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Remediation of Contaminated Subsurface Materials by a Metal Reducing Bacterium

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ABSTRACT

A biotic approach for remediating subsurface sediments and groundwater contaminated with carbon tetrachloride (CT) and chromium was evaluated. Cells of the Fe(III)-reducing bacterium strain BrY were added to sealed, anoxic flasks containing Hanford groundwater, natural subsurface sediments, and either carbon tetrachloride, CT, or oxidized chromium, Cr(VI). With lactate as the electron donor, BrY transformed CT to chloroform (CF), which accumulated to about 10 % of the initial concentration of CT. The remainder of the CT was transformed to unidentified, nonvolatile compounds. Transformation of CT by BrY was an indirect process Cells reduced solid phase Fe(III) to chemically reactive Fe(II) that chemically transformed the chlorinated contaminant. Cr(VI), in contrast, was reduced by a direct enzymatic reaction in the presence or absence of Fe(III)-bearing sediments. These results demonstrate that Fe(III)-reducing bacteria provide potential for transforming CT and for reducing Cr(VI) to less toxic Cr(III). Technologies for stimulating indigenous populations of metal-reducing bacteria or for introducing specific metal-reducing bacteria to the subsurface are being investigated.

INTRODUCTION

Soils at many of the 3,000 U.S. Department of Energy (DOE) sites are contaminated with complex mixtures of radionuclides (e.g., uranium), metals (e.g., chromium), anions (e.g., nitrate), and chlorinated solvents (e.g., CT) (Riley and Zachara, 1992). At the Hanford Site alone, more than 600 metric tons of CT were disposed to the soil in the 200-West area along with undetermined amounts of heavy metals and radionuclides (Stenner *et al.*, 1988). The DOE Office of Technology Development (OTD) now supports research and development of technologies for restoring subsurface environments to natural conditions.

Pump and treat methods for removing these contaminant are expensive and are limited by the rate of release of contaminants to the aqueous phase and by the hydraulic properties of the aquifer. Alternative technologies focus on

treating subsurface contaminants in situ. A particularly favorable approach for remediation at Hanford appears to be be chemical or microbiological reduction of both the groundwater and the solid materials within the aquifer to form a permeable treatment barrier. In situ remediation obviates the need for expensive pump-and-treat technologies and limits the exposure of personelle to hazardous chemical contaminants. This paper describes the potential for certain bacteria, namely dissimilatory Fe(III)-reducing bacteria, to form a permeable bioremediation barrier in the Hanford subsurface.

Dissimilatory Fe(III)-reducing bacteria, such as the facultatively anaerobic strain BrY (Caccavo *et al.*, 1992), couple the reduction of a wide range of multivalent metals, including iron, chromium, and uranium, to the oxidation of reduced organic matter or hydrogen (Lovley, 1993). These bacteria gain energy for growth by this enzymatic process under anoxic conditions and can affect the environmental fate of inorganic contaminants. For example, oxidized uranium, U(VI), is highly soluble and moves freely in groundwater. Reduced uranium, U(IV), produced by microbial reduction is highly insoluble and precipitates from solution (Lovley *et al.*, 1991). In aqueous waste streams and simulated aquifers, U(IV) readily precipitated as the pure uraninite (Gorby and Lovley, 1992). By this microbial process, the concentration of uranium in the aqueous phase was decreased from ca. 100 ppm to below regulatory concern.

Fe(III)-reducing bacteria can degrade a variety of organic contaminants. In contaminated aquifer material, Fe(III)-reducing bacteria coupled the oxidation of aromatic compounds to the reduction of Fe (Lovley *et al.*, 1989). Moreover, pure cultures of the Fe(III)-reducing bacterium *Geobacter metallireducens* strain GS-15 oxidized benzoate, toluene, phenol, and p-cresol. Recently, transformation of CT and 4-chloronitrobenzene by Fe(III)-reducing bacteria was reported (Heijman *et al.*, 1993; Picardal *et al.*, 1993).

In the work presented here, Fe(III)-reducing bacteria transformed CT and reduced Cr(VI) in natural Hanford subsurface materials. The results provide important potential for in situ remediation at the Hanford Site.

MATERIALS AND METHODS

Bacteria and Culture Conditions. The facultative Fe(III)-reducing bacterium, strain BrY (Caccavo *et al.*, 1992) was cultured aerobically in 100 ml of tryptic soy broth without dextrose (DIFCO Laboratories, Detroit, MI). After incubating for 16 hours at 30°C on a rotary shaker at 100 rpm, cells in the late log phase of growth were aseptically harvested by centrifugation (6000 x g, 15 min, 5°C) and then washed three times in sterile 10 mM piperazine-N-N'-bis 2 ethanesulfonic acid (PIPES) buffer (pH 7.0) that was previously made anoxic by purging with N_2 gas. The cells were suspended to a final density of ca. 10^9 cells · ml^{-1}, sealed in a stoppered serum bottle under a headspace of N_2 gas, and stored at 4°C for no longer than 30 min until needed.

