspension which reduce nitrogen concentrations, abbreviated OFB) and in effluent slightly fluctuated because the increase in flow rate impacted the biofilm reactor when HRT was changed. Nitrate removal efficiency achieved 100% after a short-term adaption when the HRT was longer than 8 h.

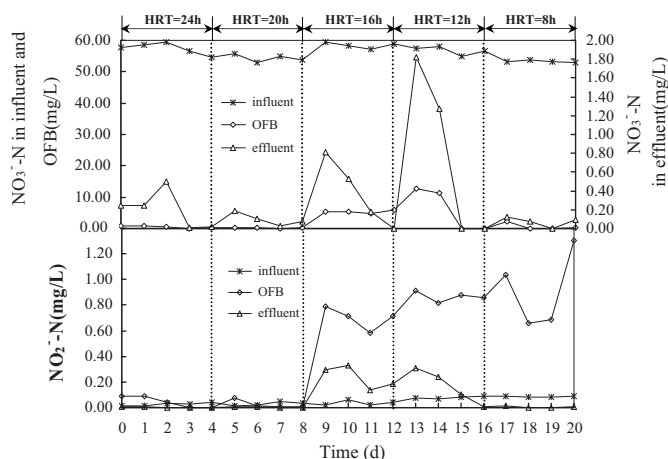


Fig. 2. Effect of HRTs on nitrate and nitrite removal. NO_3^- -N = 50 mg L^{-1} , C/N = 3, $I = 10 \text{ mA}$ with initial applied voltage 2.50 V .

During the initial stage of the experiment, there were almost no denitrifying bacteria in influent water tank. NO_2^- -N in influent increased with time because a few denitrifying bacteria began to grow in the bucket wall. Nitrate was partially transformed to nitrite before flowing into the reactor. Therefore, the influent tank was cleaned regularly, so as to keep nitrate loading above 47 mg L^{-1} .

NO_2^- -N in OFB was obviously higher than that in influent and effluent and fluctuated with a slight upward trend. NO_2^- -N in effluent remained stable at almost 0 at the first two HRTs (24 and 20 h) but accumulated to about 0.30 mg L^{-1} when HRT was changed to 16 and 12 h, and finally became constantly undetectable at HRT = 8 h. NO_2^- -N in effluent decreased when HRT was shortened in the range of 16–8 h. This behavior is non-intuitive but indicates that the biofilm grown to maturity in the course of gradual acclimation and the denitrification efficiency was accordingly greatly improved.

The increasing trend of nitrite in OFB and decreasing trend of nitrite in effluent shows that nitrate was first transformed to nitrite, which is an intermediate product, and then nitrite was deoxidized to nitrogen gas. Compared with our previous study [27], the application of current shortened the acclimation periods in HRTs to 20 days. Denitrifying bacteria could acclimatize to alterations in HRT rapidly in the case of mild electric current and adequate organic carbon source.

HRT is one of the most important parameters for BERs and determines the treatment capacity of reactors at a certain influent velocity. Increasing residence times in the reactor allowed the bacteria to degrade the organic matter and to consume nitrate to greater extents. Based on our previous research [27], the appropriate HRT was at 8 h when NO_3^- -N in influent was about 50 mg L^{-1} . Wu [28] reported the same appropriate HRT by electrode–biomembrane method.

3.2. Effect of C/Ns on nitrate and nitrite removal

Fig. 3 shows that the concentration of NO_3^- -N in effluent increased from 0.01 to 18.57 mg L^{-1} when C/N was reduced from 2.00 to 0.75 . NO_3^- -N was close to 0.01 mg L^{-1} at C/N = 1 which

