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ORIGINAL PAPER

Simultaneous bio-reduction of nitrate, perchlorate, selenate, chromate, arsenate, and dibromochloropropane using a hydrogen-based membrane biofilm reactor

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Abstract We tested the hypothesis that the H_2-based membrane biofilm reactor (MBfR) is capable of reducing multiple oxidized contaminants, a common situation for groundwater contamination. We conducted bench-scale experiments with three groundwater samples collected from California's San Joaquin Valley and on two synthetic groundwaters containing selenate and chromate. The actual groundwater sources had nitrate levels exceeding 10 mg-N l^{-1} and different combinations of anthropogenic perchlorate + chlorate, arsenate, and dibromochloropropane (DBCP). For all actual groundwaters, the MBfR reduced nitrate to less than 0.01 mg-N l^{-1}. Present in two groundwaters, perchlorate + chlorate was reduced to below the California Notification Level, 6 μg-ClO_4 l^{-1}. As(V) was substantially reduced to As(III) for two groundwaters samples, which had influent As(V) concentrations from 3 to 8.8 μg-As l^{-1}. DBCP, present in one groundwater at 1.4 μg l^{-1}, was reduced to below its detection limit of 0.01 μg l^{-1}, which is well below California's 0.2 μg l^{-1} MCL for DBCP. For the synthetic groundwaters, two MBfRs initially reduced Se(VI) or Cr(VI) stably to Se° or Cr(III). When we switched the influent oxidized contaminants, the new oxidized contaminant was reduced immediately, and its reduction soon was approximately the same or greater than it had been reduced in its original MBfR. These results support that the H_2-based MBfR can reduce multiple oxidized contaminants simultaneously.

Keywords Arsenate · Bioreduction · Chromate, Dibromochloropropane · Hydrogen · Membrane biofilm reactor · Oxidized contaminants · Perchlorate · Selenate

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Introduction

In recent years, several oxidized contaminants have emerged as serious drinking-water pollutants, including nitrate (NO_3^-), arsenate ($H_2AsO_4^{2-}$), perchlorate (ClO_4^-), chlorate (ClO_3^-), chromate

(CrO_4^{2-}), selenate (SeO_4^{2-}), and pesticides like dibromochloropropane (DBCP). While nitrate is a long-standing water-quality problem, the others fall into the category of emerging contaminants.

Arsenic has become a global public-health problem due to its acute and broad toxicity (Leonard 1991; Naqvi et al. 1994), and it has a complex geochemistry that governs the speciation and distribution of its two most prevalent oxidation states found in nature: arsenate (As(V)) and arsenite (As(III)) (Cullen and Reimer 1989; Jain et al. 1999; Masscheleyn et al. 1991). Sub-acute doses produce effects in the respiratory, gastrointestinal, cardiovascular, and nervous systems (Jain and Ali 2000). Recently, the USEPA reduced the maximum contaminant level (MCL) for arsenic in drinking water from 50 to 10 μg-As l^{-1}, effective in 2006 (USEPA 2001).

Perchlorate (ClO_4^-), which is the most oxidized form of chlorine (Cl(VII)), is found in groundwater throughout the United States (USEPA 2004) and interferes with iodide uptake into the thyroid gland, disrupting thyroid function (Capen and Martin 1989; Wolff 1998; Clark 2000) and making it an endocrine disruptor. The California notification level is 6 μg l^{-1} (CDHS 2005), and the USEPA is considering a similar standard.

Chlorate (Cl(V)) has been used as an herbicide to kill all terrestrial plants except mosses. The only other major source of chlorate input to the environment is pulp mill effluent, where chlorine dioxide is used for bleaching. Chlorate is a partial reduction product from perchlorate and appears to be more toxic than perchlorate (Stouthamer 1967; Anderson et al. 2000).

Selenium is widely used in a variety of industries, including production of glass, pigments, pesticides, stainless steel, and photoelectric cells (Haygarth 1994), and it can cause environmental problems: for example, bird malformations due to selenium have been reported in the San Joaquin Valley in central California (Ohlendorf and Santolo 1994; Oremland et al. 1989, 1990). Selenate (Se(VI)), the most oxidized form of selenium, and selenite (Se(IV)) are highly water soluble and are toxic to biological systems at relatively low concentrations. Elemental selenium (Se°) is highly insoluble in water and therefore has minimal toxicity (Doran 1982). The MCL for drinking water is 50 μg-Se l^{-1} total selenium (USEPA 2003a).

The widespread use of chromate in industries such as leather tanning, metallurgy, electroplating, nuclear reactor operation, and irradiated fuel processing and fabrication has resulted in large quantities of chromium being discharged into the environment (Riley and Zachara 1992; Barnhart 1997). In the natural environments, chromium is found in trivalent (Cr(III)) and hexavalent (Cr(VI)) forms. Of these, Cr(III) is far less toxic than Cr(VI) and is far less mobile in groundwater, being found either as cationic species that sorb to solids or as relatively insoluble precipitates, such as $Cr(OH)_3$ (Anderson and Kozlovsky 1985; Palmer and Wittbrodt 1991). On the other hand, Cr(VI) is highly soluble and, therefore, mobile and bioavailable in aquatic systems (Dragun 1988; Rai et al. 1987). The MCL for drinking water is 100 μg-Cr l^{-1} total chromium (USEPA 2003a).