Hanford groundwater growth medium (HGGM) was used for all experiments involving reduction of Fe(III) in Hanford subsurface sediments, reductive dechlorination of CT, and reduction of Cr(VI). The HGGM contained the following ingredients in grams · L^{-1} of filter Hanford groundwater: ammonium chloride, 0.15; monobasic sodium phosphate, 0.06; potassium chloride, 0.01. Trace vitamins and minerals (1 ml each) were added as previously described by Lovley and Phillips (1988).

Biotic reduction of Fe(III) in Hanford subsurface materials.

Fe(III)-bearing minerals from the Hanford subsurface were added to HGGM as sole source of electron acceptor. A ferruginous soil clay (R9), with and iron content of ca. 10 mmoles · g^{-1} was obtained from the upper Ringold formation of the Hanford site and sieved to obtain particles of less than 2 μm in diameter. Native material from 237 ft depth (HS237) of well 299-W11-32 located in the 200-West area was sieved to particles with less than 2 mm diameter in an anaerobic glove box to avoid oxidation of native Fe(II). The Fe(III)-bearing minerals were added to 10 ml of prereduced medium to a final Fe(III) concentration of 10 mmoles · L^{-1}. All suspensions were bubbled with O_2-free N_2 gas for an additional 5 minutes and the bottles were sealed with thick butyl rubber stoppers. When appropriate, lactate served as the electron donor and carbon source and was added from an anoxic stock solution to a final

concentration of 20 mM. Tubes serving as negative controls received no electron donor.

Concentrations of Fe(II) and total Fe in the Hanford materials were assayed by modifying the phenanthroline method of Stucki (1981). Briefly, 2.5 ml of each clay suspension, taken through the stopper using a needle and syringe, were added to 15 ml of a hot digestion mixture in amber bottles that contained 12 ml of 3.6 N H_2SO_4, 2 ml of 10% phenanthroline in 95% ethanol, and 1 ml of 48% hydrofluoric acid. Samples were then digested for 30 min in boiling water, mixed with 10 ml of 5% H_3BO_3, and allowed to cool for 15 min. Digested samples were then diluted with 90 ml of distilled, deionized water. Exactly 1 ml of the diluted samples was transferred to amber bottles containing 10 ml of 1% sodium citrate and the concentration of Fe(II) was determined spectrophotometrically at 512 nm. To determine the concentration of total Fe, 1 ml of the diluted sample was added to a clear scintillation vial containing 10 ml of 1% sodium citrate. This solution was irradiated with ultraviolet light for 45 min to reduce Fe(III) to Fe(II) and read at 512 nm.

Iron reduction was also followed by a modification of the ferrozine method (Stookey, 1970). Tubes were prepared as described above and inoculated with BrY to a final cell concentration of ca. 10^5 cells per ml. Tubes were sacrificed in triplicate and acidified with 0.4 ml of concentrated HCl. Acidified samples (1 ml) were diluted with 5 ml of 0.5 M HCl and 1 ml of the diluted sample was then transferred to 5 ml of ferrozine (1 g $\cdot$ L^{-1} in 10 mM n-2-hyroxyethylpiperizine-n'-2-ethane-sulfonic acid buffer, pH 7.0). The concentration of Fe(II)-ferrozine complex was read at 562 nm on a Beckman DU 70 spectrophotometer.

Abiotic reduction of Fe(III) in Hanford sediments. In some experiments, Fe(III) in Hanford sediments was chemically reduced using sodium dithionite for comparison with the microbial reduction experiments. In an anaerobic glove box, 40 mg of sodium dithionite was added to reaction vessels containing 10 ml of anoxic groundwater and either HS237 or R9 clay. The final concentration of Fe in each vessel was 10 mmol $\cdot$ L^{-1}. After incubating for 48 hours, unreacted dithionite was removed by washing the reduced materials 5 times with an anoxic solution of 100 mM NaCl followed by 3 washes with anoxic groundwater. The volume of the aqueous phase was adjusted to

10 ml before using these preparations in dechlorination experiments described below.

Degradation of carbon tetrachloride. The ability of BrY to dehalogenate CT in the presence and absence of Hanford subsurface materials was tested. In an anaerobic glove box, 20-ml headspace vials received 10 ml of anoxic HGGM and either HS237 or R9 clay to a final Fe(III) concentration of 10 mmol · L^{-1}. Lactate at 20 mM served as the electron donor, when appropriate. Washed cells of BrY were then added to each vial to a final density of ca. 10^5 cells · ml^{-1}. Vials without cells served as negative controls. Each bottle received 5 µL of a stock solution of CT (1000 ppm in methanol) and was immediately sealed with teflon-coated butyl rubber stoppers and aluminum crimps.

