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Complete bromate and nitrate reduction using hydrogen as the sole electron donor in a rotating biofilm-electrode reactor

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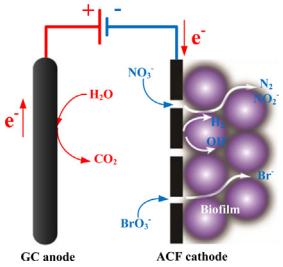
HIGHLIGHTS

- Cathode of RBER was designed to automatically rotate.
- Simultaneous bromate and nitrate removal was achieved by auto-hydrogenotrophic reduction.
- The maximum bromate reduction rate estimated by the Monod equation was 109.12 μg/L h.
- An electron transfer process and main reaction mechanism in RBER was explored.

GRAPHICAL ABSTRACT

Main mechanism of simultaneous bromate and nitrate removal in the RBER.



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ABSTRACT

Simultaneous reduction of bromate and nitrate was investigated using a rotating biofilm-electrode reactor (RBER) with graphite carbon (GC) rods as anode and activated carbon fiber (ACF) bonded with steel ring as cathode. In RBER, the community of denitrifying bacteria immobilized on the cathode surface could completely utilize hydrogen (H_2) as the electron donor, which was internally produced by the electrolysis of water. The short-term test confirmed that the RBER system could reduce 150–800 μg/L bromate to below 10 μg/L under autotrophic conditions. The reduced bromate was considered to be roughly equivalent to the amount of bromide in effluent, indicating that bromate was completely reduced to bromide without accumulation of by-products. The long-term test (over 120 days) showed that the removal fluxes of bromate and nitrate could be improved by increasing the electric current and decreasing the hydraulic retention time (HRT). But nitrite in effluent was significantly accumulated when the electric current was beyond 10 mA and the HRT was less than 6 h. The maximum bromate reduction rate estimated by the Monod equation was 109.12 μg/L h when the electric current was 10 mA and HRT was 12 h. It was proposed that the electron transfer process in RBER produced H_2 on the surface of the ACF cathode, and the microbial cultures attached closely on the cathode which could completely utilize H_2 as electron donors for reduction of bromate and nitrate.

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1. Introduction

Bromate (BrO_3^-) is a disinfection by-product from the ozonation or advanced oxidation of water containing bromide (Br^-). It has been experimentally demonstrated that $KBrO_3$ induces renal

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cell tumors, peritoneal mesothelioma, and follicular cell tumors of the thyroid [1,2]. Hence, the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) has classified bromate as a group B-2 carcinogen (a suspected human carcinogen). Currently, the European Union and the U. S. Environmental Protection Agency (EPA) have set a maximum contaminant level of 10 μg/L (0.078 μmol/L) for bromate in drinking water [3]. Therefore, it is necessary to control or remove bromate from bromate-contaminated water. In the past decades, a lot of methods have been successfully implemented to remove bromate, such as adsorption process [4,5], membrane separation [6,7], catalytic decomposition [8–10] and biological reduction [11,12]. Among them, biological reduction is considered to be the a promising alternative due to its ability of reducing bromate to relatively innocuous bromide in a cost-effective way [13].

Previous research showed that bromate could be reduced to bromide by denitrifying bacteria like *Pseudomonas* spp. possibly via co-metabolic action of nitrate reductase [14]. The biological denitrification, either autotrophic or heterotrophic, has been proposed for nitrate and/or bromate removal [15,16]. The heterotrophic denitrification requires the organic carbon sources, such as ethanol, glucose and acetate, as electron donor for the respiration and growth of microorganisms. These organic substrates could potentially leave organic residuals which are easy to form the toxic disinfection byproducts during chlorination [17]. By contrast, autotrophic denitrification does not require organic supplementation, in which some inorganic compounds act as electron donor instead of organic substrates. The elemental sulfur (S°) and sulfide have been reported to be used as the electron donor for the reduction of bromate and/or nitrate in sulfur-based autotrophic denitrification due to its low cost and availability [18,19], but it need some extra steps to remove the reaction residues. Comparing to the other electron donors, the hydrogen gas (H_2) as an attractive alternative electron donor possess the characteristic of harmless and naturally cleaning, and hence, it has been widely applied in the microbial reduction of oxidized inorganic compounds, including perchlorate [20,21], nitrate [22,23], sulfate [24] and their mixture [25–27].

Although H_2 has been proven to be an effective electron donor, the present application in practice is subject to some limit owing to its low solubility in water (1.6 mg/L at 20 °C) and explosion characteristic. To overcome these shortcomings, Nerenberg et al. designed a new reactor, the hollow-fiber membrane biofilm reactor (MBfR), to safely and efficiently deliver H_2 to the biofilm for reduction of oxidized contaminants in drinking water [28]. In the MBfR, an autotrophic biofilm naturally grows on the outside wall of the membrane fibers, and the H_2 electron donor diffuses through the wall of membrane fibers to meet the contaminant electron acceptor at the interface. Because of the counter-current transport of H_2 and the oxidized contaminant in the biofilm, H_2-utilization efficiency could achieve nearly 100% [29]. However, H_2 has to be supplied externally in MBfR and the risk of hydrogen storage still exists. In addition, some reports showed that the internal (electrochemical or chemical) production of H_2 could be more effectively utilized by bacteria than that of external supplied [30]. To overcome the above-mentioned disadvantages, biofilm-electrode reactor (BER) combining biological and electrochemical method has developed and employed to remove nitrate based on the hydrogen autotrophic denitrification. In BER, H_2 is generated on site by electrolysis of water and completely utilized as the electron donor by the denitrifying bacteria immobilized on the cathode surface, avoiding the waste of excessive H_2 in the case of external addition [31]. It has been demonstrated that BER is a promising method for nitrate removal from drinking water [30,32,33], but none of studies have been dedicated to the simultaneous removal of bromate and nitrate by BER.

In this research, an auto-hydrogenotrophic rotating biofilm-electrode reactor (RBER) was developed to remove bromate and nitrate simultaneously by autotrophic denitrification using H_2 as the sole electron donor. The cathode of RBER was designed to automatically rotate so as to enhance the immobilization of biofilm on the cathode and achieve the complete mixing condition. The main objectives of this research were to (1) determine whether the autotrophic denitrifying bacteria in RBER could reduce bromate to below the emission standard of 10-μg/L treatment objective; (2) investigate the effect of electric current and HRT on bromate and nitrate reduction; and (3) explore the electron transfer process and the mechanism of simultaneous reduction of bromate and nitrate in the RBER.