DBCP is a pesticide that is a persistent problem in many agricultural areas, such as the San Joaquin Valley of California. DBCP was used extensively as a nematocide for soil fumigation from the 1960s until it was banned in 1977. DBCP is a potent carcinogen and perhaps the most powerful testicular toxin ever made (Environmental Working Group 1999). Other adverse effects from exposure to levels above 0.2 parts per billion for ''relatively short periods of time'' include liver and kidney damage (USEPA 2003a).

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. Oxidative processes, such as chlorine oxidation or ozonation, are ineffective. Conventional water-treatment processes, such as coagulation and precipitation, are effective, but limited to low ferric- or alum-dose applications, because high coagulant dose will result in too-frequent backwashes (USEPA 2003b). Some advanced separation treatment processes, such as reverse osmosis, ion exchange, membrane filtration, and electrodialysis, are effective, but are expensive and generate concentrated wastes that require subsequent disposal (Komori et al. 1990; Srivastava et al. 1986).

A promising approach to detoxify all of the oxidized contaminants together is biological

reduction to harmless or immobile forms. For example, biological reduction of ClO_4^- and ClO_3^- produces harmless Cl^- and H_2O, while reductions of AsO_4^{2-}, CrO_4^{2-}, and SeO_4^{2-} can lead to insoluble and immobile As_2S_3, $Cr(OH)_3$, and Se°, respectively. Reduction of DBCP creates harmless Cl^- and Br^-, as well as non-toxic and biodegradable propane (Castro and Belser 1968; Babich et al. 1981). For biological reduction, a microbiologically available electron donor must be added. Hydrogen (H_2) is the ideal electron donor, because it is non-toxic, relatively inexpensive, and sparingly soluble, and it supports autotrophic bacteria, which require no organic-C source (Lee and Rittmann 2002; Nerenberg et al. 2002). Furthermore, research over the past 10–15 years suggests that H_2 is the one electron donor that bacteria can use to reduce all of the oxidized contaminants (Rittmann et al. 2004). Thus, using H_2 opens the door to simultaneous reduction of mixtures of oxidized contaminants.

The membrane biofilm reactor (MBfR) is a recent technological advance that 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; Nerenberg and Rittmann, 2004). Here, we test the hypothesis that H_2-based, denitrifying MBfRs simultaneously reduces combinations of nitrate, selenate, chromate, perchlorate, chlorate, arsenate, and DBCP found in contaminated groundwater.

Materials and methods

Experimental set-up

A schematic and characteristics of the MBfRs used in this study are shown in Fig. 1. Each MBfR system consisted of two membrane modules connected in a recirculation loop. Three MBfRs were fed with different media: MBfR A received actual contaminated groundwaters, MBfR B initially received a selenate-contaminated medium, and

Item	Value	Unit
Active Length (vertical portion)	25	cm
Cross Sectional Area	0.28	cm^2
Active Volume (vertical portion)	7.07	cm^3
Number of fibers	32	
Total Surface area of fibers	70.4	cm^2

