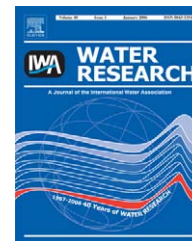


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# Bio-reduction of soluble chromate using a hydrogen-based membrane biofilm reactor

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## ABSTRACT

Hexavalent chromium (Cr(VI)) is a mutagen and carcinogen that is a significant concern in water and wastewater. A simple and non-hazardous means to remove Cr(VI) is bioreduction to Cr(III), which should precipitate as Cr(OH)<sub>3(s)</sub>. Since Cr(VI)-reducing bacteria can use hydrogen (H<sub>2</sub>) as an electron donor, we tested the potential of the H<sub>2</sub>-based membrane biofilm reactor (MBfR) for chromate reduction and removal from water and wastewater. When Cr(VI) was added to a denitrifying MBfR, Cr(VI) reduction was immediate and increased over 11 days. Short-term experiments investigated the effects of Cr(VI) loading, H<sub>2</sub> pressure, and nitrate loading on Cr(VI) reduction. Increasing the H<sub>2</sub> pressure improved Cr(VI) reduction. Cr(VI) reduction also was sensitive to pH, with an optimum near 7.0, a sharp drop off below 7.0, and a gradual decline to 8.2. Cr(III) precipitated after a small upward adjustment of the pH. These experiments confirm that a denitrifying, H<sub>2</sub>-based MBfR can be used to reduce Cr(VI) to Cr(III) and remove Cr from water. The research shows that critical operational parameters include the H<sub>2</sub> concentration, nitrate concentration, and pH.

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## 1. Introduction

The widespread use of chromate in industries such as leather tanning, metallurgy, electroplating, petroleum refining, textile manufacturing, and pulp production has resulted in large quantities of chromium being discharged into the environment (Barnhart, 1997). In the natural environment, chromium is found in trivalent (Cr(III)) and hexavalent (Cr(VI)) forms. Cr(III) has relatively low toxicity and tends to form insoluble complexes with hydroxides at neutral pH (Anderson and Kozlovsky, 1985; Palmer and Wittbrodt, 1991). On the other hand, Cr(VI) is highly soluble and, therefore, mobile and bio-available in aquatic systems (Dragun, 1988). At relatively high concentrations, Cr(VI) compounds are potent irritants whose acute effects include ulceration of skin, eyes, mucous

membranes, and the gastrointestinal tract. At low concentrations, typical of those found in the environment, Cr(VI) has mutagenic and carcinogenic effects (DeLeo and Ehrlich, 1994; National Toxicology Program, 1991; US EPA, 1992). The maximum contaminant level (MCL) for drinking water is 100 µg/l total chromium in the United States (US EPA, 2003).

Conventional drinking water treatment is not effective for removing chromate. Advanced treatment techniques, such as reverse osmosis, ion exchange, membrane filtration, and electrodialysis, are more effective for removing Cr(VI), but are expensive and generate concentrated wastes that require subsequent treatment and disposal (Komori et al., 1990; Srivastava et al., 1986).

Biological reduction may provide a suitable means for Cr(VI) removal from water and wastewater (Lovley and Coates, 1997;

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Rittmann et al., 2004). Once biologically reduced, Cr(III) can precipitate as  $\text{Cr}(\text{OH})_3(\text{s})$ , which can be removed by filtration.

Cr(VI) is bio-reduced to Cr(III) under aerobic (Bopp and Ehrlich, 1988; Gopalan and Veeramani, 1994) and anaerobic (Lovley, 1993; Wang et al., 1989) conditions. Direct reduction of Cr(VI) in the absence of sulfide positively correlated with nitrate reduction by a nitrate-reducing consortium when straw of cattail was used as the organic carbon source (Vainshtein et al., 2003), and the addition of molasses and nitrate to a microcosm stimulated chromate reduction (Oliver et al., 2003). Chen and Hao (1996) and Wang (2000) reported that environmental factors, including pH, temperature, and other electron acceptors, affected Cr(VI) reduction in an anaerobically enriched mixed culture. Cervantes (1991) indicated that chromate reduction did not stimulate growth. If growth with chromate were possible, the yields would be low since chromate reduction provides less free energy per electron than sulfate (Marsh and McInerney, 2001).

Some Cr(VI)-reducing bacteria can use hydrogen ( $\text{H}_2$ ) as an electron donor (Marsh and McInerney, 2001), and the  $\text{H}_2$ -based membrane biofilm reactor (MBfR) (Lee and Rittmann, 2000, 2002, 2003; Nerenberg and Rittmann, 2002; 2004; Nerenberg et al., 2002; Rittmann et al., 2004; Ergas and Reuss, 2001) is an ideal biological reactor configuration for autotrophic bioreduction of chromate.  $\text{H}_2$  is non-toxic to humans, is inexpensive compared to organic donors, leaves no residuals that could cause bacterial re-growth or add oxygen demand, and is used with nearly 100% efficiency in the MBfR setting (Lee and Rittmann, 2002; Nerenberg et al., 2002; Rittmann et al., 2004).  $\text{H}_2$ -based bio-reduction also supports autotrophic microorganisms that have inherently low biomass yields (Rittmann and McCarty, 2001). In addition, screening studies (Nerenberg and Rittmann, 2004) demonstrated that Cr(VI) was reduced without lag in MBfRs in which oxygen or nitrate was the primary electron acceptor.

In this study, we examine chromate reduction in a denitrifying,  $\text{H}_2$ -based MBfR. We use a denitrifying reactor because nitrate is a common co-contaminant in surface and ground waters and because nitrate can serve as a primary electron acceptor for chromate-reducing bacteria. In particular, we investigate factors that ought to affect the kinetics of Cr(VI) reduction or the removal of the resulting Cr(III):  $\text{H}_2$  pressure, chromate surface loading, the addition of other electron acceptors, and pH. The results provide information important for optimizing chromate reduction and removal in a  $\text{H}_2$ -based MBfR.

