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2000

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PII S0016-7037(99)00427-5

## The effect of biogenic Fe(II) on the stability and sorption of Co(II)EDTA<sup>2-</sup> to goethite and a subsurface sediment

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(Received May 4, 1999; accepted in revised form December 1, 1999)

**Abstract**—Laboratory experiments were conducted with suspensions of goethite ( $\alpha$ -FeOOH) and a subsurface sediment to assess the influence of bacterial iron reduction on the fate of Co(II)EDTA<sup>2-</sup>, a representative metal-ligand complex of intermediate stability ( $\log K_{\text{Co(II)EDTA}} = 17.97$ ). The goethite was synthetic (ca. 55 m<sup>2</sup>/g) and the sediment was a Pleistocene age, Fe(III) oxide-containing material from the Atlantic coastal plain (Milford). *Shewanella alga* strain BrY, a dissimilatory iron reducing bacterium (DIRB), was used to promote Fe(III) oxide reduction. Sorption isotherms and pH adsorption edges were measured for Co<sup>2+</sup>, Fe<sup>2+</sup>, Co(II)EDTA<sup>2-</sup>, and Fe(II)EDTA<sup>2-</sup> on the two sorbents in 0.001 mol/L Ca(ClO<sub>4</sub>)<sub>2</sub> to aid in experiment interpretation. Anoxic suspensions of the sorbents in PIPES buffer at pH 6.5–7.0 were spiked with Co(II)EDTA<sup>2-</sup> (10<sup>-5</sup> mol/L, <sup>60</sup>Co and <sup>14</sup>EDTA labeled), inoculated with BrY (1–6 × 10<sup>8</sup> organisms/mL), and the headspace filled with a N<sub>2</sub>/H<sub>2</sub> gas mix. The experiments were conducted under non-growth conditions. The medium did not contain PO<sub>4</sub><sup>3-</sup> (with one exception), trace elements, or vitamins. The tubes were incubated under anoxic conditions at 25°C for time periods in excess of 100 d. Replicate tubes were sacrificed and analyzed at desired time periods for pH, Fe(II)<sub>TOT</sub>, Fe<sub>(aq)</sub><sup>2+</sup>, <sup>60</sup>Co, and <sup>14</sup>EDTA. Abiotic analogue experiments were conducted where Fe<sub>(aq)</sub><sup>2+</sup> was added in increasing concentration to Co(II)EDTA<sup>2-</sup>/mineral suspensions to simulate the influence of bacterial Fe(II) evolution. The DIRB generated Fe(II) from both goethite and the Milford sediment that was strongly sorbed by mineral surfaces. Aqueous Fe<sup>2+</sup> increased during the experiment as surfaces became saturated; Fe<sub>(aq)</sub><sup>2+</sup> induced the dissociation of Co(II)EDTA<sup>2-</sup> into a mixture of Co<sup>2+</sup>, Co(II)EDTA<sup>2-</sup>, and Fe(II)EDTA<sup>2-</sup> ( $\log K_{\text{Fe(II)EDTA}} = 15.98$ ). The extent of dissociation of Co(II)EDTA<sup>2-</sup> was greater in the subsurface sediment because it sorbed Fe(II) less strongly than did goethite. The post dissociation sorption behavior of Co<sup>2+</sup> was dependent on pH and the intrinsic sorptivity of the solid phases. Dissociation generally lead to an increase in the sorption (e.g., K<sub>d</sub>) of Co<sup>2+</sup> relative to EDTA<sup>4-</sup> (form unspecified). Sorbed biogenic Fe(II) competed with free Co<sub>(aq)</sub><sup>2+</sup> and reduced its sorption relative to unreduced material. It is concluded that cationic radionuclides such as <sup>60</sup>Co or <sup>239/240</sup>Pu, which may be mobilized from disposed wastes by complexation with EDTA<sup>4-</sup>, may become immobilized in groundwater zones where dissimilatory bacterial iron reduction is operative. Copyright © 2000 Elsevier Science Ltd

### 1. INTRODUCTION

Organic ligand complexation can modify the aqueous geochemical behavior of metal ions by either increasing or decreasing the tendency of the metal ion to associate with particle surfaces. Complexing ligands such as ethylenediaminetetraacetic acid (EDTA<sup>4-</sup>) decrease metal ion sorption in circumneutral waters by suppressing surface coordination reactions of the metal ion with hydroxylated surface sites on reactive Fe and Al oxides (Bowers and Huang, 1986; Girvin et al., 1993; Girvin et al., 1996). Such metal ion complexation has been cited as causal for the far field groundwater migration of radioactive cations (<sup>60</sup>Co and <sup>239/240</sup>Pu) from subsurface disposal sites (Means et al., 1978; Killely et al., 1984). In this communication we investigate the geochemical behavior of a organic metal complex [Co(II)EDTA<sup>2-</sup>] selected to represent those of intermediate to high stability (e.g.,  $\log K_{\text{ML}} > 15$ ). EDTA<sup>4-</sup> is a commonly used industrial complexant and food additive, is relatively recalcitrant to microbial degradation, has been cited as metal ion mobilizer in subsurface systems, and is an ubiqu-

itous minor component of many diverse waste streams including low and high level nuclear waste. NiEDTA<sup>2-</sup> released from wastewater treatment plants, for example, has been observed in San Francisco Bay (Bedsworth and Sedlak, 1999).

Under oxidizing conditions where solid phase Fe(III)-oxides are stable, Me(n)EDTA<sup>(4-n)-</sup> complexes undergo a complex reaction suite. It includes:

1. Adsorption to hydroxylated surface sites on Fe and Al oxides (e.g., SOH);
2. Dissociation promoted by soluble Fe<sup>3+</sup> and Al<sup>3+</sup> that compete for EDTA<sup>4-</sup>, and hydroxylated surface sites that compete for the metal; and
3. For Co(II) specifically, oxidation to a weakly reactive, highly stable form [Co(III)EDTA<sup>-</sup>;  $\log K = 41.2$ ] (Zachara et al., 1995a; Brooks and Jardine, 1996).

Rapid and slow reactions exist in the reaction suite (Szecsody et al., 1998a,b), and these may impart strong kinetic behavior to the phase distribution of the complex over time periods of hours to days (Szecsody et al., 1994; Zachara et al., 1995a,b). The concentrations of the ligands (EDTA<sup>4-</sup>, SO<sup>-</sup>, SOH<sub>2</sub><sup>-</sup>) relative to the metal (Co<sup>2+</sup>), their stability constants ( $\log K$ 's for both aqueous and surface species), and the solubility of the

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Fe and Al containing phases are determining factors of the extent of dissociation, adsorption, and retardation.

Far less is known about the geochemical behavior of organic-metal complexes in anoxic environments. Ferrogenic conditions, the subject of this paper, are characterized by the absence of bacterial sulfate reduction and the presence of significant  $\text{Fe}_{(\text{aq})}^{2+}$  (Lyngkilde and Christensen, 1992a,b; Lovley and Chapelle, 1995). Ferrogenic groundwaters are common (Chapelle, 1993), and result primarily from the enzymatic reduction of Fe(III) oxides coupled with organic matter or  $\text{H}_{2(\text{g})}$  oxidation by dissimilatory iron reducing bacteria (DIRB; Lovley et al., 1990; Lovley, 1993; Albrechtsen and Christensen, 1994). Significant changes occur to aquifer solids in transition from oxic to ferrogenic conditions including a depletion of poorly crystalline and crystalline Fe(III) oxides and an increase in solid associated Fe(II) (Heron et al., 1994; Heron and Christensen, 1995). The presence of  $\text{Fe}_{(\text{aq})}^{2+}$ , the depletion of reactive Fe(III) oxides and alteration of their surface properties, and the potential saturation of surfaces by sorbed Fe(II) characterize ferrogenic groundwaters/aquifer solids and are factors that may have a profound, and as yet unexplored, influence on the chemical behavior of  $\text{Me}(\text{n})\text{EDTA}^{(4-\text{n})-}$  complexes.

Studies with microbial enrichment cultures have shown that DIRB require direct contact with the Fe(III) oxide surface for enzymatic reduction of the solid phase (Arnold et al., 1988; Lovley et al., 1991; Myers and Nealson, 1988). Amorphous, poorly crystalline, and crystalline Fe(III) oxides are all vulnerable to DIRB reduction (Arnold et al., 1988; Phillips et al., 1993; Roden and Zachara, 1996; Fredrickson et al., 1998; Zachara et al., 1998). Surface chemistry appears to influence the bioreduction of goethite and hematite, as the microbial reduction rate slows as the surface reaches apparent saturation with sorbed  $\text{Fe}^{2+}$  (Roden and Zachara, 1996). Aqueous complexants stimulate bacterial reduction of crystalline Fe(III) oxides by drawing Fe(II) from the surface (Urrutia et al., 1998; Urrutia et al., 1999). In subsurface materials, fine grained amorphous and cryptocrystalline Fe(III) oxides may be preferentially dissolved by DIRB, while the surfaces of more crystalline phases may be transformed to a Fe(II)-like phase.

We have previously investigated the influence of adsorption, dissociation, and oxidation on the geochemical behavior of  $\text{Co}(\text{II})\text{EDTA}^{2-}$  in oxidized subsurface sediments (Zachara et al., 1995a,b). Here, we extend those findings to anoxic conditions representative of  $\text{Fe}^{2+}$  containing groundwaters. Ferrogenic conditions were generated in anoxic suspensions of goethite and a goethite-containing subsurface sediment through the addition of  $\text{Fe}_{(\text{aq})}^{2+}$ , and biotically through inoculation with DIRB (*S. alga*, strain BrY) and an electron donor ( $\text{H}_{2(\text{g})}$ ). Chemically unreactive electrolytes, buffer, and media were chosen to minimize complexity. The biologic experiments were conducted under non-growth conditions by withholding phosphate (except in one case), trace metals, and vitamins to avoid complications resulting from abiotic reactions with nutrients and cell division, etc. The geochemical behavior of  $\text{Co}(\text{I})\text{EDTA}^{2-}$  was determined as a function of time and  $\text{Fe}^{2+}$  concentration, emphasizing surface complexation and aqueous dissociation reactions controlling chemical distribution between the aqueous and solid phase.

## 2. EXPERIMENTAL PROCEDURES

### 2.1. Stock Solutions and Sorbates

Stock solutions of  $10^{-2}$  mol/L  $\text{Co}^{2+}$  and  $10^{-1}$  mol/L  $\text{EDTA}^{4-}$  were prepared using  $\text{Co}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  (Pfaltz and Bauer, Inc., Waterbury, CT) and  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$  [EDTA, disodium salt, dihydrate (JT Baker Chemical Co., Phillipsburg, NJ)] and deionized, distilled water (DDW). The concentrations were verified by ICP analysis of Co and Na, respectively. Dilutions were prepared to yield solutions of  $10^{-3}$  mol/L  $\text{Co}^{2+}$ ,  $\text{EDTA}^{4-}$  or  $\text{Co}(\text{II})\text{EDTA}^{2-}$ . Radioactive cobalt ( $^{60}\text{Co}$  as  $\text{CoCl}_2$ , 90.55 mCi/mg, 99% radiochemical purity, DuPont, Wilmington, DE) and  $\text{EDTA}^{4-}$  ( $^{14}\text{C}$  as ethylenediaminetetraacetic acid [acetic- $1\text{-}^{14}\text{C}$ ], 4.5 mCi/mmol, >99% purity, ICN Biomedicals, Inc., Irvine, CA) were added to yield  $10^6$  cpm/mL for radiochemical determination of aqueous phase  $^{60}\text{Co}$  and/or  $^{14}\text{C}$ .