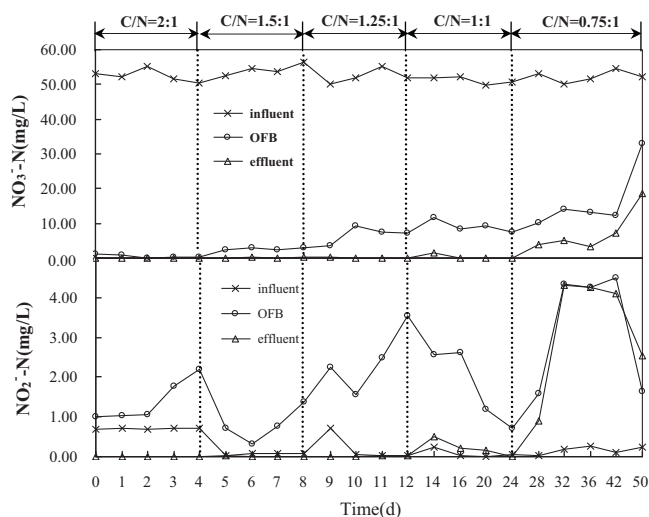
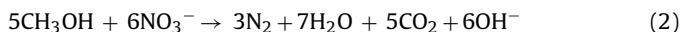


Fig. 3. Effect of C/Ns on nitrate and nitrite removal. $\text{NO}_3^- - \text{N} = 50 \text{ mg L}^{-1}$, HRT = 8 h, $I = 10 \text{ mA}$.

is superior to our previous research using single heterotrophic denitrification, in which $\text{NO}_3^- - \text{N}$ was $0.36 \pm 0.36 \text{ mg L}^{-1}$ at $\text{C/N} = 1$ [27]. The concentration of $\text{NO}_2^- - \text{N}$ in effluent was lower than 0.02 mg L^{-1} when C/N was decreased from 3.00 to 1.00, while the concentration of $\text{NO}_2^- - \text{N}$ was $0.83 \pm 0.16 \text{ mg L}^{-1}$ at $\text{C/N} = 1.00$ in our previous research [27]. The concentration of $\text{NO}_2^- - \text{N}$ exceeded 2.5 mg L^{-1} at $\text{C/N} = 0.75$. Four days were required to achieve complete denitrification when the C/N was greater than 1.25. However, the reactor took 12 days to reach the same goal at $\text{C/N} = 1$. The denitrifying bacteria therefore apparently required a longer adaptive process when C/N was relatively low.

High C/N ratios could accelerate the growth of heterotrophic denitrifying bacteria in the biofilm and thus promote the total denitrification rate [24]. The dependence on the organic carbon source determined the high sensitivity to changes of organics during heterotrophic denitrification. This explains why the concentration of $\text{NO}_3^- - \text{N}$ and $\text{NO}_2^- - \text{N}$ in OFB always exceeded that in effluent since C/Ns were decreased and simply indicates the fact that, in our cooperative heterotrophic and autotrophic denitrification system, heterotrophic denitrification dominated in the OFB, whereas autotrophic denitrification played a leading role inside the biofilm. The concentration of $\text{NO}_3^- - \text{N}$ and $\text{NO}_2^- - \text{N}$ in OFB began to rise in the initial few days. However, significant variation of $\text{NO}_3^- - \text{N}$ and $\text{NO}_2^- - \text{N}$ was observed at $\text{C/N} = 0.75$ in effluent. This further confirms that autotrophic denitrification occupied a more important position inside the biofilm than in OFB, which directly affected the denitrification efficiency in effluent. The results may be attributed mainly to the design of reactor, in which the cathode was embedded in the fibrous carrier. Such a structure guaranteed that more heterotrophic and autotrophic bacteria adhered to the surface of the biofilm. Meanwhile, high mass transfer efficiency for hydrogen produced by the cathode was obtained inside the biofilm.

In single heterotrophic denitrification, as shown in Eq. (2), the C/N ratios for complete denitrification were established as 0.71 according to theoretical stoichiometric equations when the carbon source was methanol [29].