## 2. Materials and methods

### 2.1. Experimental setup

A schematic of the MBfRs used in this study is shown in Fig. 1, and reactor characteristics are provided in Table 1. The MBfR system consisted of two membrane modules connected in a recirculation loop. The system behaved as a completely mixed biofilm reactor because of the high recirculation ratio (150:1), which also provided a high-flow velocity that helped maintain a consistent biomass thickness on the hollow fibers. The main membrane module contained a bundle of 32

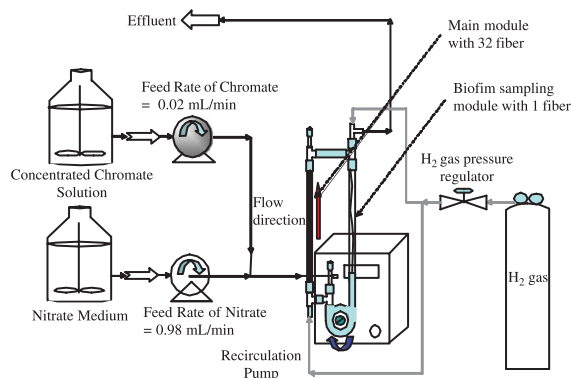


Fig. 1 - Schematic of the bench-scale MBfR used to investigate chromate reduction.

Table 1 - Physical characteristics of the main module of the MBfR

	Units	Value
Tube length	cm	27
Tube inside diameter	cm	0.6
Tube cross-sectional area	$\text{cm}^2$	0.28
Fiber outside diameter	$\mu\text{m}$	280
Fiber cross-sectional area	$\mu\text{m}^2$	61,544
Number of hollow fibers	—	32
Active length of fiber	cm	25
Area of single fiber	$\text{cm}^2$	0.06
Fiber surface area	$\text{cm}^2$	70.4
Feed flow rate	ml/min	1
Recirculation rate	ml/min	150
Volume	ml	23.9
Retention time	min	23.9

hydrophobic hollow-fiber membranes (Model MHF 200TL, Mitsubishi Rayon) inside a glass pipe shell. The other module contained a single, sacrificial fiber that was used to collect biofilm samples for electron microscopy. Two peristaltic manifold pumps (Gilson Minipuls 3, Middleton, WI) were used to blend two feed stocks: a nitrate medium, supplied at a rate of 0.98 ml/min, and a chromate stock solution, supplied at rate of 0.02 ml/min. The total feed flow rate was 1 ml/min. Pure hydrogen was supplied to the inside of the hollow fibers through the manifold at the base, and the standard  $\text{H}_2$  pressure for both reactors was 2.5 psi (0.17 atm). In order to evaluate the effect of  $\text{H}_2$  availability, the  $\text{H}_2$  pressure was increased to 4 psi (0.27 atm).

### 2.2. Feed medium, stock solutions, and mixed influent

The composition of the feed medium was (g/l):  $\text{KH}_2\text{PO}_4$ , 0.128;  $\text{Na}_2\text{HPO}_4$ , 0.434;  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.2;  $\text{NaNO}_3$  as N, 0.03;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.001;  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.001; and 1 ml of trace mineral solution. The trace mineral solution (mg/l) consisted of:  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 100;  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 30;  $\text{H}_3\text{BO}_3$ , 300;

CoCl<sub>2</sub>•6H<sub>2</sub>O, 200; CuCl<sub>2</sub>•2H<sub>2</sub>O, 10; NiCl<sub>2</sub>•6H<sub>2</sub>O, 10; Na<sub>2</sub>MoO<sub>4</sub>•2H<sub>2</sub>O, 30; and Na<sub>2</sub>SeO<sub>3</sub>, 30. It was prepared in an 8-l glass bottle (Pyrex) and filter sterilized into another sterile 8-l glass bottle using a capsule filter (Pall SuporCap 100, Pall Corporation, Ann Arbor, MI). All feed media were purged with N<sub>2</sub> gas to eliminate dissolved O<sub>2</sub> in the influent. The chromate stock solution contained deionized water with 50 mg/l of Cr(VI) (from K<sub>2</sub>CrO<sub>4</sub>). When the nitrate medium and the chromate stock solutions were mixed, the influent concentrations were 5 mg NO<sub>3</sub><sup>-</sup>-N/l, 78.5 mg SO<sub>4</sub><sup>2-</sup>/l, and 1000 µg/l CrO<sub>4</sub><sup>2-</sup> as Cr. In order to determine the effect of Cr(VI) loading, the influent concentration of chromate was reduced to 250 µg Cr/l in the presence of the same concentrations of nitrate and sulfate. The chromate concentration was set 7 to 30 times the MCL for municipal drinking water in order to enhance our ability to study its fate and kinetics (US EPA, 2005).

### 2.3. Inoculum, start up, and steady states

The MBfR was inoculated with mixed-culture biofilm collected from a pilot-scale MBfR used for perchlorate and nitrate reduction at La Puente, California (Rittmann et al., 2004; Nerenberg, 2003). The pilot-scale MBfR treated groundwater containing approximately 5 mgN/l of nitrate and 60 µg/l of perchlorate, and it had no exposure to chromate. The biofilm developed from indigenous bacteria present in the aquifer. The biofilm was preserved at -80 °C in a 25% glycerol solution prior to use. For inoculating the MBfR, biomass from the mixture was thawed, washed twice by centrifuging for 15 min at 5000 g, then resuspended in 10 ml of sterile minimal medium without electron donor. A 1.5-ml aliquot of the washed biofilm suspension was added into reactor.

Initially, H<sub>2</sub> was supplied to the lumen of the fibers at 2.5 psi (0.17 atm), and nitrate medium was recirculated for 24 h to

establish a biofilm. Then, nitrate medium was fed at a rate of 0.2 ml/min. The effluent nitrate concentration reached steady-state after around three days, and then the feed rate of influent medium was increased to 1.0 ml/min. After nitrate was completely removed (c. 20 days), chromate was added to the influent of the MBfR at 1000 µg-Cr/l. After a steady state was reached, the H<sub>2</sub> pressure was increased to 4 psi (0.27 atm) until a second steady state was attained. A third steady state was then achieved with a H<sub>2</sub> pressure of 2.5 psi and a lower chromate feed concentration of 250 µg Cr/l.