Stock solutions of  $10^{-2}$  mol/L  $\text{Fe}^{2+}$  and  $10^{-1}$  mol/L  $\text{EDTA}^{4-}$  were prepared in an anoxic chamber using  $\text{Fe}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  (Johnson Matthey Co. Inc., Ward Hill, MA) and  $\text{C}_{10}\text{H}_{14}\text{N}_2\text{Na}_2\text{O}_8 \cdot 2\text{H}_2\text{O}$  [EDTA, disodium salt, dihydrate (JT Baker Chemical Co., Phillipsburg NJ)] and degassed, DDW. The solution pH was adjusted to 6.5 using  $\text{CO}_2$ -free NaOH and passed through a  $0.2 \mu\text{m}$  filter. The concentrations were verified by ICP analysis of Fe and Na. Dilutions were prepared to yield the desired concentrations of  $\text{Fe}^{2+}$ ,  $\text{EDTA}^{4-}$  or  $\text{Fe}(\text{II})\text{EDTA}^{2-}$ . Radioactive  $\text{Fe}^{2+}$  ( $^{59}\text{Fe}$  as  $\text{FeSO}_4$ , 23.76 mCi/mg, 99% radiochemical purity, DuPont, Wilmington, DE) and  $\text{EDTA}^{4-}$  ( $^{14}\text{C}$  as ethylenediaminetetraacetic acid [acetic- $1\text{-}^{14}\text{C}$ ], 4.5 mCi/mmol, >99% purity, ICN Biomedicals, Inc., Irvine, CA) were added to yield  $10^6$  cpm/mL.

### 2.2. Sorbents

Goethite was synthesized by combining 0.2 L of 1 mol/L  $\text{Fe}(\text{NO}_3)_3$  with 1.8 L of 1 mol/L KOH (Schwertmann et al., 1985) in an acid-washed 4 L HDPE Nalgene® bottle. The bottle was placed in a static position in an oven at  $75^\circ\text{C}$  for 7 d.

The goethite was washed to remove residual  $\text{NO}_3^-$  and extracted five times with acidified (0.25 mol/L HCl) hydroxylamine ( $\text{NH}_2\text{OH} \cdot \text{HCl}$ ) at  $50^\circ\text{C}$  to remove residual ferrihydrite. After extraction, the goethite was washed 2 $\times$  with 0.03 mol/L  $\text{Ca}(\text{ClO}_4)_2$  resuspended in DDW, transferred to SpectraPor® 1000 MWCO tubing and dialyzed against DDW until the conductivity of the outside solution was  $<5 \mu\text{mho/cm}$ . The suspension was lyophilized, and lightly crushed to pass an 850-mesh sieve. The resulting goethite had a surface area of  $55.4 \text{ m}^2/\text{g}$  measured by the  $\text{N}_2$  B.E.T. method.

A bulk sample of subsurface sediment was obtained in a sand pit at a depth of 2 m from the Pleistocene-age, Columbia formation near Milford, DE. The sand textured sediment was weakly cohesive with grains cemented by Fe(III) oxides (primarily goethite). The sand mineralogy was dominated by quartz and weathered feldspars. The sediment was air dried and sieved to  $<2 \text{ mm}$ . Selected characteristics of the sediment are shown in Table 1. A more complete description of the origin, sampling, and mineralogic analyses of this sediment were presented in Zachara et al. (1995b; Zachara et al., 1998).

### 2.3. Abiotic Experiments

#### 2.3.1. pH-dependent Fe(II) sorption

A known mass of Milford sediment was placed in 50 mL polycarbonate centrifuge tubes or Nalgene HDPE bottles and passed into an anoxic chamber. The anoxic chamber used throughout this study was equipped with an oxygen meter. Oxygen sensitive experiments were performed when the  $\text{O}_2$  meter read 0.0 ppm  $\text{O}_2$ . Dissolved  $\text{O}_2$  measurements were not made. Degassed, electrolyte solution [0.003 mol/L  $\text{Ca}(\text{ClO}_4)_2$ ] was added to achieve the desired solid:solution ratio.  $\text{HClO}_4$  or NaOH were added to duplicate tubes to achieve eleven target pH values between 3.5 and 9.5. Following overnight equilibration, the suspensions were re-adjusted to their target pH. This cycle was repeated until pH drift was  $<0.5$  pH unit during the overnight equilibration. Vigorous, prolonged mixing of the suspensions was minimized. Once pH equilibration was achieved, the suspensions were washed twice with degassed, pH-adjusted 0.003 mol/L  $\text{Ca}(\text{ClO}_4)_2$  to reduce the concentration of dissolution products in solution. Following the last wash, pH-adjusted electrolyte was quantitatively added to each tube to

Table 1. Selected properties of the Milford sediment.

N <sub>2</sub> -surface area (m <sup>2</sup> /g)	Extractable Fe			Silt and Clay %	Mineralogy
	NH <sub>2</sub> OH · HCl <sup>a</sup>	NH <sub>4</sub> Ox <sup>b</sup> (umol/g)	DCB <sup>c</sup>		
6.83	0.87	2.78	40.2	3.2	Quartz, K-feldspar, <sup>d</sup> trace rutile, and magnetite; <sup>d</sup> kaolinite and goethite <sup>e</sup>

<sup>a</sup> Acidified hydroxylamine hydrochloride.

<sup>b</sup> Ammonium oxalate in dark.

<sup>c</sup> Dithionite-citrate-bicarbonate.

<sup>d</sup> Sand sized fraction.

<sup>e</sup> Silt and clay.

yield the desired solid:solution ratio. A variety of different solids concentrations were used depending on the sorbate. These ranged between 33 g/L and 500 g/L with specific values noted in the figures and captions.

A stock suspension of goethite was prepared in an anoxic chamber using degassed electrolyte solution [0.003 or 0.03 mol/L Ca(ClO<sub>4</sub>)<sub>2</sub>]. Aliquots of the suspension were transferred to tarred 50 mL Oakridge tubes and the suspension mass determined. HClO<sub>4</sub> and NaOH were added to duplicate tubes to achieve target pH's ranging from 2.0 to 9.5. Typically 0.5 g/L goethite was used for the sorption experiments. Changes from this concentration are noted when they occurred. The pH adjustment process followed that described for the Milford sediment.

Ferrous iron was added to the pH adjusted suspensions as 10<sup>-3</sup> mol/L Fe<sup>2+</sup> or the preformed Fe(II)EDTA<sup>2-</sup> complex to yield a diluted initial concentration of 10<sup>-5</sup> mol/L. The suspensions were equilibrated by rotating at 80 rpm for 4 to 16 h in a light-excluded environmental chamber (25°C). Phase separation was accomplished by centrifugation at 5000 rcf for 30 min. Aqueous concentrations of <sup>14</sup>C and <sup>59</sup>Fe were determined in the supernatant. Because dual-label counting techniques could not be used for the simultaneous determination of <sup>14</sup>C and <sup>59</sup>Fe, duplicate experiments were conducted with either <sup>14</sup>C-labelled EDTA<sup>4-</sup> and stable Fe<sup>2+</sup>, or non-labeled EDTA<sup>4-</sup> and <sup>59</sup>Fe<sup>2+</sup>. A Ross semimicro combination pH electrode was used to determine the pH of the equilibrated solution.

Aqueous concentrations of Al, Fe, and Si in the Milford supernatants and Fe in the goethite supernatants were determined using ICP-AES on subsamples that had been passed through a 18 Å filter (Amicon Centriflo® membrane cones; Danver, MA). Prior to sample filtration, the membrane cones were soaked in pH 2 DDW (pH adjusted with HNO<sub>3</sub>) for 1 h and rinsed twice with centrifugation using DDW. The first 4 mL of sample filtrate were discarded and the following 4 mL was combined with 200 µL of concentrated Ultrex® HNO<sub>3</sub> for sample preservation.

### 2.3.2. pH-Dependent adsorption of Co(II)EDTA<sup>2-</sup>

The pH variable adsorption of Co(II)EDTA<sup>2-</sup> at 10<sup>-5</sup> mol/L was measured identically to Fe(II)EDTA<sup>2-</sup> except that dual label counting (<sup>60</sup>Co, <sup>14</sup>C) was used to simultaneously quantify sorption of both analytes.

### 2.3.3. Adsorption isotherms of Fe<sup>2+</sup> and Co<sup>2+</sup>

Adsorption isotherms were measured on the Milford sediment and goethite over a concentration range of 10<sup>-7</sup> to 10<sup>-2</sup> mol/L at pH values relevant to the biotic reduction experiments. A 0.003 mol/L Ca(ClO<sub>4</sub>)<sub>2</sub> background electrolyte was used that was buffered at pH 6.5 in some cases with 0.01 mol/L PIPES [piperazine-N, N'-bis(2-ethanesulfonic acid)]. Electrolyte and sorbate solutions were prepared in an anoxic chamber using degassed, DDW. Ferrous iron solutions spiked with <sup>59</sup>Fe<sup>2+</sup> were prepared as previously described. All stages of preparation and sampling were conducted in an anoxic chamber. Sorbents were degassed in the anoxic chamber for at least one week prior to contact with Fe<sup>2+</sup><sub>(aq)</sub>. Various sorbent concentrations were used depending on the

sorbate and method of analysis. The synthetic goethite was used at 0.05, 0.1, 0.25, and 0.5 g/L and the Milford sediment at 10, 50, and 500 g/L. Only sealed reaction vessels were removed from the anoxic chamber for determination of mass or during equilibration (under an N<sub>2</sub> atmosphere). Suspensions were equilibrated at 25°C for 16 h on a tabletop shaker (80 rpm), and were sampled and analyzed as described for the pH edges.

### 2.3.4. Effect of Fe<sup>2+</sup><sub>(aq)</sub> on Co(II)EDTA<sup>2-</sup> sorption

To simulate the potential effects of bacterial Fe(III) oxide reduction on Co(II)EDTA<sup>2-</sup> sorption, Fe<sup>2+</sup><sub>(aq)</sub> was metered [at concentrations of 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, and 10<sup>-6</sup> mol/L Fe<sup>2+</sup>] into Co(II)EDTA<sup>2-</sup> (10<sup>-5</sup> mol/L) suspensions with goethite and the Milford sediment at two different initial pH values. The pH values and the sorbent concentrations were selected so that approximately 75–80% of the Co(I-EDTA)<sup>2-</sup> would be adsorbed at the lower pH and <50% would be adsorbed at the higher pH. All stages of preparation and sampling were conducted in an anoxic chamber.

A sorbent concentration of 0.5 g/L for goethite and 500 g/L for the Milford sediment was used which included either 0.003 mol/L Ca(ClO<sub>4</sub>)<sub>2</sub> as the background electrolyte or 0.003 mol/L Ca(ClO<sub>4</sub>)<sub>2</sub> and 0.01 mol/L PIPES as a pH buffer. The suspension pH values were adjusted to values of approximately 5.5 and 7.0 for goethite and 6.0 and 7.5 for the Milford, both of which were re-adjusted for several days to ensure initial pH stability. The electrolyte was replaced before sorbate spiking first with Fe<sup>2+</sup><sub>(aq)</sub> and second [after 1 h equilibration with Fe<sup>2+</sup>] with Co(II)EDTA<sup>2-</sup>. Suspensions were equilibrated for 16 h shaken at 80 rpm and 25°C before sampling and analyzing as previously described. Aqueous Fe<sup>2+</sup> concentrations in the equilibrium solution were determined by ICP-AES in 18 Å filtrates.

## 2.4. Microbiologic Experiments

### 2.4.1. Bacterial media, cultivation, and cell preparation

The dissimilatory iron reducing bacteria, *S. alga*, strain BrY (Cac-cavo et al., 1992; Roselló-Mora et al., 1994; Roden and Zachara, 1996; Urrutia et al., 1998), was used to promote Fe(III) oxide reduction. *S. alga* was maintained on tryptic soy agar slants [30 g/L tryptic soy broth (TSB), 15 g/L agar; Difco Laboratories, Detroit, MI] incubated aerobically at 30°C, and cultured for routine use in TSB medium on a rotary shaker (200 rpm) at 37°C. The cell suspension was grown for 16 h to late log phase. Two hours prior to inoculation of the mineral suspension, the bacterial culture was harvested by centrifugation at 6930 rcf for 15 min at 10°C. The cell pellet was resuspended in sterile deionized water and centrifuged again. Following the second centrifugation, the cell pellet was resuspended in sterile DDW to yield the desired cell concentration. The bacterial cell density was determined using the A<sub>600</sub> of a 4-fold dilution.

