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Simultaneous removal of selected oxidized contaminants in groundwater using a continuously stirred hydrogen-based membrane biofilm reactor

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Abstract

A laboratory trial was conducted for evaluating the capability of a continuously stirred hydrogen-based membrane biofilm reactor to simultaneously reduce nitrate (NO_3^--N), sulfate (SO_4^{2-}), bromate (BrO_3^-), hexavalent chromium (Cr(VI)) and *para*-chloronitrobenzene (*p*-CNB). The reactor contained two bundles of hollow fiber membranes functioning as an autotrophic biofilm carrier and hydrogen pipe as well. On the condition that hydrogen was supplied as electron donor and diffused into water through membrane pores, autohydrogenotrophic bacteria were capable of reducing contaminants to forms with lower toxicity. Reduction occurred within 1 day and removal fluxes for NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI), and *p*-CNB reached 0.641, 2.396, 0.008, 0.016 and 0.031 g/(day·m^2), respectively after 112 days of continuous operation. Except for the fact that sulfate was 37% removed under high surface loading, the other four contaminants were reduced by over 95%. The removal flux comparison between phases varying in surface loading and H_2 pressure showed that decreasing surface loading or increasing H_2 pressure would promote removal flux. Competition for electrons occurred among the five contaminants. Electron-equivalent flux analysis showed that the amount of utilized hydrogen was mainly controlled by NO_3^--N and SO_4^{2-} reduction, which accounted for over 99% of the electron flux altogether. It also indicated the electron acceptor order, showing that nitrate was the most prior electron acceptor while sulfate was the second of the five contaminants.

Key words: oxidized contaminant; groundwater; simultaneous removal; hydrogen-based membrane biofilm reactor

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Introduction

Being the main source of drinking water in many regions, groundwater and its corresponding contamination problems are of worldwide concern. During recent decades, several oxidized contaminants such as nitrate (NO_3^--N), sulfate (SO_4^{2-}), bromate (BrO_3^-), hexavalent chromium (Cr(VI)) and aromatic nitro compounds like para-chloronitrobenzene (*p*-CNB) have been widely detected in groundwater as long-standing water-quality problems or emerging pollutants.

Nitrates are introduced into groundwater from a variety of sources such as agricultural activities, poor sewer systems, wastewater, and industrial activities (Alabdula'aly et al., 2010). Nitrate in drinking water and groundwater is suspected to be a possible cause of methaemoglobinaemia in infants (Johns and Lawrence, 1973; George et al., 2001; Abu Naser et al., 2007). As an intermediate in denitrification, nitrite has been verified to be toxic to human and animals as well. Worldwide nitrate contaminations with concentrations exceeding the permissible limit of the World Health Organization (10 mg/L) have received attention since 1973 (Johns and Lawrence, 1973; Showers et al., 2008; Yang and Liu, 2010). China has set a threshold nitrate (as nitrogen) concentration for groundwater of 2.0 mg/L (GB/T 14848-93).

Sulfate naturally exists in water systems through geochemical processes such as mineral dissolution. The content of sulfate in lakes and rivers ranges from a few tens to several hundred mg/L. Sulfate deteriorates the taste of water and causes laxation and decline of gastric juice acidity in human (Safe Drinking Water Committee, 1986). The results of an animal trial with nursery pigs indicated that pigs drinking high sulfate water had an increased prevalence of nonpathogenic diarrhea (Veenhuizen et al., 1992). The threshold groundwater sulfate concentration in China is 50 mg/L (GB/T 14848-93).

Bromate (BrO_3^-) contamination is commonly associated with disinfection byproduct formation during the treatment by ozonation of potable water containing back-

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ground bromide (Br^-), which is not prescribed and is found naturally within most water systems (Hutchinson et al., 1997; Michalski, 2003). In contrast to bromide, bromate is not reported as occurring naturally in surface waters (Hutchinson et al., 1997) and is not normally present in aquifers. Some clinical cases have suggested an association between acute renal failure and bromate ingestion (DeVriese et al., 1997; Sashiyama et al., 2002). In addition, bromate is classified as a potential carcinogen based on rodent studies (Butler et al., 2005). China specified a maximum bromate concentration of 10 μg/L in 2006 (GB/T 5749-2006).

Chromium and its compounds are extensively employed in leather processing and finishing, in the production of refractory steel, drilling mud, electroplating cleaning agents, catalytic manufacture and in the production of chromic acid and specialty chemicals (Shanker et al., 2005). These anthropogenic activities along with unreasonable discharge have resulted in beyond-standard Cr existence in the environment. Inorganic Cr is relatively insoluble and nontoxic when present in the trivalent form, Cr(III), rather than in the more soluble and more toxic hexavalent form, Cr(VI) (Becker et al., 2006). Cr(III) is considered as an essential trace element ion (50–200 μg/day) while Cr(VI) is regarded as an environmental toxicant (CieślakGolonka, 1996). Cr(VI) is harmful to vital organs (Mishra and Mohanty, 2008) and is widely recognized to be carcinogenic, mutagenic and redox active (Krumschnabel and Nawaz, 2004). Accordingly, many countries have limited the Cr(VI) concentration in water. For instance, China has restricted Cr(VI) concentrations to below 5 μg/L in groundwater (GB/T 14848-93).

Chloronitrobenzenes are widely used as intermediates for chemical syntheses of drugs, herbicides, dyes, etc., and are known to be very toxic and resistant to microbial degradation due to the electron-withdrawing properties of nitro and chlorine groups (Park et al., 1999; Wu et al., 2006). Chloronitrobenzenes exist in three isomers: *ortho*-chloronitrobenzene (*o*-CNB), *meta*-chloronitrobenzene (*m*-CNB) and *para*-chloronitrobenzene (*p*-CNB), among which *p*-CNB is the most toxic isomer (Davydova, 1967; Watanabe et al., 1976). *p*-CNB is a hazardous material that can cause methemoglobinemia and malignant tumors in human beings and animals (Linch, 1974; Matsumoto et al., 2006) and is weakly mutagenic, carcinogenic and of chronic toxity (Weisburger et al., 1978; Shimizu et al., 1983; Matsumoto et al., 2006). To minimize the potential for adverse health effects, the *p*-CNB concentration in drinking water supply source is regulated at 50 μg/L in China (GB/T 5749-2006).