### 2.4. Short-term experiments

Short-term experiments were conducted to investigate systematically how H<sub>2</sub> availability, chromate loading, nitrate loading, and pH affected MBfR performance. The experiments were organized in a matrix with four series of experiments, whose conditions are listed in Table 2. Prior to each experiment, the reactor was returned to the steady state condition of an influent with 1000 µg Cr/l of chromate, 5 mgN/l of nitrate, 78.5 mg SO<sub>4</sub><sup>2-</sup>/l, and a 2.5 psi H<sub>2</sub> pressure.

For each short-term experiment, the conditions were run for 2 h before samples were taken. With a liquid retention time of 24 min in the MBfR, 2 h (more than five liquid retention times) was long enough for the system to reach a pseudo-steady-state, which is defined as a condition in which the liquid concentration reach a stable state, while the biofilm accumulation and biomass are not changed significantly from the true steady state. After each short-term test was completed, the system was returned to the steady-state before the next short-term experiment began. In order to see the effect of the chromate loading, the influent concentration of Cr(VI) was varied from 100 to 1000 µgCr/l, while nitrate concentration, H<sub>2</sub> pressure, and initial pH were fixed

**Table 2 – System conditions for short-term experiments**

Experimental series	H <sub>2</sub> pressure (psi) (1 psi = 0.068 atm)	Influent concentration		Influent pH					
		Chromate (µg Cr/l)	Nitrate (µg N/l)						
Series #1	2.5	1000 <sup>a</sup>	5000	7.5					
		500 <sup>a</sup>							
		250 <sup>a</sup>							
		100 <sup>a</sup>							
Series #2	2.5 <sup>a</sup> 4.0 <sup>a</sup> 5.5 <sup>a</sup>	1000	5000	7.5					
					Series #3	2.5	1000	10,000 <sup>a</sup>	7.5
					5000 <sup>a</sup>				
2500 <sup>a</sup>									
Series #4	2.5	1000	5000	6.5 <sup>a</sup>					
				7.1 <sup>a</sup>					
				7.6 <sup>a</sup>					
				8.2 <sup>a</sup>					
				8.9 <sup>a</sup>					

<sup>a</sup> The parameters changed in each item with the other parameters held constant.

at 5 mgN/l, 2.5 and 7.5 psi, respectively. In the second series, the  $H_2$  pressure was 2.5, 4.0, or 5.5 psi, with fixed influent chromate concentration (1000  $\mu\text{gCr/l}$ ), nitrate (5 mgN/l), and initial pH = 7.5. To investigate the effect of nitrate (Series #3), its influent concentration was varied from 0 to 10 mgN/l, while maintaining the chromate,  $H_2$  pressure, and the input pH at 1000  $\mu\text{gCr/l}$ , 2.5, and 7.5 psi, respectively. Finally, to test the pH effect, pH was set at 6.5, 7.1, 7.6, 8.2, or 8.9, while chromate concentration, nitrate concentration, and  $H_2$  pressure were fixed at 1000  $\mu\text{gCr/l}$ , 5 mgN/l, and 2.5 psi, respectively.

### 2.5. Cr(III)-precipitation test

A Cr(III)-precipitation test was carried out in a 250-ml glass batch reactor. The liquid contents were MBfR effluent, which was well-mixed with a stir bar and contained 95  $\mu\text{gCr/l}$  of Cr(VI) and 153  $\mu\text{gCr/l}$  of Cr(III). The average nitrate and sulfate concentrations in effluent were 0 and 74 mg/l, respectively. The temperature and pH was 21 °C and 7.5. First, 0.5-mM hydrochloric acid was added to lower the pH to 3; then, 0.5-mM sodium hydroxide was titrated to increase the pH, with each pH held constant for 20–30 min. For each pH, 10 ml of the solution was withdrawn and used to measurement of optical density (OD) (BioSpec-1601 Spectrophotometer, Shimadzu) at a wavelength of 600 nm. The sample was then passed immediately through a 0.2- $\mu\text{m}$  membrane filter, acidified with concentrated nitric acid, and analyzed by inductively coupled plasma/mass spectrometer (ICP-MS: PQExCell, VG Elemental) analysis to determine the concentration of soluble Cr.

### 2.6. Sampling and analysis

The running performance of the reactor was monitored by analyzing influent and effluent samples on a daily basis. After samples were filtered through a 0.2- $\mu\text{m}$  membrane filter (Pall Corp., Ann Arbor, MI) and centrifuged (15,000 g, 10 min), total soluble chromium (Cr(III)+Cr(VI)) was determined by ICP-MS (PQExCell, VG Elemental) according to the manufacturer's instructions. In order to investigate the redox speciation, the Cr(VI) concentration was analyzed by a diphenyl carbazide method (Method 3500-Cr D, APHA 1998). The Cr(III) concentration was determined by subtracting Cr(VI) from total chromium. Analyses for nitrate and nitrite were carried out by ion chromatography using an AS-11 column, an AG-11 pre-column, and a 200- $\mu\text{g/l}$  injection loop, as described in Nerenberg et al. (2002). The sulfate concentration was measured using a capillary ion analyzer (CIA, Millipore Corp., Milford, MA), and the dissolved sulfide concentration in the aqueous phase was measured using a colorimetric method based on methylene blue (Cline, 1969).

