Original article

Biological activation of hydrous ferric oxide for reduction of hexavalent chromium in the presence of different anions

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Abstract

Shewanella alga BrY, a dissimilatory iron reducing bacterium (DIRB), transformed inert ferric oxides that are common in sediments, aquifer material and passivated permeable reactive iron barriers (PRBs), producing dissolved and sorbed Fe(II) capable of rapidly reducing and immobilizing Cr(VI). The effect of groundwater chemistry on the formation and reactivity of such microbial-produced, abiotic reductants was investigated. Batch reactors with high carbonate concentration (10 mM) were the most reactive, removing $66.0\% \pm 2.8$ of Cr (VI) (76 mg/l) from liquid phase within 5 min. Treatments with high concentrations of sulfate (5.2 mM), chloride (10 mM), phosphate (1 mM) or silica (0.75 mM) were less reactive (about 40% removal). Loss of reactivity was observed possibly due to oxidation of Fe(II) (sorbed and dissolved) by Cr(VI). Normalization of Cr(VI) removal to the mass of biogenic solid present showed the following molar Cr/Fe ratios in solid phase: 0.185 ± 0.041 (carbonate), 0.146 ± 0.013 (sulfate), 0.092 ± 0.010 (silica), 0.075 ± 0.012 (phosphate) and 0.062 ± 0.012 (chloride). Overall, these results show that bacterial transformation of inert ferric oxides can contribute to the (abiotic) natural attenuation of Cr(VI) in and around PRBs, and that groundwater chemistry is an important determinant of biogenic solids reactivity.

Keywords: Shewanella alga BrY; Iron reduction; Groundwater; Permeable reactive barriers

1. Introduction

Hexavalent chromium generated in tanning and plating industries is a contaminant of significant concern due to its high toxicity and mobility in groundwater and soil [39]. Common treatment technologies reduce Cr(VI) to Cr(III), which is then precipitated as $Cr(OH)_3$ at high pH [13]. Cr(VI) in groundwater can also be reduced by organic and inorganic matter present in the soil [6,33], or by microorganisms [34,45,46], although these natural attenuation processes are rarely sufficient to remove Cr(VI) contamination to acceptable levels.

Permeable reactive barriers (PRBs) are a cost-effective alternative to remove Cr(VI) and other pollutants in situ as groundwater moves through reactive material placed perpendicular to the flow path of the plume [37]. Most PRBs use granular zero-valent iron (ZVI) as reactive material [37,44]. ZVI is a powerful reductant

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$(E_o' = -0.44$ V) that can be used to treat a wide variety of oxidized pollutants, including chlorinated solvents, nitrate, nitro-aromatics, and U(VI) [37,44,53]. In the case of chromium, treatment involves Cr(VI) reduction coupled to ZVI oxidation to Fe(II) and Fe(III) [5], resulting in the formation of an insoluble crystalline solid [38]. Reaction kinetics is very fast, proportional to iron surface concentration and slower at high pH values [2]. Fe-Cr solids are very stable, as Cr associated to ferric oxides does not dissolve easily with changes in pH [4, 13].

A potential concern regarding ZVI-PRBs is their long-term performance, which can be hindered by passivation and clogging of the iron media. Precipitation of iron-bearing solids (oxides, carbonates, and sulfides) has been reported for 26% of the installed barriers, accounting for losses on porosity ranging from 1.5 to 15% per year [53]. The amount, characteristics and spatial distribution of precipitates are influenced by the flow rate, groundwater chemistry, and microbial processes [15,20,26,44]. Passivation increases with the tendency of corrosion products to precipitate and occlude reactive sites [15,26]. Inert precipitates that have been found in ZVI PRBs include lepidocrocite, akaganeite, mackinawite, ferrihydrite, goethite, and siderite [16,20]. Some of these inert precipitates in and around PRBs can be dissolved and/or transformed by chemical or enzymatic reactions to become powerful reductants and enhance the overall reduction capacity of the system [11,18, 51]. For example, dissimilatory iron reducing bacteria (DIRB) could be stimulated to reductively dissolve passivating Fe(III)-oxide layers and/or generate reactive, surface-associated Fe(II) species, to enhance PRB treatment efficiency [18,28,30,32].