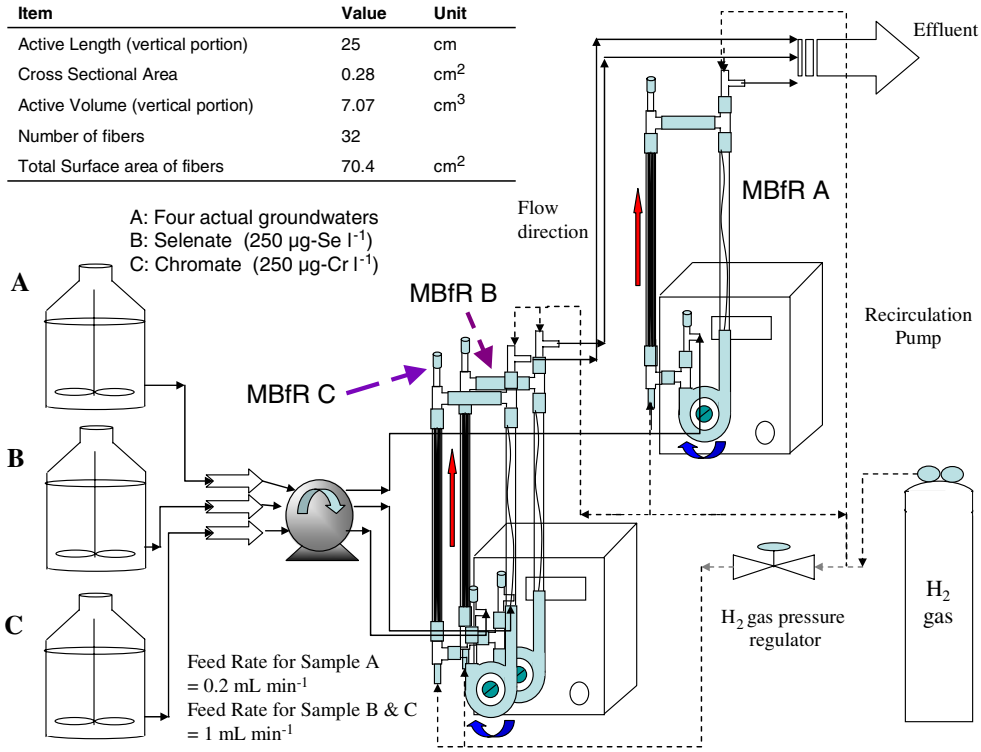

Fig. 1 Schematic and physical characteristics of the bench-scale MBfRs used in this study

MBfR C initially received a chromate-contaminated medium. The main membrane module contained a bundle of 32 hydrophobic hollow-fiber membranes (Model MHF 200TL, Mitsubishi Rayon) inside a polyvinyl chloride pipe shell, while the other module contained a single fiber used to take biofilm samples without disrupting the biofilm in the main module. A manifold peristaltic pump (Gilson Minipuls 3, Middleton, WI) was used with PVC tubing to give feed rate of 0.2 and 1 ml min^{-1} for actual groundwaters and the synthetic media containing selenate and chromate, respectively. The system behaved as a completely mixed biofilm reactor because of the high recirculation rate (150 ml min^{-1}), and the high recirculation rate helped maintain a consistent biomass accumulation on the hollow-fibers. The supplied H_2 was transferred through the fiber walls to the surrounding biofilm and the standard H_2 pressure was 2.5–4 psi (0.17–0.31 atm).

Inoculation, start up, and steady states

The inoculum was obtained from the pilot-scale MBfR operated at La Puente, California (Rittmann et al. 2004). The pilot-scale reactor treated groundwater with approximately 5 mg-N l^{-1} nitrate and 60 μg-ClO_4 l^{-1} perchlorate. It had two modules in series; most of the nitrate was removed in the first module and most of the perchlorate in the second. Biofilm samples were taken from both modules and mixed together. This mixture was added to a sterile glycerol solution, with a final concentration of 25% glycerol, and stored at −80°C. For inoculating the MBfRs, biofilm samples were thawed, washed twice by centrifuging for 15 min at 5,000 g, and resuspended in 10-ml of sterile minimal medium without electron donor. A 1.5-ml aliquot of the washed biofilm suspension was added into the reactor.

Start up of each system began when H_2 gas was supplied, and the liquid in the reactor was recirculated for 24 h to establish a biofilm. Then, the first groundwater sample (denoted 061004) was fed at a rate of 0.2 ml min^{-1} to MBfR A. This condition was maintained until a steady state was achieved. Later, two other groundwaters (denoted 062204 and 062304) were applied sequentially to MBfR A until each achieved steady-state performance. Synthetic feed medium without selenate or chromate was applied at a rate of 0.2 ml min^{-1} to start up MBfRs B and C. The concentration of nitrate in the effluent declined rapidly in B and C and approached steady state after around 3 days, and then the feed rate was increased to 1.0 ml min^{-1}. After nitrate was completely removed with the higher influent flow rate (20–25 days), selenate and chromate were added to the influent of MBfR B and C at 250 μg-(Se for B or Cr for C) l^{-1}. After about 40 days from when Se(VI) and Cr(VI) feeding began, the reduction of Se(VI) and Cr(VI) reached steady-state conditions.

Groundwater and synthetic selenate/chromate media

The three contaminated-groundwater samples (identified as 061004, 062204, and 062304) were collected from California's Central San Joaquin Valley, packaged in insulated containers with blue-ice, and shipped by overnight to the authors' laboratory and to the Clinical Laboratories of San Bernadino, California. Water quality assays were carried out at both laboratories, as described below Table 1. Once received at the authors' laboratory,

Table 1 Summary of the analytical characterization of the three groundwaters in terms of oxidized components

	Unit	Method	061004	062204	062304
Nitrate-N	mg-N l^{-1}	EPA 300.0	10.8	13.1	21.4
Nitrite-N	mg-N l^{-1}	EPA 300.0	N.D.	N.D.	N.D.
Sum of perchlorate and chlorate	μg-ClO_4 l^{-1}	EPA 300.1	82	34	N.D.
Sulfate	Mg l^{-1}	EPA 300.0	63	27	34
Arsenic	μg l^{-1}	SM 3113B	N.D.	8.8	3.0
DBCP	μg l^{-1}	EPA 504.1	1.4	N.D.	0.014

N.D.: Not detected at or not above the detection limit for reporting

the water was stored in a cold room at 4°C and brought to room temperature (20°C) before it was fed into MBfR A. No chemical additions were made during the use of actual contaminated groundwaters. MBfR A was sequentially fed groundwaters 061004, 062204, and 062304 in this order.