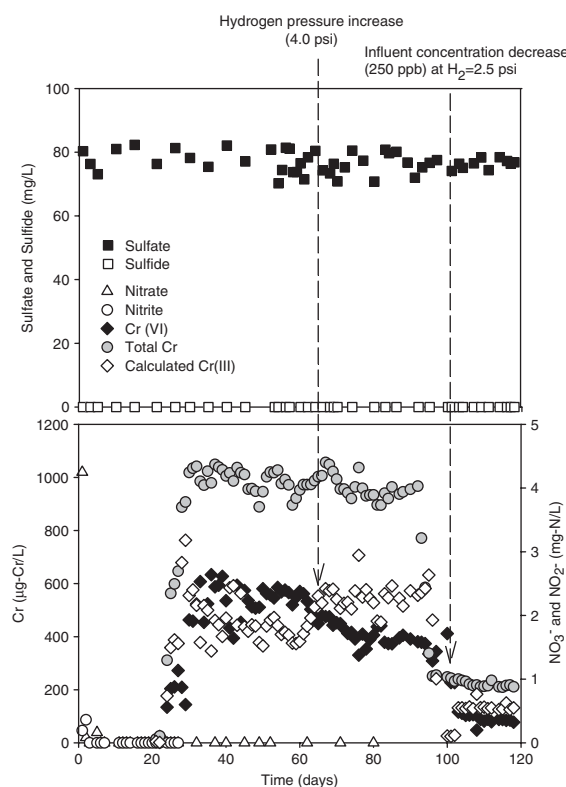
A headspace analysis method was used for dissolved  $H_2$  (Schmidt and Ahring, 1993). A 1-ml liquid sample was transferred from the reactor to a 160-ml serum vial with a thick butyl-rubber stopper previously out-gassed with nitrogen. The vial was shaken vigorously to liberate the dissolved  $H_2$ . A gas-tight syringe was used to sample the headspace (1 ml), and  $H_2$  in the headspace was assayed by a reduction gas analysis (RGA3, Trace Analytical, Menlo Park, CA). Once the headspace  $H_2$  concentration was known, Henry's law and

mass balance were used to determine the dissolved  $H_2$  concentration (Tchobanoglous and Burton, 1991).

## 3. Results and discussion

### 3.1. Start up and steady states

Fig. 2 summarizes the start-up and steady-state results for  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ , Cr(VI), Cr(III), and total Cr. In the first few days of operation, some nitrate was converted only to nitrite, but the nitrate and nitrite concentrations in the effluent dropped to less than 15  $\mu\text{gN/l}$  within 10 days. On day 21, chromate was added to the feed at 1000  $\mu\text{gCr/l}$ . The reduction of Cr(VI) to Cr(III) began within three days, indicated by 179  $\mu\text{gCr/l}$  of Cr(III) in the second sampling after Cr(VI) addition. Steady-state reduction of Cr(VI) to Cr(III) was evident by day 32, or after 11 days of feeding Cr(VI). The average reduction of Cr(VI) to Cr(III) was 45  $\pm$  6%, yielding an effluent Cr(III) concentration of 440  $\pm$  60  $\mu\text{gCr/l}$ . The effluent pH (7.7) was slightly higher than the influent pH (7.5). The effluent concentration of total Cr reached approximately 1000  $\mu\text{gCr/l}$ , which indicates that the reduced Cr(III) was not a solid that was removed by filtration+centrifugation. To confirm this, effluent samples from MBfR were assayed for total Cr concentration without



**Fig. 2 – Nitrate, nitrite, sulfate, sulfide, total Cr, Cr(VI), and calculated Cr(III) concentrations in the effluent from the MBfR that was fed 5 mg/l of  $\text{NO}_3^-$ -N, 78.5 mg  $\text{SO}_4^{2-}$ /l, and 1000  $\mu\text{gCr/l}$  of  $\text{CrO}_4^{2-}$  (upper left Y-axis: sulfate and sulfide in mg/l, lower left Y-axis: Cr in  $\mu\text{g/l}$ , and lower right Y-axis:  $\text{NO}_3^-$  and  $\text{NO}_2^-$  in mg N/l).**

filtration; the difference between filtered and unfiltered samples was minimal ( $<5 \mu\text{gCr/l}$ ) (not shown). Thus, the reduced Cr(III) in the effluent was either soluble or present in colloids smaller than  $0.2 \mu\text{m}$ .

The  $\text{H}_2$  pressure was increased to 4.0 psi (0.27 atm) on day 65. The Cr(VI) concentration in the effluent gradually decreased and reached a steady state from day 68, or 10 days after the pressure change. From day 68 to day 90, the average concentrations of Cr(VI) and Cr(III) in the effluent were  $406 \pm 35 \mu\text{gCr/l}$  and  $549 \pm 51 \mu\text{gCr/l}$ , respectively, and the average reduction of Cr(VI) was  $57 \pm 4\%$ . These results indicate that chromate reduction was controlled by  $\text{H}_2$  availability, and the time to reach the new steady state suggests that improved performance was associated with growth of chromate-reducing bacteria.

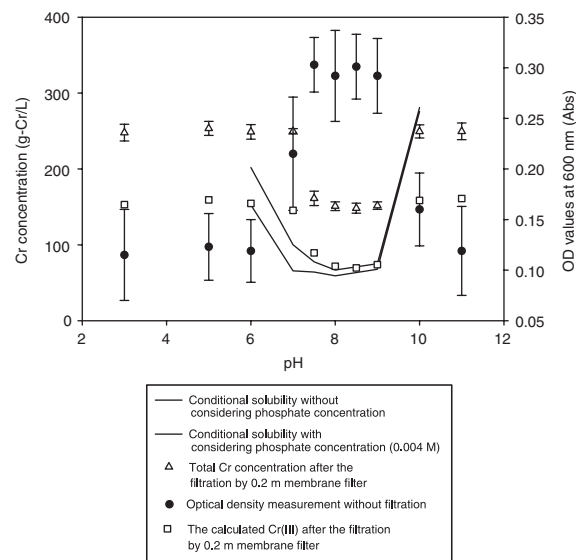
Once Cr(III) reached steady-state removal (by day 92), the influent Cr(VI) loading was reduced to a fourth of the initial Cr(VI) concentration (i.e., to  $250 \mu\text{gCr/l}$ ), while the  $\text{H}_2$  pressure was returned at 2.5 psi (0.17 atm). The Cr(VI) and Cr(III) concentrations in the effluent reached steady state by day 106, or 14 days after the influent loading decreased. From day 106 to day 118, the average level of Cr(III) was  $136 \pm 16 \mu\text{gCr/l}$ , and the average reduction of Cr(VI) was  $63 \pm 4\%$ . This result confirms the expected trend that Cr(VI) reduction was more complete with a lower Cr(VI) loading, although this combination of loading and  $\text{H}_2$  pressure did not lead to 100% reduction.