2. Materials and methods

2.1. Experimental set-up

The schematic view of rotating biofilm-electrode reactor (RBER) is shown in Fig. 1. The RBER consisted of polyethylene plastic cylinder (12 cm in diameter and 14 cm in height) as an electrolytic tank, an activated carbon fiber (ACF) bonded with steel ring as cathode (8 × 8 cm) and two graphite carbon (GC) rods as anode (ϕ5 × 150 mm). The cathode was designed to automatically rotate through the motor stirrer and the rotating rate is constant at 14 ± 1 r/min, which enhances the immobilization of biofilm on the cathode and achieve the complete mixing condition. The reactor was tightly sealed to maintain anaerobic condition with the effective working volume of 1.21 L. Electric current or electrode potential was applied by using a DC power supply (Rek PS-303D, China) with a voltage range of 0–30 V. The pH of influent solution was adjusted to 7.2 ± 0.2 with either 0.5-M NaOH or 0.5-M H_2SO_4.

2.2. Synthetic medium

The medium (per liter of ultrapure water) contained following mineral salts (analytical grade): 1.55 g/L K_2HPO_4, 0.85 g/L KH_2PO_4, 0.10 g/L $MgSO_4{\cdot}7H_2O$, 0.25 g/L $NaHCO_3$. The aliquots of trace mineral containing metal-chelator has been described previously [34]. $NaNO_3$, $NaBrO_3$ and the synthetic medium were added in tap water as synthetic water to achieve the concentrations which are given in Table 1. The medium was maintained at 35 ± 2 °C via water bath during all periods. Before feeding, nitrogen (N_2) gas was continuously sprayed to make sure the solution under low DO condition (0.4 ± 0.1 mg/L).

The feed solution is deoxygenated passing through N_2 gas only at the early period (0–15 days) in order to accelerate the anaerobic bacteria growth and bifilm formation. After the acclimation accomplished, the synthetic medium flowed into the RBER directly without deoxygenating by N_2 gas.

2.3. Inoculum and acclimation

200 mL anaerobic sludge was taken from the second municipal wastewater treatment plant (Changsha, China) which was used as the inoculums. The inoculums were acclimated in RBER for 5 days in the batch mode, feeding the above-mentioned medium every day. Then, the microbe was further acclimated in continuous mode, feeding synthetic water containing 25 mg/L nitrate. The hydraulic retention time (HRT) and an applied electric current was set at 12 h and 10 mA, respectively. About 15 days later, the nitrate removal efficiency was higher than 75% and a dark grey biofilm covered the activated carbon fibers, indicating that the acclimation accomplished.

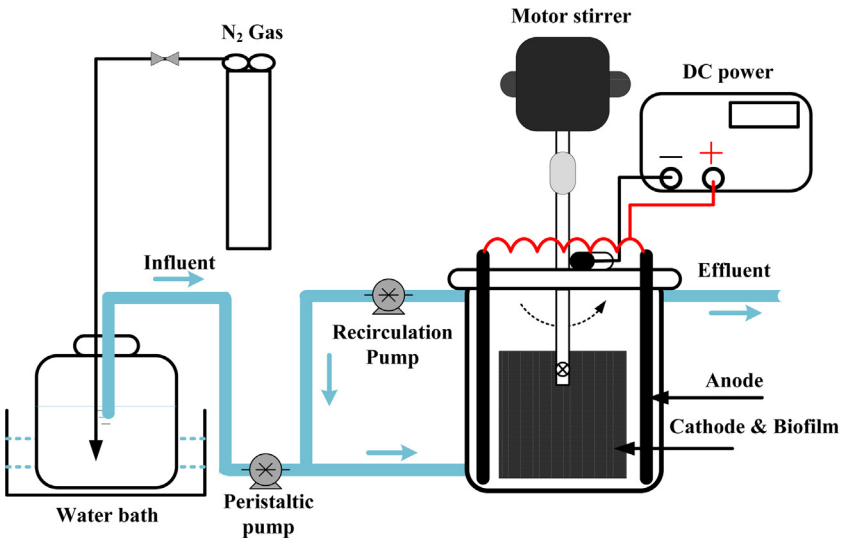


Fig. 1. A schematic view of RBER system.

Table 1
Experimental conditions used in this study.

Experiments[a]	Operating mode	Performance period (d)	HRT (h)	BrO_3^- feed (μg/L)	NO_3^- feed (mg/L)	Electric current (mA)
Inoculation	–	15 (0–15)	12	0	25	10
Short-term test	Phase A	8(16–23)	12	150	25	10
	Phase B	17(24–40)	12	400	25	10
	Phase C	15(41–55)	12	800	50	10
	Phase D	15(56–70)	12	800	25	10
Long-termtest	Baseline	2	12	800	25	10
	Stage 1	60	12	800	25	5–30
	Stage 2	60	6–24	800	25	10

[a] Other experimental conditions: temperature 35 ± 2 °C; initial pH 7.2 ± 0.2; DO 0.4 ± 0.1 mg/L.

2.4. Short-term test

Short-term test was operated to evaluate the RBER ability to reduce bromate to below the 10 μg/L standard and explore the interactions between bromate and nitrate. According to the different influent bromate (150–800 μg/L) and nitrate (25–50 mg/L) concentration, the test was divided into four phases (A–D) and each phase lasted 8, 17, 15 and 15 days, respectively. The influent conditions and operation parameters are listed in Table 1.

2.5. Long-term test

To determine the effects of electric current and HRT on bromate and nitrate reduction, long-term test was carried out over a period of 120 days. An influent of 800 μg/L bromate plus 25 mg/L nitrate was maintained as a baseline condition. Before changing the operation conditions, RBER returned to baseline influent conditions for 48 h. In stage 1, electric current gradually increased from 5 to 30 mA and each interval period lasted 15 days until a steady state was achieved. In stage 2, HRT raised from 6 to 24 h at an interval of 6 h unless otherwise stated. Influent conditions and other operation parameters are listed in Table 1.

2.6. Analysis and calculation

Samples collected daily were filtered with 0.22 mm membrane filter (LC + PVDF membrane, ANPEL Laboratory Technologies Inc., China). Bromate, bromide, nitrate, and nitrite in influent and effluent were analyzed using ion chromatography (Dionex ICS-900, USA) with an AS19 analytical column and AG19 precolumn. The mobile phase (flow rate 1 mL/min) was set with a 9.4 mM solution of Na_2CO_3 and 1.8 mM solution of $NaHCO_3$. Dissolved oxygen was measured by a DO meter (HACH, USA) and the pH was monitored with a standard glass electrode (pHS-3C model, Leici, China). The morphology of cathode surface before and after biofilm forming was examined using a scanning electron microscopy (SEM, JSM-6700F, Japan).