The composition of the synthetic feed medium applied to MBfRs B and C was (g l^{-1}): KH_2PO_4 0.128, 100, Na_2HPO_4 0.434, $MgSO_4 \cdot 7H_2O$ 0.2, $NaNO_3$ as N 0.03, $CaCl_2 \cdot 2H_2O$ 0.001, $FeSO_4 \cdot 7H_2O$ 0.001, and 1 ml of trace mineral solution. The trace mineral solution (mg l^{-1}) consisted of $ZnSO_4 \cdot 7H_2O$ 100, $MnCl_2 \cdot 4H_2O$ 30, H_3BO_3 300, $CoCl_2 \cdot 6H_2O$ 200, $CuCl_2 \cdot 2H_2O$ 10, NiCl2 $\cdot$ $6H_2O$ 10, $Na_2MoO_4 \cdot 2H_2O$ 30, and Na_2SeO_3 30. The were 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). The nitrate concentration was 5 mg-N l^{-1} and 78.5 mg SO_4^{2-} l^{-1}; these nitrate and sulfate are concentrations representative of selenate-contaminated groundwater in the San Joaquin Valley (Presser and Ohlendorf 1987; Ohlendorf 1989; Cantafio et al. 1996; Zhang et al. 2003). When selenate and chromate were added to media, the influent concentrations were 1,000 μg-Se l^{-1} or 1,000 μg-Cr l^{-1} for MBfRs B and C, respectively.

Sampling and analyses performed at Northwestern University

Groundwater quality and the performance of the reactors were monitored by analyzing effluent samples taken on a daily basis and immediately filtered through a 0.2-μm membrane filter (Pall Corp., Ann Arbor, ML). Analyses for nitrate, nitrite, chlorate, and perchlorate were carried out by ion chromatography (Dionex 4500) using an AS-11 column, an AS-11 pre-column, and a 200-μg l^{-1} injection loop, as described in Nerenberg et al. (2002). Perchlorate and chlorate eluted at the same time; thus, the concentration is reported as perchlorate + chlorate in μg-ClO_4 l^{-1}. The sulfate concentration was measured using a capillary ion analyzer (CIA, Millipore Corp., Milford, MA).

After sample centrifugation (15,000 g, 10 min), total soluble selenium (Se(IV) + Se(VI)) and chromium (Cr(III) + Cr(VI)) were determined by ICP-MS (PQExCell, VG Elemental). In order to investigate the degree of reduction of oxidized contaminants, Se(IV) and Cr(VI) concentrations were analyzed by a fluorometric method for Se(IV) (Standard method 3500-Se E) and diphenyl carbazide method (3500-Cr D) for Cr (VI). The Cr(III) concentration was determined by subtracting Cr(VI) from total chromium. The concentration of Se(VI) was calculated by subtracting Se(IV) from total Se in the effluent. This approach assumes that selenium reduced to Se° results in a solid that is removed by centrifugation. The concentration of selenium removed as Se° is computed as the difference between influent and effluent total Se.

The procedure for arsenic speciation was as follows: A 250-ml bottle (identified as bottle A) was used to contain an unfiltered sample, which was analyzed to determine the total arsenic concentration (soluble + particulate). The soluble portion of the sample was obtained after filtration through a 0.45-μm screw-on disc filter to remove any particulate arsenic; the filtrate was collected in a 125-ml bottle (identified as bottle B). Bottle B contained 0.05% (V/V) pure sulfuric acid to acidify the sample to about pH 2. At this pH, As(III) was completely protonated to H_3AsO_3, and As(V) was present in ionic and protonated forms. A portion of the acidified sample in bottle B was run though the resin column. The resin retained the As(V) and allowed As(III) to pass through the column (Note that the resin retains only $H_2AsO_3^-$ and that H_3AsO_4, when passing through the column was ionized to $H_2AsO_3^-$ due to elevated pH values in the column caused by the buffer capacity of acetate exchanged from the resin). The column was eluted by draining, and the elutant of the column was collected in another bottle (identified as bottle C). Samples in bottles A, B, and C were analyzed for total arsenic using ICP-MS (PQExCell, VG Elemental). The As(III) concentration was the total arsenic concentration of the resin-treated sample in bottle C. The As(V) concentration was calculated by subtracting As(III) from the total soluble arsenic concentration of the sample in bottle B (USEPA 2000).

Analysis of DBCP

All analyses for DBCP were performed by the Clinical Laboratories of San Bernadino according to EPA method 504.1. DBCP was concentrated by micro-extraction into hexane: 35 ml of sample were extracted with 2 ml of hexane in sample/hexane mixed vials using an auto-injector. 2 µl of the extract are then injected into a GC equipped with an electron capture detector for separation and analysis. Aqueous calibration standards were extracted and analyzed in an identical manner as the samples in order to compensate for possible extraction losses. The extraction and analysis time was 30–50 min per sample.