### 3.2. Influence of chromate on sulfate reduction

The influence of chromate on sulfate reduction could be investigated in the Cr-reducing MBfR, since sulfate was in the influent at  $78.5 \text{ mg/l}$ . As shown in Fig. 2, chromate may have significantly inhibited the reduction of sulfate, since the amount of sulfate reduction was small (averaging  $4 \text{ mgSO}_4/\text{l}$ ), and sulfide in the effluent was not detectable. Another MBfR run in parallel with the same conditions, except that it received selenate instead of chromate, showed major sulfate reduction (Chung et al., 2006). The lack of soluble sulfide in the effluent probably was due to precipitation of metal-sulfide solids with influent cations Fe, Zn, Cu, and Ni. The results in Fig. 2 imply that chromate acted as an inhibitor of sulfate reduction, perhaps like molybdate, which forms unstable analogues of active sulfate, depleting cells of ATP (Postgate, 1984; Smith and Gadd, 2000). Chromate anions have the same charge and a similar physical size to the sulfate anion and also have the potential to enter into sulfate transport pathways in the biological system (Smith and Gadd, 2000).

### 3.3. Precipitation test

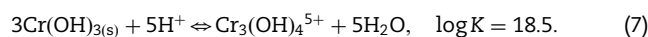
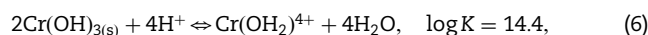
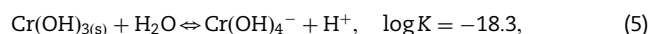
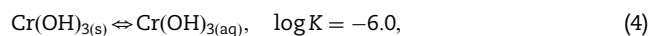
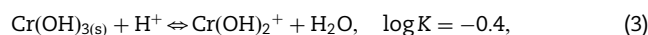
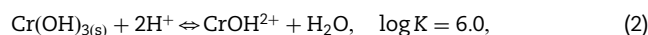
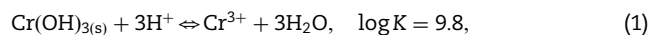
In order to investigate the character of the reduced Cr(III), a precipitation test was performed using the effluent from the chromate-reducing MBfR. The effluent contained  $95 \mu\text{gCr/l}$  of Cr(VI) and  $154 \mu\text{gCr/l}$  of Cr(III) for this test, giving total Cr of  $249 \mu\text{gCr/l}$ . Precipitation of  $\text{Cr(OH)}_{3(s)}$  should be enhanced by a higher pH, since  $\text{HO}^-$  is a ligand for  $\text{Cr(OH)}_3$ . The test results are shown in Fig. 3, which shows the remaining dissolved concentrations of total chromium and Cr(III) after filtration and OD at 600nm. Both results show that precipitation of



**Fig. 3 - Dissolved concentrations of Cr(III) and total chromium, along with OD levels, in the effluent and calculated conditional solubilities considering or not considering phosphate complexes for various pH between 6 and 10 (left Y-axis: Cr in  $\mu\text{gCr/l}$ , and right Y-axis: OD at 600 nm).**

Cr(III) started at a pH of about 7.0, and the greatest precipitation ( $\sim 33\%$ ) occurred at pH of 7.5–9.0, where total Cr was approximately  $150 \mu\text{gCr/l}$  and Cr(III) was about  $55 \mu\text{gCr/l}$ . The solid re-dissolved at  $\text{pH} \geq 10$ , probably due to formation of  $\text{Cr(OH)}_4^-$ . These results demonstrate the potential to remove reduced Cr(III) as  $\text{Cr(OH)}_{3(s)}$  through a small adjustment of the pH.

Although it is generally thought that Cr(III) is immobile from pH values of about 5.5–11, due to  $\text{Cr(OH)}_{3(s)}$  precipitation, the degree of immobilization depends on how the conditional solubility compares to the target Cr(III) concentration (Ziemiak et al., 1998; Cherry, 1982). The conditional solubility of Cr(III) was for different pH values using the following reactions and equilibrium constants (at  $25^\circ\text{C}$ ) for the Cr(III)-hydroxide complexes (Rai et al., 1987):



The solid line in Fig. 3 shows the conditional solubility of Cr(III) based on reactions (1)–(7) for pH from 6 to 10. It shows a solubility minimum at  $59 \mu\text{gCr/l}$  at  $\text{pH} = 8$ , a value that agrees closely with the measured Cr(III) concentration in the

precipitation tests,  $\sim 55 \mu\text{gCr/l}$ . The predicted conditional solubility increases strongly for pH less than 7 and greater than 7, which is consistent with the experimental trends.

Cr(III) also can complex with phosphate, sulfate, nitrate, fluoride, citrate, or other soluble organic ligands (Evanko and Dzombak, 1997; Armienta and Quere, 1995; Ziemniak et al., 1998; Rai et al., 2004). Of particular significance in our study are the Cr(III)-phosphate species, since the medium contained 0.004 M phosphate. Cr(III)-phosphate complexation species, reactions, and logK values are (Rai et al., 2004)

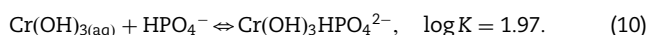
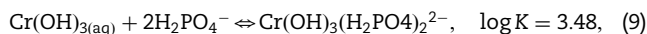
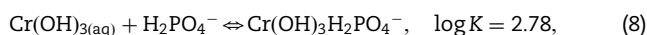


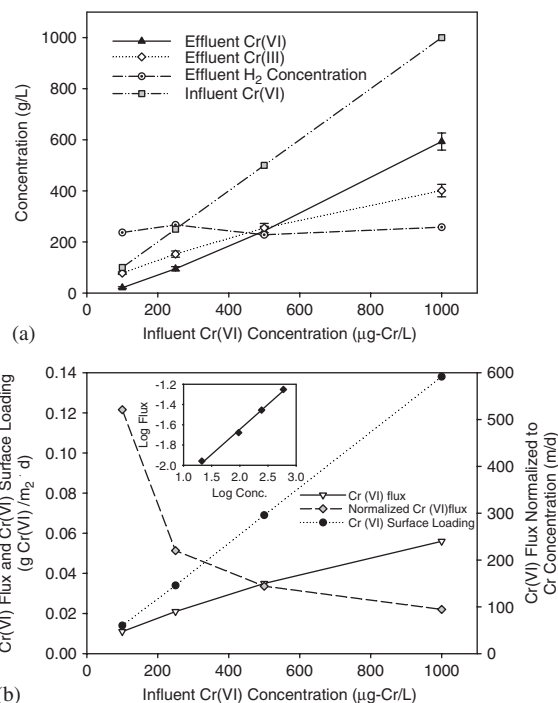
Fig. 3 also shows the conditional solubility of Cr(III) based on reactions (1)–(10). The dashed line shows a solubility minimum of  $67 \mu\text{gCr/l}$  at pH = 8; this value is only slightly larger than the conditional solubility that does not consider phosphate complexes and indicates that the predominant control of the solubility of Cr(III) normally is by the hydroxide complexes. Fortunately, the conditional solubility of Cr(III) is lower than the MCL of  $100 \mu\text{gCr/l}$  and underscores the value of reducing Cr(VI) to Cr(III).