In numerous cases, two or more of the oxidized contaminants occur together, and a treatment technology that can detoxify all of them simultaneously would be of high value (Chung et al., 2007). Effective separation treatment processes such as reverse osmosis, ion exchange, membrane filtration and electrodialysis are expensive and generate concentrated wastes that require subsequent disposal (Komori et al., 1990). In contrast, bioreduction is a promising approach for simultaneous removal of mixtures of oxidized contaminants. With an electron donor provided, microorganisms are able to reduce contaminants to nontoxic and immobile forms. For instance, complete reduction of NO_3^--N generates nitrogen, the major component of air, and the reductions of SO_4^{2-}, BrO_3^- and *p*-CNB produce low toxicity S^{2-}, Br^-, Cl^- and aniline respectively (Tuttle et al., 1969; Heijman et al., 1993; Hijnen et al., 1995). Cr(III) generated from Cr(VI) reduction easily transforms to insoluble $Cr(OH)_3$ in alkaline conditions (Komori et al., 1990). Due to the oligotrophic condition of groundwater, it is generally necessary to externally add an electron donor for microbiological treatment. Compared with conventional electron donors such as methane and ethanol, hydrogen (H_2) is a superior electron donor with favorable properties including being nontoxic, relatively inexpensive and non-residual, and it also supports autotrophic bacteria which require no organic-C source (Lee and Rittmann, 2002; Nerenberg and Rittmann, 2004). However, the explosion and safety concerns associated with H_2 have prevented widespread acceptance of hydrogenotrophic reduction as a remediation technology (Haugen et al., 2002).

The hollow fiber membrane biofilm reactor (MBfR) combines the advantages of hydrogen-based autotrophic bioreduction and hollow fiber aeration. In the MBfR, H_2 gas diffuses through the wall of a composite membrane, and an autotrophic biofilm naturally develops on the outside of the membrane, where the bacteria's electron acceptor is an oxidized contaminant (e.g., NO_3^- or ClO_4^-) supplied from the water (Rittmann et al., 2004). The MBfR makes it possible to deliver H_2 gas to bacteria efficiently and safely, despite hydrogen's low water solubility and risk of forming a combustible atmosphere when mixed with air (Lee and Rittmann, 2002; Rittmann et al., 2004). The MBfR's capacity for removing oxidized contaminants when only one or two sorts of pollutants exist in water has already been explored in some former studies. For example, NO_3^--N was thoroughly reduced to H_2 without NO_2^--N remaining (Tang et al., 2010; Xia et al., 2010); Cr(VI) was bioreduced to Cr(III) and eventually precipitated as $Cr(OH)_3$ (Chung et al., 2006); BrO_3^- was reduced to Br^- (Downing and Nerenberg, 2007); *p*-CNB was reduced to aniline via *para*-chloroaniline (*p*-CAN) as intermediate (Xia et al., 2011). However, few studies have focused on simultaneous reduction under complex conditions as in this study. Therefore, we applied an MBfR aiming at: (1) demonstrating the feasibility of simultaneous bioreduction of nitrate, sulfate, bromate, hexavalent chromium and *p*-CNB by a MBfR; (2) investigating the effect of surface loading and H_2 pressure on the MBfR's performance; (3) determining the order of the five contaminants as electron

acceptors.

1 Materials and methods

1.1 Experiment setup

A schematic diagram of the lab-scale continuously-stirred hydrogen-based membrane biofilm reactor (CS-MBfR) used in this study is shown in **Fig. 1**. The MBfR system consisted of a transparent plastic main tube, a magnetic stirrer, silicone pipelines and pumps. Both ends of the main tube were sealed for anaerobic reduction. Two bundles of hydrophobic polyvinyl chloride hollow fiber membranes (Litree Company, Suzhou, China) were fixed inside the main tube with their upper ends connected with H_2 pipelines and lower ends plugged. Hydrogen was supplied by a high-pressure H_2 tank under a controlled pressure. Given a constant flow by a peristaltic manifold pump (Longer Company, Baoding, China), the synthetic groundwater was continuously pumped into the bottom of the main tube and the effluent overflow with the same flow through a short pipe on the top. Due to the uninterrupted stirring by the magnetic stirrer below the tube, the liquid in reactor was considered as completely mixed. The whole system was shaded with an aluminum foil bag to avoid algae growth. Detailed physical parameters are as following: number of hollow fibers 96, fiber inner diameter 0.085 cm, fiber external diameter 0.15 cm, fiber pore diameter 0.01 μm, fiber surface area 633.3 cm^2, fiber specific surface 113.1 m^2/m^3, tube length 22 cm, tube inner diameterr 6 cm, tube effective volume 560 mL.

1.2 Inoculation and start up

The inoculum was obtained from anaerobic active sludge in a treatment plant treating municipal sewage (Quyang Sewage Treatment Plant, Shanghai, China). The treatment process included an anaerobic pond where denitrification took place, so the active sludge was expected to contain denitrifying strains and form a denitrifying biofim. Another consideration was that the biofilm formed from this sort of inoculum was considered as a mixed culture which was capable of reducing different kinds of oxidized contaminants. Anaerobic active sludge of 20 mL (MLSS around 3000 mg/L) was injected into the reactor with a sterilized syringe. After that, the reactor was fed by 5 mg/L NO_3^--N with flow of 0.2 mL/min for 24 hr. The H_2 supply was shut off during this period. Then, an initial biofilm attached to the surface of the membrane fibers was observed. Then the MBfR system was started up when H_2 was supplied to the inside of the hollow fiber membranes. In advance of adding the target contaminants, the reactor was fed with 5 mg/L NO_3^--N at a flow of 1.0 mL/min and H_2 pressure of 0.04 MPa (5.8 psi) for biofilm accumulation. During this period, effluent NO_3^--N and NO_2^--N concentrations were monitored daily for determining the formation of the mature biofilm. On the first several days, NO_2^--N accumulation was detected in the effluent. However, within 6 days effluent NO_2^--N decreased to below its detection limit of 0.003 mg/L and held at this level until day 20. Meanwhile, effluent NO_3^--N decreased gradually and was below its detection limit of 0.08 mg/L on day 10. After 20 days, the nitrogen removal reached 100% (data not shown). The biofilm was considered as mature and ready for subsequent operation with the appearance of a tan film attached to the surface of the membrane fibers (**Fig. 2**).