Nitrate and bromate removal fluxes (mg/cm^2 d) were calculated from the following Eq. (1) [27,35]:

$$J = (C_0 - C)\frac{Q}{A} \quad (1)$$

in which C_0 and C are the influent and effluent bromate/nitrate concentration (mg/L), Q is the influent flow rate to the RBER system (L/d), and A is the cathode surface area (m^2). Meanwhile, the actual hydrogen (H_2) flux was calculated based on the removal fluxed and reaction stoichiometry shown in Eqs. (2–3) [36,37]:

$$BrO_3^- + 3H_2 \rightarrow Br^- + 3H_2O \quad (2)$$

$$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^- \quad (3)$$

It is assumed that current efficiency for H_2 production is 100%, according to Faraday's law of electrolysis [38], the theoretical maximum of H_2 flux produced by electrolysis water could be calculated from the following Eq. (4):

$$n = \frac{It}{zF} \quad (4)$$

where n is the amount of produced H_2 (mole); I is the applied current (A); t is the total time with constant current was applied (s); z is the valency number of ions of the substance (electrons trans-

ferred per ions); F is the Faraday constant (96,485 C/mol). Actual H_2 flux was compared with the maximum H_2 flux to indicate whether H_2 delivery was limiting.

3. Results and discussion

3.1. Formation of biofilm on cathode

The morphologies of activated carbon fiber before and after biofilm forming were examined by SEM (Fig. 2). As shown in Fig. 2a, a lot of micro-monofilaments (5–10 μm diameters) interlaced together with the activated carbon fiber. These interconnected structure and ample inner space could provide adequate area for the growth of bacteria. After inoculation of 15 days, it could be observed that clear biofilm covered the activated carbon fiber (Fig. 2b). Since typical bacterial size was larger than the nanopore of the activated carbon fiber, nearly all microorganisms appeared in direct contact with the electrode surface. This indicated that H_2 generated on the site of the cathode could be immediately utilized by immobilized autotrophic denitrifying bacteria to reduce bromate and nitrate.

In the inoculation period, more than 75% of nitrate was removed after inoculation of 15 days, and only less 0.5 mg/L nitrite was accumulated in effluent (Fig. 3). Meanwhile, a visible grey-colored biofilm layer completely covered on the cathode surface. It suggested that denitrifying bacteria was growing in a good condition.

3.2. Short-term test

A four phase short-term test was carried out to evaluate the RBER ability to reduce bromate and nitrate under variable influent concentrations and explore the interactions between bromate and nitrate (Fig. 3).

In phase A, when 150 μg/L of bromate was added to the 25 mg/L nitrate medium, the removal efficiency of nitrate still maintained at high level (95%) and bromate in effluent was immediately reduced to below the 10 μg/L standard (Fig. 3a). However, no bromide was detected in effluent and the nitrite concentration increased from less 0.5 mg/L to 1.0 mg/L (Fig. 3b). This phenomenon indicated that the low concentration of bromate was removed by adsorption instead of biological reduction and the initial existence of bromate affected the autotrophic denitrification.

With the influent bromate up to 400 μg/L (in phase B), the complete bromate reduction was achieved and stoichiometric amounts of bromide were also found in effluent, which suggested that denitrifying bacteria had adapted the bromate load and the microbial bromate reduction by denitrifying bacteria had occupied a predominant position in RBER. This result was in agreement with bromate reduction in a hydrogen-oxidizing, denitrifying biofilm reactor, where adding bromate in a mixed-culture denitrifying bioreactor has resulted in a gradual decrease of bromate concentration in effluent [39].

For studying the impact of high concentration bromate and nitrate on the performance of RBER, influent concentration of bromate and nitrate was further raised to 800 μg/L and 50 mg/L (phase C in Fig. 3a), respectively. The effluent bromate concentration immediately exceeded 80 μg/L, which was significantly higher than 10-μg/L treatment objective. Obvious nitrite accumulation (effluent nitrite over 6 mg/L) was also observed in effluent (Fig. 3b). The corresponding reduction efficiency for bromate and nitrate declined to 90% and 91%, respectively. This result was similar to the interactions between perchlorate (ClO_4^-) and nitrate reductions in hydrogen-based membrane biofilm reactor [27]. High nitrate load could slow perchlorate reduction by competing for the common donor when H_2 was the electron donor.

Considering the competing of nitrate to electron donor, the influent nitrate decreased to 25 mg/L and bromate maintained at 800 μg/L in phase D in Fig. 3a. The effluent bromate concentration was below 10 μg/L again and nitrite accumulation also disappeared, illustrating that the competition of bromate and nitrate for the electron donor did not exist with the decreasing of influent nitrate. Bromate reduction efficiency increased to 95% at this phase, which should be contributed to bromate self-inhibition. However, the influent bromate (800 μg/L) was at a low level in this study, so the bromate self-inhibition was not evident. Downing and his co-workers reported that the bromate self-inhibition would be significant due to the formation of bromide or other toxic intermediates when the influent bromate increased to 30 and 50 mg/L [39].

The comparison between the actual H_2 consumption and the maximum H_2 flux could be utilized to judge whether H_2 delivery was limiting. The removal fluxes of bromate and nitrate, along with the stoichiometric fluxes of H_2 were calculated and listed in Table 2. As shown in Table 2, the maximum H_2 delivery fluxes (0.070 e^- eq/cm^2 d) calculated from Eq. (4) might be excessive. As actual H_2 consumption was 0.034–0.039 e^- eq/cm^2 d in phase A, phase B and phase D, which is only about 50% of maximum H_2 delivery fluxes. It indicated that H_2 delivery should not be limiting in these phases. The perfect performance of bromate and nitrate reduction in Fig. 3 also confirmed it.

However, when influent nitrate increased to 50 mg/L in phase C, actual H_2 consumption (0.077 e^- eq/cm^2 d) was larger than the maximum H_2 flux (0.070 e^- eq/cm^2 d). The percentage removals of bromate and nitrate all declined due to the H_2 delivery limiting (Fig. 3). The competition for the electron donor H_2 appeared between nitrate and bromate. In the investigation of bromate reduction in MBfR, earlier researchers reported that H_2-utilization of NO_3^- was prior to BrO_3^- when H_2 was limiting [40], and they also suggested that full NO_3^- removal was essential before ClO_4^- removal started in an MBfR [41].