Loss of CT and formation of volatile degradation products were determined using a Hewlett Packard 5890 series II gas chromatograph equipped with an electron capture detector. Operating conditions were as follows: 100-m Vocal fused capillary column (Supelco, Bellefonte, PA); injector temperature, 250°C; detector temperature, 200°C; initial oven temperature, 50°C for 1 minute, initial ramp up rate 7°C · min^{-1} to 140°C, then 25°C · min^{-1} to 200° for 1 minute. Helium was the carrier gas and argon/methane was the make up gas.

Abiotic dechlorination of CT was demonstrated using Hanford sediments that were reduced with sodium dithionite, as described above. Following extensive washing to remove unreacted sodium dithionite, reduced sediments suspensions received CT from a stock solution to a final aqueous concentration of 0.5 ppm and the reaction vials were immediately sealed with teflon coated butyl rubber stoppers and aluminum crimp seals. Loss of CT was monitored by gas chromatography.

Reduction of chromate. Hanford groundwater medium was amended with 0.1 mM potassium chromate and made anoxic by bubbling with O_2-free N_2 gas for 10 minutes. HS237 was added to 2 out of every 3 bottles to a final Fe(III) concentration of 10 mmoles · L^{-1}. All tubes were sealed with thick butyl rubber stoppers. Lactate at 20 mM served as the electron donor and was added to appropriate tubes from an anoxic stock using a needle and syringe. Washed

BrY cells were added to the vials to a final density of ca. 10^5 cells · ml^{-1}. Vials lacking either cells or lactate served as negative controls.

Loss of Cr(VI) was assayed by the spectrophometric method of Urone (1955). Samples (0.5 ml) were taken through the stoppers using a needle and syringe and added to 5 ml of 0.2 N H_2SO_4. The colorimetric reagent s-diphenyl carbazide (1 ml of 0.25% stock in acetone) was added to each acidified sample and the absorbance of the resulting solution was determined at 540 nm using a Beckman DU70 spectrophotometer. Potassium dichromate was used as the standard.

RESULTS AND DISCUSSION

Reduction of Fe(III) in Hanford sediments. BrY reduced over 10% of the total Fe(III) in the R9 clay within 290 hours (Fig. 1). Reduction was qualitatively identified by a color change from light brown to an olive grey. A small amount of Fe(III) was reduced in the lactate negative control, probably due to small amount of H_2 gas originating from the atmosphere of the anaerobic glove boxserving as an alternate physiological electron donor.

Microbial reduction of structural Fe(III) in phyllosilicates has previously been reported (Stucki *et al.*, 1987; Wu *et al.*, 1988; Gates *et al.*, 1993) Although those studies provided important information concerning the effects of Fe(III) reduction on the physico-chemical properties of the clay minerals, they did not demonstrate that reduction of structural Fe(III) was related to cellular bioenergetics or that it supported microbial growth. Growth of Fe(III)-reducing bacteria with structural Fe(III) as the electron acceptor is important for establishing and maintaining an immobile redox buffer in subsurface environments in the absence of alternative terminal electron acceptors.

BrY grew with Fe(III) in R9 as the sole terminal electron acceptor (Fig. 2). For each millimole that was reduced, an average of 3.2×10^6 cells were produced. Cultures were successively transferred at least 5 times under anoxic conditions. Similar results were obtained with other smectitic clays (Gorby, unpublished data). These results represent the first direct evidence that

structural Fe(III) in phyllosilicates can support the growth of bacteria. The ability of Fe(III)-reducing bacteria to grow with structural Fe(III) as the electron acceptor is an important finding because it provides a mechanism by which an immobile redox buffer could be established and maintained in subsurface environments.

Clay minerals are common components of subsurface sediments. Sieved (<2 mm) sediment material HS237 from the Hanford subsurface contains ca. 8% by weight of clay-sized material. Of this fraction, over 60% is Fe-bearing smectite (Amonette, unpublished data). The total amount of Fe in these sediments was ca. 3% by weight.

BrY reduced about 10% of available Fe(III) in untreated native material (HS237) from the Hanford subsurface (Fig. 3). Nearly 95% of the Fe that was reduced remained associated with the sediment and did not partition into the aqueous phase (data not shown). Immobile Fe(II) in these sediments is vital for establishing a permeable, in situ redox barrier. Oxidized organic and inorganic contaminants could be transported to this reducing environment through natural groundwater flow and react, either chemically or enzymatically, with reduced materials or viable bacterial populations in this zone. The remediation potential of these reduced materials is examined in the following set of experiments.

Biotic transformation of CT. In anoxic HGGM containing BrY, HS237, and lactate, over 98% of the initial concentration of CT (0.5 ppm) was degraded within 24 days (Fig. 4a). A small amount (less than 10%) was transformed in tubes that lacked either bacteria or HS237. Chloroform (CF) accumulated as one of the degradation products of CT transformation to a concentration that was ca. 25% of the initial concentration of CT (Fig. 4b). In a separate experiment using ^{14}C-labelled CT, the remaining 75% of the CT was detected as unidentified soluble organics (data not shown).