In this study, a C/N ratio of 0.75 was already very close to the theoretical value of single heterotrophic denitrification. Completed denitrification at $\text{C/N} = 1$ has outperformed many other systems reported [28]. It is also consistent with that of Bao and Hao [30], who found that the electrode biofilm method obtained higher denitrification efficiency than a single biofilm method when the C/N

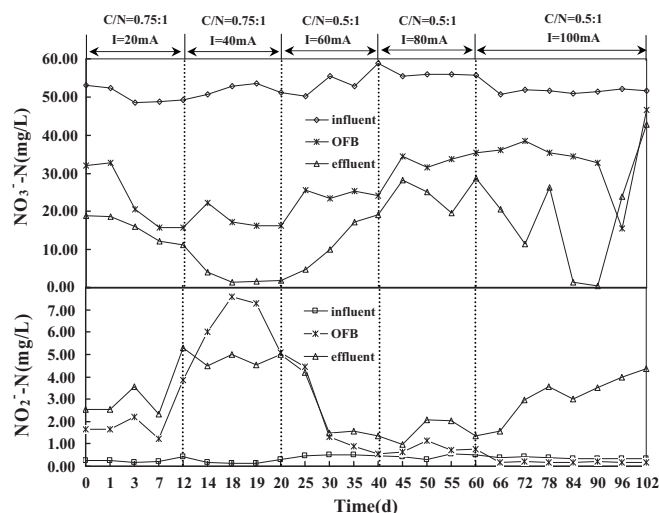


Fig. 4. Effect of applied current on nitrate and nitrite removal. $\text{NO}_3^- - \text{N} = 50 \text{ mg L}^{-1}$, HRT = 8 h.

was relatively low ($\text{C/N} = 1$). This desirable result was inseparable from the effect of autotrophic denitrification at $I = 10 \text{ mA}$.

3.3. Effect of applied current on nitrate and nitrite removal

To ascertain the contribution of the electrochemical effect, a background experiment was carried out before inoculation with bacteria. With carbon electrode, the maximum removal was only 6.80%, which is consistent with the results obtained by Dash [31] that graphite electrode showed 8% nitrate reduction.

As shown in Fig. 4, the concentration of $\text{NO}_3^- - \text{N}$ in OFB and the effluent decreased, while the concentration of $\text{NO}_2^- - \text{N}$ in OFB and the effluent rose with applied current increasing from 20 to 40 mA at $\text{C/N} = 0.75$. When the removal efficiency attained equilibrium at $I = 40 \text{ mA}$, $\text{NO}_3^- - \text{N}$ in the treated water was 1.75 mg L^{-1} . The biofilm-electrode reactor lived up to the expected high efficiency, with over 96.8% nitrate removal.

To clarify the roles of heterotrophic and autotrophic denitrification in the presence of applied current, the differences in nitrate removal rate in OFB and the effluent were studied. In the current range of 20–40 mA, nitrate in OFB was degraded from 32.85 to 16.33 mg L^{-1} , with a removal rate of 43.47%, while nitrate in effluent was reduced from 18.57 to 1.75 mg L^{-1} with a removal rate of 90.58%. As shown in Fig. 3, heterotrophic denitrification dominated in OFB and autotrophic denitrification dominated inside the biofilm near the outlet. Apparently, apart from a slight electrochemical effect, the promoting effect of current on autotrophic denitrification was vastly greater than that on heterotrophic denitrification. This outcome demonstrates that the presence of current is more conducive to the growth of autotrophic denitrification bacteria and that the autotrophic denitrification rate was accelerated as denitrifying bacteria adapted to the environment. Nitrite was accumulated to a concentration of 4.75 mg L^{-1} at $I = 40 \text{ mA}$. Similar literature reports found an optimal electrical intensity at 20 mA and if the current was higher than this value, a high concentration of nitrite would be left in treated water [21,32].

Changing the C/N ratio to 0.5 and increasing the applied current from 60 to 100 mA, caused the nitrate removal rate to progress in three stages of falling, steady, and unstable at $I = 60, 80$ and 100 mA , respectively. The carbon source was completely insufficient at a $\text{C/N} = 0.5$ for heterotrophic denitrification and consequently, the nitrate in OFB was maintained at an elevated level in the current range 60–100 mA. In the falling region at $I = 60 \text{ mA}$, $\text{NO}_3^- - \text{N}$ in effluent increased to 20 mg L^{-1} , while $\text{NO}_2^- - \text{N}$ decreased to 1.2 mg L^{-1} .