3.3. Long-term test

Fig. 4 shows the effect of electric current on bromate and nitrate reduction in RBER system. With the increase of electric current, higher bromate and nitrate removal was achieved, and the bromate and nitrate in the effluent declined significantly. When the electric current increased from 5 to 10 mA, the effluent nitrate declined from 10.5 to 2.5 mg/L, and effluent bromate decreased from 215 to 15 μg/L. The corresponding maximum H_2 flux increased from 0.035 to 0.070 e^- eq/cm^2 d, which suggested that more H_2 produced at higher of electric current and supplied more electron donor for bromate and nitrate reduction. With the electric current exceeding 10 mA, the nitrate and bromate concentration in effluent was almost kept lower levels of 2.0 mg/L and 10 μg/L, respectively. However, the significant nitrite accumulation appeared in effluent (over 10 mg/L) (Fig. 4b). This variation trend was consistent with the results in nitrate removal by BER [33,38]. These results indicated that the electric current on the impact of microorganism was basically similar in different BER. Namely, an optimal electric current usually existed in BER. No matter higher or lower than this value, the removal efficiency would decline. It might be because the low current could not produce the required sufficient amount of hydrogen. In this study, we found that the actual H_2 consumption was closed to maximum H_2 flux when the electric current was 5 mA. The high electric current accelerated electrolytic action and might inhibit microbial activities because of so-called hydrogen depression effect [42,43]. An excessively high concentration of H_2 has been declared as an inhibitor of denitrification to cause the nitrite accumulation [33,38]. So in this study, 10 mA should be an optimal electric current.

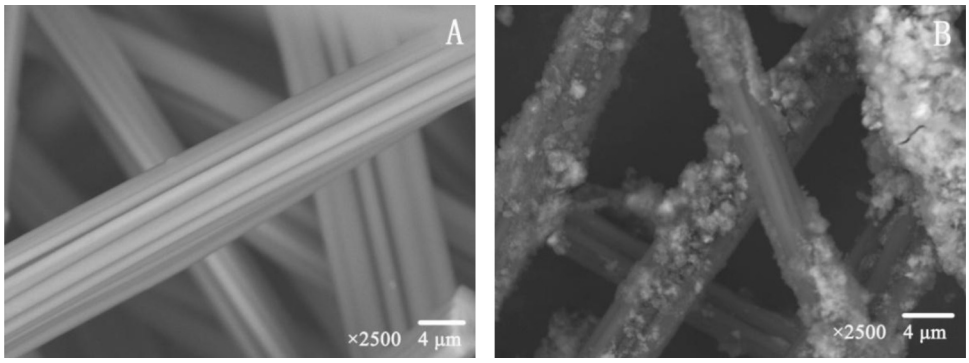


Fig. 2. The SEM images of activated carbon fiber cathode inoculated with anaerobic sludge. (A) Before biofilms forming (2500 times), and (B) after biofilms forming (2500 times).

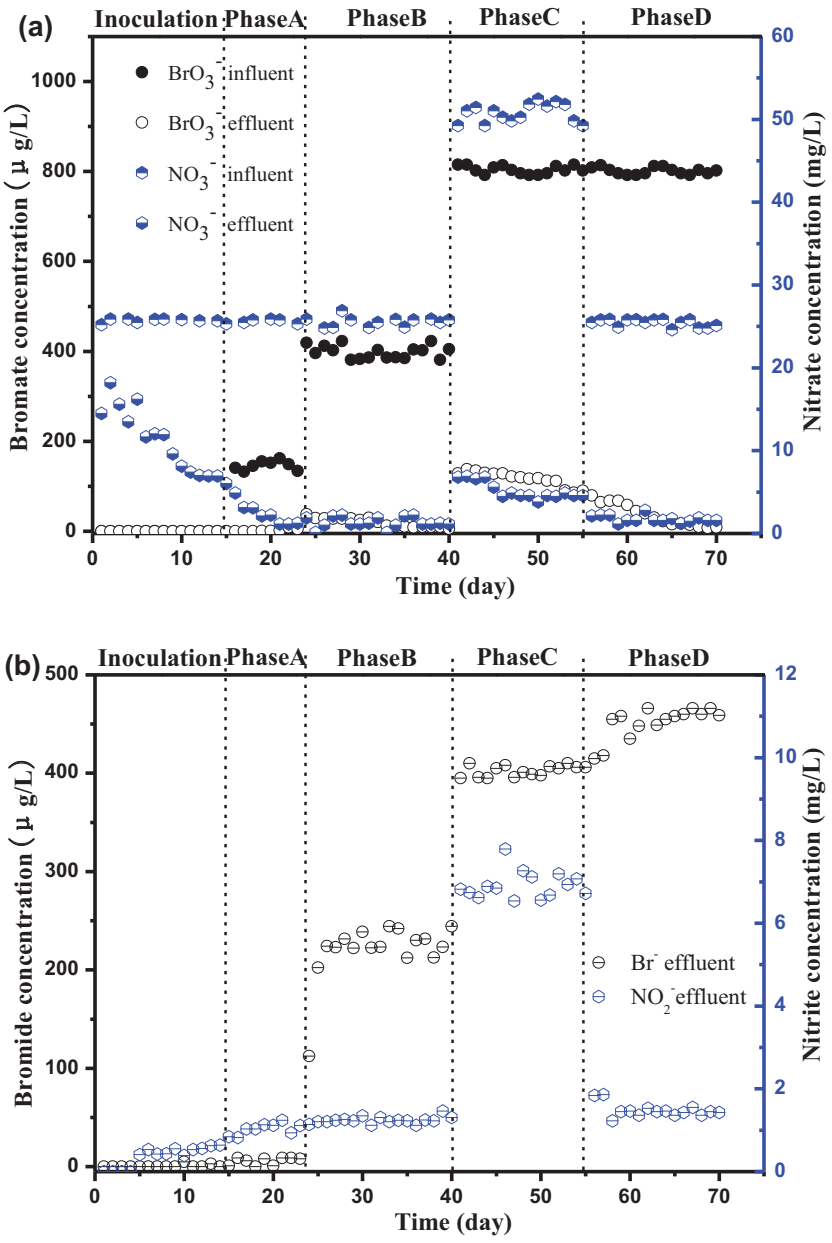


Fig. 3. (a) Influent and effluent concentration of bromate and nitrate in RBER, and (b) effluent concentration of bromide and nitrite. Experimental conditions: temperature 35 ± 2 °C; pH 7.2 ± 0.2; DO 0.4 ± 0.1 mg/L; electric current 10 mA.