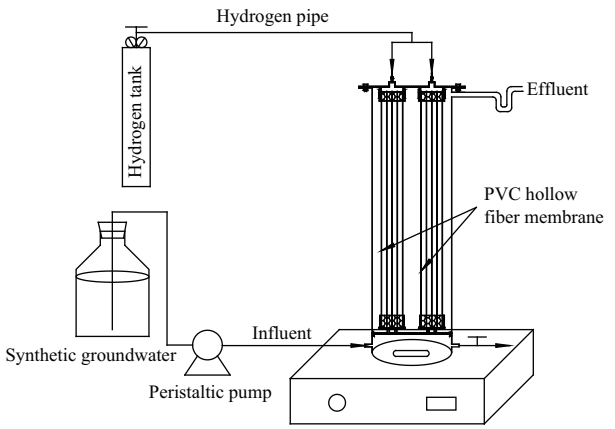

Fig. 1 Schematic diagram of the CS-MBfR.

1.3 Feed medium

The synthetic groundwater consisted of a feed medium and five target contaminants. The composition of the feed medium was (mg/L): KH_2PO_4 181, Na_2HPO_4 379, $NaHCO_3$ 80, $FeSO_4{\cdot}7H_2O$ 1, $MgSO_4{\cdot}7H_2O$ 200, $CaCl_2{\cdot}2H_2O$ 1, $ZnSO_4{\cdot}7H_2O$ 0.1, H_3BO_3 0.3, $CuCl_2{\cdot}2H_2O$ 0.01, $Na_2MoO_4{\cdot}2H_2O$ 0.03, $MnCl_2{\cdot}4H_2O$ 0.03, $CoCl_2{\cdot}6H_2O$ 0.2, $NiCl_2{\cdot}6H_2O$ 0.01, and Na_2SeO_3 0.03. $NaHCO_3$ was used as inorganic carbon source and phosphate buffer (KH_2PO_4+Na_2HPO_4) was used to stabilize the system pH value at approximately 7.2, which is within the optimum range for bioreduction.

Fig. 2 Appearance of the biofilm attached to the surface of membrane fibers.

Table 1 Detailed operational conditions for the four phases

Phase	H_2 pressure (MPa)	Flow rate (mL/min)	Influent concentration (mg/L)					Surface loading[a] (g/(day·m^2))				
			NO_3^--N	SO_4^{2-}	BrO_3^-	Cr(VI)	*p*-CNB	NO_3^--N	SO_4^{2-}	BrO_3^-	Cr(VI)	*p*-CNB
1	0.06	2.0	10	100	0.1	0.25	0.5	0.455	4.548	0.00455	0.0114	0.0227
2	0.06	2.0	20	200	0.2	0.5	1.0	0.910	9.095	0.00910	0.0227	0.0455
3	0.06	1.4	20	200	0.2	0.5	1.0	0.637	6.367	0.00637	0.0159	0.0637
4	0.08	1.4	20	200	0.2	0.5	1.0	0.637	6.367	0.00637	0.0159	0.0637

[a] Surface loading = $\frac{S_0 Q}{A}$, where, S_0 (g/m^3) is influent concentration; Q (m^3/day) is flow; and A (m^2) is fiber area.

Five chemicals: nitrate-nitrogen (from sodium nitrate), sulfate (from sodium sulfate), bromate (from potassium bromate), Cr(VI) (from potassium chromate) and *p*-CNB were chosen as target contaminants. The concentrations and proportions were set close to what they would be in actual groundwater. The influent was prepared in distilled water and stored in a 10 L glass bottle also shaded with aluminum foil. Dissolved oxygen in the influent was removed by pure nitrogen aeration every time a new bottle of influent came into use.

1.4 Operational conditions

The contaminants were added into the influent as soon as the biofilm was mature. In the reduction reactions, hydrogen functioned as electron donor and the contaminants were utilized by the microorganisms as substrates and electron acceptors as well. Therefore we considered H_2 availability and contaminant surface loading as factors that might influence reduction reactions. The contaminant surface loading referred to the mass rate of contaminant entering the system normalized to the biofilm surface area. It has also been demonstrated that hydrogenotrophic reductions strongly depend on H_2 availability (Chung et al., 2006; Xia et al., 2010). When hydrogen limitation existed in a reactor, a slight increase in H_2 pressure could drastically promote reduction (Lee and Rittmann, 2002). H_2 availability was controlled by the H_2 pressure applied to the reactor. Changing the H_2 pressure might affect the delivery efficiency of H_2. The higher the pressure was, the more H_2 transfer could occur across the membrane fiber walls. Due to those reasons, the experiment was organized into four successive phases in operational conditions varying in H_2 pressure and contaminant surface loading to explore the effects of the two factors. The contaminant surface loadings were changed by raising influent concentrations or decreasing flow or both. Each phase lasted for a sufficient period for the reactor to reach a steady state, which was 30–60 days in this study. Phases 1, 2 and 3 varied in surface loadings while phases 3 and 4 involved different H_2 pressures. Detailed operational conditions are shown in **Table 1**.

1.5 Sampling and analysis

We monitored the performance of the MBfR by analyzing influent and effluent samples for concentrations of soluble NO_3^--N, NO_2^--N, SO_4^{2-}, BrO_3^-, Br^-, Cr(VI), total Cr, *p*-CNB, *p*-CAN and aniline. The effluent samples were daily taken and immediately filtered through a 0.45 μm polyether sulfone syringe filter (Anpel Company, Shanghai, China) to eliminate any possibly detached biofilm. Analysis of NO_3^--N, NO_2^--N and Cr(VI) were carried out with spectrophotometry as described in standard methods (Ministry of Environmental Protection of the People's Republic of China, 2002). SO_4^{2-}, BrO_3^- and Br^- were analyzed by ion chromatography (Dionex, USA) with an AS-19 column, an AG-19 precolumn and a 250 μL injection loop. Total Cr was analyzed by inductively coupled plasma (Optima, USA). We detected *p*-CNB, *p*-CAN and aniline using high performance liquid chromatography (Agilent, USA). Analysis parameters are listed as follows: column: Polaris C18, 5 μm, 4.6 mm × 250 mm; mobile phase: acetonitrile/H_2O = 60/40 (*V/V*), flow: 1.0 mL/min; detector: UV at 254 nm, column temperature at 25°C. The retention times of *p*-CNB, *p*-CAN and aniline were around 4.1, 5.1 and 8.0 min, respectively (Xia et al., 2011).