Results and discussion

Groundwater quality

For experiments with different combinations of NO_3^-, ClO_4^-, ClO_3^-, AsO_3^{2-}, and DBCP, the influents were the three actual groundwaters contaminated with different mixtures of the emerging oxidized contaminants and NO_3^-. Table 1 summarizes the water quality in terms of oxidized component for the three groundwaters. All the groundwaters had at least two oxidized contaminants, as well as significant sulfate. Three samples exceeded the 45 mg l^{-1} nitrate concentration (or 10 mg-N l^{-1}) MCL in the United States (USEPA 2003c). Besides nitrate, sample 061004 contained 82 µg-ClO_4 l^{-1} perchlorate + chlorate, 1.4 µg l^{-1} of DBCP, and 63 mg l^{-1} sulfate; sample 062204 contained 34 µg-ClO_4 l^{-1} perchlorate + chlorate, 8.8 µg-As l^{-1} total arsenic, and 27 mg l^{-1} sulfate; and sample 062304 had 1.8 µg-As l^{-1} total arsenic and 34 mg l^{-1} sulfate.

Simultaneous bio-reduction in the groundwaters

Naturally occurring mixed-culture biofilms developed in the MBfR were sequentially fed to groundwaters 061004, 062204, and 062304. The MBfRs were operated at a pH of 7, a H_2 pressure of 3.0 psi (0.21 atm), and a 100-min hydraulic retention time.

The first groundwater sample (061004) contained 11 mg-N l^{-1} nitrate, 82 µg-ClO_4 l^{-1} perchlorate + chlorate, and 1.4 µg l^{-1} of DBCP. Within 1 day after feeding sample 061004, 38% of the nitrate was reduced to nitrite or nitrogen gas, as shown in Fig. 2. Perchlorate + chlorate removal was 12% by day one. Reductions of nitrate and perchlorate increased gradually, and, by 50 days, the MBfR removed the nitrate, nitrite, perchlorate, and chlorate completely. Once nitrate had been reduced to non-detect levels (0.01 mg-N l^{-1}) in the effluent (day 36), influent and effluent sampled were submitted for DBCP analysis. The MBfR was operated continuously for an additional 2 weeks, with influent and effluent samples collected and submitted for a second DBCP analysis on day 50. The concentration of DBCP in both effluent samples was below the 0.01-µg l^{-1}, the detection limit of EPA method 504.1, compared to the influent concentration of 1.4 µg l^{-1}, which was maintained for all influent samples. These results indicate that the MBfR effectively reduced DBCP. Since partial dehalogenation products were not assayed, full reduction to propane cannot be proven.

The second groundwater (062204) contained 13 mg-N l^{-1} nitrate, 34 µg-ClO_4 l^{-1} perchlorate + chlorate, 27 mg-SO_4^{2-} l^{-1} sulfate, and 7.3 µg-As l^{-1} arsenate. Figure 3 shows the perchlorate + chlorate, nitrate, nitrite, and arsenic species (total, soluble As(V), and soluble As(III)) in the effluent from MBfR. Nitrate, perchlorate,

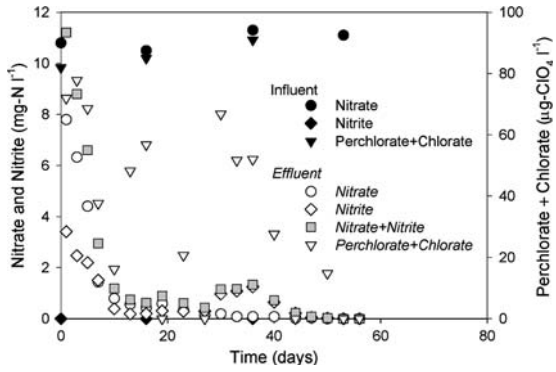

Fig. 2 Concentrations of nitrate, nitrite, and perchlorate + chlorate in groundwater 061004 before and after treatment by MBfR A. (Left Y-axis: Nitrate and nitrite (mg-N l^{-1}), Right Y-axis: Perchlorate + Chlorate (µg-ClO_4 l^{-1}))

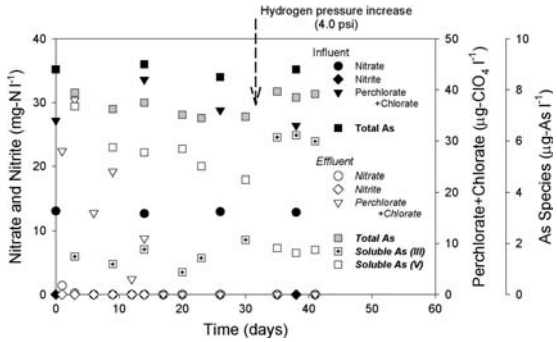

Fig. 3 Concentrations of contaminants in groundwater 062204 before and after treatment by MBfR A (Left Y-axis: Nitrate and nitrite (mg-N l^{-1}), right Y-axis: Perchlorate + chlorate (µg-ClO_4 l^{-1}), and Right-end Y-axis: Total As, soluble As(III) and As(V) (µg-As l^{-1}))