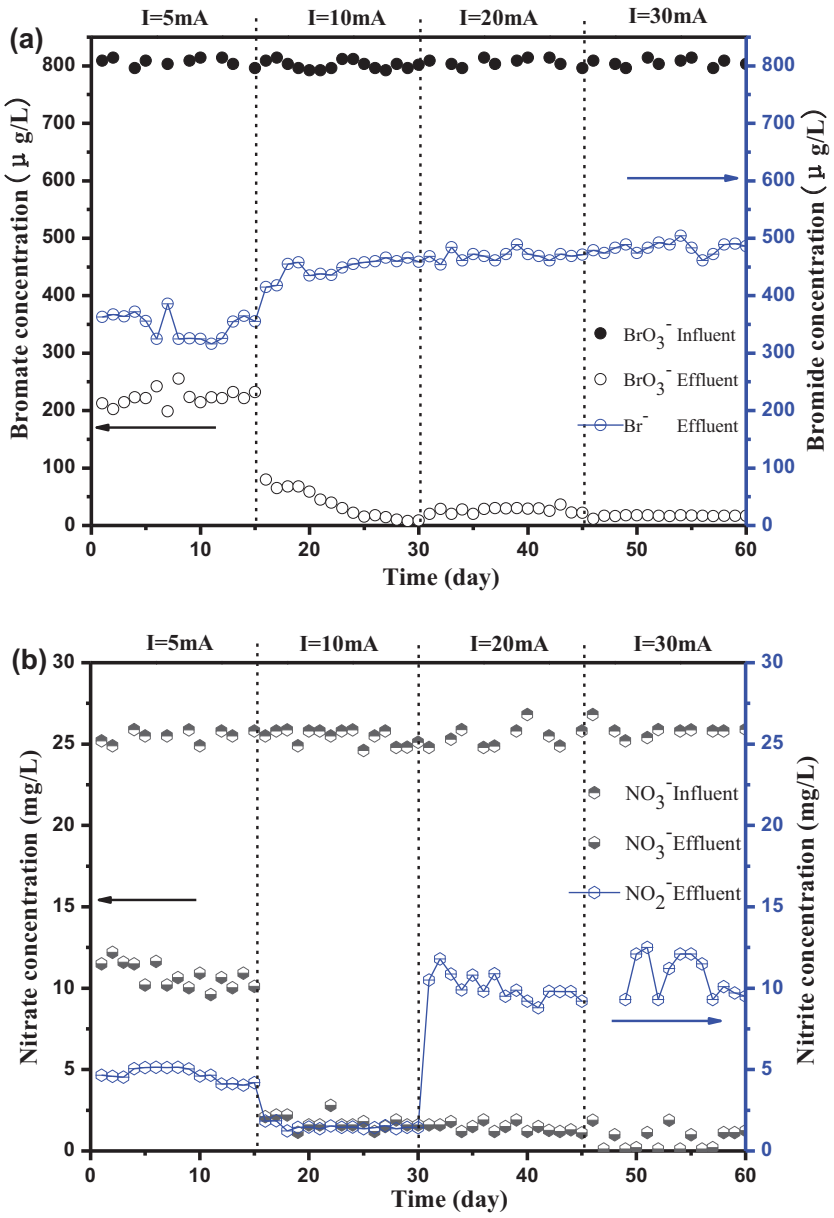


Fig. 4. Performance of RBER system at different electric current. Influent and effluent concentration of (a) bromate and bromide, and (b) nitrate and nitrite in the RBER. Experimental conditions: temperature 35 ± 2 °C; pH 7.2 ± 0.2; DO 0.4 ± 0.1 mg/L.

The bromate and nitrate removal fluxes at different HRT are presented at Table 2. The removal fluxes of bromate and nitrate decreased with HRT prolonging while maximum H_2 flux did not change due to the constant electric current (10 mA). The effluent bromate and nitrate slightly fluctuated when HRT decreased from 24 to 6 h. When the HRT was 6 h, little nitrite accumulation was observed in effluent (data were not shown), which should be explained by H_2 delivery limiting. Under this HRT condition, the actual H_2 flux 5(0.043 e^- eq/cm^2 d) accounted for 61.42% of the theoretical maximum H_2 flux (0.070 e^- eq/cm^2 d). With HRT increasing, the bromate and nitrate load decreased and the H_2 delivery limiting was released accordingly. Bromate and nitrate removal efficiency achieved 100% and effluent nitrite was not monitored when the HRT exceeded 12 h. Previous researches reported that the appropriate HRT was at 8 h in BER [33,38]. This difference might be attributed to the different electric current and influent substrate concentration.

Kinetic analysis for bromate reduction in RBER was carried out at HRT 12 h and electric current 10 mA with influent bromate concentration 800 μg/L and nitrate concentration 25 mg/L. The maximum bromate reduction rate in RBER were estimated by the Monod equation as following Eq. (5).

$$V = -\frac{dS}{dt} = \frac{kXS}{Ks + S} \tag{5}$$

where V is the bromate reduction rate (μg/L h), S is the bromate concentration (μg/L), X is the biomass concentration (mg VSS/L), k is the maximum specific bromate reduction (μg/(mg VSS h)) and Ks is half-maximum rate constant (μg/L). The biomass concentration can be assumed to be constant in bioflm system. Then Eq. (5) can be simplified to:

$$V = -\frac{dS}{dt} = \frac{V_{max}S}{Ks + S} \tag{6}$$

Table 2
Average acceptor removal fluxes and donor fluxes for each stage at short-term test and long-term test.

Experiment	Operating mode	Condition		BrO_3^-		NO_3^-		Electron donor (H_2)	
		HRT(h)	Electric current (mA)	Removal flux (mg/cm^2 d)	Electron donor consumed (e^- eq/cm^2 d)	Removal flux (mg/cm^2 d)	Electron donor consumed (e^- eq/cm^2 d)	[a] Actual H_2 consumption (e^- eq/cm^2 d)	[b] Maximum H_2 flux (e^- eq/cm^2 d)
Inoculation	–	12	10	0	0	0.191	0.034	0.034	0.070
Short-term test	Phase A	12	10	0.001	0.538×10^{-4}	0.220	0.039	0.039	0.070
	Phase B	12	10	0.004	0.139×10^{-3}	0.220	0.039	0.039	0.070
	Phase C	12	10	0.008	0.281×10^{-3}	0.430	0.077	0.077	0.070
	Phase D	12	10	0.007	0.280×10^{-3}	0.220	0.039	0.039	0.070
Long-term test	Stage 1	12	5	0.006	0.215×10^{-3}	0.153	0.027	0.027	0.035
		12	10	0.007	0.280×10^{-3}	0.210	0.038	0.038	0.070
		12	20	0.007	0.281×10^{-3}	0.220	0.039	0.039	0.140
		12	30	0.008	0.283×10^{-3}	0.225	0.040	0.040	0.210
	Stage 2	6	10	0.012	0.442×10^{-3}	0.239	0.042	0.043	0.070
		12	10	0.007	0.280×10^{-3}	0.220	0.039	0.039	0.070
		18	10	0.005	0.188×10^{-3}	0.150	0.027	0.027	0.070
		24	10	0.004	0.142×10^{-3}	0.115	0.020	0.021	0.070

[a] Actual H_2 consumption calculated from Eqs. (1)–(3), and.
[b] Maximum H_2 flux calculated from Eq. (4).