Shewanella species are facultative DIRB that can grow anaerobically utilizing either H_2 (commonly produced in PRBs during anaerobic ZVI corrosion) or lactate as an electron donor, and either fumarate, citrate, or malate as a carbon source. These bacteria can respire with a wide variety of electron acceptors, including O_2, nitrate, Fe(III), Mn(IV), fumarate, and thiosulfate [8,9,18,57]. Due to their metabolic versatility, *Shewanella* sp. have been the subject of much research for environmental remediation applications. For example, *Shewanella alga* BrY is known to reduce a substantial fraction (8–18%) of the inert iron(III) oxide content of a variety of soil and subsurface materials [42], generating ferrous solids that can dechlorinate carbon tetrachloride and reduce Cr(VI) [3,18,32,54]. Bioaugmentation of iron columns with BrY has also been reported to enhance the removal of nitrate [17], which can also be

respired by BrY. However, strategies to exploit the reduction capabilities of *Shewanella* sp. in complex environmental systems have not yet been developed, and little is known about how groundwater chemistry affects their Fe(III) reducing activity and the reactivity of the resulting Fe(II)-bearing solids.

This paper investigates the ability of *Shewanella alga* BrY to transform inert ferric precipitates into reactive solids and dissolved Fe(II) capable of reducing Cr(VI). Emphasis was placed on characterizing how different anions commonly found in groundwater affect the formation and reactivity of such reductants. In doing so, information was obtained on the effect of groundwater chemistry on microbial-promoted abiotic reductive processes that contribute to natural attenuation of oxidized pollutants. This information also enhances our understanding of the roles of facultative DIRB in pollution control in and around PRBs.

2. Materials and methods

2.1. Fe(III) oxide synthesis

A hydrous ferric hydroxide was used as a model of ferric precipitates. It was synthesized by neutralizing a $FeCl_3$ solution (108 g/l of distilled water) with 10 N NaOH [41]. The oxide was washed six times (20 min each) with DI water by centrifuging at 10,000 rpm. The resulting sludge was preserved at 4 °C, and then it was re-suspended in anoxic water, under N_2/CO_2 (80:20) atmosphere, to achieve a final concentration of 1 M.

2.2. Shewanella alga BrY culture

Shewanella alga BrY (ATCC 51181) was obtained from American Type Culture Collection (Manassas, VA). Cells were grown in 500 ml of tryptic soy broth (30 g/l) and incubated on a rotary shaker at 30 °C and 90 rpm for 15 hours. Cells were then centrifuged at 10,000 rpm and 4 °C for 20 min and washed three times in PIPES (piperazine-1,4-bis(2-ethanesulfonic acid)) solution (20 mM).

2.3. Biogenic solids generation

One hundred ml of ferrihydrite solution (1 M) was added to 800 ml of fresh water medium, containing (in mg/l): K_2SO_4 (40), $NaNO_3$ (16), $MgCl_2·6H_2O$ (12), $CaCl_2$ (6.7), $Ni(NO_3)_2·6H_2O$ (0.002), $CuSO_4·5H_2O$ (0.002), $ZnSO_4·7H_2O$ (0.002), $CoSO_4·7H_2O$ (0.002), $(NH_4)6Mo_7O_{24}$ (0.001), H_3BO_3 (0.0004). 10 ml of

Table 1
Anions added in excess for biogenic solids preparation

Set	Factor	Elevated concentration (mM)	Rationale
1	Carbonate (HCO_3^-/CO_3^{2-})	10	Background carbonate concentration reported for Copenhagen Freight Yard [25]; expected to be at the upper limit of typical groundwater carbonate concentrations
2	Silica (SiO_2)	0.75	Upper limit reported for typical groundwater [12]
3	Phosphate (PO_4^{3-})	1	Average high groundwater concentration [41,50]
4	Chloride (Cl^-)	10	Level used by Klausen et al. [26]; effects of this concentration on TCE reductive dechlorination were mixed.
5	Sulfate (SO_4^{2-})	5.2	Average high groundwater concentration [15,20,55, 60]