### 3.4. Short-term experiments

In the first short-term series, the influent chromate concentration was 100, 250, 500, or  $1000 \mu\text{gCr/l}$ , with applied  $\text{H}_2$  pressure of 2.5 psi (0.17 atm) and influent nitrate and sulfate at 5 mgN/l and  $78.5 \text{ mgSO}_4^{2-}/\text{l}$ , respectively. Results are shown in Fig. 4. Nitrate reduction (not shown) was  $>99.5\%$  and not affected by the influent chromate load. With a fixed  $\text{H}_2$  availability (2.5 psi or 0.17 atm) and biofilm accumulation, the Cr(VI) concentration in the effluent from MBfR increased with loading up  $593 \pm 34 \mu\text{gCr/l}$ . The percent chromate removal declined from  $78 \pm 4\%$  at the lowest chromate input,  $100 \mu\text{g/l}$ , to 40% at  $1000 \mu\text{gCr/l}$ . The effluent  $\text{H}_2$  concentration was nearly constant at  $248 \pm 18 \mu\text{g/l}$  during this set of short-term experiments, which means that chromate reduction was not controlling overall  $\text{H}_2$  utilization. This feature is explored in detail below.

The Cr(VI) fluxes (denoted as  $J$ ) ranged from 0.011 to  $0.056 \text{ g Cr(VI)}/\text{m}^2 \text{ d}$  and gradually increased when the influent concentration increased from 100 to  $1000 \mu\text{gCr/l}$ . Although Cr(VI) fluxes were smallest for the lowest Cr(VI) loading and concentration, the Cr(VI) flux normalized to effluent Cr(VI) concentration (denoted as  $S$ ), i.e., a pseudo-first-order rate coefficient (m/d) for Cr(VI) reduction, was significantly higher for the lowest influent Cr(VI) concentration.

In order to estimate the reaction order,  $\log J$  versus  $\log S$  is plotted in the inset of Fig. 4. The slope is 0.49, which is virtually equal to the well-known half-order kinetics for deep biofilms in which the reaction is zero in substrate concentration (Harremoës, 1976; Rittmann and McCarty, 2001). A reaction order smaller than one means that  $J$  increases less than proportionally with  $S$  increases, and the percentage removal declines for higher loading. This trend is the observed result shown in Fig. 4 and suggests that the rate

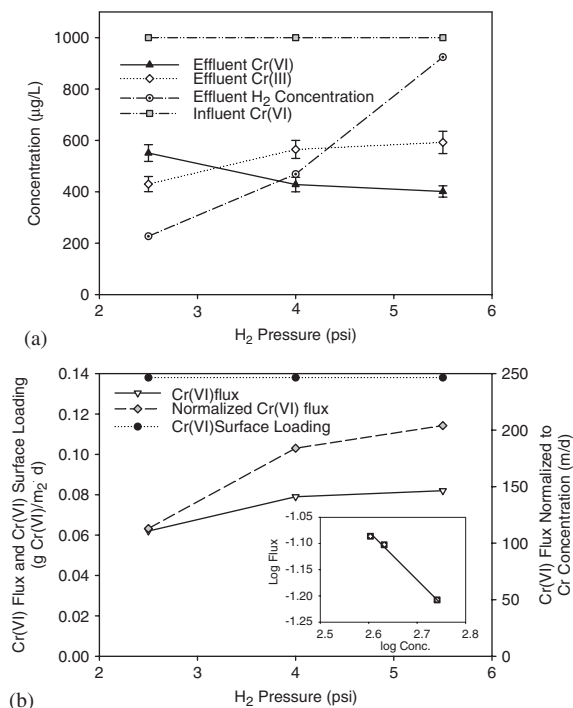


**Fig. 4 – Results for the first short-term series (chromate load variable) :** (a) Cr(VI), Cr(III), and  $\text{H}_2$  concentration in the effluent; (b) Cr(VI) flux, Cr(VI) flux normalized to effluent Cr(VI) concentration, and Cr(VI) surface loading; (inset) logarithm of Cr(VI) flux plotted against the logarithm of the effluent Cr(VI) concentration (Cr species concentration in  $\mu\text{g Cr/l}$ ,  $\text{H}_2$  concentration in  $\mu\text{g/l}$ , Cr(VI) flux and Cr(VI) surface loading in  $\text{g Cr(VI)}/\text{m}^2 \text{ d}$ , Cr(VI) flux normalized to Cr(VI) concentration in m/d).

of Cr(VI) reduction was largely diffusion limited in a deep biofilm.