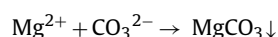
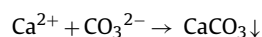
Although the applied current was increased, autotrophic bacteria could not be acclimatized so rapidly, so the nitrate removal rate dropped and nitrite accumulation also declined. Another possible cause of the behavior might be that the increase in current oxidized nitrite to nitrate.

At $I=80$ mA, nitrate and nitrite removal in effluent and OFB remained comparatively steady but a small amount of white powder began to appear in effluent.

X-ray diffractometry (D/MAX 2500 or TTR) was used to identify the specific components of the white powder. The result indicated that the main components of the powder were CaCO_3 , MgCO_3 and $\text{Ca}(\text{Mg})\text{CO}_3$. Meanwhile, small quantities of ferric carbonate (FeCO_3) and carbon (C) powder were detected. Calcium and magnesium ions exist in tap water and these would deposit onto the surface of the cathode during electrolysis. CO_2 was dissolved in water and CO_3^{2-} was generated according to Eqs. (3) and (4):



Sediment was produced as follows:



Unfortunately, the sediment could adversely affect electrolysis at the cathode. Although the deposited calcium and magnesium could be re-dissolved by changing the polarity of the electrodes [33], inevitable sedimentation caused biological carrier caking and this directly impacted microbial activity, leading to a decline in nitrate removal.

Fe ion would be dissolved out by microorganisms corrosion in aquatic environment [34]. Moreover, this was an anaerobic system with ions HCO_3^- and CO_3^{2-} , which provided favorable conditions for forming FeCO_3 .



Carbon from the anode could be stripped out into water in minor quantities. When the current was increased, the color of the effluent would deepen, as mentioned elsewhere [32].

When the current was increased to 100 mA, the nitrate levels in effluent became unstable, while nitrite accumulation increased linearly. This indicates that increased current accelerated electrolytic action. An excessively high concentration of hydrogen has been reported as an inhibitor of denitrification and a cause of nitrite accumulation [21,32,35]. On day 90, nitrate removal efficiency reached 100% because of the embedding of additional stainless wire (cathode material) in the carriers. However, the effect this produced as not sustained for a long time. Less than 10% nitrate removal in effluent marked the end of the experiment. This outcome shows that such a high current is not suitable for the cooperative heterotrophic and autotrophic denitrification system.

3.4. pH variation under different applied currents

As shown in Fig. 5, pH in influent was stabilized between 7.5 and 7.7 throughout the experiment. The pH in OFB decreased from 7.6 to 7.1 when the applied current was set from 10 to 100 mA. The majority of researchers admittedly agree that the optimum pH for denitrifying bacteria is between 7.0 and 7.5 in the environment. Therefore, no additional reagents were needed for pH adjustment, which is superior to many other systems [21,31,32,36]. pH in effluent increased significantly with increasing current. At an applied current of 100 mA, the pH in effluent reached 11.7.

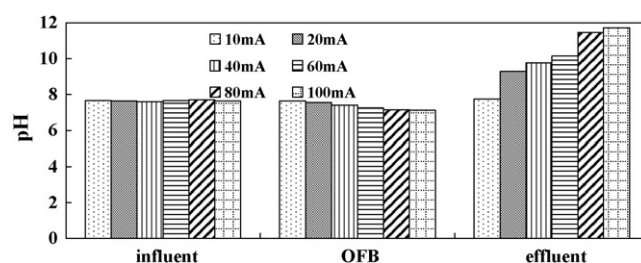
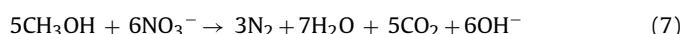


Fig. 5. pH variation under different applied current.

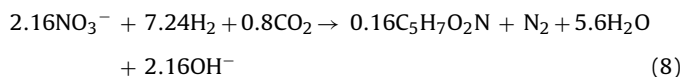
The degradation mechanism for the denitrification process could be deduced from the pH variation.

The process of heterotrophic denitrification process is



OH^- was produced in the degradation reaction, which caused the rise in pH in accordance with the theory. However, the pH in the environment of biofilm growth did not increase; on the contrary, it was slightly decreased in OFB. This phenomenon mainly occurred due to the autotrophic denitrification. Specific causes can be explained as follows.