and chlorate were completely reduced by 20 days. Reduction of As(V) to As(III) began within 2 days, and As(V) reduction to As(III) was 40% by day 24, yielding an effluent soluble As(V) concentration of 5.4 µg-As l^{-1}. Effluent soluble As(III) was 1.6 µg-As l^{-1}, making total As at day 24 approximately 7 µg-As l^{-1}, and the average sulfate concentration in effluent somewhat decreased to 22 mg-SO_4^{2-} l^{-1}. This result means that total-As removal, presumably as $As_2S_{3(s)}$, was approximately 20% as a maximal value. The H_2 pressure was increased to 4.0 psi (from 2.5 psi) on day 31 to increase H_2 availability. With the first sample after the pressure change (day 35), the As(V) concentration in the effluent decreased significantly and then remained steady at about 1.8 µg-As l^{-1}, or 80% reduction Effluent As(III) was 6.1 µg-As l^{-1}, total soluble As in the effluent was around 8 µg-As l^{-1}, and sulfate was 20 mg-SO_4^{2-} l^{-1}. These results confirm that As(V) reduction was controlled by H_2 availability and that the likely sink for total As was $As_2S_{3(s)}$.

The third groundwater (062304) had relatively high nitrate (21 mg-N l^{-1}) and low arsenate (3 µg-As l^{-1}). DBCP also was present at 0.014 µg l^{-1}, a value barely above the detection limit of 0.01 µg l^{-1}. Due to the very low DBCP concentration in this sample, effluent samples were not submitted for DBCP analysis. The H_2 pressure was 4.0 psi (0.31 atm) over the entire experimental period. Figure 4 shows the effluent results for sample 062304. Even though a relatively high nitrate loading were applied in the influent, nitrate decreased to essentially zero within 9 days, and nitrite never appeared. By Day 7, As(V) was being reduced and to As(III). Steady-state reduction of As(V) to As(III) was evident by day 18, and the average effluent levels of As(III) and As(V) after day 18 were 1.8 ± 0.2 and 0.64 ± 0.2 µg-As l^{-1}, respectively. Thus, the average reduction of As(V) to As(III) was around 60%. Also, the sulfate concentration contained in this groundwater was 34 mg-SO_4^{2-} l^{-1}, and the average concentration in effluent was 28 mg-SO_4^{2-} l^{-1}. Effluent total As was approximately 2.4 µg-As l^{-1}, which indicates that only a small amount of As was precipitated as $As_2S_{3(s)}$ or adsorbed as As(V) in the biofilm.

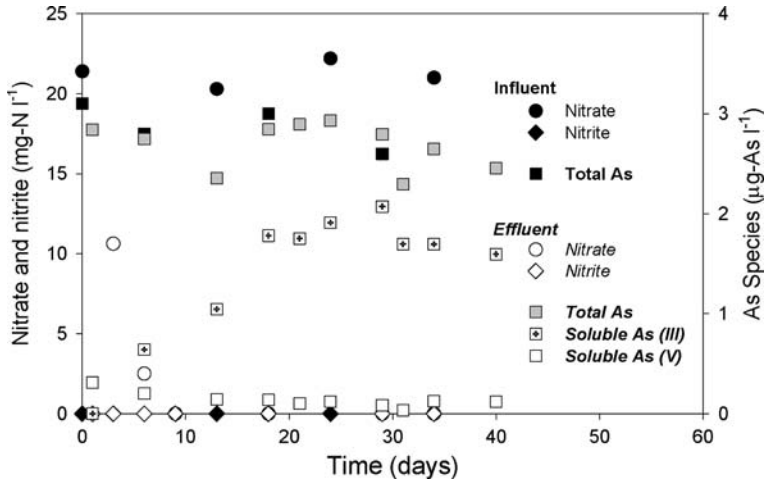

Fig. 4 Concentrations of contaminants in groundwater 062304 before and after treatment by MBfR A (Left Y-axis: Nitrate and nitrite (mg-N l^{-1}) and right Y-axis: Total As, soluble As(III) and As(V) (µg-As l^{-1}))

Removals of selenate and chromate

For experiments with selenate and chromate, a synthetic influent included 5 mg-N l^{-1} nitrate and 78.5 mg l^{-1} sulfate as potential primary electron acceptors, plus 250 μg l^{-1} of one of the oxidized contaminants. MBfR B initially received selenate, and MBfR C initially received chromate. The H_2 pressure was 2.5 psi (0.17 atm). For both MBfRs, nitrate and nitrite concentrations in the effluent dropped to less than 15 μg-N l^{-1} within 10 days and were stable at this level for the remainder of the experiments. Addition of selenate or chromate began on day 50.