Reductive transformation of CT by an Fe(III)-reducing bacterium was previously demonstrated (Picardal *et al.*, 1993). Thick cell suspensions of *Shewanella putrefaciens* 200 transformed CT in an aqueous medium under anoxic conditions, with CF and unidentified nonvolatile organic compounds as the predominant transformation products. The physiological mechanism of dechlorination in *S. putrefaciens* was not determined, although reduction by c-type cytochromes was suspected.

It is noteworthy that, in the present study, dechlorination occurred only in tubes that contained both bacteria and HS237. BrY did not transform CT by a direct enzymatic mechanism. The results suggested that dechlorination under these condition was an indirect process that involved 1) the reduction of Fe(III) with 2) subsequent chemical reaction between Fe(II) and CT. This hypothesis was supported by results from a separate experiment described below.

Abiotic transformation of CT. Transformation of CT was tested in sealed, anoxic vials containing either 1) HS237 that was chemically reduced with sodium dithionite or 2) untreated HS237. Transformation occurred only in vials containing treated materials (Fig. 5). CF accumulated as a transformation product to ca. 10% of the initial concentration of CT. Dechlorination was not detected and CF did not accumulate in anoxic suspensions of untreated HS237. Similar results were obtained using a variety of purified phyllosilicate clay that contained structural Fe (Gorby, unpublished data).

Abiotic transformation of CT by sheet silicates that were treated with sulfide has been reported (Kriegman-King and Reinhard, 1992). The authors hypothesized that either 1) structural Fe(II) in biotite and vermiculite transferred electrons to CT, 2) CT reacted with sulfide that was absorbed onto the treated sheet silicate, or 3) sulfide reacted with dissolved Fe and the resulting Fe-sulfide mineral reacted with CT. In our experiments, sediments were washed free of unreacted dithionite. Fe-sulfide minerals might have been formed in the HS237 material, but not in purified phyllosilicates that were tested in a separate study. It is more than likely, reactive Fe(II) in phyllosilicates or associated with sediment surfaces in HS237 transformed CT under the conditions of these experiments.

Reduction of Cr(VI). Cell suspensions of BrY containing lactate reduced Cr(VI) in either the presence or absence of HS237 (Fig. 6). When lactate was excluded from suspension, less than 20% of the Cr(VI) was reduced over the 7-day incubation period. This background activity was attributed to stores of endogenous reductant with washed cells of strain BrY. No Cr(VI) was reduced when BrY was excluded from the suspensions.

Reduction of Cr(VI) decreases the solubility of this highly toxic contaminant. Microbial reduction of Cr(VI) can lead to the formation of $Cr(OH)_3$ in aqueous media and is recognized as a potential means of remediating contaminated waters and subsurfaces (Palmer and Wittbrodt, 1991). By stimulating and maintaining a localized populations of Fe(III)-reducing bacteria in the flow path of a dilute contaminant plume, we propose that this metabolism will immobilize and concentrate chromium in a designated zone.

SUMMARY

The ability of microorganisms to degrade or influence the environmental fate of natural and anthropogenic compounds is widely recognized. Fe(III)-reducing bacteria may provide distinct advantages over other metabolically active groups of bacteria for the in situ remediation of subsurfaces containing radionuclides or heavy metals and chlorinated hydrocarbons as contaminants. First, Fe(III)-reducing bacteria can use native forms of Fe(III) as the sole terminal electron acceptor for growth and are found naturally in a wide variety of soils and anoxic sediments (Lovley, 1991). With Fe(III) as the fourth most abundant element on earth and the most abundant electron acceptor following the onset of anoxic condition, populations of Fe(III)-reducing organisms can be maintained without need for expensive electron acceptor supplements. Nitrate reducers, in comparison, that are able to dechlorinate CT and reduce Cr(VI) in aquifer materials require additional nitrate to completely degrade CT (Skeen *et al.*, 1993).

Another desirable trait of Fe(III)-reducing organisms, from a remediation standpoint, is that they produce reactive, reduced materials in subsurface sediments. The enzymatic reduction of structural Fe(III) in phyllosilicate clay minerals and non-structural Fe(III) in amorphous and crystalline oxides can form immobile forms of reactive Fe(II) that transform CT. A recent report provided evidence that 4-chloronitrobenzene was degraded to 4-chlorobenzene by a similar mechanism (Heijman *et al.*, 1993). Based upon the activation energies required for dehalogenating other substituted organics, compounds such as PCBs and PCP should also be treatable by this process.