In autotrophic denitrification, the process is as shown in Eq. (8)



A small amount of carbon dioxide arose from products of heterotrophic denitrification but was mainly generated by oxidation of the carbon rods. The main reaction at the anode was:



Hydrogen gas was produced by electrolysis of water. The main reaction at the cathode was:



H_2CO_3 and HCO_3^- were present in the water, as CO_2 was dissolved. H^+ , H_2 , H_2CO_3 , and HCO_3^- were then efficiently utilized by bacteria in the biofilm. Carbon dioxide and bicarbonate diffused to the biofilm and this process buffered the pH value. Moreover, increased applied current caused a higher CO_2 yield, so as to decrease the pH values out of the biofilm. Meanwhile, H^+ moved from inside to outside the biofilm under the external electric field. Therefore, bacteria attached to the biofilm could use H^+ effectively for denitrification. The spread of hydrogen gas produced an anoxic environment around the biofilm, which was conducive to the growth of denitrifying bacteria. Conversely, efficient utilization of hydrogen gas avoided the difficulties in transportation and low efficiency. Nevertheless, the pH value in effluent increased partly because of the OH^- generated in the overall reaction (Eq. (8)) when effluent water was pumped from the water collector. Also, the cathode was located near the water collector, so part of the OH^- produced according to Eq. (10) would have entered the effluent by convective flow. The increase of pH to 11.7 at the cathode zone inhibited nitrite reduction, as shown in Fig. 4, in agreement with other work [21].

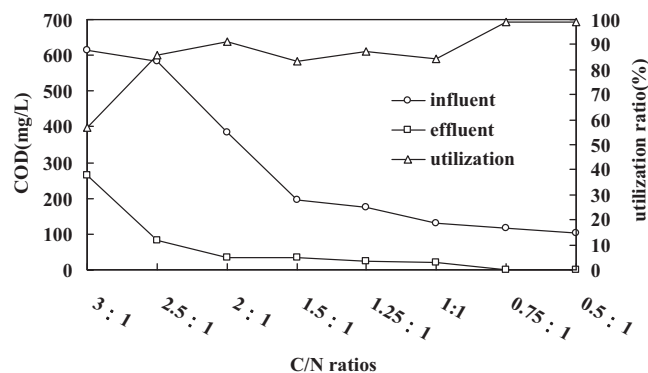
3.5. Utilization of organic carbon source at different C/N ratios

Methanol was used as the carbon source for heterotrophic denitrification in this study, while carbon dioxide was utilized for autotrophic denitrification. Autotrophic denitrification consumed carbon dioxide, which was intermediate product from heterotrophic denitrification, to decrease the demand for methanol. In our previous study [27], COD was down from 180.2 to 20.4 mg L^{-1}

Table 1
Comparison with other denitrification systems.

Reactors	Effective volume (L)	H/A	Initial (NO_3^- -N) (mg L^{-1})	Carbon source	Current (mA)	C/N	HRT (h)	Removal efficiency (%)	Nitrogen volume-reduction rates ($\text{gN/m}^3\text{d}$)	Reference
Multi-cathode BER combined with microfiltration (MF)	0.6	A	25	–	60	0	2.88	80	167	[23]
Multi-cathode BER	36	A	20	–	960	0	6	80	64	[22]
A packed bed BER	0.33	A	40	–	14	0	8.25	100	116	[36]
A novel upflow BER	0.8	A	20	–	10–17	0	14–41	100	34	[21]
Combined bioelectrochemical and sulfur autotrophic denitrification system (CBSAD)	33.47	A	20.9–22.0	–	1000	0	2.1	95	227	[37]
BER	0.205	A	22.5	–	5	0	10	100	54	[33]
A three-dimensional (3D) bio-electrochemical reactor	0.6	H&A	20	$\text{C}_2\text{H}_5\text{OH}$	1.5	0.95	4	100	120	[24]
BER	2.5	H&A	35	CH_3COONa	80	1	8	90	105	[30]
BER	0.216	H&A	100	CH_3COONa	20	3	10	93	223	[38]
Membrane bioreactor	4	H	20	CH_3OH	–	Enough	5.3	89	81	[39]
A fiber-based biofilm reactor	12	H	50–100	CH_3OH	–	1.25	8	99	149	[27]
Intensified BER	12	H&A	50	CH_3OH	40	0.75	8	97	146	This study

Note: H, heterotrophic; A, autotrophic.