each, Wolfe's vitamin and mineral solutions [24] and sodium lactate as electron donor (20 mM) were added. The pH was adjusted to 7.0. Aerobically grown bacteria (BrY) were transferred to 50 ml of described medium, and added to the system. The final volume was adjusted to 1 l. All procedures were performed inside a Coy anaerobic chamber. The original medium was modified by addition of one of the next anions in high concentration to test its effect on production and reactivity of the biogenic reduced solids: carbonate (10 mM), silica (0.75 mM), phosphate (1 mM), chloride (10 mM) and sulfate (5.2 mM), as summarized in Table 1. The media were kept in the anaerobic chamber for 45 days, when a noticeable change in color was evident from reddish to dark brown in at least two biogenic solids. Survival of BrY was tested by plating particles of the reduced solids in marine agar, under aerobic conditions.

2.4. Chromium reduction in batch reactors

Batch experiments were conducted to characterize the Cr(VI)-reduction capability of biogenic solids formed by BrY under different water chemistry conditions. Sets of five replicate anaerobic batch reactors containing a 10 ml aliquot of biogenic solids suspension and hexavalent chromium were prepared for each of the five treatments (subsequently called carbonate, chloride, phosphate, silica and sulfate solids), for a total of 25 reactors. Two control sets were also prepared: a no-treatment control set containing freshwater medium and no solids to discern Cr(VI) losses by sorption, and a set containing the starting ferric oxide not exposed to BrY. No attempt was made to eliminate bacteria from the solids. Reactors were prepared in 15 ml serum bottles, caped and crimped with aluminum seal. Prior to sealing, the reactors were amended with potassium dichromate (115 mg/l as Cr(VI)) and deoxygenated by purging with N_2 for 20 min in the liquid phase and 10 min in the headspace. Liquid samples were withdrawn after 5, 20 and 35 min of contact time and filtered (0.2 μm) to measure residual Cr(VI) in solution. Dissolved iron was also measured in these samples. All reactors were then opened and solids were washed five times with DI water, to remove water-soluble species. The solids were then dried, weighted and digested with HCl solution (40% vol.), for total Cr and Fe analysis by atomic absorption spectroscopy.

2.5. Analytical methods

The starting ferric oxide was characterized using Mössbauer spectroscopy, measuring the % absorbed γ-wave radiation from a [57]Co source to the iron solids sample [56]. Samples for Mössbauer were prepared by filtering the iron solids onto 13 mm, 0.45 micron filter paper and sealing between two layers of Kapton® tape (which has very low O_2 permeability, thus retaining anoxic conditions). Spectra were obtained after 8 hours and fit with Recoil ® software.

Cr(VI) was measured spectrophotometrically at 540 nm by the S-diphenyl carbazide method [10] in a UV-Vis spectrometer Varian Cary 50. Total chromium and iron, in solid and liquid phase, were determined by flame atomic absorption spectroscopy (AAS) in a Varian SpectrAA 200. Cr(III) was estimated as the difference between total and hexavalent chromium.

2.6. Chemicals

All solutions were prepared in DI water. All chemicals were HPLC or ACS grade (Sigma, St. Louis, MO). N_2 and N_2/CO_2 (80/20%) gases were purchased from Air Products, Inc.

3. Results and discussion

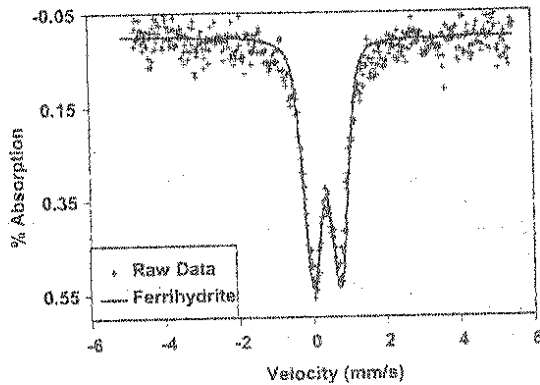Fig. 1 shows the Mössbauer spectra for the amorphous ferric oxide used as the starting material that

