

Hydrogen-based, hollow-fiber membrane biofilm reactor for reduction of perchlorate and other oxidized contaminants

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Abstract Many oxidized pollutants, such as nitrate, perchlorate, bromate, and chlorinated solvents, can be microbially reduced to less toxic or less soluble forms. For drinking water treatment, an electron donor must be added. Hydrogen is an ideal electron donor, as it is non-toxic, inexpensive, and sparsely soluble. We tested a hydrogen-based, hollow-fiber membrane biofilm reactor (MBfR) for reduction of perchlorate, bromate, chlorate, chlorite, chromate, selenate, selenite, and dichloromethane. The influent included 5 mg/L nitrate or 8 mg/L oxygen as a primary electron accepting substrate, plus 1 mg/L of the contaminant. The mixed-culture reactor was operated at a pH of 7 and with a 25 minute hydraulic detention time. High recirculation rates provided completely mixed conditions. The objective was to screen for the reduction of each contaminant. The tests were short-term, without allowing time for the reactor to adapt to the contaminants. Nitrate and oxygen were reduced by over 99 percent for all tests. Removals for the contaminants ranged from a minimum of 29% for chlorate to over 95% for bromate. Results show that the tested contaminants can be removed as secondary substrates in an MBfR, and that the MBfR may be suitable for treating these and other oxidized contaminants in drinking water.

Keywords Biofilm reactor; denitrification; hollow-fiber membrane; hydrogen; secondary substrate

Introduction

In recent years, several oxidized contaminants have emerged as drinking water pollutants, including arsenate (H_2AsO_4^-), chromate (CrO_4^{2-}), selenate (SeO_4^{2-}), bromate (BrO_3^-), and, most recently, perchlorate (ClO_4^-). In many cases, conventional water treatment processes, as well as oxidative processes such as chlorine-oxidation or ozonation, are ineffective. Advanced separation processes, such as reverse osmosis, ion exchange, membrane filtration, and electrodialysis, can be effective, but are expensive and generate concentrated wastes that require proper disposal. Biological reduction may provide a more suitable treatment alternative, especially when the oxidized contaminant is reduced to a less toxic species (Lovley and Coates, 1997).

Many oxidized contaminants are reduced in thermodynamically favorable reactions (Table 1) and have been shown to support bacterial growth. However, in some cases the treatment standards may be below bacterial growth thresholds (S_{\min}) (Rittmann and McCarty, 2001). In such cases, reduction may occur in parallel with reduction of more amply available “primary” electron acceptors, such as nitrate or oxygen. In this research, we tested a hollow-fiber membrane biofilm reactor (MBfR) for reduction and removal of several oxidized contaminants when nitrate or oxygen served as primary electron acceptors. The MBfR is ideal for treating oxidized compounds in drinking water, as it uses hydrogen as an electron donor (Ergas and Reuss, 2001; Lee and Rittmann, 2002; Nerenberg *et al.*, 2002). Hydrogen is non-toxic, inexpensive compared to organic donors, and leaves no residuals that could cause bacterial regrowth. Table 1 lists important oxidized contaminants, their reduction reactions with hydrogen as the electron donor, and their Gibbs free energy. Oxygen and sulfate are shown for reference.

Table 1 Potential reactions and their free energies for reduction of selected oxidized compounds with concurrent oxidation of hydrogen