Remediation of contaminated sites by Fe(III)-reducing bacteria has not been tested in the field. However, considering the ability of these organisms to reduce and precipitate radionuclides and heavy metals, to transform chlorinated compounds, and to degrade a wide range of anthropogenic organic contaminants, evaluating the remediation potential of these organisms is critical for determining the best available option for treatment of contaminated subsurface environments.

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REFERENCES

Caccavo, J., F, R. P. Blakemore and D. R. Lovley. 1992. A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. *Appl. Environ. Microbiol.* 58:

Gates, W. P., H. T. Wilkinson and J. W. Stucki. 1993. Swelling properties of microbially reduced ferruginous smectite. *Clays & Clay Minerals.* 41:360-364.

Gorby, Y. A. and D. R. Lovley. 1992. Enzymatic uranium precipitation. *Environ. Sci. Technol.* 26:205-207.

Heijman, C. G., C. Holliger, M. A. Glaus, R. P. Schwarzenbach and J. Zeyer. 1993. Abiotic reduction of 4-chloronitrobenzene to 4-chloroaniline in a dissimilatory iron-reducing enrichment culture. *Appl. Environ. Microbiol.* 59:4350-4353.

Kriegman-King, M. R. and M. Reinhard. 1992. Transformation of carbon tetrachloride in the presence of sulfide, biotite, and vermiculite. *Environ Sci Technol.* 26:2198-2206.

Lovley, D. R. 1991. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* 55:259-287.

Lovley, D. R. 1993. Dissimilatory metal reduction. *Annu. Rev. Microbiol.* 47:263-290.

Lovley, D. R., M. J. Baedecker, D. J. Lonergan, I. M. Cozzarelli, E. J. P. Phillips and D. I. Siegel. 1989. Oxidation of aromatic contaminants coupled to microbial iron reduction. *Nature.* 339:297-299.

Lovley, D. R. and E. J. P. Phillips. 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* 54:1472-1480.

Lovley, D. R., E. J. P. Phillips, Y. A. Gorby and E. R. Landa. 1991. Microbial reduction of uranium. *Nature*. 350:413-416.

Palmer, C. D. and P. R. Wittbrodt. 1991. Processes affecting the remediation of chromium-contaminated sites. *Environ Health Perspect*. 92:25-40.

Picardal, F. W., R. G. Arnold, H. Couch, A. M. Little and M. E. Smith. 1993. Involvement of cytochromes in the anaerobic biotransformation of Tetrachloromethane by Shewanella putrefaciens 200. *Appl. Environ. Microbiol.* 59:3763-3770.

Riley, R. G. and J. M. Zachara. 1992. *Chemical Contaminants on DOE Lands and Selection of Contaminant Mixtures for Subsurface Science Research*, DOE/ER-0547T. U.S. Department of Energy, Washington, D.C.

Skeen, R. S., S. P. Luttrell, T. M. Brouns and B. S. Hooker. 1993. In-situ bioremediation of Hanford groundwater. *Remediation*. 353-367.

Stenner, R. D., K. H. Cramer, K. A. Higley, S. J. Jette, D. A. Lamar, T. J. McLaughlin, D. R. Sherwood and N. C. Van Houten. 1988. *Hazard Ranking System Evaluation of CERCLA Inactive Waste Sites at Hanford*, PNL-6456. Pacific Northwest Laboratory, Richland, Washington.

Stookey, L. L. 1970. Ferrozine - a new spectrophotometric reagent for iron. *Anal. Chem.* 42:779-781.

Stucki, J. W. 1981. The quantitative assay of minerals for Fe(II) and Fe(III) using 1,10-phenanthroline: II. A photochemical method. *Soil Sci. Soc. Am. J.* 45:638-641.

Stucki, J. W., P. Komadel and H. T. Wilkinson. 1987. Microbial reduction of structural iron (III) in smectites. *Soil Sci. Soc. Am. J.* 51:1663-1665.

Urone, P. F. 1955. Stability of colorimetric reagent for chromium, s-diphenylcarbazide, in various solvents. *Anal. Chem.* 27:1354-1355.

Wu, J., C. B. Roth and P. F. Low. 1988. Biological reduction of structural iron in sodium-nontronite. *Soil Sci. Soc. Am. J.* 52:295-296.

FIGURE LEGENDS

Fig. 1. Reduction of Fe(III) in Hanford soil clay (R9). BrY reduced ca. 95 μmol of Fe(III) in each gram of R9 soil clay within 260 hours. Reduction of Fe(III) was minimal in control tubes that contained no electron donor.