**Fig. 6.** Utilization of organic carbon source at different C/N ratios.

with a C/N from 3.00 to 1.50, and COD in effluent was undetectable at C/N = 1.25 and 1.00. However, in this study, COD was 265.7 and 32.7 mg L^{-1} when the C/N ratios were 3 and 1.5, respectively, and the COD in effluent was higher than 20.0 mg L^{-1} at C/N = 1.25 and 1.00, as shown in Fig. 6. This means that the organic carbon source was sufficient for heterotrophic denitrification when the C/N ratio was 1.00, which is superior to the performance reported in earlier research [27]. COD was not detected in effluent until the C/N ratio decreased to 0.75. When the organic carbon source was adequate at C/N of greater than 1, heterotrophic denitrifying bacteria were dominant. Autotrophic denitrifying bacteria began to play a more important role when the C/N was 0.75. This clearly indicates that the cooperation of heterotrophic denitrification and autotrophic denitrification enhanced the removal efficiency of nitrate in the intensified biofilm electrode reactor. Furthermore, the maximum utilization of methanol was 91.3% at C/N=2 when the organic carbon source was adequate. When the organic carbon source insufficient (C/N=0.75 and 0.5), COD was undetectable in effluent because the organic carbon source was completely used by the heterotrophic bacteria and therefore there was no residual methanol in effluent. This outcome avoids the disadvantage of incomplete use of methanol causing secondary pollution to the environment. Consequently, the cooperative process is superior to single heterotrophic denitrification.

3.6. Comparison with other denitrification systems

To allow a comparison with other biological denitrification systems (including single heterotrophic, single autotrophic, bio-electrochemical, three-dimensional, and combined reactors), the main operational parameters at the best removal capacity for each reactor type are summarized in Table 1.

It can be seen from Table 1 that the cooperative heterotrophic and autotrophic denitrification has significant advantages. Compared with autotrophic denitrification, the obvious superiority lies in a larger treatment capacity and high removal efficiency. Lower organic carbon source utilization and the absence of organic pollution make the process better than heterotrophic denitrification and traditional BERs.

The operating cost of this system mainly includes carbon and energy consumption. 126.40 mL methanol was consumed and 0.22 Chinese yuan (0.034 dollar) was cost to dispose 1 m^3 of water. 0.08 kWh electricity was required and 0.04 Chinese yuan (0.006 dollar) was spent to treat 1 m^3 of water. Therefore, on the basis of China's price, 0.04 dollar was cost for continuous treatment of 1 m^3 of 50 mg L^{-1} nitrate contaminated water. It indicated that a little electricity was consumed but C/Ns was largely decreased, and operating cost was accordingly reduced. It was apparent that the IBER is favorable for treatment of nitrate contaminated groundwater.

4. Conclusions

To effectively treat nitrate polluted groundwater, an intensified biofilm–electrode reactor (IBER) using cooperation between heterotrophic and autotrophic denitrification has been developed. The performance of the IBER was investigated using a laboratory-scale apparatus and based on the experimental results, the following conclusions were obtained.

Nitrate removal efficiency was higher than 97% at the ranges of C/N (3–0.75), HRT (24–8 h), and I (10–40 mA). The optimum parameters were C/N=0.75, HRT=8 h, and I =40 mA, and there was no nitrite accumulation until the C/N was decreased to 0.75. $\text{NH}_3\text{-N}$ and residual organic carbon (methanol) were not detected at the best condition.

It is concluded that the cooperation of heterotrophic and autotrophic denitrification in the IBER is a promising configuration for enhanced treatment of nitrate-contaminated water. The isolation and identification of denitrification bacteria will be studied in the future.

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