2 Results and discussion

2.1 Steady states

After the accumulation of the biofilm, we raised influent NO_3^--N from 5 to 10 mg/L, added the other four contaminants into the influent, and raised the H_2 pressure to 0.06 MPa (5.8 psi) to start phase 1 from day 21. As shown in **Fig. 3**, within one day the MBfR reduced all contaminants to some degree immediately: 87.7% NO_3^--N, 5.9% SO_4^{2-}, 14.3% BrO_3^-, 20.2% Cr(VI) and 75.1% *p*-CNB. As operation continued, the effluent concentrations of all contaminants showed similar trends in that they all gradually decreased before reaching steady states. On day 31 of phase 1, the effluent concentration of NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI), and *p*-CNB was stable at about 0 (non-detected level), 80.7, 0.007, 0.036 and 0.070 mg/L, respectively. As for trends of end products and intermediates, effluent NO_2^--N was below the detection limit since operation started, which suggested the complete reduction of NO_3^--N to N_2. As reported in earlier research (Hijnen et al., 1995; Butler et al., 2005), complete bioreduction of BrO_3^- generated Br^-. In this study effluent Br^- increased from day 1 and steadily remained at 0.13 mg/L on day 52. We calculated the ratio of the actual effluent Br^- in the steady-state of phase 1 to a theoretical

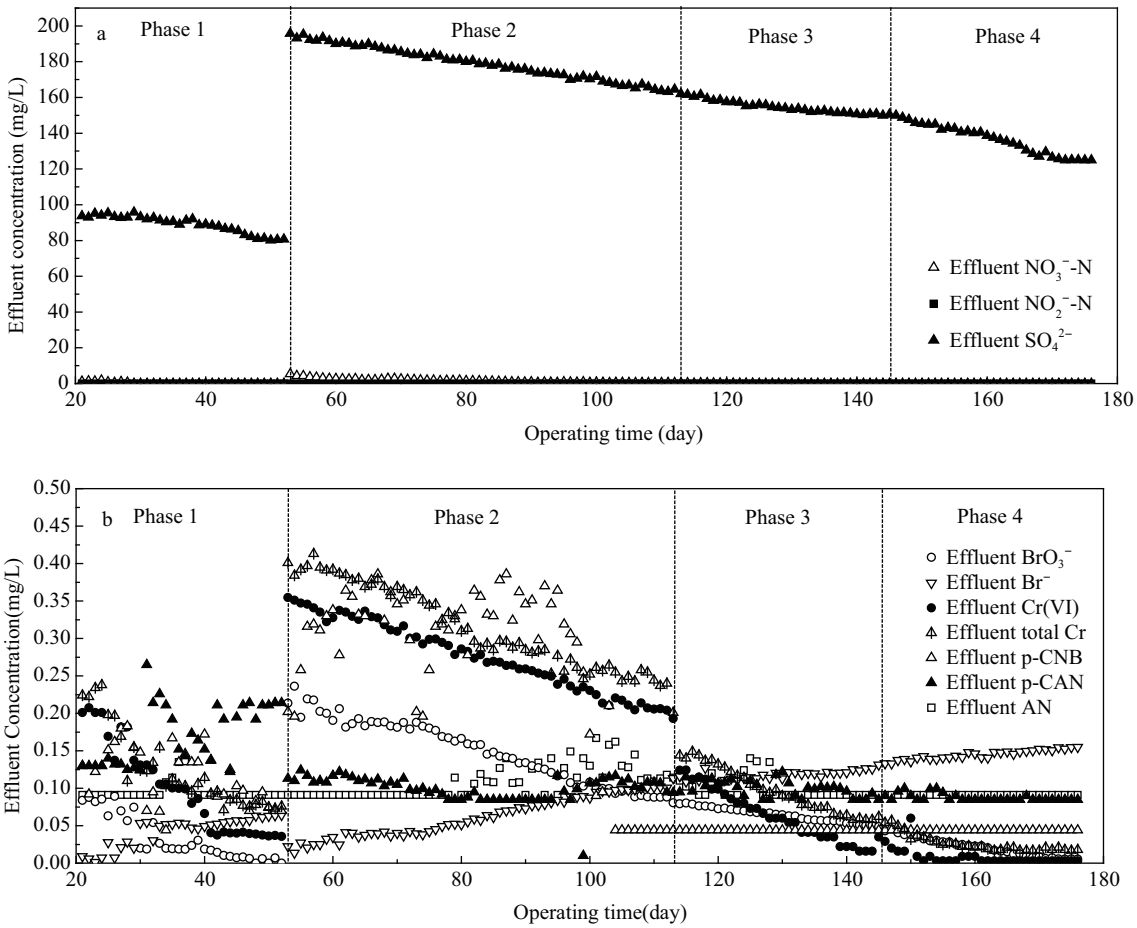

Fig. 3 Effluent concentrations of NO_3^--N, NO_2^--N and SO_4^{2-} (a); and effluent concentrations of BrO_3^-, Br^-, Cr(VI), total Cr, *p*-CNB, *p*-CAN and aniline (b).

value under the hypothesis that BrO_3^- was completely reduced to Br^- (1 mg BrO_3^- produces 0.625 mg Br^-), and the results ranged from 0.99 to 1.00 (data not shown), demonstrating that BrO_3^- was entirely reduced to Br^- in this study. The concentration of Cr(III), the end product of Cr(VI) reduction, was calculated by subtracting Cr(VI) from total Cr in the effluent. The comparison of the trends of effluent total Cr and effluent Cr(VI) (**Fig. 3b**) indicated that the majority of Cr(III) formed insoluble $Cr(OH)_3$ at pH 7.2 and was removed from the effluent after filtration. Chloronitrobenzenes are readily biodegraded to aniline through nitro reduction and successive dechlorination under anaerobic conditions as previously reported (Heijman et al., 1993; Susarla et al., 1996; Katsivela et al., 1999). It is conjectured that this mechanism of *p*-CNB reduction occurred in this study as well and was evident by effluent *p*-CAN and AN accumulation as shown in **Fig. 3b**.