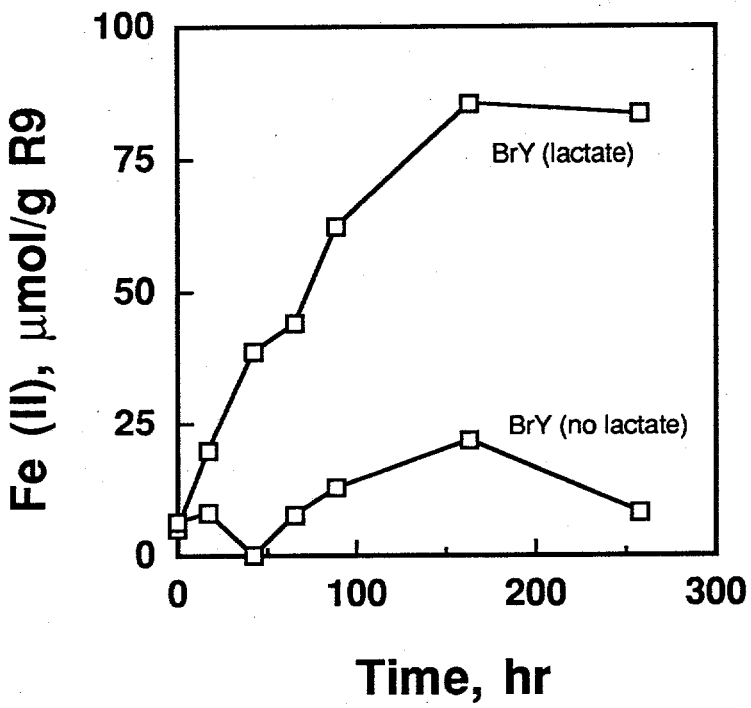
Fig. 2. Cell growth with R9 clay as the terminal electron acceptor. Fe(III) reduction in Hanford soil clay R9 supported growth of BrY in an anoxic medium. 3.2×10^6 cells were produced for each millimole of Fe(III) that was reduced.

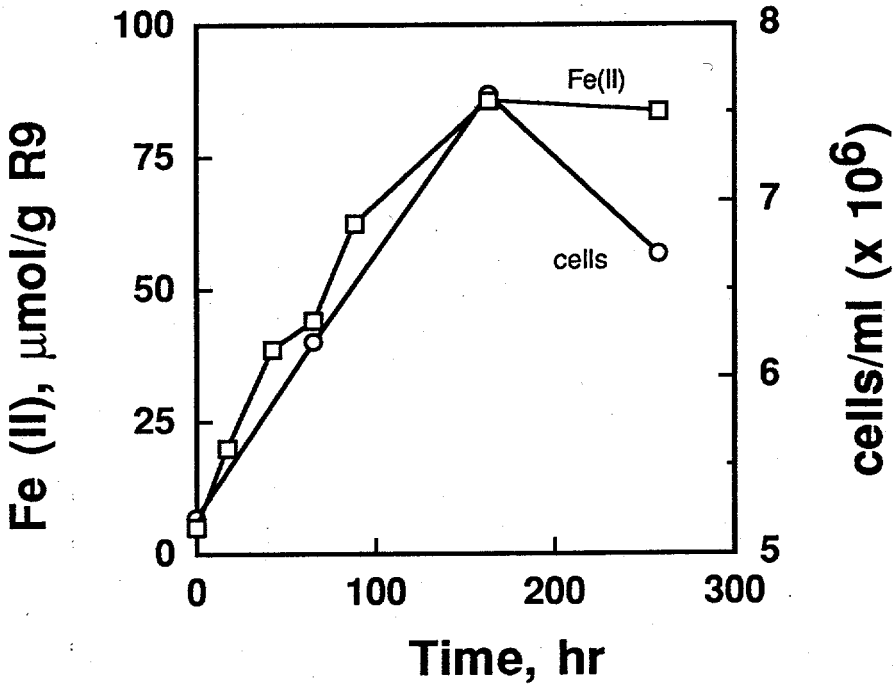
Fig. 3. Reduction of Fe(III) in Hanford sediment. BrY coupled the reduction of Fe(III) in Hanford subsurface material HS237 to the oxidation of lactate. No reduction was detected in tubes that contained no electron donor.

Fig. 4. Biotic transformation of CT. Carbon tetrachloride was lost (a) and CF accumulated (b) from anoxic groundwater in the presence of BrY, lactate, and HS237. No transformation was detected in tubes that lacked either cells or subsurface sediments.

Fig. 5. Abiotic transformation of CT. Carbon tetrachloride was lost and CF accumulated in sealed pressure tubes containing anoxic groundwater and HS237 that was previously reduced with sodium dithionite. Carbon tetrachloride was not transformed in tubes that contained untreated HS237.

Fig. 6. Enzymatic reduction of Cr(VI). BrY reduced Cr(VI) to Cr(III) with lactate as the electron donor. Reduction proceeded in the presence or absence of HS237. Cr(VI) was not reduced in tubes that contained no bacteria. Minimal amount of reduction was detected in tubes that contained bacteria but no lactate. This was attributed to endogenous stores of reductant within cells of BrY.