To further prepare the cells for inoculation into mineral suspensions, the culture was transferred to an N<sub>2</sub>-sparged, autoclaved serum bottle. A sterile, 15 cm spinal tap needle (18 gauge) connected to a sterile,

cotton-filled syringe through which  $N_2$  was flowing was inserted through the stopper of the serum bottle until the tip was submerged in the cell culture medium. The spinal needle and serum stopper was swabbed with ethanol as the needle was pushed into the bottle. Another needle, connected to a sterile, cotton-filled syringe was then pushed through the stopper to provide a gas outlet, and the medium in the bottle was purged for about 20 min. Aliquots of 0.5 mL cell culture were added to the sediment suspensions to initiate the experiments. Unless specified otherwise, the initial cell density in the sediment suspensions was about  $10^8$  cells/mL. All cell additions were performed with sterile syringes and needles that had been flushed with sterile,  $O_2$ -free  $N_2$ .

#### 2.4.2. Incubation of $Co(II)EDTA^{2-}$ with BrY and goethite

Two experiments were conducted with laboratory synthesized goethite incubated anaerobically with BrY in 0.003 mol/L  $Ca(ClO_4)_2$ . The experiments were performed in 30 mL Cortex<sup>®</sup> centrifuge tube with an approximate 20 mL total suspension volume after inoculation and spiking. The suspension pH was adjusted to 6.5 using NaOH. In the first experiment, goethite was used at 1.5 g/L and BrY at a cell density of  $1 \times 10^8$  cells/mL. The second experiment used 1.0 g/L goethite and a BrY density of about  $6 \times 10^8$  cells/mL.

The glass reaction vessels with goethite suspension were closed with septum caps fitted with an inverted #20 Bellco rubber septum. To selected tubes, a 0.5 mL aliquot of BrY cell suspension and/or 0.1 mL of equimolar  $Co(II)EDTA^{2-}$  solution labeled with  $^{14}C$  and  $^{60}Co$  were added. Experimental controls included treatments without the BrY inoculum and/or treatments without radio-labeled  $Co(II)EDTA^{2-}$ . The target  $Co(II)EDTA^{2-}$  concentration was  $10^{-5}$  mol/L. After the addition of bacteria and  $Co(II)EDTA^{2-}$  to the suspensions, approximately 9 mL of headspace atmosphere was aseptically removed and replaced with 10 mL of sterile, ultrapure  $H_2$ . The headspace in the tubes was maintained anaerobic throughout the experiment. Incubation occurred in the dark at 25°C with occasional shaking.

At the desired time intervals, tubes were centrifuged at 5000 rcf for 10 min and the supernatant was sampled for  $^{14}C$  and  $^{60}Co$  and other analytes (e.g., Fe and Al after 18 Å filtration). The pH of the supernatant was measured using a combination pH electrode (Microelectrodes, Inc.; Londonderry, NH) in  $O_2$ -free atmosphere.

Inoculated and non-inoculated control suspensions were sampled for HCl-extractable Fe(II) at each sampling point, a measure of total biogenic Fe(II) (Fredrickson et al., 1998). The oxide/sediment suspension was combined with Ultrex<sup>®</sup> HCl to yield a 0.5 mol/L HCl concentration, mixed and allowed to sit overnight (16 h). The suspension was re-mixed and filtered through a 0.22 μm syringe filter. The first 5 mL of filtrate were discarded. The Fe(II) concentration of the filtrate was measured by adding an aliquot of the filtrate with ferrozine in 50 mmol/L HEPES (*N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid) buffer. The  $A_{562}$  of the sample was determined and compared to Fe(II) standards prepared from ferrous ammonia sulfate and treated similarly.

#### 2.4.3. Incubation of $Co(II)EDTA^{2-}$ with BrY and the Milford sediment

Six g of Milford sediment were added to 30 mL Corex<sup>®</sup> centrifuge tubes with 13.9 mL of anaerobic solution containing 0.003 mol/L  $Ca(ClO_4)_2$ . The pH of the sediment suspension was adjusted to 6.5. The tubes were closed with septum caps fitted with an inverted #20 Bellco<sup>™</sup> rubber septum. The suspensions were allowed to rest for 48 h to ensure pH stability. The tubes were inoculated with BrY ( $6 \times 10^8$  cells/mL), spiked with  $Co(II)EDTA^{2-}$ , incubated, and sampled/analyzed as described for goethite in 2.4.2.

#### 2.4.4. Incubation of $Co(II)EDTA^{2-}$ with BrY, malate, and the Milford sediment

This experiment was performed like 2.4.3 with the exceptions that pH was adjusted with  $NH_4OH$ , and 0.03 mol/L sodium malate and  $5 \times 10^{-5}$  mol/L  $KH_2PO_4$  were added to the anaerobic media. In this case only, an attempt was made to stimulate growth by addition of a C-source (malate) and supplementary N and P.

#### 2.4.5. pH-dependent sorption of $Co(II)EDTA^{2-}$ on microbially-reduced Milford sediment

Suspensions containing 6 g of Milford sediment and 12 mL of 0.003 mol/L  $Ca(ClO_4)_2$  were prepared, and their pH was adjusted to ~6.5. BrY cells were inoculated to yield  $1 \times 10^8$  cells/mL,  $H_{2(g)}$  was added as electron donor, and the tubes equilibrated as described previously. The HCl-extractable Fe(II) of the suspension was monitored with time to establish the extent of Fe(III) reduction. At 14 d after inoculation with BrY, it was determined that the extent of Fe(III) reduction was maximized at approximately 12% of the total goethite Fe(III). An identical series of tubes containing Milford sediment and solution were mixed and equilibrated without *S. alga*. This treatment allowed comparison of the sorption characteristics of the reduced to the unreduced material.

On day 14, the suspensions were washed with 0.3 mol/L  $Ca(ClO_4)_2$  to remove loosely attached bacterial cells and aqueous/exchangeable  $Fe^{2+}$ . The washing procedure involved centrifugation; removal of supernatant solution under anaerobic conditions; replacement of the supernatant with fresh, anoxic electrolyte; resuspension of the sediment; and sonication for 5 min. The washing step was performed 3 times. After the final wash, the electrolyte was replaced and pH-adjustment of the suspensions was initiated. The suspension pH was adjusted to a range of values (between pH = 3.5 and 9.5) daily using either NaOH or  $HClO_4$ . After five days, the desired pHs were approximately achieved. Radio-labeled, equimolar  $Co(II)EDTA^{2-}$  was added to yield  $10^{-5}$  mol/L. After a 16 h equilibration with gentle mixing in the dark, the suspensions were centrifuged at 650 rcf for 1 h. The supernatant was sampled for  $^{14}C$  and  $^{60}Co$  and pH as described previously.

### 3. RESULTS

#### 3.1. Identification of Chemical Components and Species

*S. alga*, strain BrY, produces Fe(II) in anoxic suspensions of goethite and the Milford sediment in the presence of an appropriate e-donor (Roden and Zachara, 1996; Urrutia et al., 1998). As  $Fe_{(aq)}^{2+}$  is evolved, the speciation of  $Co(II)EDTA_{(aq)}^{2-}$  may change. Shown in Table 2 are the computed effects of  $Fe_{(aq)}^{2+}$  on the species distribution of  $Co(II)EDTA_{(aq)}^{2-}$  ( $1.0 \times 10^{-5}$  mol/L) at pH 6.75 using the aqueous complexation constants in Table 3. The range in  $[Fe^{2+}]_T$  represents that observed by Roden and Zachara (1996). Increasing concentrations of  $Fe_{(aq)}^{2+}$  induce  $Co(II)EDTA_{(aq)}^{2-}$  dissociation at equilibrium, yielding a mixture of  $Co(II)EDTA_{(aq)}^{2-}$ ,  $Co_{(aq)}^{2+}$ ,  $Fe_{(aq)}^{2+}$ , and  $Fe(II)EDTA_{(aq)}^{2-}$ . The dissociation of  $Co(II)EDTA_{(aq)}^{2-}$  is not complete in spite of the molar excess of  $Fe_{(aq)}^{2+}$  at its higher concentrations ( $10^{-4}$ – $10^{-3}$  mol/L) because of the higher stability constant of  $Co(II)EDTA_{(aq)}^{2-}$  (Table 3).

The dissociative effects of  $Fe_{(aq)}^{2+}$  are shown for example only in Table 2, as the actual effects will be influenced by adsorption. Sorption of  $Co^{2+}$  to goethite enhances the extent of  $Co(II)EDTA_{(aq)}^{2-}$  dissociation, as surface SOH sites strongly compete with  $EDTA^{4-}$  for the metal. Ferrous iron sorption decreases  $Co(II)EDTA_{(aq)}^{2-}$  dissociation by reducing  $Fe_{(aq)}^{2+}$  concentrations.

#### 3.2. Sorption of Individual Chemical Components and Species

Much of the experimental sorption data are presented as a concentration based distribution coefficient,  $K_d$  [ $K_d(L/g) = \text{total sorbed concentration (mol/g)/total aqueous concentration (mol/L)}$ ] to allow comparisons between experiments with different sorbent concentrations. The  $K_d$  is influenced by both

Table 2. Computed dissociation of 10<sup>-5</sup> mol/L Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> in response to variable [Fe<sup>2+</sup>]<sub>TOT</sub> concentrations.

[Fe(II)] <sub>TOT</sub> <sup>a</sup>	Co(II)EDTA <sup>2-</sup> (mol/L)	% (of [Co] <sub>TOT</sub> ) <sup>a</sup>	Co <sup>2+</sup> (mol/L)	% (of [Co] <sub>TOT</sub> ) <sup>1</sup>	Fe <sup>2+</sup> (mol/L)	% (of [Fe] <sub>TOT</sub> ) <sup>1</sup>	Fe(II)EDTA <sup>2-</sup> (mol/L)	% (of [EDTA] <sub>TOT</sub> ) <sup>a</sup>	% (of [Fe] <sub>TOT</sub> ) <sup>a</sup>
1.0 × 10 <sup>-6</sup>	9.62 × 10 <sup>-6</sup>	96.2	3.78 × 10 <sup>-7</sup>	3.80	7.92 × 10 <sup>-7</sup>	79.2	1.38 × 10 <sup>-7</sup>	1.38	20.6
5.0 × 10 <sup>-6</sup>	9.30 × 10 <sup>-6</sup>	93.0	6.93 × 10 <sup>-7</sup>	6.92	4.39 × 10 <sup>-6</sup>	87.8	6.02 × 10 <sup>-7</sup>	6.02	12.1
1.0 × 10 <sup>-5</sup>	9.05 × 10 <sup>-6</sup>	90.5	9.50 × 10 <sup>-7</sup>	9.54	9.10 × 10 <sup>-6</sup>	91.0	8.86 × 10 <sup>-7</sup>	8.86	8.90
5.0 × 10 <sup>-5</sup>	7.99 × 10 <sup>-6</sup>	80.0	1.99 × 10 <sup>-6</sup>	20.0	4.79 × 10 <sup>-5</sup>	95.9	1.96 × 10 <sup>-6</sup>	19.6	3.90
1.0 × 10 <sup>-4</sup>	7.29 × 10 <sup>-6</sup>	72.9	2.70 × 10 <sup>-6</sup>	27.0	9.71 × 10 <sup>-5</sup>	97.2	2.68 × 10 <sup>-6</sup>	26.8	2.70
5.0 × 10 <sup>-4</sup>	4.97 × 10 <sup>-6</sup>	49.7	5.02 × 10 <sup>-6</sup>	50.2	4.94 × 10 <sup>-4</sup>	98.9	5.00 × 10 <sup>-6</sup>	50.0	1.00
1.0 × 10 <sup>-3</sup>	3.79 × 10 <sup>-6</sup>	37.9	6.21 × 10 <sup>-6</sup>	62.1	9.95 × 10 <sup>-4</sup>	99.5	6.19 × 10 <sup>-6</sup>	61.9	0.50

<sup>a</sup> TOT = total component concentration.

aqueous and surface speciation; i.e.,  $K_d = \Sigma [\text{surface species}] / [\text{aqueous species}]$ . Weakly sorbing aqueous species decrease  $K_d$ , while strongly sorbing species increase  $K_d$ .

### 3.2.1. Goethite

The  $K_d$  for Co<sub>(aq)</sub><sup>2+</sup> and Fe<sub>(aq)</sub><sup>2+</sup> sorption to goethite increased with increasing pH (Fig. 1a) as a result of coordination to surface ligands (SOH) in competition with the proton (Davis and Kent, 1990; Hayes and Katz, 1996).