For MBfR B (Fig. 5), Se(VI) was reduced to Se(IV) within one day of Se(VI) addition, and Se(IV) begun to be removed through reduction to Se° at the same time. On day 18 after Se(VI) feeding began, Se(IV) in the effluent from the MBfR was below 10 μg-Se l^{-1}, and 97% of the input Se(VI) was reduced to solid Se° within 20 days. This suggests that dissimilatory selenate-reducing bacteria were present in the biofilm in significant numbers, but that continuous exposure to Se(VI) provided a selective pressure that further enriched these bacteria. The Se(VI) flux was 0.034 g-Se(VI) m^{-2} of biofilm surface area/day, and nitrate and sulfate fluxes were 0.710 g-NO_3^- m^{-2} days and 7.92 g-SO_4^{2-} m^{-2} days, respectively. Converted to common electron-equivalent units, selenate, nitrate, and sulfate fluxes were 0.48, 57.2, and 702 e^-meq m^{-2} days, respectively.

For MBfR C (Fig. 6), reduction of Cr(VI) to Cr(III) began immediately, indicated by 25 μg-Cr l^{-1} of Cr(III) in the effluent sample after 1 day of Cr(VI) addition, and steady-state reduction of Cr(VI) to Cr(III) was evident by day 13 of feeding Cr(VI). The average removal of Cr(VI) was 170 μg-Cr l^{-1}, or 65%. Effluent Cr(III) was 123 ± 18 μg-Cr l^{-1}, or 49%. The effluent concentration of total Cr reached approximately 210 μg-Cr l^{-1} (or 84%), which indicates that the most reduced Cr(III) was not a solid (likely $Cr(OH)_3$) that was removed by adsorption to the biofilm. The success of precipitation depends on how the solubility compares to the target Cr(III) concentration. The conditional solubility of Cr(III) calculated for pH = 7.3 in this study is approximately 66 μg-Cr l^{-1}, based on the following reactions and equilibrium constants (at 25°C) for the Cr(III)-hydroxide complexes (Rai et al. 1987):

$$Cr(OH)_{3(s)} + 3H^+ \Leftrightarrow Cr^{3+} + 3H_2O \quad \log K = 9.8 \tag{1}$$

$$Cr(OH)_{3(s)} + 2H^+ \Leftrightarrow CrOH^{2+} + H_2O \quad \log K = 6.0 \tag{2}$$

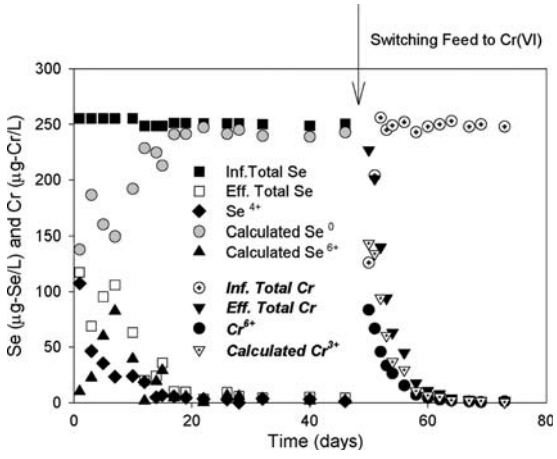

Fig. 5 Concentrations in the effluent from MBfR B. Up to day 49, 5 mg l^{-1} of NO_3^-N and 250 μg-Se l^{-1} were in the influent. From day 50, 5 mg l^{-1} of NO_3^--N and 250 μg-Cr l^{-1} were in the influent (Left Y axis: Se species in μg-Se l^{-1} and Cr species in μg-Cr l^{-1})

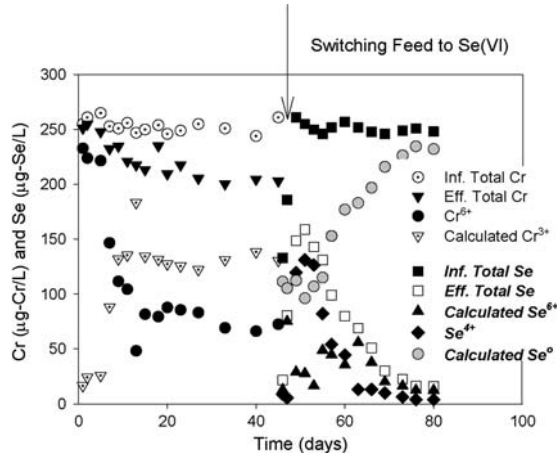

Fig. 6 Concentration in the effluent from MBfR C. Up to day 45, 5 mg l^{-1} of NO_3^--N and 250 μg-Cr l^{-1} were in the influent. From day 46, 5 mg l^{-1} of NO_3^--N and 250 μg-Se l^{-1} were in the influent. (Left Y axis: Cr species in μg-Cr l^{-1} and Se species in μg-Se l^{-1})

$$Cr(OH)_{3(s)} + H^+ \Leftrightarrow Cr(OH)_2^+ + H_2O \quad \log K = -0.4 \tag{3}$$

$$Cr(OH)_{3(s)} \Leftrightarrow Cr(OH)_{3(aq)} \quad \log K = -6.0 \tag{4}$$

$$Cr(OH)_{3(s)} + H_2O \Leftrightarrow Cr(OH)_4^- + H^+ \quad \log K = -18.3 \tag{5}$$

$$2Cr(OH)_{3(s)} + 4H^+ \Leftrightarrow Cr(OH_2)^{4+} + 4H_2O \quad \log K = 14.4 \tag{6}$$

$$3Cr(OH)_{3(s)} + 5H^+ \Leftrightarrow Cr_3(OH)_4^{5+} + 5H_2O \quad \log K = 18.5 \tag{7}$$