100
75
50
25
0
Fe (II), µmol/g R9
BrY (lactate)
BrY (no lactate)
0
100
200
300
Time, hr


100
75
50
25
0
Fe (II), µmol/g R9
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200
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Time, hr
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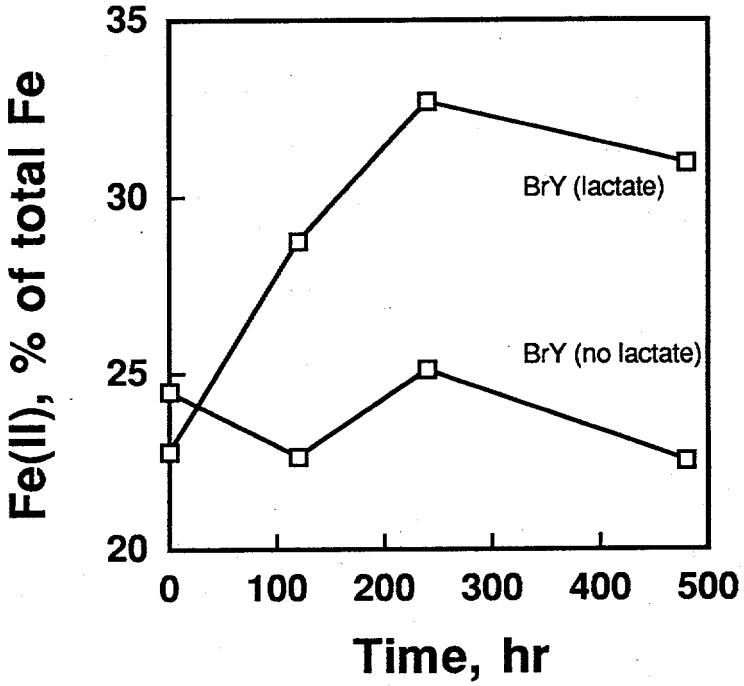
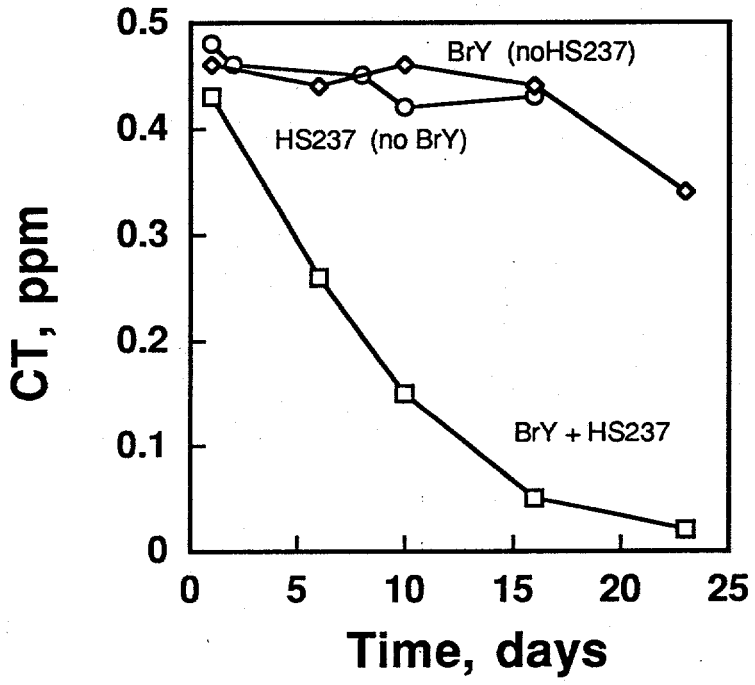
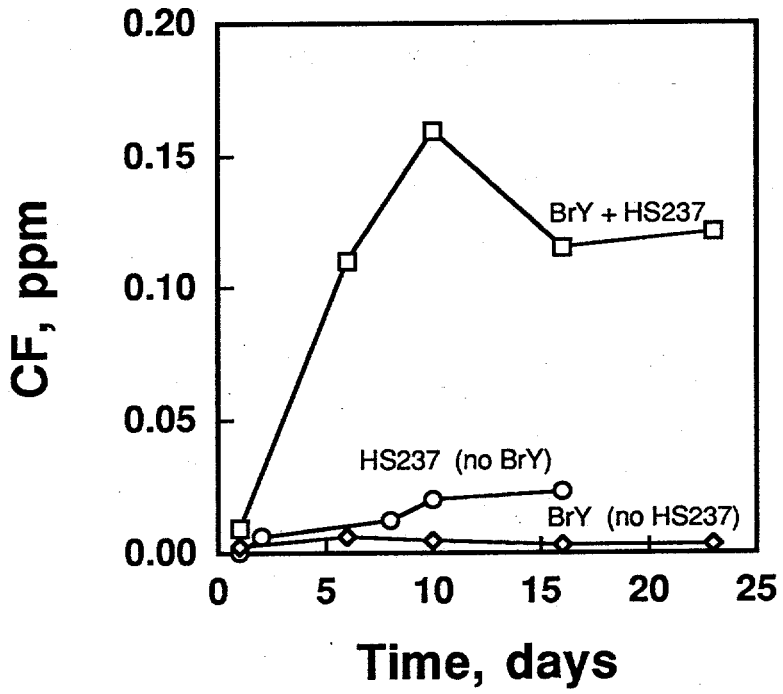
7
6
5
cells/ml (x 10^6)
Fe(II)
cells