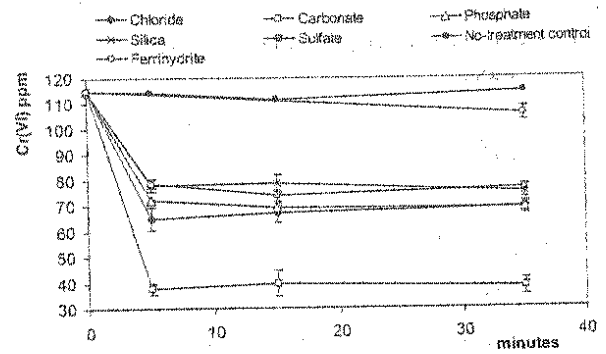Fig. 1. Mössbauer spectra for original ferric oxide.

Fig. 2. Cr(VI) reduction by Fe(II) (dissolved and associated with biogenic solids) produced by BrY from ferrihydrite in presence of different ions. Error bars represent one standard deviation from the mean of triplicate measurements.

was transformed by *Shewanella alga* BrY. The data show a doublet (two peaks), typical for ferrihydrite. A very broad distribution of crystal forms (lattice structures) is apparent in the broad peaks, reflecting a less crystalline and more amorphous nature of ferrihydrite. Ferrihydrite has been used before as a model for inert ferric oxides in PRBs [58], and has been found extensively in PRB where appropriated analytical techniques have been used [16]. Whereas ferrihydrite can adsorb Cr(III) and Cr(VI), it does not reduce Cr(VI) under subsurface conditions (absence of light) [52].

After 45 days, ferrihydrite incubated with BrY in the presence of high concentrations of chloride, carbonate or phosphate changed its color to dark brown (reflecting reduction of Fe(III) to Fe(II)), with larger particles observed in the high-chloride treatment. Solids formed with high sulfate or silica concentrations were lighter in color, suggesting differences in the structure of the microbial-produced solids.

Ferrihydrite did not remove Cr(VI) during the course of the experiments, except for a small amount (< 9%) that was reversibly adsorbed. In order to confirm that this removal was due to reversible adsorption, a concentrated phosphate solution was added to stimulate Cr(VI) displacement by ion exchange. This resulted in the recovery of the Cr(VI) that had been removed by sorption onto ferrihydrite (data not shown). In contrast, all treatments incubated with BrY removed Cr(VI) rapidly (Fig. 2) and irreversibly. For all treatments, the reaction took place in less than 5 min, with no further Cr(VI) reduction observed afterwards, probably due to depletion of reduction capacity. Whereas BrY is capable of reducing Cr(VI) directly [21,27,54] and plating of biogenic solid samples confirmed that BrY had survived during the incubation period, the rate of microbial reduction (~10^{-4} M/h) is significantly slower than that of surface-associated or dissolved Fe(II) [54]. For ex-

ample, the initial reduction rates depicted in Fig. 2 are at least one order of magnitude higher than the above microbial reduction rate. Therefore, Cr(VI) removal was primarily attributed to abiotic reduction by microbial-produced Fe(II) (dissolved and solid-associated) with subsequent precipitation of Cr(III).

Further experiments with longer storage of the biogenic solids (i.e. 180 days) showed no significant decrease in Cr(VI) removal capacity, provided that the solids be kept in anaerobic environment (data not shown). However, the reducing capacity of Fe(II)-bearing solids is rapidly depleted upon contact with an oxidant, such as O_2, Cr(VI), or other oxidized pollutants. This could be a concern regarding process sustainability. Nevertheless, it should be kept in mind that reducing equivalents are abundant in anaerobic environments, and DIRB could use many electron donors to
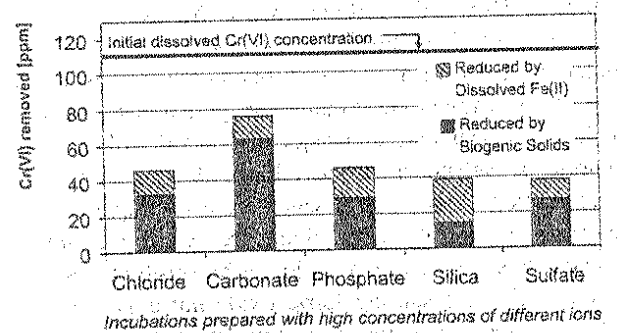