Since the measured Cr(III) concentration was substantially higher than 66 μg-Cr l^{-1}, soluble Cr(III) may have been prevented from precipitating with HO^- due to its complexation with phosphate, sulfate, or organic ligands (Evanko and Dzombak 1997; Armienta and Quere 1995). In particular, phosphate was relatively high in the synthetic groundwater (0.004 M). Such a high phosphate level is unlikely in actual groundwaters. Thus, the key finding here is that the reduction of Cr(VI) to Cr(III) gives a conditional solubility of Cr(III) lower than the MCL of 100 μg-Cr l^{-1}. The Cr(VI) flux was 0.021 g-Cr(VI) m^{-2} days, and nitrate and sulfate flux were 0.684 g-NO_3^- m^{-2} days and 0.510 g-SO_4^{2-} m^{-2} days, respectively. Converted to common e^- eq units, Cr(VI), nitrate, and sulfate fluxes were 0.54, 66.9, and 425 e^- meq m^{-2} days, respectively.

Once stable reductions of selenate and chromate were achieved in MBfRs B and C, the influent oxidized contaminant to each MBfR was switched; thus, the Se-reducing biofilm in MBfR B was exposed to chromate and vice versa. As shown in Fig. 6, Cr(VI) reduction to Cr(III) was immediate and increased to essentially 100% reduction within 20 days. Almost no Cr(III) appeared in the effluent by 20 days: all chromium species in effluent were below 10 μg-Cr l^{-1}, and the chromium reduction rate in MBfR B was higher than it had been in the Cr-reducing MBfR C. The Cr(VI) flux in Se-reducing MBfR B increased by 0.031 g-Cr(VI) m^{-2}·d, and electron-equivalent flux also increased to 0.80 e^- meq - m^{-2}·d.

As soon as Se(VI) feeding began to the Cr-reducing MBfR C (Fig. 6), Se(VI) was reduced to Se(IV) and Se°. Over the next 30 days, the amount of Se(VI) reduction increased to over 90%, and the end product was mostly Se°.

The results for switching oxidized contaminants to MBfRs B and C supports that using H_2 as the electron donor in the MBfR setting accumulated significant numbers of bacteria able to reduce selenate and chromate together. The results presented here cannot be used to determine the degree to which the same bacteria could reduce both oxidized contaminants, but the immediate onset of major reduction after the switch in both MBfRs suggests that the overlap of reduction capability is significant.

We confirmed at bench-scale that MBfRs have the potential to reduce perchlorate (+chlorate), selenate, chromate, and arsenate. A major difference between the bio-reduction of the above-mentioned contaminants and the more familiar biological denitrification is that perchlorate, chlorate, selenate, chromate, and arsenate typically are present at concentrations orders of magnitudes lower than nitrate. Consequently, these oxidized contaminants probably are reduced as secondary substrates, with another electron acceptor (oxygen, nitrate, and/or sulfate in this case) serving as the primary substrate, i.e., the acceptor whose reduction generates most of the energy for the microorganisms' synthesis and maintenance. Nerenberg and Rittmann (2002) saw secondary utilization in an MBfR when small concentrations of perchlorate were reduced concurrently with larger concentration of nitrate. Since a significant biomass can be sustained by utilization of the primary substrate, secondary utilization makes it possible to achieve very low effluent concentrations of the secondary substrate.

Conclusion

We investigated if directly using H_2 as the electron donor in an MBfR setting allows simultaneous reduction of many different combinations of oxidized contaminants. First, the H_2-based MBfR simultaneously reduced different combinations of nitrate, nitrite, perchlorate + chlorate, arsenate, and DBCP in three contaminated groundwaters from California's San Joaquin Valley. Nitrate reduction to N_2 for all groundwaters was complete, and As(V) was substantially reduced to As(III) for two samples. Perchlorate and chlorate were reduced to below the California Notification Level (6 $\mu g\ l^{-1}$). DBCP present in one groundwater was also reduced to below its detection limit of 0.01 $\mu g\ l^{-1}$. Second, switching the influent oxidized contaminant from selenate to chromate or from chromate to selenate gave immediate and significant reduction of the new contaminant in MBfRs treating one contaminant, but not having been previously exposed to the new contaminant. These results support that the H_2-based MBfR can treat multiple oxidized contaminants simultaneously, which addresses the common groundwater situation of having multiple oxidized contaminants.

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