In the second short-term experiment, the  $\text{H}_2$  pressure was 2.5 psi (0.17 atm), 4.0 psi (0.27 atm), or 5.5 psi (0.37 atm), with a fixed chromate influent of  $1000 \mu\text{gCr/l}$ . Influent  $\text{NO}_3^-$  and  $\text{SO}_4^{2-}$  were at their standard feed concentrations of 5 mgN/l and 78.5 mg/l, respectively. Denitrification was at least 99.5% for all experiments (data not shown) and not affected by  $\text{H}_2$  pressure. As shown in Fig. 5, increasing  $\text{H}_2$  pressure caused a decrease in effluent Cr(VI), and Cr(III) continually increased, reached  $592 \pm 43 \mu\text{gCr/l}$  ( $\sim 62\%$  at 5.5 psi). At 2.5 psi applied pressure, the effluent residual  $\text{H}_2$  was  $227 \mu\text{g/l}$ , and the nitrate concentration in the effluent was  $<0.02 \text{ mgN/l}$ . As the  $\text{H}_2$  pressure increased to 4.0 psi, the reduction of Cr(VI) to Cr(III) increased by 12%. Increasing the  $\text{H}_2$  pressure to 5.5 psi gave a small increase to Cr(VI) reduction, but the effluent  $\text{H}_2$  concentration rose to  $\geq 924 \mu\text{g/l}$ . In general, an increasing applied  $\text{H}_2$  pressure at given chromate, nitrate, and sulfate loadings caused an increasing effluent  $\text{H}_2$  concentration, although the results suggest that  $\text{H}_2$  limitation in the biofilm was minimal for the highest  $\text{H}_2$  pressure.

As  $\text{H}_2$  pressure increased from 2.5 to 5.5 psi, the Cr(VI) flux normalized by its effluent concentration increased from 113 to 204 m/d (Fig. 5b). This demonstrates that the Cr(VI) reduction strongly depended on  $\text{H}_2$  availability. This is shown

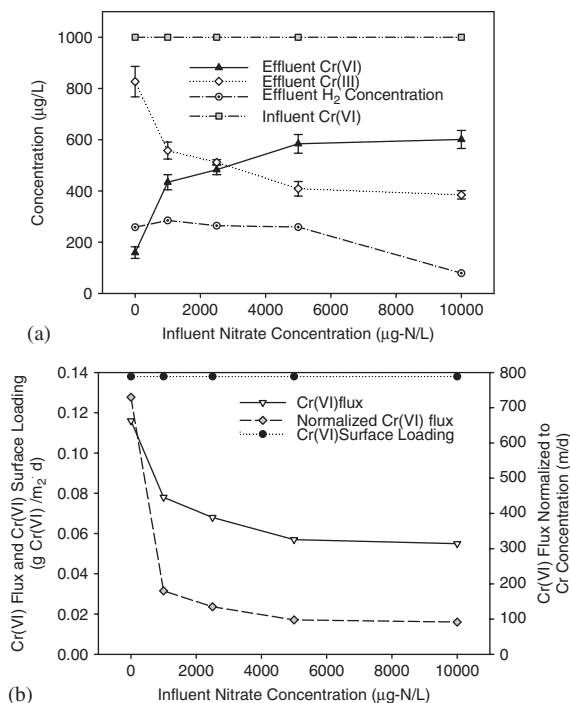


**Fig. 5 – Results for the second short-term series ( $H_2$  pressure variable): (a) Cr(VI), Cr(III), and  $H_2$  concentration in the effluent; (b) Cr(VI) flux, Cr(VI) flux normalized to effluent Cr(VI) concentration, and Cr(VI) surface loading; (inset) logarithm of Cr(VI) flux versus logarithm of effluent concentration of Cr(VI) (Cr species concentration in  $\mu\text{g Cr/l}$ ,  $H_2$  concentration in  $\mu\text{g/l}$ , Cr(VI) flux and Cr(VI) surface loading in  $\text{g Cr(VI)/m}^2 \text{d}$ , Cr(VI) flux normalized to Cr(VI) concentration in  $\text{m/d}$ ).**

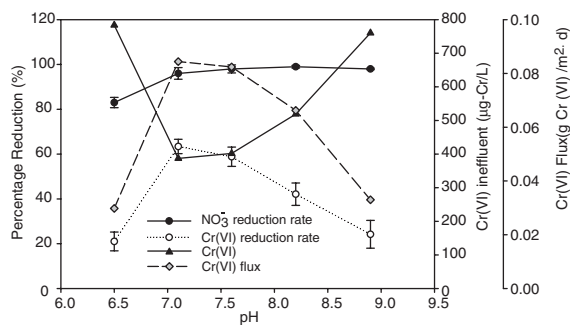
very dramatically by the negative reaction order ( $-0.90$ ) shown in the inset to Fig. 5. The flux increased with decreasing Cr(VI) concentration because of the increased  $H_2$  availability, which increased the active depth of the biofilm for Cr(VI) reduction.

In the third short-term experiment, the influent nitrate concentrations was varied from 0 to 10 mg N/l, with  $H_2$  pressure and Cr(VI) concentration at the steady-state concentrations of 2.5 psi and 1000  $\mu\text{g/l}$ , respectively. As shown in Fig. 6, Cr(VI) reduction significantly increased when no nitrate was in the influent. The amounts of reduced Cr(III) gradually decreased to  $385 \pm 17 \mu\text{g Cr/l}$  as influent nitrate concentration increased. With zero nitrate in the influent, the percentage removal of chromate increased to 84% (% data not shown). The nitrate concentration in the effluent increased only to 32  $\mu\text{g N/l}$  when 10 mg N/l of nitrate was added.

The bulk-liquid  $H_2$  concentration decreased as the nitrate loading increased, due to the greater demand for  $H_2$  (Fig. 6). The lower  $H_2$  concentration caused increasing  $H_2$  limitation for all reduction reactions. As the nitrate loading increased, the Cr(VI) flux and the Cr(VI) flux normalized to Cr(VI) effluent concentration dramatically declined. This suggests that Cr(VI) reduction was out-competed for limited  $H_2$  by nitrate reduction.