35
30
25
20
Fe(II), % of total Fe
BrY (lactate)
BrY (no lactate)
0
100
200
300
400
500
Time, hr


0.5
0.4
0.3
0.2
0.1
0
CT, ppm
BrY (noHS237)
HS237 (no BrY)
BrY + HS237
0
5
10
15
20
25
Time, days


0.20
0.15
0.10
0.05
0.00
CF, ppm
BrY + HS237
HS237 (no BrY)
BrY (no HS237)
0
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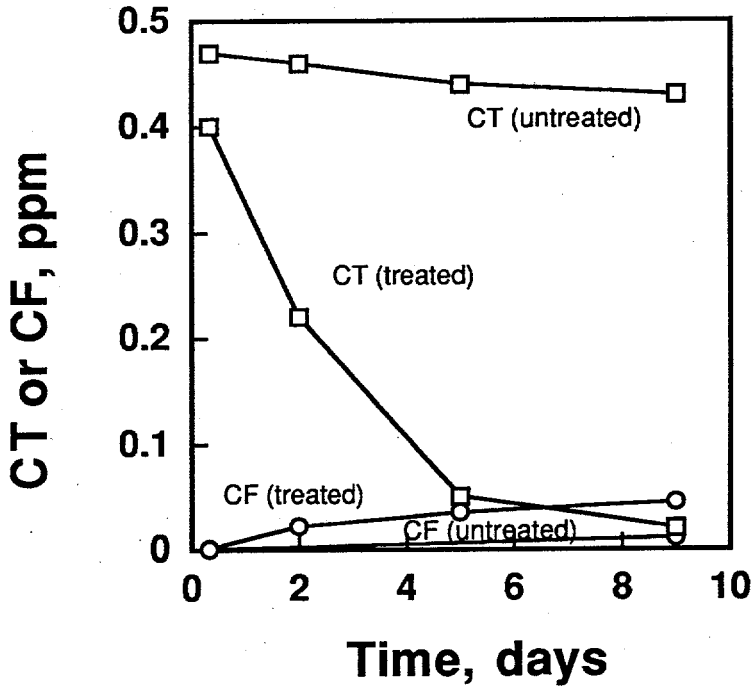
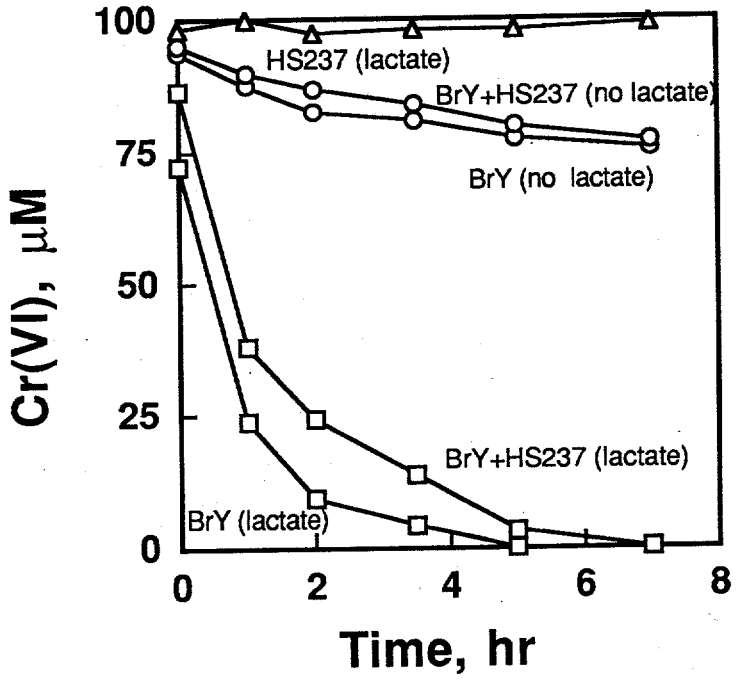
10
15
20
25
Time, days

IV B


0.5
0.4
0.3
0.2
0.1
0
CT or CF, ppm
CT (untreated)
CT (treated)
CF (treated)
CF (untreated)
0
2
4
6
8
10
Time, days


100
75
50
25
0
Cr(VI), μM
HS237 (lactate)
BrY+HS237 (no lactate)
BrY (no lactate)
BrY+HS237 (lactate)
BrY (lactate)
0
2
4
6
8
Time, hr