Compound	Probable reduction reaction(s)	ΔG° (KJ/e ⁻)
Bromate	$\text{BrO}_3^- + 3\text{H}_2 \rightarrow \text{Br}^- + 3\text{H}_2\text{O}$	-136
Oxygen	$\text{O}_2 + 2\text{H}_2 \rightarrow 2\text{H}_2\text{O}$	-123
Chlorite	$\text{ClO}_2^- + 2\text{H}_2 \rightarrow \text{Cl}^- + 2\text{H}_2\text{O}$	-123
Chlorate	$\text{ClO}_3^- + 3\text{H}_2 \rightarrow \text{Cl}^- + 3\text{H}_2\text{O}$	-118 ¹
Perchlorate	$\text{ClO}_4^- + 4\text{H}_2 \rightarrow \text{Cl}^- + 4\text{H}_2\text{O}$	-118 ¹
Nitrate	$\text{NO}_3^- + 2.5\text{H}_2 + \text{H}^+ \rightarrow 0.5\text{N}_2 + 3\text{H}_2\text{O}$	-112
Selenate	$\text{SeO}_4^{2-} + 3\text{H}_2 + 2\text{H}^+ \rightarrow \text{Se}^0 + 4\text{H}_2\text{O}$	-71
Selenite	$\text{HSeO}_3^- + 2\text{H}_2 + \text{H}^+ \rightarrow \text{Se}^0 + 3\text{H}_2\text{O}$	-65
Arsenate	$\text{H}_2\text{AsO}_4^- + \text{H}_2 + \text{H}^+ \rightarrow \text{H}_3\text{AsO}_3 + \text{H}_2\text{O}$	-45
Sulfate	$2\text{SO}_4^{2-} + 8\text{H}_2 + 3\text{H}^+ \rightarrow \text{H}_2\text{S} + \text{HS}^- + 8\text{H}_2\text{O}$	-19
Chromate	$\text{CrO}_4^{2-} + 1.5\text{H}_2 + 2\text{H}^+ \rightarrow \text{Cr}(\text{OH})_3 + \text{H}_2\text{O}$	-9

ΔG° is the standard Gibbs free energy at pH = 7

¹The free energies exclude the energy from chlorite disproportionation into chloride and oxygen, but include the energy from oxygen reduction

We now review the bases for why biological reduction of key oxidized contaminants is likely.

Arsenate

Arsenic is an inorganic contaminant that is almost exclusively found in groundwater. It can cause gastrointestinal damage and cardiac damage and is considered a human carcinogen. The current United States Environmental Protection Agency (EPA) Maximum Contaminant Level Goal (MCLG) for arsenic in drinking water is 10 µg/L. Arsenic in the environment is normally found in the As(III) and As(V) oxidation states, depending on the redox conditions. The redox state of arsenic can have an important effect on its mobility and toxicity (Cullen and Reimer, 1989). Microbial reduction of As(V) to As(III) may result from a detoxification process (Cervantes *et al.*, 1994) or dissimilatory reduction (Laverman *et al.*, 1995; Macy *et al.*, 1996; Blum *et al.*, 1998; Oremland *et al.*, 1999). Some dissimilatory arsenate-reducing bacteria can also grow with oxygen or nitrate, and many also reduce selenate (Laverman *et al.*, 1995; Macy *et al.*, 1996; Oremland *et al.*, 1999; Santini *et al.*, 2002). Santini *et al.* (2002) isolated an obligate anaerobe that reduced arsenic with hydrogen as an electron donor, and it also used nitrate as an electron acceptor. Although As(III) is more mobile and difficult to remove than As(V) when using physical-chemical processes, it can precipitate with sulfide under sulfate-reducing conditions (Wilkin *et al.*, 2003), which could be a strategy for its removal in a biological treatment system. Thermodynamically, arsenate is less energetic than nitrate, but more highly energetic than sulfate (Table 1).

Bromate

Bromate is commonly produced from bromide during the ozonation of drinking water or during water treatment with advanced oxidation processes (Myllykangas *et al.*, 2000; Pinkernell and von Gunten, 2001; von Gunten, 2003). Bromate is a genotoxic carcinogen (Kurokawa *et al.*, 1990; Umemura *et al.*, 1995) and is regulated under the Stage 1 Disinfectants/Disinfection By-Products Rule at 10 µg/L (Kirisits *et al.*, 2001). Bromate was reduced to bromide by denitrifying bacteria in a denitrifying bioreactor (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999) and in a biologically active carbon filter with NO_3^- (Krisits and Snoeyink, 1999; Kirisits *et al.*, 2001). The presence of nitrate slowed bromate reduction (Hijnen *et al.*, 1995; Hijnen *et al.*, 1999), but some reduction occurred at measurable oxygen and nitrate concentrations (Kirisits *et al.*, 2001). It was not clear whether bromate is