Fig. 3. Comparison of Cr(VI) reduction by dissolved and solid-associated Fe(II) produced by BrY from ferrihydrite in presence of different ions. Fraction reduced by dissolved Fe(II) was estimated based on stoichiometry and Fe(II) consumption measurements. Amount of Cr(VI) reduced by solids was calculated as the difference between total reduced chromium and the amount attributable to Fe(II) oxidation.

"recycle" Fe(III) into Fe(II) again, to restore the reduction capacity of the solids [32].

Fig. 3 depicts the relative contribution of dissolved iron and the biogenic Fe(II)- bearing solids towards Cr(VI) reduction. The contribution of dissolved Fe(II) was estimated based on the amount of dissolved Fe(II) consumed (Table 2) and the theoretical stoichiometric requirement of 3 mol of Fe(II) per mole of reduced Cr(VI). In all treatments, the amount of Cr(VI) removed exceeded the amount possibly reduced by the available dissolved Fe(II), and the difference was attributed to reduction by the biogenic Fe(II)- bearing solids. In general, the contribution of biogenic solids was higher than that of dissolved Fe(II), possibly because the reducing capacity of Fe(II) is favored thermodynamically and kinetically by adsorption to surfaces due to generation of an efficient electron carrier system [7,14,48]. Surface-associated Fe(II) can reduce priority pollutants such as halomethanes, RDX, Cr(VI) and carbon tetrachloride [3,7,19,23]. The oxide surface that acts as support can also serve as a catalyst by fixing the position of the two charged reactants in a suitable geometry for reaction. In fact, unreacted ferrihydrite has been found to enhance Cr(VI) reduction by Fe(II) [7]. Thus, it is likely that unreacted ferrihydrite also contributed (indirectly) to Cr(VI) removal in these experiments.

The molar ratios of precipitated Cr to solid iron (Cr/Fe) were calculated (Table 2). The estimated stoichiometric ratios are smaller than those reported in the literature, which typically range between 0.5 and 1 [10, 54]. The smaller values obtained in these experiments suggest that reactive surface sites were not readily available for chromium reduction and/or fixation into the solid phase. Based on final measured Cr/Fe ratios (0.185 for carbonate and 0.146 for sulfate) and the initial presence of ferrihydrite in our systems, we hypothesize that formation of Cr-substituted ferrihydrite occurs as a result of chromium reduction for biogenic solids produced in the presence of high concentrations of sulfate and carbonate. Cr-substituted ferrihydrite has

been found in Cr-reduction by green rust (a mixed valence iron oxide a.k.a. fougerite), with a 0.16–0.22 Cr/Fe ratio [29]. This solid has a very low solubility, similar to that of the Cr(III)-hydroxide precipitate.

Regarding the effect of common ions, reactors with high carbonate concentrations were the most reactive, removing $66.0 \pm 2.8\%$ of the added Cr(VI) from the liquid phase, while all the other reactors removed about 40% (Table 2). High concentrations of chloride, phosphate or silica resulted in microbial-produced solids with smaller reduction capabilities than those generated in the presence of high concentrations of carbonate and sulfate (Table 2). BrY requires direct contact with the iron mineral during electron transport [28]. Phosphate and silica likely blocked access of BrY to iron surfaces, through the formation of surface complexes at the particle–water interface [12,26,40,43,47,49]. These compounds could block both access of BrY to solids (hindering reduction) and sorption/incorporation of Fe(II) on the solid phase. It is more difficult to predict or explain the effect of chloride. Although chloride is generally considered as a corrosion promoter [22], which could be conducive to enhanced electron transfer, chloride has been reported to inhibit perchlorate reduction by iron surfaces [31]. Sulfate also can be a corrosion promoter, and its presence might have also enhanced solids reactivity by serving as a constituent of sulfate green rust. Sulfate green rust is less stable than carbonate green rust [50] but is nonetheless a powerful reductant. This hypothesis, however, could not be validated because the presence of green rust was not investigated in this experiment.