Aiming to explore the MBfR's reducing capacity, on day 53 (day 1 of phase 2) we doubled the influent concentrations with flow unchanged, thus doubling the surface loadings of all contaminants. A drastic increase of the effluent concentrations of all contaminants except for NO_3^--N were observed within one day, then gradual decline began subsequently. The effluent concentrations of NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI), and *p*-CNB increased to 5.34, 195.73, 0.214, 0.355 and 0.202 mg/L, respectively. After gradual decline and stabilizing within 60 days, the effluent still contained 0.41 mg/L NO_3^--N, 164.53 mg/L SO_4^{2-}, 0.088 mg/L BrO_3^-, 0.20 mg/L Cr(VI) and 0.044 mg/L *p*-CNB, which was much higher than in phase 1 except that *p*-CNB remained the same. This result suggested that the MBfR system showed shock loading resistance to a certain extent, yet effluent concentrations and percentage removals were inevitably impacted by high surface loading.

Therefore we decreased the flow from 2.0 to 1.4 mL/min on day 113 (day 1 of phase 3), reducing surface loadings by 30%. A sudden drawdown of effluent Cr(VI) occurred within one day after flow change. Thereafter, except for *p*-CNB, the effluent concentrations of all contaminants kept gradually decreasing and the new steady-state effluent concentrations were much lower than those before flow adjustment: NO_3^--N 0 mg/L, SO_4^{2-} 150.0 mg/L, BrO_3^- 0.050 mg/L , Cr(VI) 0.016 mg/L and *p*-CNB 0.044 mg/L. On day 130 (18 days into phase 3) NO_3^--N was entirely reduced, which indicated that the quickest adjustment of the MBfR was for NO_3^--N reduction. It seemed that NO_3^--N reduction was not affected by the complex co-

existing conditions of several contaminants. The reason for this might be the first-electron-accepter role of NO_3^--N according to previous studies (Nerenberg and Rittmann, 2004; Ziv-El and Rittmann, 2009), which is also supported by electron-equivalent flux analysis in this study.

On day 145, the H_2 pressure was raised from 0.06 MPa (8.7 psi) to 0.08 MPa (11.6 psi) to increase H_2 availability. Although the reactor had already reached steady state in the former phase, the effluent concentration gradually decreased within one day after H_2 pressure enhancement except that effluent NO_3^--N remained at the non-detected level (**Fig. 3a**). This result indicated the existence of H_2 limitation in phase 3. On day 176 the effluent concentrations of NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI), and *p*-CNB steadily declined to 0, 125.0, 0.005, 0.003 and 0.044 mg/L, respectively. All contaminants were at least 95% reduced except for SO_4^{2-} which was 37.6% removed under a relative high loading. We did not apply a further rise of H_2 pressure in this study in consideration of safety, but we conjectured it might result in higher removal percentage.

2.2 Removal flux analysis

To evaluate the reduction rate, we introduced the removal flux (J, g/(day·m^2)), which defined as the mass of reduced target contaminant per square meter of biofilm per hour:

$$J = \frac{Q(S_0 - S_e)}{A}$$

where, Q (m^3/day) refers to flow; S_0 (g/m^3) refers to influent concentration; S_e (g/m^3) refers to effluent concentration and A (m^2) refers to fiber area. We compared the removal fluxes and percentage removals of the five contaminants in phases 1–3, or under different surface loadings. Results are shown in **Fig. 4**. Except for Cr(VI), all contaminants showed a similar trend, with removal flux increasing as surface loading increased. However, the removal flux of Cr(VI) was the lowest for the lowest surface loading (in phase 1) and was the highest for the second highest surface loading (in phase 3). A probable explanation was that the long-term Cr(VI) feeding in phases 1 and 2 had caused appreciable enrichment of specific Cr(VI)-reducing strains in the biofilm, which resulted in a larger capacity of the reactor for Cr(VI) reduction in phase 3. But this conjecture could not be confirmed in this paper due to the lack of biofilm population structure data. The comparison of removal fluxes between phase 3 and phase 4 (**Table 2**) showed that the effluent concentration decreased while the removal percentage and removal flux both increased as H_2 pressure increased, indicating that H_2 availability was limited in phase 3. As phase 4 reached steady state, the removal fluxes for NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI) and *p*-CNB were 0.641, 2.396, 0.008, 0.016 and 0.031 g/(day·m^2).

2.3 Electron-equivalent flux analysis

The essence of the reduction process was electron transport, and the continuous operation results have revealed the competition for electron donors among the five contaminants at a given H_2 pressure. Although the comparison of removal fluxes explained this competition to some degree, the value of the removal flux was strongly affected by the molecular weight of the relevant contaminant. Thus the electron-equivalent flux was introduced. The unit of the electron-equivalent flux was e^-/(day·m^2). It was defined by replacing "the mass of contaminant" in the index removal flux with "the moles of electrons transported to the reduction of the contaminant", that is to say, it referred to the moles of electrons transported to the reduction of a certain contaminant per square meter of biofilm per hour. It was calculated as removal flux divided by the equivalent factor. The equivalent factor was introduced to eliminate the mentioned effect of molecular weight on the removal flux. It was defined as the mass of target contaminant that a mole of electrons could reduce, and calculated by dividing the molecular weight of contaminant by the number of electrons used for reducing a mole of this contaminant. For example, the equivalent factor of NO_3^--N was 14/5 = 2.8 g/e^-. This factor varied with the contaminants and was 2.8 g/e^- for NO_3^--N, 12 g/e^- for SO_4^{2-}, 13.3 g/e^- for BrO_3^-, 17.3 g/e^- for Cr(VI) and 19.7 g/e^- for *p*-CNB. **Table 3** shows the electron-equivalent flux of each contaminant in different phases along with the percentage distribution of each flux. The data was obtained from the average results of the last three days' steady operation in every phase.