reduced cometabolically by the nitrate reductase enzyme or by specialized bacteria with a bromate-reduction pathway. Bromate reduction under hydrogen-oxidizing conditions has not been investigated. However, since many denitrifiers can use hydrogen as an electron donor, it is reasonable to presume that some bromate-reducing denitrifiers may be able to use hydrogen. The thermodynamics for bromate reduction are very favorable, even higher than oxygen reduction (Table 1). Reduction of bromate produces bromide, an innocuous product.

Perchlorate, chlorate, and chlorite

Perchlorate in water mainly comes from improperly disposed-of rocket fuel. It inhibits thyroid function at low levels (Espenson, 2000). Although there is no standard for perchlorate in the United States, California has set an action level of 4 µg/L. Chlorate and chlorite are two-electron reduction products from perchlorate. Perchlorate, chlorate, and chlorite can be reduced to chloride by (per)chlorate-reducing bacteria (PCRB) (Logan, 1998; van Ginkel *et al.*, 1998; Coates *et al.*, 1999; Nerenberg *et al.*, 2002). Table 1 shows that the per-electron energy yield for all of these acceptors is very high, comparable to denitrification and oxygen reduction. The product of perchlorate, chlorate, and chlorite reduction is innocuous chloride.

Chromate

Chromium is an inorganic contaminant released to drinking water sources from electroplating facilities, old mining operations, and fossil-fuel power plants. Chromium can cause liver and kidney damage, and the maximum contaminant level for drinking water is 0.1 mg/L total chromium. The toxicity and mobility of chromium depends on its oxidation state. Cr(VI) is carcinogenic and highly soluble in water (Flores and Perez, 1999), while Cr(III) tends to form insoluble complexes with hydroxides and is considered much less toxic (Palmer and Wittbrodt, 1991). Cr(VI) is reduced under aerobic and anaerobic conditions, and some Cr(VI)-reducing bacteria can use hydrogen as an electron donor (Marsh and McInerney, 2001). Reduction of chromate did not stimulate growth (Cervantes, 1991). However, direct reduction of Cr(VI) (i.e., reduction not associated with sulfide) correlated with nitrate reduction by a nitrate-reducing consortium when straw was used as an organic carbon source (Vainshtein *et al.*, 2003), and the addition of molasses and nitrate to a microcosm stimulated chromate reduction (Oliver *et al.*, 2003). If growth with chromate were possible, the yields would be low, as chromate reduction provides less per-electron free energy than sulfate. If biologically reduced and precipitated, Cr(III) presumably should be removed by filtration.

Dichloromethane (DCM)

DCM (CH₂Cl₂), also known as methylene chloride, is a common industrial solvent that is carcinogenic, highly soluble in water, and a common groundwater contaminant (Kohlerstaub *et al.*, 1995). Dichloromethane can be transformed microbially by four mechanisms: hydrolytic dehalogenation, oxidative metabolism, reductive dehalogenation, and fermentation (Rittmann *et al.*, 1994; Magli *et al.*, 1998). DCM can serve as an electron donor for growth and can be completely dehalogenated under denitrifying conditions (Leisinger *et al.*, 1994; Kohlerstaub *et al.*, 1995).

Selenate/selenite

Selenium is commonly found in the effluents of coal-burning power plants, oil refineries, and metal smelting plants (Lawson and Macy, 1995). It is also widely used in industry, including for production of pigments and semiconductors. Selenium also can be leached

from soils, a particular problem in the Central Valley of California. Selenium can be reduced to selenite or to elemental selenium by bacteria that also use nitrate as an electron acceptor (Fujita *et al.*, 1997; Rege *et al.*, 1999a). For example, SeO_4^- was removed concurrently with nitrate by a denitrifying consortia or pure cultures (Macy *et al.*, 1993; Rege *et al.*, 1999b; Kashiwa *et al.*, 2000). Some of the bacteria are facultative anaerobes (Fujita *et al.*, 1997). In all cases, the reduction was dissimilatory. Elemental selenium is toxic, but is insoluble and can be removed by filtration.