**Fig. 6 – Results for the third set of short-term experiments (nitrate load variable): (a) nitrate, Cr(VI), Cr(III), and  $H_2$  concentration in the effluent; (b) Cr(VI) flux and Cr(VI) flux normalized to effluent Cr(VI) concentration (Cr species concentration in  $\mu\text{g Cr/l}$ ,  $H_2$  concentration in  $\mu\text{g/l}$ , nitrate concentration in  $\mu\text{g N/l}$ , Cr(VI) flux and Cr(VI) surface loading in  $\text{g Cr(VI)/m}^2 \text{d}$ , Cr(VI) flux normalized to Cr(VI) concentration in  $\text{m/d}$ ).**



**Fig. 7 – Results for the fourth set of short-term experiments (influent pH variable). Percentage reduction, effluent concentration of Cr(VI), and Cr(VI) flux (left Y-axis: reduction percentage in %, right Y-axis: Cr(VI) concentration in effluent ( $\mu\text{g Cr/l}$ ), and right off axis: Cr(VI) flux in  $\text{g Cr(VI)/m}^2 \text{d}$ ).**

In the fourth short-term experiment, the influent pH values were set to 6.5, 7.1, 7.6, 8.2, or 8.9, with applied  $H_2$  pressure, influent Cr(VI) concentration, influent nitrate, and influent sulfate concentration at their steady-state values of 2.5 psi, 1000  $\mu\text{g Cr/l}$ , 5 mg N/l, and 78.5 mg  $\text{SO}_4^{2-}/\text{l}$ , respectively. In this short-term test, along with the previous three short-term experiments, the effluent pH was only slightly higher

**Table 3 – Electron-equivalent fluxes for chromate, sulfate, and nitrate in the short-term experiments**

Unit	Influent concentration		Electron-equivalent flux			Sum up the fluxes in electron equivalents	Distribution of fluxes			
	$\mu\text{g-Cr/l}$	$\text{psi}$	$\text{Cr}^{\text{a}}$	Nitrate	Sulfate		Cr	Nitrate	Sulfate	
				$\frac{\text{eg}}{\text{m}^2\text{d}}$		$\frac{\text{eg}}{\text{m}^2\text{d}}$	%			
Cr (VI) conc. loading	1000	2.5	0.00145	0.0547	0.0458	0.1020	1.4	53.7	44.9	
	500		0.00091	0.0552	0.0432	0.0993	0.9	55.6	43.5	
	250		0.00054	0.0569	0.0425	0.0994	0.5	56.9	42.6	
	100		0.00028	0.0582	0.0450	0.1035	0.3	56.2	43.5	
H <sub>2</sub> pressure	1000	2.5	0.00160	0.0529	0.0442	0.0987	1.6	53.6	44.8	
		4.0	0.00204	0.0570	0.0458	0.1048	1.9	54.4	43.7	
		5.5	0.00212	0.0544	0.0438	0.1003	2.1	54.2	43.7	
NO <sub>3</sub> <sup>-</sup> (mgN/l)	10	1000	2.5	0.00142	0.108	0.0452	0.1546	0.9	69.9	29.2
	5			0.00147	0.0543	0.0417	0.0975	1.5	55.7	42.8
	2.5			0.00176	0.0272	0.0442	0.0732	2.4	37.2	60.4
	1			0.00202	0.0106	0.0425	0.0551	3.7	19.2	77.1
	0			0.00300	—	0.0433	0.0463	6.5	—	93.6

<sup>a</sup> calculated by:  $J_{\text{Cr}}/\text{EW}_{\text{Cr}} = \frac{\text{Influent flowrate}(Q) \times \text{removed Cr(VI)} (\Delta S)}{\text{Total biofilm surface}(aV) \times \text{EW}_{\text{Cr}}}$ , where Q is in m<sup>3</sup>/d,  $\Delta S$  is in g-Cr/m<sup>3</sup>, aV is in m<sup>2</sup>, EW is in gCr/e<sup>-</sup> equivalent for reduction of Cr(VI) to Cr(III), and J is in gCr/m<sup>2</sup> d.

( $\leq 0.2$  pH units) than the influent pH. As shown in Fig. 7, Cr(VI) reduction was highly sensitive to pH, with a maximum removal percentage and flux at a pH of approximately 7.0. Cr(VI) reduction to Cr(III) declined sharply for pH = 6.5, but had a shallow decline from 7.0 to 8.2. The fairly shallow pH optimum between 7.0 and 8.2 for Cr(VI) reduction may be fortunate, because denitrification and sulfate reduction add base, which can cause a pH increase that might slow Cr(VI) reduction if the optimum were sharper.

### 3.5. Flux analysis

Table 3 shows the electron-equivalent fluxes of electron acceptors Cr(VI), nitrate, and sulfate, along with the percentage distribution of each flux. Nitrate and sulfate fluxes together accounted for at least 93.6% of the total electron flux, and they averaged 98%. Conversely, Cr(VI) reduction was always a small percentage of the electron flux, less than 6.5% and averaging only 2%. This means that the total demand for H<sub>2</sub> was largely controlled by sulfate and nitrate reduction, never Cr(VI) reduction, and explains why Cr(VI) loading had no impact on the H<sub>2</sub> concentration (first short-term experiment; Fig. 4). Cr(VI) reduction was significantly sensitive to H<sub>2</sub> pressure based on the normalized Cr(VI) fluxes (Fig. 5 and Table 3). However, the nitrate and sulfate fluxes, along with the total electron-equivalent flux, were not affected by H<sub>2</sub> pressure (Table 3).

## 4. Conclusions

The reduction of Cr(VI) to Cr(III) occurred rapidly under normal MBfR denitrifying conditions and with an environmental inoculum. As the H<sub>2</sub> pressure increased or influent loading decreased, the average reduction of Cr(VI) increased.

Short-term experiments confirmed that influent chromate loading, H<sub>2</sub> pressure, and nitrate concentration significantly affected the rate and extent Cr(VI) reduction. In particular, increasing the H<sub>2</sub> pressure or reducing the competition for H<sub>2</sub> from denitrification gave a higher rate and extent of Cr(VI) reduction to Cr(III). Cr(VI) reduction also depended on pH, with an optimum near 7.0, a sharp drop off below 7.0, and a gradual decline to 8.2. Cr(VI) seemed to inhibit sulfate reduction, which was minimal throughout the experiments. Precipitation tests demonstrated that Cr(III) can be precipitated by a small upward adjustment of the pH and removed by filtration, although the extent of Cr removal as Cr(OH)<sub>3(s)</sub> seems to be limited by conditional solubility controlled mainly by Cr(III)-hydroxide complexes. These results show that the H<sub>2</sub>-based MBfR is a promising treatment technology for treating Cr(VI) in drinking water and wastewater. For effective Cr(VI) removal, the critical operational parameters for maximizing reduction of Cr(VI) to Cr(III) are the H<sub>2</sub> concentration, nitrate concentration, and pH.

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