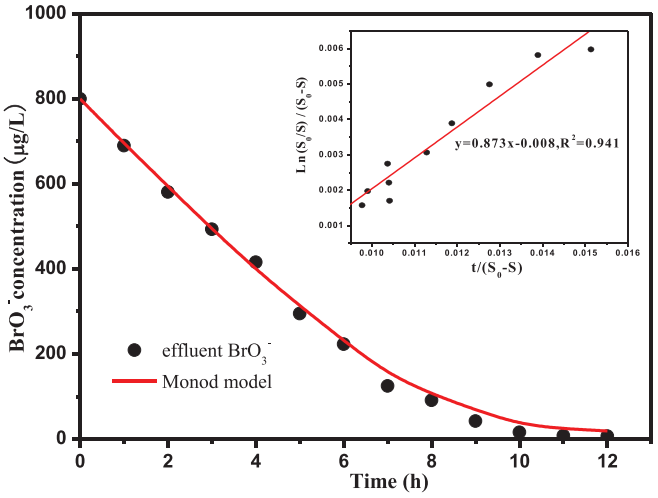


Fig. 5. Application of Monod equation to fit the bromate reduction date. Insert: modeling of the kinetics by fitting to the Monod equation. Experimental conditions: temperature 35 ± 2 °C; pH 7.2 ± 0.2; DO 0.4 ± 0.1 mg/L; electric current 10 mA; and HRT 12 h.

where V_{max} is the maximum reduction rate (μg/L h) at a given temperature. Its integrated form can be obtained as follows:

$$\frac{Ln(S_0/S)}{S_0 - S} = \frac{V_{max}}{Ks} \times \frac{t}{S_0 - S} - \frac{1}{Ks} \quad (7)$$

where S_0 is the influent concentration of bromate. A linear plot of $Ln\ (S_0/S)/(S_0\text{-}S)$ against $t/(S_0\text{-}S)$ was employed to determine values of Ks and V_{max} from the intercepts and slopes of the plot. The kinetic analysis was conducted as shown in Fig. 5.

The results indicated that Monod kinetic model could follow bromate reduction well with a high correlation coefficient (R^2) of 0.941. The value of Ks for the bromate reducing micro-organisms was 125 μg/L and the maximum bromate reduction rate estimated by using Eq. (7) was 109.12 μg/L h at investigated condition. Compared with other biological systems summarized in Table 3, the bromate reduction rate in RBER was greater than the rates that obtained in previous studies [19,44,45]. A higher removal rate of 116 μg/L h was achieved by using anaerobic column reactor which used ethanol as electron donor [15], with a slightly higher rate of 120.5 μg/L h observed by using MBfR while hydrogen supplying from out of reactor [39]. It is noteworthy that the working volume of MBfR is only 0.065 L, this small volume possibly benefit to maintain anaerobic environment and enrich the bromate-utilizing microorganisms. In the current study, using H_2 as electron donor, especially generated on the site of cathode exhibited desirable ability to reduce bromate. Therefore, the RBER is an ideal reactor for simultaneous reduction of bromate and nitrate from water.

3.4. Possible mechanism in the RBER

During short-term test, the pH in effluent has increased slightly from 7.2 to 8.1 at the end of each phase (data were not shown). The pH variation may be ascribed to the production of hydroxyl anion in the ACF cathode, according to Reactions (3) and (8).

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (8)$$

The majority of researchers admittedly agreed that the optimum pH for denitrifying bacteria is between 7.0 and 7.5 [33]. The increasing pH in RBER may negatively affect the bromate and nitrate reduction process. Thus, 0.1 mL of H_2SO_4 (0.5 M) has been added into the solution in a period of hydraulic retention time to maintain pH around neutrality.

Table 3
Comparison of bromate removal rates with other biological systems.

Reactors	Effective volume (L)	Initial bromate (μg/L)	Initial nitrate (mg/L)	Electron donor	HRT (h)	Bromate removal rate (μg/L h)	Ref.
Denitrifying bioreactor	4.6	25–35	85	Ethanol	0.3	83–116	[15]
Fixed-film bioreactor	36	1100	30.7	Glucose	40	27.5	[44]
Ion exchange membrane bioreactor	0.7	200	60	Ethanol	8.3	24	[45]
Fixed bed column reactor	0.4	100–500	45	Sulfur	10.1	10–50	[19]
Hydrogen-based membrane biofilm reactor	0.065	100	5	Hydrogen	0.83	120.5	[39]
Rotating biofilm-electrode reactor	1.21	800	25	Hydrogen	6–12	66.7–109.1	This study

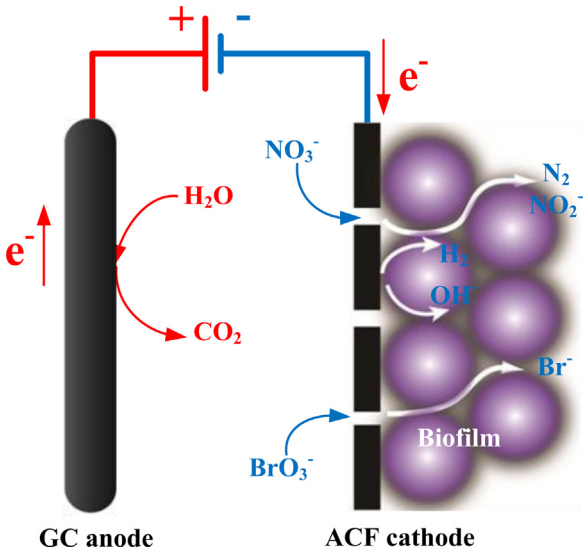
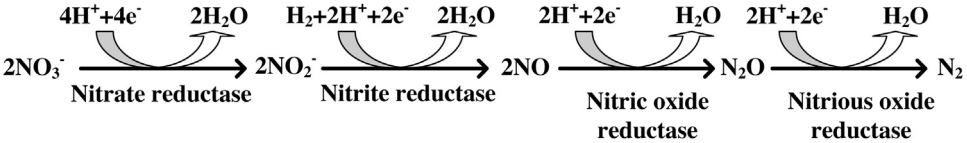


Fig. 6. Main mechanism of simultaneous bromate and nitrate removal in the RBER.