The total electron flux ranged from 0.238 to 0.458 e^-/(day·m^2) and increased with total surface loading as the data of phases 1–3 displayed. The electron flux of a single contaminant also increased with its respective surface loading. This result showed higher contaminant surface loading encouraged higher H_2 flux. This effect might be attributed to a higher biological demand for H_2, driving down the ambient dissolved H_2 concentration and accelerating gas transfer across the membrane (Haugen et al., 2002). The comparison of total electron fluxes between phase 3 and phase 4 clearly showed that the amount of electrons used by bioreduction increased by 20% as the H_2 pressure was raised from 0.06 MPa (8.7 psi) to 0.08 MPa (11.6 psi), thus confirming the H_2 limitation in phase 3. Higher pressure promoted the delivery of H_2 and thus more available electrons were provided for the biofilm.

The distributions of electron fluxes were almost stable in all four phases. Through all cases, the distribution of electron-equivalent flux for the electron acceptors NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI) and *p*-CNB were 53.04%–68.38%, 29.71%–30.86%, 0.12%–0.14%, 0.17%–0.25% and 0.36%–0.48%, respectively. This result indicated the dominant roles of NO_3^--N and SO_4^{2-} reduction among the five contaminants throughout. NO_3^--N and SO_4^{2-} reduction together, averaging 99.24%, accounted for at

Table 2 Removal fluxes and removal percentages of the five contaminants in phases 3 and 4

Phase	H_2 pressure (MPa)	Removal flux (g/(day·m^2))				
		NO_3^--N	SO_4^{2-}	BrO_3^-	Cr(VI)	*p*-CNB
3	0.06	0.631	1.573	0.006	0.015	0.030
4	0.08	0.641	2.396	0.008	0.016	0.031

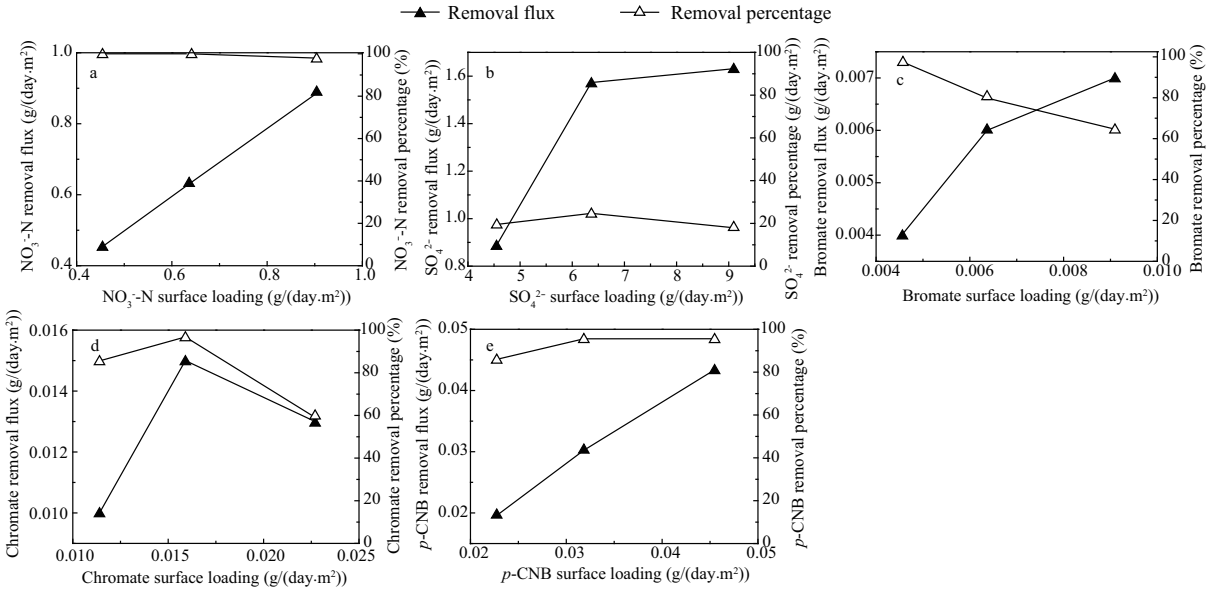

Fig. 4 Removal flux and removal percentage of NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI) and *p*-CNB in phases 1, 2, and 3.

Table 3 Electron-equivalent flux and distribution

Phase	Electron-equivalent flux (e^-/(day·m^2))						Electron-equivalent flux distribution (%)					
	NO_3^--N	SO_4^{2-}	BrO_3^-	Cr(VI)	*p*-CNB	Sum	NO_3^--N	SO_4^{2-}	BrO_3^-	Cr(VI)	*p*-CNB	Sum
1	0.162	0.074	0.00033	0.00056	0.00099	0.238	68.14	31.06	0.14	0.24	0.42	100.00
2	0.318	0.136	0.00055	0.00078	0.00220	0.458	69.53	29.70	0.12	0.17	0.48	100.00
3	0.225	0.131	0.00048	0.00089	0.00154	0.359	62.71	36.48	0.13	0.25	0.43	100.00
4	0.229	0.200	0.00059	0.00091	0.00156	0.432	53.04	46.25	0.14	0.21	0.36	100.00

least 99.18% of total electron flux. NO_3^--N was the most dominant electron consumer, which accepted more than half the electrons, and was followed by SO_4^{2-}, which consumed more than 29.71% of the electrons. Reductions of the other three contaminants were always small fractions of the electron flux, less than 1% altogether. This result clearly showed that the H_2 consumption was controlled by NO_3^--N and SO_4^{2-} reduction to a great extent in this study. The explanation might arise from two aspects: the priority of NO_3^--N and SO_4^{2-} as electron acceptors in electron competition, and the relatively higher surface loadings of them (20–1000 times that of the others), directly leading to higher removal fluxes. A general consensus considering NO_3^--N to be the first priority electron accepter was reached in previous studies (Nerenberg and Rittmann, 2004; Ziv-El and Rittmann, 2009), which was also substantiated in this study by the fact that NO_3^--N reduction accounted for a larger electron flux distribution than SO_4^{2-} reduction, with surface loading far lower than that of SO_4^{2-}. When operational conditions switched from phase 3 to phase 4, more available H_2 and therefore more electrons were provided. Since NO_3^--N was already fully reduced in phase 3 and therefore did not consume any more electron in phase 4, the additional electrons mostly went to SO_4^{2-}, BrO_3^-, Cr(VI) and *p*-CNB reduction. As shown in **Table 3**, the increase of equivalent electron flux for SO_4^{2-} was the most significant (increased by 52.33% from 0.131 to 0.200 e^-/(day·m^2) while NO_3^--N, BrO_3^-, Cr(VI) and *p*-CNB increased by 1.62%, 23.21%, 2.83% and 1.68% respectively), indicating that electrons preferentially went to SO_4^{2-} reduction. The data documented that SO_4^{2-} was the priority electron accepter among the other four contaminants in this study, following NO_3^--N.