Depending on its concentration, carbonate could either enhance or inhibit biological reduction and the reactivity of Fe(II)-bearing solids. Carbonate can precipitate as calcite, aragonite, or carbonate green rust [1, 20,35,58]. This could inhibit contaminant access to the solid surface [1,36,58], and in the case of green rust, provide a redox-active phase [55]. Solids produced in the presence of carbonate presented higher reactivity;

Table 2

Comparison of biogenic solids reactivity

Biogenic solid produced with high concentrations of[a]:	Cr(VI) removed from liquid phase (%)	Cr/Fe molar ratio[**]	Total dissolved Fe(II) before contact with Cr(VI)[**] (mg/l)	Total dissolved Fe(II) after contact with Cr(VI)[b] (mg/l)
Chloride	40.0 ± 1.3	0.062 ± 0.012	44.95 ± 4.42	0.78 ± 0.48
Carbonate	66.0 ± 2.8	0.185 ± 0.041	41.3 ± 4.09	0.13 ± 0.07
Phosphate	40.2 ± 0.2	0.092 ± 0.010	54.0 ± 4.20	0.08 ± 0.03
Silica	34.8 ± 2.0	0.075 ± 0.012	84.2 ± 7.82	4.85 ± 1.48
Sulfate	33.3 ± 1.1	0.146 ± 0.013	34.7 ± 3.05	0.00 ± 0.00

[a] See Table 1 for ion concentrations.

[b] measured by AAS. Initial Cr(VI) concentration: 65 mg/l.

thus, these solids were further analyzed by Mössbauer spectroscopy (data not shown). This analysis identified siderite ($FeCO_3$, a Fe(II)-bearing solid). Whereas siderite is unlikely to account for the high reactivity of these solids, its presence confirmed that in presence of carbonate, ferrihydrite was reduced by BrY to Fe(II)-bearing solids.

Although Cr(VI) reduction by biologically produced ferrous species has been reported before, our work shows that the generation and/or reactivity of such species could be significantly influenced by presence of different anions commonly found in groundwater. This information contributes towards delineating the role, capabilities and limitation of facultative DIRB in pollution control.

4. Conclusions

Results suggest that DIRB such as BrY could enhance the long-term performance of ZVI PRBs and contribute to abiotic natural attenuation of Cr(VI) plumes in aquifer systems. The facts that the BrY is a facultative anaerobe (i.e. tolerant to oxygen) and can utilize H_2 (a ZVI corrosion product) as electron donor make this strain an attractive candidate for PRB inoculation. Furthermore, *Shewanella* are r-strategists that are likely to thrive and "activate" inert iron oxides in contaminated aquifers under selective biostimulated conditions, using various electron donors. Nevertheless, significant improvement in performance is unlikely to be universally achieved, and pilot studies should be conducted to evaluate process sustainability (e.g. bacterial cycling of Fe(II)/Fe(III)) and identify potential critical limitations associated with scale up issues, including the effect of high pH, co-contaminants and redox conditions.

Despite the clear interest in the application of biological processes to treat contaminated aquifers, little has been done to assess the role of related chemical (or abiotic) transformation pathways and determine whether combined biological and chemical transformation process are likely to be sufficiently *sustainable* under field conditions to enhance the attenuation of Cr(VI) plumes. Our work shows factors such as groundwater chemistry will affect production and/or reactivity of biogenic solids and dissolved species generated by enzymatic activity. Carbonate-containing media promoted higher Cr(VI) removal, although further studies are needed to evaluate long-term stability and ascertain whether different processes could promote chromate leaching from the solid phase. Chloride, silica and

phosphate hindered biogenic solid production and resulting reactivity, possibly by blocking access to reactive sites. Solids prepared in presence of high sulfate concentrations exhibited a moderate Cr(VI) removal capacity. However, a complex system like this, containing different solid phases, biological residues, dissolved and sorbed Cr and Fe species will require further studies to completely characterize the distribution of the contaminant after reduction.

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