Meanwhile, the surface of graphite carbon anode was dissolved during the long-term test. When the electric current increased to 30 mA, this phenomenon was more noticeable than the other current. Feleke et al. [37]. summarized the main Reaction (9) of the GC anode when the electric current was applied.

$$C + 2H_2O \rightarrow CO_2 + 4H^+ + 4e^- \quad (9)$$

Based on the discussion above, the electron transfer process in RBER was proposed and the most likely mechanism of bromate and nitrate reduction could be deduced in Fig. 6. When the electric current is applied, the electrochemical Reactions (8) and (9) could be occurred on the ACF cathode and GC anode, respectively. Meanwhile, bromate and nitrate ions were adsorbed onto the biofilm, and microbial cultures attached closely on the cathodes completely utilize H_2 as electron donors for reduction of bromate and nitrate. The short-term tests show that when H_2 was not limiting, bromate and nitrate reduction were complete, and the RBER biofilm was composed mainly of bacteria from proteobacteria and bacteroidetes classes [46]. When H_2 was limiting, nitrate reduction was prior to bromate reduction for electrons from H_2. The reactions and enzymes of NO_3^- removal was summarized as follows [27]:



For bromate removal, the cometabolism of bromate via nitrate reductase has been postulated, but the scientific explanation of bromate removal needs further assessment to judge if there was a bromate-specific reduction pathway exists in some microorganisms. Eventually, reduction products such as bromide, nitrite and nitrogen gas released to solutions (Fig. 6).

4. Conclusions

In this study, an auto-hydrogenotrophic rotating biofilm-electrode reactor (RBER) was designed for the simultaneous removal of bromate and nitrate. Short-term test results confirmed that the RBER system could reduce 150–800 μg/L bromate to below 10 μg/L when H_2 delivery was not limiting. High nitrate load could affect bromate reduction through competing for the electron donor H_2. The long-term test (over 120 days) considered the influence of electric current and hydraulic retention time (HRT) on bromate and nitrate reduction. Kinetic parameters for bromate reduction calculated by the Monod equation were maximum bromate reduction rate 109.12 μg/L h and *Ks* 125 μg/L when the electric current was 10 mA and HRT was 12 h. Based on the analysis of reduction products, the bromate and nitrate removal in RBER might be attributed to biological reduction of denitrifying bacteria using hydrogen as the sole electron donor.

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References

[1] A.B. DeAngelo, M.H. George, S.R. Kilburn, T.M. Moore, D.C. Wolf, Carcinogenicity of potassium bromate administered in the drinking water to male B6C3F1 mice and F344/N rats, Toxicol. Pathol. 26 (1998) 587–594.
[2] M.M. Moore, T. Chen, Mutagenicity of bromate: implications for cancer risk assessment, Toxicology 221 (2006) 190–196.
[3] C. Xu, J. Shi, W. Zhou, B. Gao, Q. Yue, X. Wang, Bromate removal from aqueous solutions by nano crystalline akaganeite (β-FeOOH)-coated quartz sand (CACQS), Chem. Eng. J. 187 (2012) 63–68.
[4] R. Chen, Q. Yang, Y. Zhong, X. Li, Y. Liu, X.M. Li, W.X. Du, G.M. Zeng, Sorption of trace levels of bromate by macroporous strong base anion exchange resin: influencing factors, equilibrium isotherms and thermodynamic studies, Desalination 344 (2014) 306–312.
[5] W.f. Chen, Z.Y. Zhang, Q. Li, H.Y. Wang, Adsorption of bromate and competition from oxyanions on cationic surfactant-modified granular activated carbon (GAC), Chem. Eng. J. 203 (2012) 319–325.
[6] J.A. Wiśniewski, M. Kabsch-Korbutowicz, S. Łakomska, Removal of bromate ions from water in the processes with ion-exchange membranes, Sep. Purif. Technol. 145 (2015) 75–82.