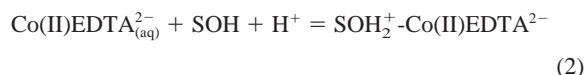
The Fe<sub>(aq)</sub><sup>2+</sup> ion was sorbed more strongly than Co<sub>(aq)</sub><sup>2+</sup>, with its sorption edge occurring 1 unit lower in pH (Fig. 1a). Both

metal ions, however, exhibited strong sorption and high  $K_d$ 's over the pH range of the biotic experiments (pH = 6–8).

Ferrous iron was more strongly adsorbed than Co<sub>(aq)</sub><sup>2+</sup> on goethite over a wide concentration range (Fig. 1b). Parallel, slightly curvilinear isotherms were observed for Co<sub>(aq)</sub><sup>2+</sup> at pH 6.6 and pH 7.3, with higher sorption noted at higher pH as expected from Figure 1a. The isotherm of Fe<sub>(aq)</sub><sup>2+</sup> was quite variable and difficult to reproduce in spite of our precautions to eliminate O<sub>2</sub> from the experiment. The best (most consistent) of these isotherms (Fig. 1b) exhibited two part behavior (L-curve; Sposito, 1984) with steep slope at low surface concentration ( $\log[\text{Me}^{2+}]_{\text{ads}} < -4.3$ ), and flat slope at high surface concentration indicating approach to surface saturation. The saturation values for Fe<sub>(aq)</sub><sup>2+</sup> estimated according to the Langmuir convention (e.g.,  $K_d$  versus the adsorbed concentration in mol/g<sup>2</sup>; Sposito, 1984) increased with increasing pH and yielded an average value of 10<sup>-3.75</sup> mol/g (3.23 × 10<sup>-6</sup> mol/m<sup>2</sup>) at the pH of the biotic reduction experiments. The surface saturation value (3.23 × 10<sup>-6</sup> mol/m<sup>2</sup>) is approximately equal to the total surface site density of goethite reported by Evanko and Dzombak, (1998) and the concentration value suggested by Davis and Kent (1990) for surface complexation modeling on mineral surfaces (3.84 × 10<sup>-6</sup> mol/m<sup>2</sup>). Lower surface saturation values for Co<sub>(aq)</sub><sup>2+</sup> were estimated from the Co<sub>(aq)</sub><sup>2+</sup> isotherms (0.875 × 10<sup>-6</sup> mol/m<sup>2</sup>).

Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> sorbed as an anion, with  $K_d$ 's (Co(II), EDTA<sup>4-</sup>) increasing with decreasing pH (Fig. 1c). Various chemical reactions are summarized below and in the following sections for interpretation of results. The reactions are intended to illustrate mass action and material balance relationships rather than to infer mechanism. Reaction sequences for the Me(n)EDTA<sup>(4-n)-</sup> series have been derived from the works of Girvin et al., (1993); Zachara et al., (1995b); Nowack et al., (1996a,b).

Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> forms a relatively weak surface complex on Fe(III) and Al(III) oxides that has been approximated for modeling purposes as both outer sphere and inner sphere (Zachara et al., 1995b; Nowack et al., 1996b).



The strength of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> sorption at its maximum ( $\log K_d = 1.25$ ), was well below that noted for the metal cations ( $\log K_d = 2.5$ , Fig 1a). Below pH = 5.5, increasing concen-

Table 3. Aqueous complexation reactions.<sup>a</sup>

Reaction	log K (I = 0, 25°C)
EDTA <sup>4-</sup> + H <sup>+</sup> = HEDTA <sup>3-</sup>	11.03
EDTA <sup>4-</sup> + 2H <sup>+</sup> = H <sub>2</sub> EDTA <sup>2-</sup>	17.78
EDTA <sup>4-</sup> + 3H <sup>+</sup> = H <sub>3</sub> EDTA <sup>-</sup>	20.89
EDTA <sup>4-</sup> + 4H <sup>+</sup> = H <sub>4</sub> EDTA	23.10
EDTA <sup>4-</sup> + Ca <sup>2+</sup> = CaEDTA <sup>2-</sup>	12.32
EDTA <sup>4-</sup> + Ca <sup>2+</sup> + H <sup>+</sup> = CaHEDTA <sup>1-</sup>	15.93
EDTA <sup>4-</sup> + Co <sup>2+</sup> = CoEDTA <sup>2-</sup>	17.97
EDTA <sup>4-</sup> + Co <sup>2+</sup> + H <sup>+</sup> = CoHEDTA <sup>-</sup>	21.40
Co <sup>2+</sup> + H <sub>2</sub> O = CoOH <sup>+</sup> + H <sup>+</sup>	-9.67
Co <sup>2+</sup> + 2H <sub>2</sub> O = Co(OH) <sub>2</sub> + 2H <sup>+</sup>	-18.76
Co <sup>2+</sup> + 3H <sub>2</sub> O = Co(OH) <sub>3</sub> + 3H <sup>+</sup>	-32.23
EDTA <sup>4-</sup> + Al <sup>3+</sup> = AlEDTA <sup>-</sup>	19.07
EDTA <sup>4-</sup> + Al <sup>3+</sup> + H <sup>+</sup> = AlHEDTA	21.78
EDTA <sup>4-</sup> + Al <sup>3+</sup> + H <sub>2</sub> O = AlOHEDTA <sup>2-</sup> + H <sup>+</sup>	12.81
EDTA <sup>4-</sup> + Al <sup>3+</sup> + 2H <sub>2</sub> O = Al(OH) <sub>2</sub> EDTA <sup>3-</sup> + 2H <sup>+</sup>	2.20
Al <sup>3+</sup> + H <sub>2</sub> O = AlOH <sup>2+</sup> + H <sup>+</sup>	-4.99
Al <sup>3+</sup> + 2H <sub>2</sub> O = Al(OH) <sub>2</sub> <sup>+</sup> + 2H <sup>+</sup>	-10.10
Al <sup>3+</sup> + 3H <sub>2</sub> O = Al(OH) <sub>3</sub> + 3H <sup>+</sup>	-16.00
Al <sup>3+</sup> + 4H <sub>2</sub> O = Al(OH) <sub>4</sub> <sup>-</sup> + 4H <sup>+</sup>	-23.00
EDTA <sup>4-</sup> + Fe <sup>2+</sup> = FeEDTA <sup>2-</sup>	15.98
EDTA <sup>4-</sup> + Fe <sup>2+</sup> + H <sup>+</sup> = FeHEDTA <sup>-</sup>	19.11
EDTA <sup>4-</sup> + Fe <sup>2+</sup> + H <sub>2</sub> O = FeOHEDTA <sup>3-</sup> + H <sup>+</sup>	6.27
Fe <sup>2+</sup> + H <sub>2</sub> O = FeOH <sup>+</sup> + H <sup>+</sup>	-9.50
Fe <sup>2+</sup> + 3H <sub>2</sub> O = Fe(OH) <sub>3</sub> + 3H <sup>+</sup>	-31.00
Mal <sup>2-</sup> + H <sup>+</sup> = HMal <sup>-</sup>	5.10
Mal <sup>2-</sup> + 2H <sup>+</sup> = H <sub>2</sub> Mal	8.56
Mal <sup>2-</sup> + Ca <sup>2+</sup> = CaMal	2.66
Mal <sup>2-</sup> + Co <sup>2+</sup> = CoMal	3.74
Mal <sup>2-</sup> + Fe <sup>2+</sup> = FeMal	3.48

<sup>a</sup> Smith and Martell (1977)

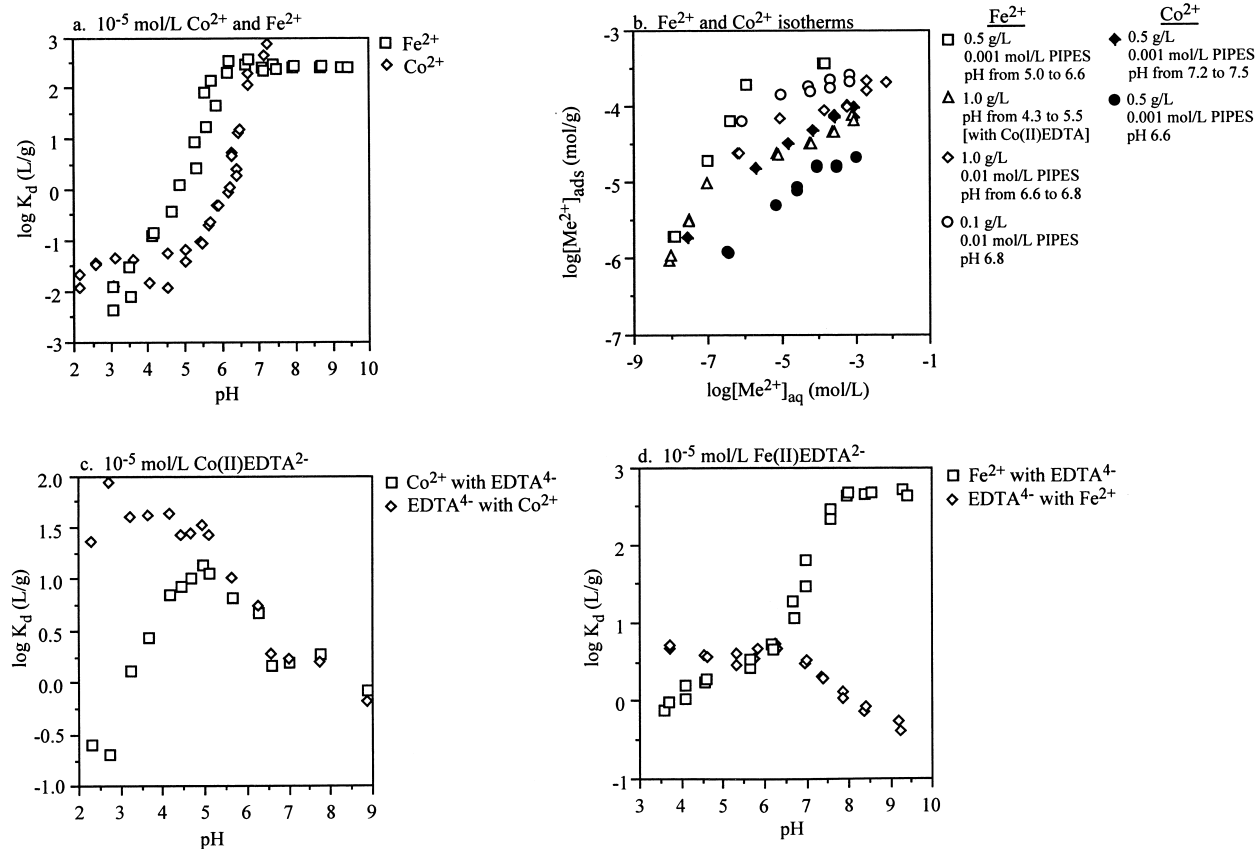


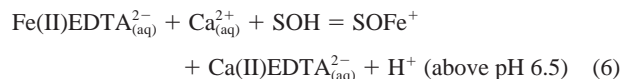
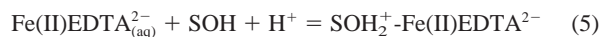
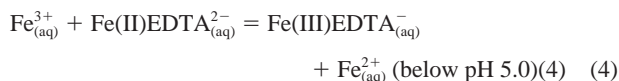
Fig. 1. Sorption behavior of  $\text{Co}_{(\text{aq})}^{2+}$ ,  $\text{Fe}_{(\text{aq})}^{2+}$ ,  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$ , and  $\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  on goethite. Electrolyte was 0.003 mol/L  $\text{Ca(ClO}_4)_2$  and goethite was 0.5 g/L unless noted otherwise. a.) pH sorption edges (as  $K_d$ ) of  $\text{Co}_{(\text{aq})}^{2+}$  and  $\text{Fe}_{(\text{aq})}^{2+}$  on goethite. b.) adsorption isotherms of  $\text{Co}_{(\text{aq})}^{2+}$  and  $\text{Fe}_{(\text{aq})}^{2+}$  on goethite at different pH and solids concentrations. c.) pH sorption edge (as  $K_d$ ) of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  on goethite; final  $\text{Co(II)}_{(\text{aq})}$  and  $\text{EDTA}_{(\text{aq})}^{4-}$  quantified simultaneously by dual label counting. d.) pH sorption edge (as  $K_d$ ) of  $\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  on goethite; results of two experiments where final  $\text{Fe(II)}_{(\text{aq})}$  and  $\text{EDTA}_{(\text{aq})}^{4-}$  were quantified independently.

trations of  $\text{Fe}_{(\text{aq})}^{3+}$  solubilized from the oxide caused disparate sorption behavior of  $\text{EDTA}_{(\text{aq})}^{4-}$  and  $\text{Co(II)}$  that were initially co-associated in the complex. The  $\text{Fe}_{(\text{aq})}^{3+}$  promoted partial dissociation of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  ( $\log K_{\text{Fe(III)EDTA}} = 27.91$ ) to a mixture of  $\text{Fe(III)EDTA}_{(\text{aq})}^{2-}$ ,  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$ , and  $\text{Co}_{(\text{aq})}^{2+}$ :



The  $K_d$ - $\text{Co(II)}$  for  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  decreased below pH 6 because of the increasing fraction of  $\text{Co}_{(\text{aq})}^{2+}$  (i.e.,  $K_d = [\text{Co(II)}]_{(\text{sorbed})} / \{[\text{Co(II)EDTA}_{(\text{aq})}^{2-}] + [\text{Co}_{(\text{aq})}^{2+}]\}$ ), which exhibited low sorptivity at these pH's (Fig. 1a).