3 Conclusions

We set up a lab-scale continuously stirred hydrogen-based membrane biofilm reactor to simultaneously reduce NO_3^--N, SO_4^{2-}, BrO_3^-, Cr(VI) and *p*-CNB in this study. H_2 was applied as electron donor for the biofilm. The

reductions started within 1 day under denitrifying conditions and with an environmental inoculum. After 112 days continuous operation, all contaminants were over 95% removed except for SO_4^{2-}, which was 37% removed under high surface loading. The detection of end products and intermediates has proved the complete reduction of NO_3^--N to N_2 via NO_2^--N, Cr(VI) to Cr(III), BrO_3^- to Br^- and *p*-CNB to aniline via *p*-CAN. Increasing surface loading promoted removal flux but decreased removal percentage. On the other hand, the reduction reactions were sensitive to H_2 pressure when the H_2 supply was limited. Under the same surface loading, an increase in H_2 pressure promoted removal flux. The result of electron-equivalent flux analysis indicated electron competition among the five contaminants. It showed that the electron consumption was controlled by NO_3^--N and SO_4^{2-} reductions with limited H_2 supply. NO_3^--N and SO_4^{2-} reductions altogether accounted more than 99% of the total electron flux. Although all contaminants accepted the electrons provided by H_2, an order existed. In this study we found that NO_3^--N was the first electron acceptor followed by SO_4^{2-}.

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References

Abu Naser A A, Ghbn N, Khoudary R, 2007. Relation of nitrate contamination of groundwater with methaemoglobin level among infants in Gaza. *Eastern Mediterranean Health Journal*, 13(5): 994–1004.

Alabdula'aly A I, Al-Rehaili A M, Al-Zarah A I, Khan M A, 2010. Assessment of nitrate concentration in groundwater in Saudi Arabia. *Environmental Monitoring and Assessment*, 161(1-4): 1–9.

Becker D S, Long E R, Proctor D M, Ginn T C, 2006. Evaluation of potential toxicity and bioavailability of chromium in sediments associated with chromite ore processing residue. *Environmental Toxicology and Chemistry*, 25(10): 2576–2583.

Butler R, Godley A, Lytton L, Cartmell E, 2005. Bromate environmental contamination: Review of impact and possible treatment. *Critical Reviews in Environmental Science and Technology*, 35(3): 193–217.

Chung J, Nerenberg R, Rittmann B E, 2006a. Bio-reduction of soluble chromate using a hydrogen-based membrane biofilm reactor. *Water Researc*h, 40(8): 1634–1642.

Chung J, Nerenberg R, Rittmann B E, 2006b. Bioreduction of selenate using a hydrogen-based membrane biofilm reactor. *Environmental Science & Technology*, 40(5): 1664–1671.

Chung J, Rittmann B E, Wright W F, Bowman R H, 2007. Simultaneous bio-reduction of nitrate, perchlorate, selenate, chromate, arsenate, and dibromochloropropane using a hydrogen-based membrane biofilm reactor. *Biodegradation*, 18(2): 199–209.

Cieslak-Golonka M, 1996. Toxic and mutagenic effects of chromium(VI): A review. *Polyhedron*, 15(21): 3667–3689.

Davydova S G, 1967. Comparative hygienic and sanitary-toxicologic characteristics of nitrochlorobenzene isomers in relation to hygienic standards for their content in bodies of water. *Gig Sanit*, 32(8): 7–11.

DeVriese A, Vanholder R, Lameire N, 1997. Severe acute renal failure due to bromate intoxication: Report of a case and discussion of management guidelines based on a review of the literature. *Nephrology Dialysis Transplantation*, 12(1): 204–209.

Downing L S, Nerenberg R, 2007. Kinetics of microbial bromate reduction in a hydrogen-oxidizing, denitrifying biofilm reactor. *Biotechnology and Bioengineering*, 98(3): 543–550.

George M, Wiklund L, Aastrup M, Pousette J, Thunholm B, Saldeen T et al., 2001. Incidence and geographical distribution of sudden infant death syndrome in relation to content of nitrate in drinking water and groundwater levels. *European Journal of Clinical Investigation*, 31(12): 1083–1094.

Haugen K S, Semmens M J, Novak P J, 2002. A novel in situ technology for the treatment of nitrate contaminated groundwater. *Water Research*, 36(14): 3497–3506.

Heijman C G, Holliger C, Glaus M A, Schwarzenbach R P, Zeyer J, 1993. Abiotic reduction of 4-chloronitrobenzene to 4-chloroaniline in a dissimilatory iron-reducing enrichment culture. *Applied and Environmental Microbiology*, 59(12): 4350–4353.

Hijnen W A M, Voogt R, Veenendaal H R, van der Jagt H, van der Kooij D, 1995. Bromate reduction by denitrifying bacteria. *Applied and Environmental Microbiology*, 61(1): 239–244.

Hutchinson T H, Hutchings M J, Moore K W, 1997. A review of the effects of bromate on aquatic organisms and toxicity of bromate to oyster (*Crassostrea gigas*) embryos. *Ecotoxicology and Environmental Safety*, 38(3): 238–243.

Johns M W, Lawrence C R, 1973. Nitrate-rich groundwater in Australia: a possible cause of methaemoglobinaemia in infants. *Medical Journal of Australia*, 2(20): 925–927.

Katsivela E, Wray V, Pieper D H, Wittich R M, 1999. Initial reactions in the biodegradation of 1-chloro-4-nitrobenzene by a newly isolated bacterium, strain LW1. *Applied and Environmental Microbiology*, 65(4): 1405–1412.