Methods

The screening experiments were carried out with two MBfRs that were smaller scale versions of the MBfRs used by Nerenberg *et al.* (2002) and Lee and Rittmann (2002). The configuration of an MBfR is shown in Figure 1. Table 2 summarizes the physical characteristics of the reactors. Each reactor consisted of two glass tubes connected with Norprene tubing and plastic “tee” fittings. One tube contained a bundle of 32 hollow-fiber membranes, each fiber with a 25-cm active length. The fibers were potted at one end, and free and individually sealed at the opposite end. The other tube contained a manifold with a single fiber. The reactors had a recirculation rate of 150 mL/min to promote completely mixed conditions. Thus, the concentration in the reactor was approximately equal to the effluent concentration.

One reactor (“nitrate reactor”) was supplied with 5 mgN/L nitrate and 6 mg/L oxygen, while the other (“oxygen reactor”) was supplied with 8 mg/L oxygen and 1 mg/L perchlorate. At the time of the screening experiments, the nitrate reactor had an effluent nitrate concentration of around 0.02 mgN/L and <0.1 mg/L oxygen, while the oxygen reactor had <0.1 mg/L oxygen in the effluent. Both reactors had been operated under these conditions for more than 50 days at the time of the screening experiments. Total biomass was not measured, but confocal laser scanning microscopy was used to determine the biofilm thickness for a short segment of the “coupon” fiber of the nitrate reactor. The thickness was 30 μm . The thickness of the oxygen fibers was visually similar to that of the nitrate reactor.

The screening experiments consisted of adding approximately 1 mg/L of one of the oxidized contaminants to a medium containing nitrate plus oxygen or oxygen alone (Nerenberg *et al.*, 2002). Each contaminant was tested sequentially in a series of short-term tests. The influent flow rates were 0.85 mL/min for the nitrate reactor and 1 mL/min for the oxygen reactor. During each experiment, the reactor medium was spiked with the contaminant for around 90 minutes (>3 detention times). After that time, 3 samples were collected at 25- to 30-minute intervals. After the samples were collected, the original medium (without the screening contaminants) was restored.

The samples were collected using a 10-mL syringe connected to the effluent port. The contaminants were tested sequentially over the course of several days. Analyses for all

Table 2 MBfR characteristics

	Units	Main tube	Coupon tube	Reactor total
Tube inside diameter	cm	0.6	0.5	–
No. of hollow fibers		32	1	33
Total fiber cross-sectional area	cm^2	0.0197	0.000616	0.0203
Feed rate	mL/min	–	–	1
Recirculation rate	mL/min	–	–	150
Net cross-sectional area	cm^2	0.26	0.20	–
Fiber surface area	cm^2	70.4	2.20	72.6
Liquid velocity	cm/min	570	766	–
Average detention time	min	–	–	23.9