$\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  exhibited sorption behavior similar, in part, to that observed for  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  (Fig. 1d), with differences resulting from the stronger affinity of the goethite surface for  $\text{Fe}_{(\text{aq})}^{2+}$  (Fig. 1a) and the smaller aqueous complexation constant ( $\log K_{\text{Fe(II)EDTA}} = 15.98$ ). The competition of  $\text{Fe}_{(\text{aq})}^{3+}$  for  $\text{EDTA}_{(\text{aq})}^{4-}$  below pH 6 and  $\text{SO}_4^-$  for  $\text{Fe}_{(\text{aq})}^{2+}$  above pH = 6.5 induced more complex dissociation than noted for  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$ . The reaction suite is approximated as:



The  $K_d$  for  $\text{Fe(II)}$  in the complex reflected aqueous speciation effects ( $K_d = [\text{Fe(II)}]_{(\text{sorbed})} / \{[\text{Fe(II)EDTA}_{(\text{aq})}^{2-}] + [\text{Fe}_{(\text{aq})}^{2+}]\}$ ) and an apparent large difference in the affinity of the surface for  $\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  versus  $\text{Fe}_{(\text{aq})}^{2+}$ . There was approximate parity in the  $K_d$ 's of  $\text{Co(II)}$  and  $\text{Fe(II)}$  in the  $\text{EDTA}_{(\text{aq})}^{4-}$  complex at pH 6 ( $\log K_d = 0.6$ ) where  $\text{Me(II)EDTA}_{(\text{aq})}^{2-}$  was believed to be the primary surface species. Thus, surface complexes of  $\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  and  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  appear to exhibit comparable surface binding strength.

### 3.2.2. Milford sediment

The chemical behavior of the four target solutes in contact with the Milford sediment showed comparable behavior with respect to pH as they did on goethite (Figs. 2a,c,d), attesting to the presence of similar sorption reactions. There were differ-

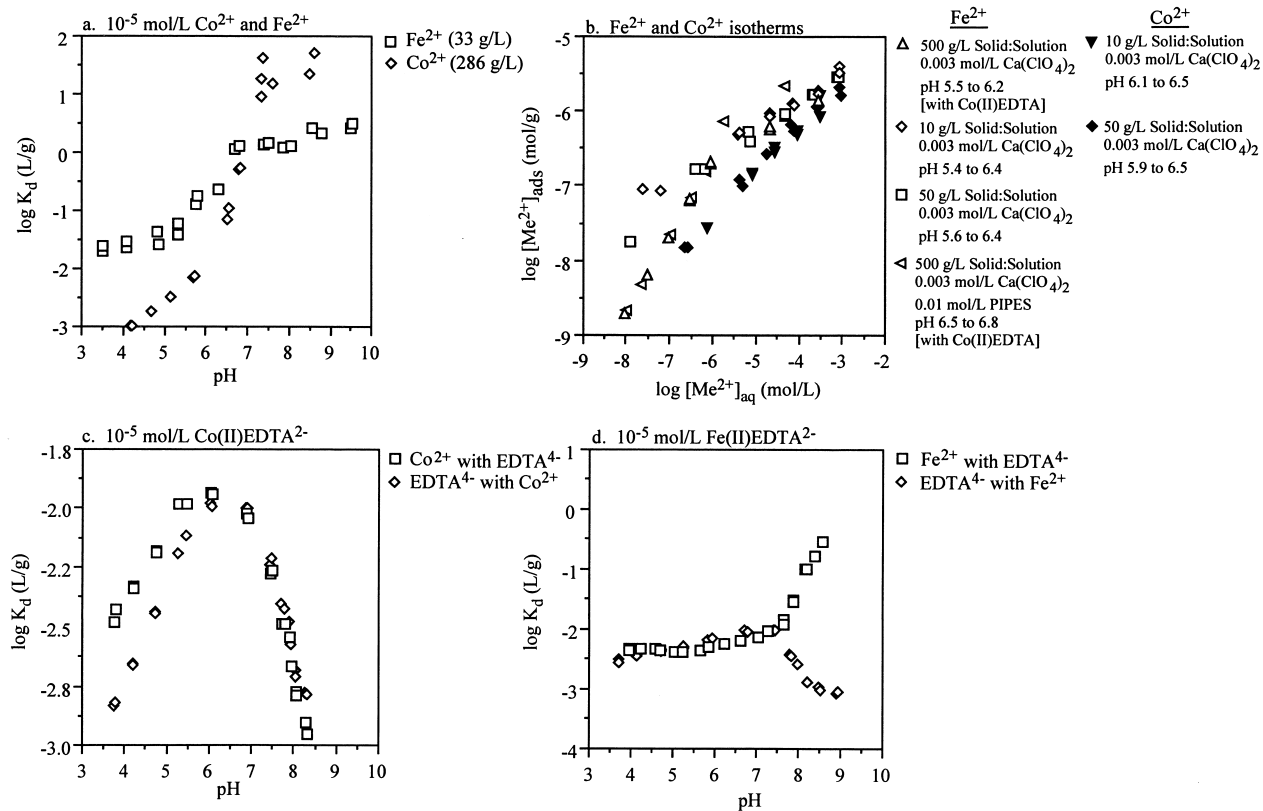


Fig. 2. Sorption behavior of  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Co}(\text{II})\text{EDTA}^{2-}$ , and  $\text{Fe}(\text{II})\text{EDTA}^{2-}$  on Milford sediment. Electrolyte was 0.003 mol/L  $\text{Ca}(\text{ClO}_4)_2$  and the Milford sediment was 500 g/L unless noted otherwise. a.) pH sorption edges (as  $K_d$ ) of  $\text{Co}^{2+}$  and  $\text{Fe}^{2+}$  on Milford sediment. b.) adsorption isotherms of  $\text{Co}^{2+}$  and  $\text{Fe}^{2+}$  on Milford sediment at different pH and solids concentrations. c.) pH sorption edge (as  $K_d$ ) of  $\text{Co}(\text{II})\text{EDTA}^{2-}$  on Milford sediment; final  $\text{Co}(\text{II})_{(\text{aq})}$  and  $\text{EDTA}^{4-}_{(\text{aq})}$  quantified simultaneously by dual label counting. d.) pH sorption edge (as  $K_d$ ) of  $\text{Fe}(\text{II})\text{EDTA}^{2-}$  on Milford sediment; results of two experiments where  $\text{Fe}(\text{II})_{(\text{aq})}$  and  $\text{EDTA}^{4-}_{(\text{aq})}$  were quantified independently.

ences, however; the  $K_d$ 's for the metal cations were approximately one log unit lower while those for the anions were in excess of two log units lower than on goethite. These differences were due to the smaller surface area of the Milford sediment, the lower exposed surface area of sorbent {[Fe(III) and Al(III) oxide]} in the sediment, and the lower intrinsic sorptivity of the natural oxide phases as compared to goethite (Zachara et al., 1995b). The dissociation front of  $\text{Fe}(\text{II})\text{EDTA}^{2-}_{(\text{aq})}$  moved approximately 1.5 units to higher pH (Fig. 2d) as compared to goethite (Fig. 1d) because of the lower affinity of the Milford sediment for  $\text{Fe}^{2+}_{(\text{aq})}$  (Fig. 2a).

Sorption isotherms of the metal cations on the Milford sediment were analogous, in part, to goethite, with  $\text{Fe}^{2+}_{(\text{aq})}$  being more strongly sorbed than  $\text{Co}^{2+}_{(\text{aq})}$  (Fig. 2b). As on goethite,  $\text{Co}^{2+}_{(\text{aq})}$  sorption was curvilinear, while  $\text{Fe}^{2+}_{(\text{aq})}$  displayed two-part isotherm behavior. Sorption affinity for both cations was much less on the Milford sediment; so much less, in fact that surface sites were not saturated at  $\log[\text{Me}^{2+}]_{\text{aq}} = -3$  mol/L (Fig. 2b). When plotted in Langmuir format (e.g.,  $K_d$  versus mol/g), the isotherms yielded estimated surface saturation values ( $\text{Fe}^{2+}_{(\text{aq})} = 7.20 \times 10^{-7}$  mol/m<sup>2</sup>;  $\text{Co}^{2+}_{(\text{aq})} = 3.53 \times 10^{-7}$  mol/m<sup>2</sup>) that were below those of goethite (3.2.1).

### 3.3. Sorption Behavior During Bacterial Fe(III) Oxide Reduction

Masses of goethite (1.0 g/L and 1.5 g/L) and the Milford sediment (500 g/L) were used in the experiments with bacterial inoculation that would yield approximately 75–90% adsorption of  $\text{Co}(\text{II})\text{EDTA}^{2-}_{(\text{aq})}$  at pH 6.75. It was presumed that sorption would decrease through bacterial activity, in part because of Fe(III) reduction and loss of Fe(III) oxide mass and surface area. Large mass differences were used for the two sorbents because of their difference in sorption affinity (Figs. 1 and 2).

#### 3.3.1. Fe(II) generation

Biogenic Fe(II) was strongly sorbed by both goethite and the Milford sediment in presence of  $\text{Co}(\text{II})\text{EDTA}^{2-}_{(\text{aq})}$  (Fig. 3). Sorption, included Fe(II) adsorbed to the Fe(III) oxide, complexed to cell materials, and bound to accessory sediment mineral phases. Sorbed Fe(II) exceeded  $\text{Fe}^{2+}_{(\text{aq})}$  concentration by over an order of magnitude. Fe(II) reached asymptotic values after approximately 20 d for goethite and 75 d for the Milford. At the asymptote, 2.24% of the goethite and 10.4% of the DCB extractable Fe(III) in the Milford was reduced. We have ob-



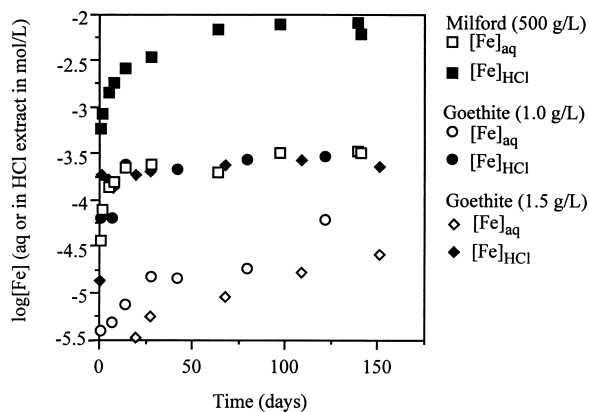


Fig. 3. Concentration of aqueous and total (HCl-extracted) Fe(II) in bioreduction experiments of goethite and the Milford sediment with BrY and Co(II)EDTA<sup>2-</sup> ( $10^{-5}$  mol/L). Solid concentrations as noted. The HCl-extracted Fe(II) represents sorbed plus aqueous Fe(II).

served here, and with another *Shewanella* strain (*S. putrefaciens*; Zachara et al., 1998), that many natural crystalline Fe(III) oxides are more reducible than goethite. The total amount of Fe(III) reduced in the Milford suspension (e.g., mol/L Fe(II) in HCl extract; Fig. 3) was larger than goethite because of the greater bioavailability of the natural Fe(III) oxide fraction and the higher mass concentration of reducible Fe(III) oxide in the suspension [ $9.15 \times 10^{-2}$  mol/L Fe(III) in the Milford as compared to  $1.13 \times 10^{-2}$  to  $1.7 \times 10^{-2}$  mol/L Fe(III) in the goethite suspensions].