Komori K, Rivas A, Toda K, Ohtake H, 1990. A method for removal of toxic chromium using dialysis-sac cultures of a chromate-reducing strain of *Enterobacter cloacae*. *Applied Microbiology and Biotechnology*, 33(1): 117–119.

Krumschnabel G, Nawaz M, 2004. Acute toxicity of hexavalent chromium in isolated teleost hepatocytes. *Aquatic Toxicology*, 70(2): 159–167.

Lee K C, Rittmann B E, 2002. Applying a novel autohydrogenotrophic hollow-fiber membrane biofilm reactor for denitrification of drinking water. *Water Research*, 36(8): 2040–2052.

Linch A L, 1974. Biological monitoring for industrial exposure to cyanogenic aromatic nitro and amino compounds. *American Industrial Hygiene Association Journal*, 35(7): 426–432.

Matsumoto M, Aiso S, Senoh H, Yamazaki K, Arito H, Nagano K et al., 2006. Carcinogenicity and chronic toxicity of para-chloronitrobenzene in rats and mice by two-year feeding. *Journal of Environmental Pathology Toxicology and Oncol-*

ogy, 25(3): 571–584.

Michalski R, 2003. Toxicity of bromate ions in drinking water and its determination using ion chromatography with post column derivatisation. *Polish Journal of Environmental Studies*, 12(6): 727–734.

Ministry of Environmental Protection of the People's Republic of China, 2002. Monitoring Method for Water and Wastewater. China Environmental Science Press, Beijing.

Mishra A K, Mohanty B, 2008. Acute toxicity impacts of hexavalent chromium on behavior and histopathology of gill, kidney and liver of the freshwater fish, *Channa punctatus* (Bloch). *Environmental Toxicology and Pharmacology*, 26(2): 136–141.

Nerenberg R, Rittmann B E, 2004. Hydrogen-based, hollow-fiber membrane biofilm reactor for reduction of perchlorate and other oxidized contaminants. *Water Science and Technology*, 49(11-12): 223–230.

Park H S, Lim S J, Chang Y K, Livingston A G, Kim H S, 1999. Degradation of chloronitrobenzenes by a coculture of *Pseudomonas putida* and a *Rhodococcus* sp. *Applied and Environmental Microbiology*, 65(3): 1083–1091.

Rittmann B E, Nerenberg R, Lee K C, Najm I, Gillogly T E, Lehman G E et al., 2004. Hydrogen-based hollow-fiber membrane biofilm reactor (MBfR) for removing oxidized contaminants. *Creative Water and Wastewater Treatment Technologies for Densely Populated Urban Areas*, 4(1): 127–133.

Safe Drinking Water Committee, 1986. Drinking Water and Health. National Academies Press, Washington DC.

Sashiyama H, Irie Y, Nakajima K, Yoshida H, Sakai T, Okuda K, 2002. Acute renal failure and hearing loss due to sodium bromate poisoning: a case report and review of the literature. *Clinical Nephrology*, 58(6): 455–457.

Shanker A K, Cervantes C, Loza-Tavera H, Avudainayagam S, 2005. Chromium toxicity in plants. *Environment International*, 31(5): 739–753.

Shimizu M, Yasui Y, Matsumoto N, 1983. Structural specificity of aromatic compounds with special reference to mutagenic activity in *Salmonella typhimurium* – a series of chloro-or fluoro-nitrobenzene derivatives. *Mutation Research*, 116(3-4): 217–238.

Showers W J, Genna B, McDade T, Bolich R, Fountain J C, 2008. Nitrate contamination in groundwater on an urbanized dairy farm. *Environmental Science & Technology*, 42(13): 4683–4688.

Susarla S, Masunaga S, Yonezawa Y, 1996. Transformations of chloronitrobenzenes in anaerobic sediment. *Chemosphere*, 32(5): 967–977.

Tang Y, Ziv-El M, Zhou C, Shin J H, Ahn C H, Meyer K et al., 2010. Bioreduction of nitrate in groundwater using a pilot-scale hydrogen-based membrane biofilm reactor. *Frontiers of Environmental Science & Engineering in China*, 4(3): 280–285.

Tuttle J H, Dugan P R, Randles C I, 1969. Microbial sulfate reduction and its potential utility as an acid mine water pollution abatement procedure. *Applied Microbiology*, 17(2): 297–302.

Veenhuizen M F, Shurson G C, Kohler E M, 1992. Effect of concentration and source of sulfate on nursery pig performance and health. *Journal of the American Veterinary Medical Association* , 201(8): 1203–1208.

Watanabe T, Ishihara N, Ikeda M, 1976. Toxicity of and biological monitoring for 1,3-diamino-2,4,6-trinitrobenzene and other nitro-amino derivatives of benzene and chlorobenzene. *International Archives of Occupational and Environmental Health*, 37(3): 157–168.

Weisburger E K, Russfield A B, Homburger F, Weisburger J H, Boger E, Van Dongen C G et al., 1978. Testing of twenty-one environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity. *Journal of Environ Pathology Toxicology*, 2(2): 325–356.

Wu J F, Jiang C Y, Wang B J, Ma Y F, Liu Z P, Liu S J, 2006. Novel partial reductive pathway for 4-chloronitrobenzene and nitrobenzene degradation in *Comamonas* sp. strain CNB-1. *Applied and Environmental Microbiology*, 72(3): 1759–1765.

Xia S Q, Li H X, Zhang Z Q, Zhang Y H, Yang X, Jia R Y et al., 2011. Bioreduction of para-chloronitrobenzene in drinking water using a continuous stirred hydrogen-based hollow fiber membrane biofilm reactor. *Journal of Hazardous Materials*, 192(2): 593–598.

Xia S Q, Zhong F H, Zhang Y H, Li H X, Yang X, 2010. Bio-reduction of nitrate from groundwater using a hydrogen-based membrane biofilm reactor. *Journal of Environmental Sciences*, 22(2): 257–262.

Yang R, Liu W J, 2010. Nitrate contamination of groundwater in an agroecosystem in Zhangye Oasis, Northwest China. *Environmental Earth Sciences*, 61(1): 123–129.

Ziv-El M C, Rittmann B E, 2009. Systematic evaluation of nitrate and perchlorate bioreduction kinetics in groundwater using a hydrogen-based membrane biofilm reactor. *Water Research*, 43(1): 173–181.

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