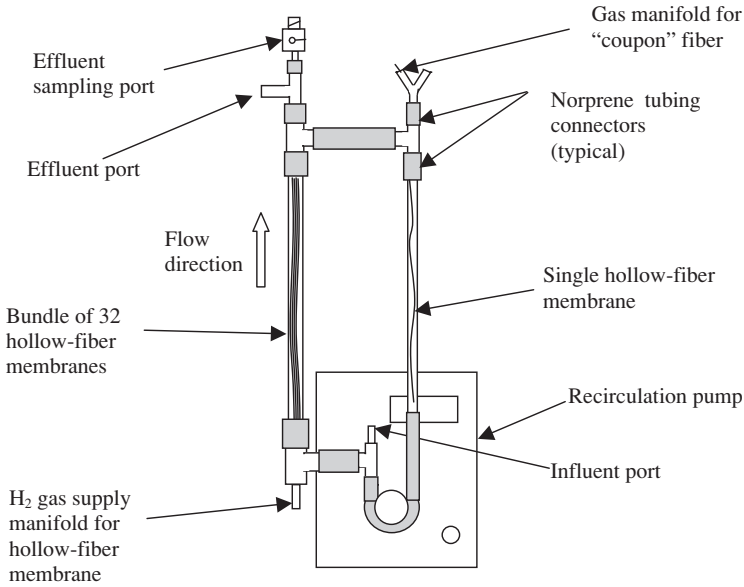


Figure 1 Bench-scale MBfR configuration

anions were carried out by ion chromatography using an AS-11 column and a AG-11 pre-column, as described in Nerenberg *et al.* (2002). For dichloromethane, the samples were collected in 40-mL VOA bottles, packed on ice, and sent to an external laboratory for analysis. Dichloromethane was analyzed by GC-MS following EPA method 8260.

Results and discussion

The screening results are summarized in Table 3. In all the tests, over 99 percent of the influent nitrate was reduced, and oxygen was reduced to below the detection limit (not shown). The influent concentration was approximately 1 mg/L in each case, and the table lists the results as percentage removals, since all experiments had nearly the same 1-mg/L influent concentration. A value reported as >xx means the effluent concentration was below the detection limit for that contaminant; therefore, the actual removal was at least as great as the removal for an effluent concentration of the detection limit.

Perchlorate, chlorate, and chlorite were more highly removed in the oxygen reactor, which previously had been exposed to perchlorate. Continued exposure to perchlorate significantly improves perchlorate removals, probably due to an enrichment for specialized bacteria (Nerenberg *et al.*, 2002) that can also remove chlorate and chlorite.

In both reactors, bromide accumulated in amounts stoichiometric to the influent bro-

Table 3 Results of screening tests

Compound	Removal efficiency (%)	
	O ₂ reactor	NO ₃ ⁻ reactor
Perchlorate	>98	36
Chlorate	>95	29
Chlorite	>75	67
Bromate	>95	>95
Chromate	>75	>75
Selenate	67	74
Selenite	93	57
Arsenate	>50	>50
Dichloromethane	38	45

mate, demonstrating full reduction. Chromate removal was over 75% in both reactors, and the likely end product was insoluble $\text{Cr}(\text{OH})_3$. For selenate, the total removal in the nitrate reactor was 74%, and 5% accumulated as SeO_3^{2-} (on a molar basis). Therefore, most of the removed selenate probably was precipitated as Se^0 . 67% of selenate was removed in the oxygen reactor. Over 90% of selenite was removed in the oxygen reactor, while 57% was removed in the nitrate reactor. Arsenate was removed by at least 50% in both reactors. Dichloromethane removal ranged from 38% in the oxygen reactor to 45% in the nitrate reactor.

Conclusions

Previous research suggested that all of the tested contaminants should be reduced in the MBfR using hydrogen as the electron donor. The results of the screening tests confirm that the wide variety of oxidized contaminants can be reduced and removed *immediately* in a hydrogen-oxidizing MBfR with oxygen or nitrate as primary acceptors. In some cases, removals were greater than 75% without any community adaptation. Previous experiments for perchlorate showed that the reduction rate increased to much higher levels with continued exposure to perchlorate (Nerenberg *et al.*, 2002); thus, removals are likely to improve with continued exposure. Further tests are being carried out to evaluate this hypothesis. Most reduction products are innocuous (e.g., N_2 , Cl^- and Br^-) or insoluble (e.g., $\text{Cr}(\text{OH})_3$ and Se^0). The insoluble products are likely to remain in the biofilm or be removed by filtration.

This research shows that the hydrogen-based, hollow-fiber membrane biofilm reactor is effective in removing a wide range of oxidized drinking water contaminants. While many of the contaminants can serve as primary acceptors at higher concentrations, nitrate or oxygen can serve as primary electron acceptors when the contaminants need to be reduced to very low levels.

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