### 3.3.2. Co(II)EDTA<sup>2-</sup> distribution in goethite/BrY suspensions

The Fe(II) generated by BrY did not strongly effect the sorption behavior of Co(II)EDTA<sup>2-</sup> in goethite suspensions (Fig. 4). While the two experiments with different goethite and organism concentrations differed slightly in magnitude and

Table 4. Measured pH in sorption/reduction experiments with goethite.

Time (d)	1.0 g/L goethite		1.5 g/L goethite		
	Without BrY	With BrY	Time (d)	Without BrY	With BrY
1	5.62	6.39	1	5.69	6.00
6.8	5.75	6.49	2	5.83	5.91
13.8	5.70	7.19	5	5.82	5.92
42.0	5.20	6.36	7.9	5.83	6.10
79.9	5.66	7.59	19.7	5.64	6.50
122	5.81	7.23	28.1	5.79	6.14
			68.1	5.26	6.20
			109	6.29	7.39
			151	6.03	7.20

trend (i.e., Figs. 4a,b), the overall results were similar. That is, the controls (without BrY) evolved to a point where  $K_d\text{-Co(II)} = K_d\text{-EDTA}^{4-} = 10$ . In the biotic experiments, the  $K_d$ 's for both Co(II) and EDTA<sup>4-</sup> were lower than the controls. Also, the  $K_d\text{-Co(II)}$  increased above the  $K_d\text{-EDTA}^{4-}$  with time, signifying partial dissociation of the complex according to the following presumed relationship:



Differences between the controls and the biotic experiments resulted from pH, which differed from target values (Table 4). The pH of the control experiments was lower than targeted (pH = 5.2–6.0), and fell in a range where Co(II)EDTA<sup>2-</sup> sorption was higher than in the biotic experiments, and where partial dissociation by Fe<sup>3+</sup> occurs (Reaction 3; Fig. 1c). This lower pH promoted the partial dissociation of the complex in the control experiments (0–50 d, Figs. 4a,b). In spite of the presence of buffer, the pH of the biotic experiments increased with time because of proton consumption during bioreduction:

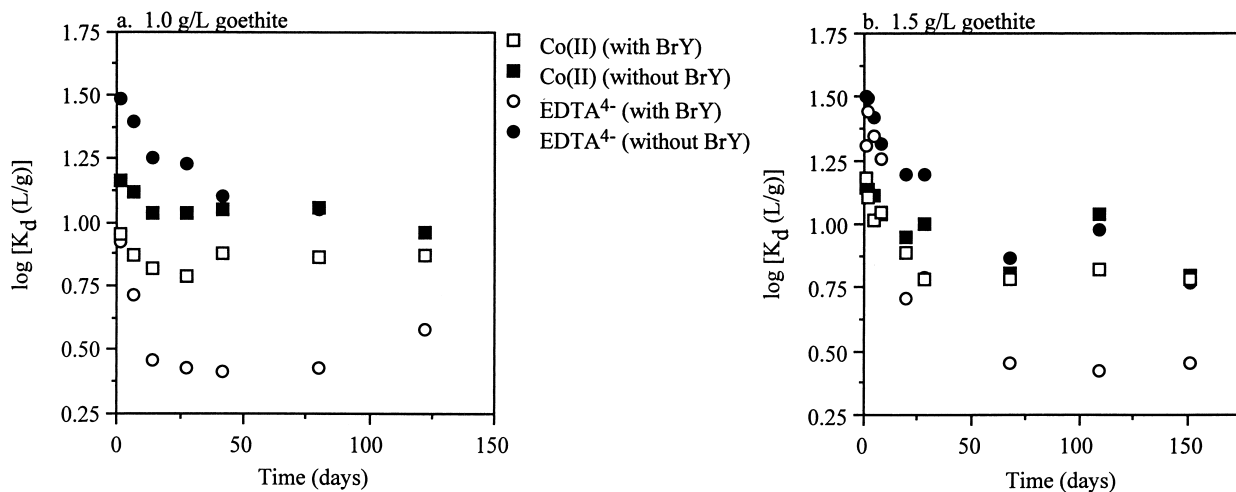


Fig. 4. Solid-liquid distribution of  $10^{-5}$  mol/L Co(II)EDTA<sup>2-</sup> (as  $K_d$ ) in goethite suspensions [a.] 1.0 g/L and b.) 1.5 g/L] with e-donor ( $\text{H}_2$ ) and variable BrY inoculum in 0.003 mol/L  $\text{Ca}(\text{ClO}_4)_2$ . The distribution of Co(II)EDTA<sup>2-</sup> was determined by dual-label counting of <sup>60</sup>Co and <sup>14</sup>EDTA. Note measured pH values in Table 4.

Table 5. Computed aqueous speciation of Fe(II), Co(II), and EDTA<sup>4-</sup> in biotic experiment with goethite.

Sorbent concentration/ time in days	Fe(II)				Co(II)			EDTA	
	Fe(II) <sub>(aq)-TOT</sub> <sup>a</sup>	Fe <sub>(aq)</sub> <sup>2+</sup> (mol/L)	Fe(II)EDTA <sub>(aq)</sub> <sup>2-</sup>	Co(II) <sub>(aq)-TOT</sub> <sup>a</sup>	Co <sub>(aq)</sub> <sup>2+</sup> (mol/L)	Co(II)EDTA <sub>(aq)</sub> <sup>2-</sup>	EDTA <sub>(aq)-TOT</sub> <sup>a</sup>	Fe(II)EDTA <sub>(aq)</sub> <sup>2-</sup>	Co(II)EDTA <sub>(aq)</sub> <sup>2-</sup>
		[C]/%			[C]/%			[C]/%	
1.0 g/L/79.9d	1.88 × 10 <sup>-5</sup>	1.71 × 10 <sup>-5</sup>	1.48 × 10 <sup>-6</sup>	1.31 × 10 <sup>-6</sup>	1.39 × 10 <sup>-7</sup>	1.17 × 10 <sup>-6</sup>	2.72 × 10 <sup>-6</sup>	1.48 × 10 <sup>-6</sup>	8.12 × 10 <sup>-7</sup>
		(91) <sup>b</sup>	(7.8) <sup>b</sup>		(10.6) <sup>b</sup>	(89.3) <sup>b</sup>		(54.2) <sup>b</sup>	(43.0) <sup>b</sup>
1.5 g/L/109d	1.61 × 10 <sup>-5</sup>	1.48 × 10 <sup>-5</sup>	1.16 × 10 <sup>-6</sup>	9.20 × 10 <sup>-7</sup>	1.07 × 10 <sup>-7</sup>	8.12 × 10 <sup>-7</sup>	2.03 × 10 <sup>-6</sup>	1.16 × 10 <sup>-6</sup>	8.12 × 10 <sup>-7</sup>
		(92.2) <sup>b</sup>	(7.2) <sup>b</sup>		(11.6) <sup>b</sup>	(88.3) <sup>b</sup>		(57) <sup>b</sup>	(40) <sup>b</sup>

<sup>a</sup> TOT = total component concentration.

<sup>b</sup> ( ) = % of aqueous component.



Higher pH promoted greater initial stability of the complex, weaker sorption of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup>, and stronger sorption of evolved Co(II).

The computed aqueous speciation in the biotic experiments (at 75 d for 1.0 g/L and 109 d for 1.5 g/L, Table 5) indicated that Fe<sub>(aq)</sub><sup>2+</sup> was high enough in concentration to induce some dissociation of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup>. The nominal concentration of Fe<sub>(aq)</sub><sup>2+</sup> was slightly in excess of Co(II)EDTA<sub>TOT</sub><sup>2-</sup> (1.6–1.9 × 10<sup>-5</sup> mol/L, Table 5), but was greater than 10× higher than the remaining aqueous concentrations of Co(II) (form unspecified), Table 5. The calculations indicated that Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> was the predominant Co(II) aqueous species, but that Fe(II)EDTA<sub>(aq)</sub><sup>2-</sup> was the predominant EDTA<sup>4-</sup> species. The computed effects of Fe(II) on Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> were modest because most of the evolved Fe(II) was sorbed to the goethite surface (Fig. 3). The total extent of dissociation (i.e., of Co(II)EDTA<sub>TOT</sub><sup>2-</sup> rather than Co(II)EDTA<sub>(aq)</sub><sup>2-</sup>) was not readily computed, however, as most of the chemical mass of both Co(II)<sub>TOT</sub> and EDTA<sub>TOT</sub><sup>4-</sup> (>75%) was surface associated. It is not known whether the surface speciation of Co(II) and EDTA<sup>4-</sup> matched that in the aqueous phase, but such parity is unlikely. More probable is that the surface ratio of Co<sup>2+</sup>:Co(II)EDTA<sup>2-</sup> exceeded that in

solution because: Co<sup>2+</sup> is strongly preferred by the surface at this pH; and excess Fe<sub>(aq)</sub><sup>2+</sup> was present to promote the dissociation of Co(II)EDTA<sup>2-</sup>.

### 3.3.3. Co(II)EDTA<sup>2-</sup> Distribution in Milford/BrY suspensions

**3.3.3.1. Without Malate.** The distribution of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> changed markedly in the Milford sediment after BrY inoculation (Fig. 5a). The inoculated experiment differed from the controls (without BrY) at <50 d, after which both systems displayed analogous behavior. Because the Milford sediment was not autoclaved (to prevent change in the Fe(III) oxide fraction), we surmised that indigenous H<sub>2</sub>-utilizing Fe(III)-reducers were present in the sediment that began reducing Fe(III) after an approximate 50 d lag period.

The following comments pertain to the inoculated system; they are, however, equally applicable to the uninoculated control after 50 d. Cobalt(II) and EDTA<sup>4-</sup> showed disparate sorption behavior indicating dissociation of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> (Fig. 5a). The increase in K<sub>d</sub>-Co(II) (Fig. 5a) between 0 and 25 d closely paralleled the evolution of Fe(II) (Fig. 3). The K<sub>d</sub> values for Co(II) (0.03, log K<sub>d</sub> = -1.52) and EDTA<sup>4-</sup> (0.002, log K<sub>d</sub> = -2.69) after 50 d approached those for Co<sub>(aq)</sub><sup>2+</sup> (log K<sub>d</sub> = -1.2, Fig. 2a) and Fe(II)EDTA<sub>(aq)</sub><sup>2-</sup> (log K<sub>d</sub> = -2.5, Fig.