[7] M. Moslemi, S.H. Davies, S.J. Masten, Rejection of bromide and bromate ions by a ceramic membrane, Environ. Eng. Sci. 29 (2012) 1092–1096.
[8] J. Restivo, O. Soares, J. Órfão, M. Pereira, Metal assessment for the catalytic reduction of bromate in water under hydrogen, Chem. Eng. J. 263 (2015) 119–126.
[9] X. Liu, T. Zhang, Y. Shao, Aqueous bromate reduction by UV activation of sulfite, Clean-Soil Air Water 42 (2014) 1370–1375.
[10] X. Huang, L. Wang, J. Zhou, N. Gao, Photocatalytic decomposition of bromate ion by the UV/P25-graphene processes, Water Res. 57 (2014) 1–7.
[11] A. Assunção, M. Martins, G. Silva, H. Lucas, M.R. Coelho, M.C. Costa, Bromate removal by anaerobic bacterial community: mechanism and phylogenetic characterization, J. Hazard. Mater. 197 (2011) 237–243.
[12] A.N. Davidson, J. Chee-Sanford, H.Y.M. Lai, C.h. Ho, J.B. Klenzendorf, M.J. Kirisits, Characterization of bromate-reducing bacterial isolates and their potential for drinking water treatment, Water Res. 45 (2011) 6051–6062.
[13] K.J. Martin, L.S. Downing, R. Nerenberg, Evidence of specialized bromate-reducing bacteria in a hollow fiber membrane biofilm reactor, Water Sci. Technol. 8 (2008) 473.
[14] W. Hijnen, R. Voogt, H. Veenendaal, H. Van der Jagt, D. Van Der Kooij, Bromate reduction by denitrifying bacteria, Appl. Environ. Microb. 61 (1995) 239–244.
[15] W. Hijnen, R. Jong, D. Van der Kooij, Bromate removal in a denitrifying bioreactor used in water treatment, Water Res. 33 (1999) 1049–1053.
[16] S. Demirel, I. Bayhan, Nitrate and bromate removal by autotrophic and heterotrophic denitrification processes: batch experiments, J. Environ. Health 11 (2013) 27.
[17] X. Ju, J.A. Field, R. Sierra-Alvarez, M. Salazar, H. Bentley, R. Bentley, Chemolithotrophic perchlorate reduction linked to the oxidation of elemental sulfur, Biotechnol. Bioeng. 96 (2007) 1073–1082.
[18] M. Chairez, Reduction of bromate by biogenic sulfide produced during microbial sulfur disproportionation, Biodegradation 21 (2010) 235–244.
[19] S. Demirel, İ. Uyanık, A. Yurtsever, H. Çelikten, D. Uçar, Simultaneous bromate and nitrate reduction in water using sulfur-utilizing autotrophic and mixotrophic denitrification processes in a fixed bed column reactor, Clean-Soil Air Water 42 (2014) 1185–1189.
[20] R. Nerenberg, Microbial ecology of a perchlorate-reducing, hydrogen-based membrane biofilm reactor, Water Res. 42 (2008) 1151–1159.
[21] J.D. Shrout, T.E. Scheetz, T.L. Casavant, G.F. Parkin, Isolation and characterization of autotrophic, hydrogen-utilizing, perchlorate-reducing bacteria, Appl. Microbiol. Biotechnol. 67 (2005) 261–268.
[22] D. Chen, K. Yang, H. Wang, B. Lv, Nitrate removal from groundwater by hydrogen-fed autotrophic denitrification in a bio-ceramsite reactor, Water Sci. Technol. 69 (2014) 2417–2422.
[23] S. Xia, F. Zhong, Y. Zhang, H. Li, X. Yang, Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor, J. Environ. Sci-China 22 (2010) 257–262.
[24] J.A.B. Sousa, C.M. Plugge, A.J.M. Stams, M.F.M. Bijmans, Sulfate reduction in a hydrogen fed bioreactor operated at haloalkaline conditions, Water Res. 68c (2014) 67–76.
[25] H.P. Zhao, A. Ontiveros-Valencia, Y. Tang, B.O. Kim, Z.E. Ilhan, R. Krajmalnik-Brown, B. Rittmann, Using a two-stage hydrogen-based membrane biofilm reactor (MBfR) to achieve complete perchlorate reduction in the presence of nitrate and sulfateg a two-stage hydrogen-based membrane biofilm reactor (MBfR) to achieve complete perchlorate reduction in the presence of nitrate and sulfate, Environ. Sci. Technol. 47 (2013) 1565–1572.
[26] A. Ontiveros-Valencia, M. Ziv-El, H.P. Zhao, F. Liang, B.E. Rittmann, R. Krajmalnik-Brown, Interactions between nitrate-reducing and sulfate-reducing bacteria coexisting in a hydrogen-fed biofilm, Environ. Sci. Technol. 46 (2012) 11289–11298.
[27] H.P. Zhao, S. Van Ginkel, Y. Tang, D.W. Kang, B. Rittmann, R. Krajmalnik-Brown, Interactions between perchlorate and nitrate reductions in the biofilm of a hydrogen-based membrane biofilm reactor, Environ. Sci. Technol. 45 (2011) 10155–10162.
[28] R. Nerenberg, B. Rittmann, Hydrogen-based, hollow-fiber membrane biofilm reactor for reduction of perchlorate and other oxidized contaminants, Water Sci. Technol. 49 (2004) 223–230.
[29] B.E. Rittman, R. Nerenberg, K.C. Lee, I. Najm, T.E. Gillogly, G.E. Lehman, S.S. Adham, Hydrogen-based hollow-fiber membrane biofilm reactor (MBfR) for removing oxidized contaminants, in: International Conference on Creative Water and Wastewater Treatment Technologies for Densely Populated Urban Areas, Hong Kong, P.R. China, 2004.
[30] M. Prosnansky, Y. Sakakibara, M. Kuroda, High-rate denitrification and SS rejection by biofilm-electrode reactor (BER) combined with microfiltration-rate denitrification and SS rejection by biofilm-electrode reactor (BER) combined with microfiltration, Water Res. 36 (2002) 4801–4810.
[31] Y. Sakakibara, M. Kuroda, Electric prompting and control of denitrification, Biotechnol. Bioeng. 42 (1993) 535–537.
[32] H.I. Park, D.K. Kim, Y.J. Choi, D. Pak, Nitrate reduction using an electrode as direct electron donor in a biofilm-electrode reactor, Process Biochem. 40 (2005) 3383–3388.
[33] Y. Zhao, C. Feng, Q. Wang, Y. Yang, Z. Zhang, N. Sugiura, Nitrate removal from groundwater by cooperating heterotrophic with autotrophic denitrification in a biofilm-electrode reactor, J. Hazard. Mater. 192 (2011) 1033–1039.
[34] D.B. Wang, X.M. Li, Q. Yang, G.M. Zeng, D.X. Liao, J. Zhang, Biological phosphorus removal in sequencing batch reactor with single-stage oxic process, Bioresour. Technol. 99 (2008) 5466–5473.
[35] M. Zhou, W. Fu, H. Gu, L. Lei, Nitrate removal from groundwater by a novel three-dimensional electrode biofilm reactor, Electrochim. Acta 52 (2007) 6052–6059.
[36] R. Nerenberg, B. Rittmann, Hydrogen-based, hollow-fiber membrane biofilm reactor for reduction of perchlorate and other oxidized contaminants, Water Sci. Technol. 49 (2004) 223–230.
[37] Z. Feleke, K. Araki, Y. Sakakibara, T. Watanabe, M. Kuroda, Selective reduction of nitrate to nitrogen gas in a biofilm-electrode reactor, Water Res. 32 (1998) 2728–2734.
[38] Y. Zhao, B. Zhang, C. Feng, F. Huang, P. Zhang, Z. Zhang, Y. Yang, N. Sugiura, Behavior of autotrophic denitrification and heterotrophic denitrification in an intensified biofilm-electrode reactor for nitrate-contaminated drinking water treatment, Bioresour. Technol. 107 (2012) 159–165.
[39] L.S. Downing, R. Nerenberg, Kinetics of microbial bromate reduction in a hydrogen-oxidizing, denitrifying biofilm reactor, Biotechnol. Bioeng. 98 (2007) 543–550.
[40] L.S. Downing, R. Nerenberg, Bromate reduction to bromide in a hydrogen-oxidizing bioreactor, in: American Water Works Association Inorganic Contaminants conference, Austin, TX, USA, 2006.
[41] R. Nerenberg, B. Rittmann, I. Najm, Perchlorate reduction in a hydrogen-based membrane-biofilm reactor, J. Am. Water Works Assoc. 94 (2002) 103–114.
[42] J. Flora, M. Suidan, S. Islam, P. Biswas, Y. Sakakibara, Numerical modeling of a biofilm-electrode reactor used for enhanced denitrification, Water Sci. Technol. 29 (1994) 517–524.
[43] Y. Sakakibara, J.R. Flora, M.T. Suidan, M. Kurodo, Modeling of electrochemically-activated denitrifying biofilms, Water Res. 28 (1994) 1077–1086.
[44] R. Butler, S. Ehrenberg, A. Godley, R. Lake, L. Lytton, E. Cartmell, Remediation of bromate-contaminated groundwater in an ex situ fixed-film bioreactor, Sci. Total Environ. 366 (2006) 12–20.
[45] C.T. Matos, S. Velizarov, M.A.M. Reis, J.G. Crespo, Removal of bromate from drinking water using the ion exchange membrane bioreactor concept, Environ. Sci. Technol. 42 (2008) 7702–7708.
[46] A.N. Davidson, J. Chee-Sanford, H.Y. Lai, C.h. Ho, J.B. Klenzendorf, M.J. Kirisits, Characterization of bromate-reducing bacterial isolates and their potential for drinking water treatment, Water Res. 45 (2011) 6051–6062.