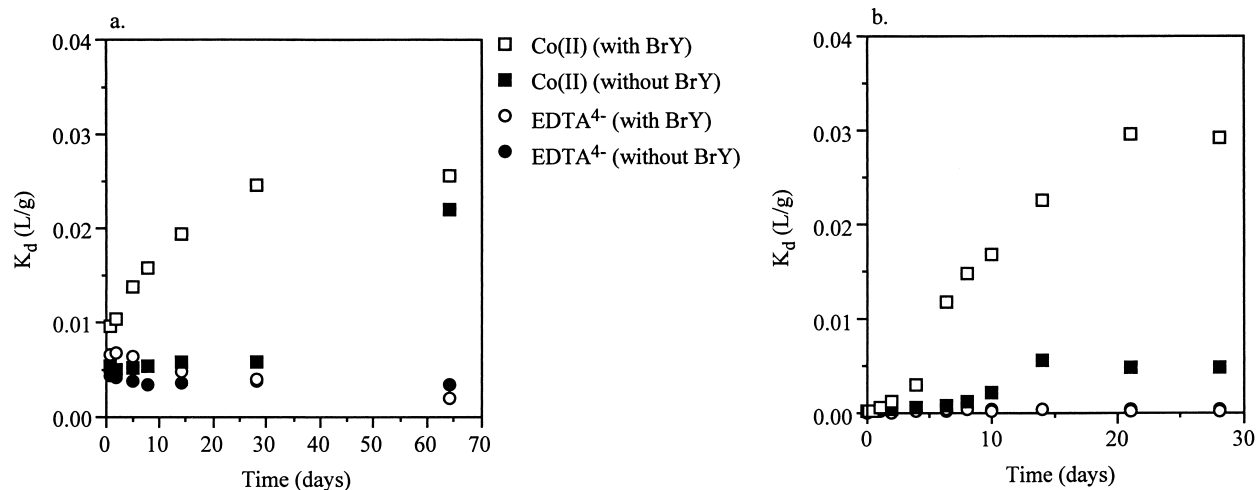


Fig. 5. Solid-liquid distribution of 10<sup>-5</sup> mol/L Co(II)EDTA<sup>2-</sup> (as K<sub>d</sub>) in Milford suspensions (500 g/L) with e-donor (H<sub>2</sub>) and BrY inoculum. The distribution of Co(II)EDTA<sup>2-</sup> was determined by dual-label counting of <sup>60</sup>Co and <sup>14</sup>EDTA. a.) without malate. b.) with 30 mM malate. Note measured pH values for the experiment without malate in Table 6.

Table 6. Computed distribution of aqueous species in the biotic experiment with the Milford sediment without malate.

Time (d)	pH	Co(II) <sub>aq-TOT</sub> <sup>a</sup>		Fe(II) <sub>aq-TOT</sub> <sup>a</sup>		EDTA <sub>aq-TOT</sub> <sup>-</sup>	
		Co <sub>(aq)</sub> <sup>2+</sup>	Co(II)EDTA <sub>(aq)</sub> <sup>2+</sup> (% of total)	Fe <sub>(aq)</sub> <sup>2+</sup>	Fe(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (% of total)	Co(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (% of total)	Fe(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (% of total)
1	5.85	25	74.6	97	2.9	53.3	45.6
2	5.99	37.8	62.0	98.3	1.6	43.1	56.3
5	6.25	45.0	54.9	98.7	1.2	28.7	70.8
8	6.43	43.3	56.6	98.6	1.3	23.6	76.0
14	6.54	47.7	52.3	98.8	1.1	16.1	83.6
28	6.55	44.7	55.3	98.7	1.2	12.0	87.6
64	6.58	30.3	69.6	97.6	2.3	10	89.6

<sup>a</sup> TOT = total component concentration.

2d) measured as individual solutes at pH 6.5. The computed speciation of the aqueous phase (Table 6) generally affirmed that Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> was being dissociated to yield Co<sub>(aq)</sub><sup>2+</sup> and Fe(II)EDTA<sub>(aq)</sub><sup>2-</sup>. Toward the conclusion of the experiment, 89.6% of the total EDTA<sub>(aq)</sub><sup>4-</sup> was computed to exist as Fe(II)EDTA<sub>(aq)</sub><sup>2-</sup>. The inequality of log K<sub>d</sub>-Co(II) at the end of this experiment (-1.52) with that at comparable pH in the sorption experiment (Fig. 2a, -1.2) may be attributed to the lingering, but small concentration of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> (Table 6), or to a competitive effect of sorbed Fe(II) which was approximately at surface saturation.

The effects of BrY on the distribution of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> were greater for the Milford sediment than for goethite because the resulting Fe<sub>(aq)</sub><sup>2+</sup> concentration was larger (Fig. 3) in the sediment. The larger Fe<sub>(aq)</sub><sup>2+</sup> concentration resulted from two factors:

1. Fe(III) oxides in the Milford sediment were more extensively reduced than was goethite; and
2. The Milford sediment did not sorb Fe(II) as strongly as goethite.

**3.3.3.2. With Malate.** The presence of 0.030 mol/L malate, added as an assimilable carbon source, suppressed the initial sorption of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> (Fig. 5b). The suppression was attributed to an anion competition effect from malate, as the sorption of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> is weak and decreases with increasing electrolyte concentration (Girvin et al., 1993; Zachara et al., 1995b; and unpublished data). The KH<sub>2</sub>PO<sub>4</sub> (5 × 10<sup>-5</sup> mol/L) present in the media of this one experiment may also have acted to suppress Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> sorption. Aqueous phase measurements (not shown) indicated that most of the PO<sub>4</sub> was sorbed. EDTA<sup>4-</sup> sorption remained low throughout the experiment implying that the sorption of Fe(II)EDTA<sub>(aq)</sub><sup>2-</sup> was suppressed as well.

The K<sub>d</sub>-Co(II), however, increased with time (Fig. 5b) paralleling the reduction of Fe(III) and the release of Fe<sub>(aq)</sub><sup>2+</sup> (data not shown). After approximately 20 d, K<sub>d</sub>-Co(II) reached a final value (0.03, log K<sub>d</sub> = -1.52) close to that in the experiment without malate (Fig. 5a). The computed aqueous speciation at that point (Table 7), indicated that close to 67.3% of the total Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> concentration had been dissociated to Co(II) and Fe(II)EDTA<sub>(aq)</sub><sup>2-</sup>. The sorption of free Co<sup>2+</sup> led to the noted increase in K<sub>d</sub>-Co(II). As in the preceding experiment, the lack of parity in log K<sub>d</sub>-Co(II) at 20 d (-1.52) versus that

at pH 7 in Fig. 2a (-0.5) may have resulted from the effects of aqueous complexation (by both EDTA<sup>4-</sup> and malate) and/or the competitive effects of sorbed Fe(II).

### 3.3.4. Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> sorption on the biotically reduced Milford sediment

Approximately 10–20% of the DCB-extractable Fe(III)-oxides in the Milford sediment were reduced during the biotic experiments described above. This amount of reduction, however, had minimal impact on the sorptivity of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> to the sediment that had been washed with Ca(ClO<sub>4</sub>)<sub>2</sub> to displace sorbed Fe(II) (Fig. 6.). Fe(III) and Al(III) oxides are the primary sorbents for Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> in the Milford sediment (Zachara et al., 1995b). The approximate parity in sorption of the original and reduced material suggests that either the reactive surface area of Fe(III) oxide fraction was conserved during reduction or that a commensurate amount of Al(III) oxides were exposed. Chemical extractions of soils and subsurface sediments have shown that Fe(III) oxide removal need not lead to a decrease in metal ion sorption if Al oxides are also present (Zachara et al., 1992; Zachara et al., 1994).

## 3.4. Abiotic Simulation by Fe<sub>(aq)</sub><sup>2+</sup> Titration

Ferrous iron was metered into Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> suspensions with goethite and the Milford sediment to abiotically simulate the effect of Fe(II) evolution on Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> stability and sorption. Note that surface reactions of Fe<sub>(aq)</sub><sup>2+</sup> had a significant impact on solution pH (Table 8 and 9) and these changes must be considered along with the pH sorption trends in Figs. 1 and 2 (e.g., of K<sub>d</sub>-Co(II) and K<sub>d</sub>-EDTA<sup>4-</sup>) to understand the results that follow.

### 3.4.1. Goethite

The addition of increasing concentrations of Fe<sub>(aq)</sub><sup>2+</sup> to goethite suspensions had differing impacts on the distribution of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> depending on initial pH (Fig. 7). At lower pH (5.5–4.3) where neither Fe<sub>(aq)</sub><sup>2+</sup> or Co<sub>(aq)</sub><sup>2+</sup> were strongly sorbed (Fig. 1), increasing Fe<sub>(aq)</sub><sup>2+</sup> concentrations led to a systematic decrease in K<sub>d</sub>-Co(II) after a threshold value was achieved. The behavior was consistent with Fe<sub>(aq)</sub><sup>2+</sup> saturation of the goethite surface followed by the Fe<sub>(aq)</sub><sup>2+</sup> induced dissociation of Co(II)EDTA<sub>(aq)</sub><sup>2-</sup> (Reaction 7) to a mixture of Co<sup>2+</sup>, Fe<sup>2+</sup>,

Table 7. Computed aqueous speciation in the Milford-BrY-malate experiment. The TOT values were analytically determined and chemical species are computed. pH = 7.07, Na<sub>(a)</sub>-TOT = 6.0 × 10<sup>-2</sup> mol/L, and malate = 3.0 × 10<sup>-2</sup> mol/L.

Fe(II) <sub>aq-TOT</sub> (mol/L)	Fe <sub>(a)</sub> <sup>2+</sup> (mol/L)	Fe(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (mol/L)	Fe-mal <sub>(aq)</sub> <sup>a</sup> (mol/L)	Co(II) <sub>aq-TOT</sub> <sup>b</sup> (mol/L)	Co <sub>(aq)</sub> <sup>2+</sup> (mol/L)	Co(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (mol/L)	Co-mal <sub>(aq)</sub> <sup>a</sup> (mol/L)	EDTA <sub>aq-TOT</sub> <sup>4-</sup> (mol/L)	Co(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (mol/L)	Fe(II)EDTA <sub>(aq)</sub> <sup>2-</sup> (mol/L)
1.46 × 10 <sup>-4</sup>	1.05 × 10 <sup>-5</sup> (7.2) <sup>c</sup>	6.73 × 10 <sup>-6</sup> (4.6) <sup>c</sup>	1.29 × 10 <sup>-4</sup> (88.2) <sup>c</sup>	4.41 × 10 <sup>-6</sup>	5.11 × 10 <sup>-8</sup> (1.2) <sup>c</sup>	3.21 × 10 <sup>-6</sup> (7.29) <sup>c</sup>	1.14 × 10 <sup>-6</sup> (26.0) <sup>c</sup>	1.0 × 10 <sup>-5</sup>	3.21 × 10 <sup>-6</sup> (32.1) <sup>c</sup>	6.73 × 10 <sup>-6</sup> (67.3) <sup>c</sup>

<sup>a</sup> Mal = malate<sup>2-</sup>.

<sup>b</sup> This concentration equals approximately 30% to the total Co(II)EDTA<sup>2-</sup> pool originally added to the experiment.

<sup>c</sup> ( ) = % of aqueous component.

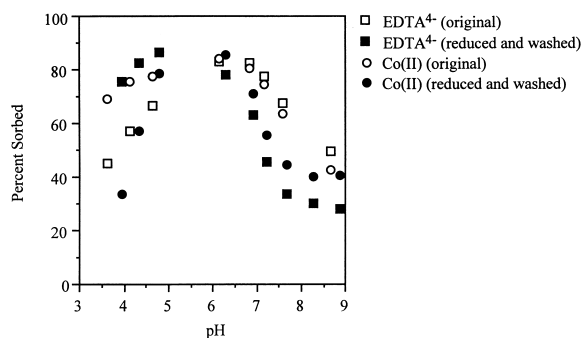


Fig. 6. Percent sorption of  $10^{-5}$  mol/L Co(II)EDTA $^{2-}$  on the natural and bio-reduced Milford sediment. The distribution of Co(II)EDTA $^{2-}$  was determined by dual-label counting of  $^{60}\text{Co}$  and  $^{14}\text{EDTA}$ . The bio-reduced sediment was washed free of sorbed Fe(II) with  $\text{Ca}(\text{ClO}_4)_2$ .

Co(II)EDTA $^{2-}$ , and Fe(II)EDTA $^{2-}$ . Aqueous speciation calculations like those in Tables 3, 5, 6, and 7 demonstrated this effect (not shown). The general constancy of  $K_d$ -EDTA $^{4-}$  as the initial concentration of Fe $^{2+}_{(\text{aq})}$  was increased reflected the rather high and comparable sorptivity of both Co(II)EDTA $^{2-}_{(\text{aq})}$  and Fe(II)EDTA $^{2-}_{(\text{aq})}$  at these pH's (Fig. 1).

At higher pH (6.83–5.85), Co(II)EDTA $^{2-}_{(\text{aq})}$  was less strongly sorbed, but Fe $^{2+}_{(\text{aq})}$  was more strongly sorbed than at lower pH. The slight tendency for both  $K_d$ -Co(II) and  $K_d$ -EDTA $^{4-}$  to increase with increasing Fe $^{2+}_{(\text{aq})}$  may result from enhanced sorption of the intact complex promoted by adsorbed positive charge density ( $\text{SO}-\text{Fe}^+$ , reaction 1). Surface saturation occurred above an initial concentration of  $10^{-3.5}$  mol/L, allowing Fe $^{2+}_{(\text{aq})}$  to increase. This, in turn, promoted complex dissociation and the decrease of  $K_d$ -Co(II) consistent with the sorption data in Figure 1. The relationship between pH, final Fe $^{2+}_{(\text{aq})}$ , and the apparent dissociation of Co(II)EDTA $^{2-}$  is summarized for the two experiments with different pH values in Table 8. Dissociation extent correlated with the appearance and concentration of Fe $^{2+}_{(\text{aq})}$ .

Table 8. Ferrous iron concentrations in Fe $^{2+}_{(\text{aq})}$  metering experiment with goethite.

Initial Fe $^{2+}_{(\text{aq})}$ (mol/L)	Final Fe $^{2+}_{(\text{aq})}$ (mol/L)	Final pH	Dissociation <sup>a</sup>
$10^{-6.0}$	DL	5.45	N
$10^{-5.5}$	DL	5.33	N
$10^{-5.0}$	DL	5.06	N
$10^{-4.5}$	$10^{-5.85}$	4.74	Y
$10^{-4.0}$	$10^{-4.20}$	4.55	Y
$10^{-3.5}$	$10^{-3.58}$	4.42	Y
$10^{-3.0}$	$10^{-3.06}$	4.31	Y
$10^{-6.0}$	DL	6.83	N
$10^{-5.5}$	DL	6.84	N
$10^{-5.0}$	DL	6.83	N
$10^{-4.5}$	DL	6.83	N
$10^{-4.0}$	DL	6.81	N
$10^{-3.5}$	$10^{-5.7}$	6.70	N
$10^{-3.0}$	$10^{-3.25}$	6.65	Y

DL = below the analytical detection limit  $10^{-6.25}$  mol/L.

<sup>a</sup> As implied by difference in  $K_d$ -Co(II) versus  $K_d$ -EDTA $^{4-}$ .

Table 9. Ferrous iron concentrations in Fe $^{2+}_{(\text{aq})}$  metering experiment with Milford sediment.

Initial Fe $^{2+}_{(\text{aq})}$ (mol/L)	Final Fe $^{2+}_{(\text{aq})}$ (mol/L)	Final pH	Dissociation <sup>a</sup>
$10^{-6.0}$	DL	6.13	N
$10^{-5.5}$	DL	6.14	N
$10^{-5.0}$	DL	6.21	N
$10^{-4.5}$	DL	6.19	N
$10^{-4.0}$	DL	6.08	N
$10^{-3.5}$	$10^{-4.66}$	5.63	Y
$10^{-3.0}$	$10^{-3.54}$	5.42	Y
$10^{-6.0}$	DL	6.79	N
$10^{-5.5}$	DL	6.81	N
$10^{-5.0}$	DL	6.83	N
$10^{-4.5}$	DL	6.81	N
$10^{-4.0}$	DL	6.81	N
$10^{-3.5}$	$10^{-5.75}$	6.75	Y
$10^{-3.0}$	$10^{-4.30}$	6.53	Y

DL = below the analytical detection limit of  $10^{-6.25}$  mol/L.

<sup>a</sup> As implied by difference in  $K_d$ -Co(II) versus  $K_d$ -EDTA $^{4-}$ .

### 3.4.2. Milford sediment

The sorption behavior of Co(II)EDTA $^{2-}$  in the Milford suspension with Fe $^{2+}_{(\text{aq})}$  titration (Fig. 8) was, in part, analogous

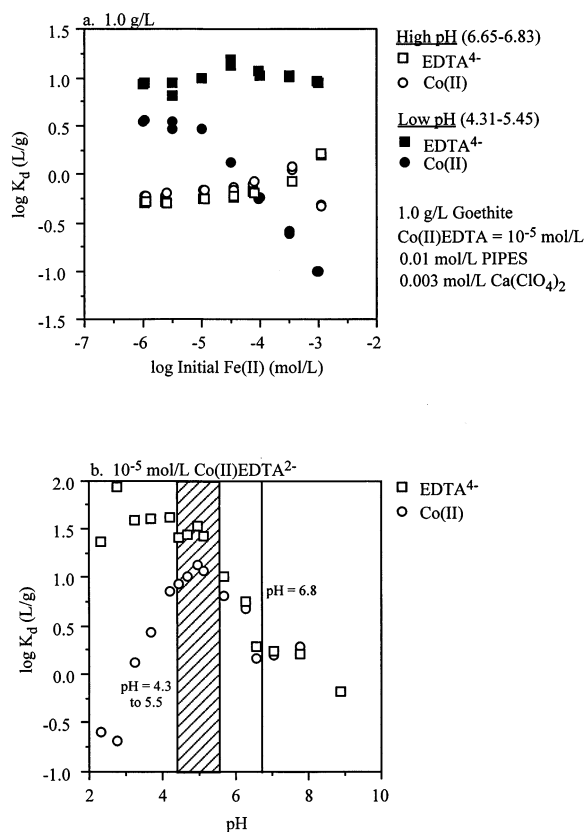


Fig. 7. a.) Influence of Fe $^{2+}_{(\text{aq})}$  addition on the distribution of Co(I-EDTA) $^{2-}$  in goethite suspensions at two different initial pH values. Final pH values are noted in Table 8. b.)  $K_d$ 's for Co(II) and EDTA $^{4-}$  in Co(II)EDTA $^{2-}$  from Fig. 2 with the pH range for the experiments in a.) noted.

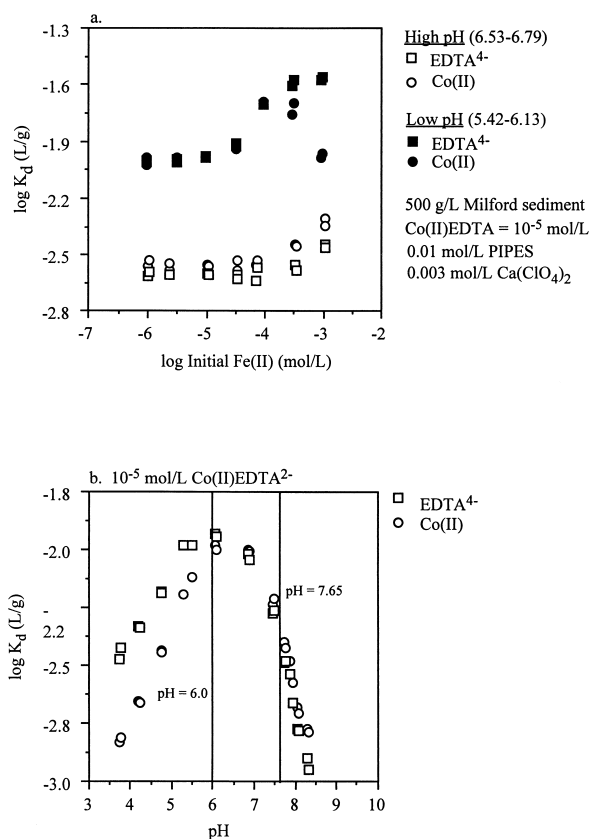


Fig. 8. a.) Influence of Fe<sub>(aq)</sub><sup>2+</sup> addition on the distribution of Co(II)EDTA<sup>2-</sup> in Milford sediment suspensions at two different pH values. The distribution of Co(II)EDTA<sup>2-</sup> was determined by dual-label counting of <sup>60</sup>Co and <sup>14</sup>EDTA. b.) K<sub>d</sub>'s for Co(II) and EDTA<sup>4-</sup> in Co(II)EDTA<sup>2-</sup> with the pH range for the experiments in a.) noted.

to that of goethite (Fig. 7). Differences were observed, however, that resulted from the solid-to-solution ratio (500 g/L) and pH of the Milford suspensions that strongly affected the extent of Fe<sub>(aq)</sub><sup>2+</sup> sorption and its final aqueous concentration. The similarity in the Milford data between pH = 6.13 to 5.42 with that of goethite between pH = 6.83 to 5.85 implied that the same reaction suite was operative, including the apparent enhancement of Co(II)EDTA<sup>2-</sup> sorption by sorbed Fe<sub>(aq)</sub><sup>2+</sup>. As noted for goethite, the observation of dissociation and its extent correlated with the appearance and concentration of Fe<sub>(aq)</sub><sup>2+</sup> (Table 9).

#### 4. DISCUSSION

##### 4.1. The Sorbing Surface in Bioreduced Materials

Recently, we showed that siderite (FeCO<sub>3</sub>) and vivianite [Fe<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub> · 8H<sub>2</sub>O] were biomineralization products resulting from the reduction of crystalline Fe(III) oxides by DIRB (Zachara et al., 1998). The formation of vivianite requires P addition to the media (often in mM concentration) while siderite formation is promoted by bicarbonate typically included to buffer pH in the medium. P was excluded from all experiments herein except one (Fig. 5b) and HCO<sub>3</sub><sup>-</sup> was not used as a buffer. Therefore, significant carbonate or phosphate biomin-

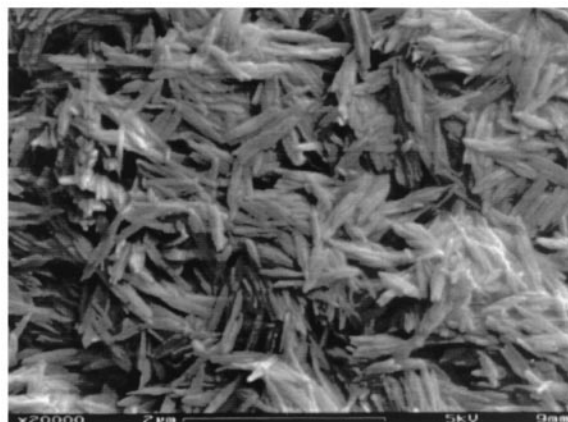


Fig. 9. Scanning electron micrograph of the goethite used in this study after bioreduction by *Shewanella putrefaciens*, strain CN-32 in pipes buffer. 2 μm scale bar as noted.

eralization was not expected. X-ray diffraction analyses of this same goethite and Milford sediment that were bioreduced by *Shewanella putrefaciens* strain CN-32 under culture conditions identical to those used here showed no discernable biomineralization products. In PIPES buffer, the bioreduced goethite appeared identical to the starting goethite (Fig. 9). When present, biomineralization products are generally clearly visible by SEM (Fredrickson et al., 1998; Zachara et al., 1999) and were not observed here.

The asymptotic values of the sorbed Fe(II) concentrations [Fe(II)-HCl] in both the bioreduced goethite (10<sup>-3.6</sup> mol/L) and Milford (10<sup>-2.15</sup> mol/L) suspensions (Fig. 3) when normalized to initial surface area (Fig. 10) were close to one another and were within a factor of 1.5–2.75 times the Fe<sup>2+</sup> sorption capacities estimated from the isotherms (3.2). These data support speculation that, under the specific media and substrate conditions used here, that saturation of adsorbing surfaces with Fe(II) has controlled and limited the bioreduction of the Fe(III) oxides by *S. alga*. The apparent excess saturation of the goethite and Milford surfaces may result from:

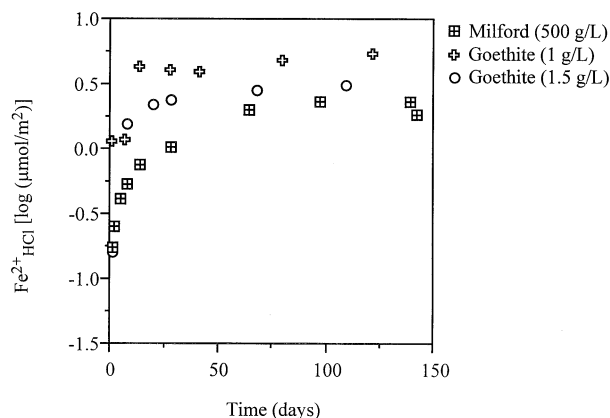


Fig. 10. The sorbed concentration of Fe(II) (in μmol/m<sup>2</sup>) in suspensions of goethite and the Milford sediment with e-donor (H<sub>2</sub>) and BrY inoculum. Data transformed from Fig. 3.

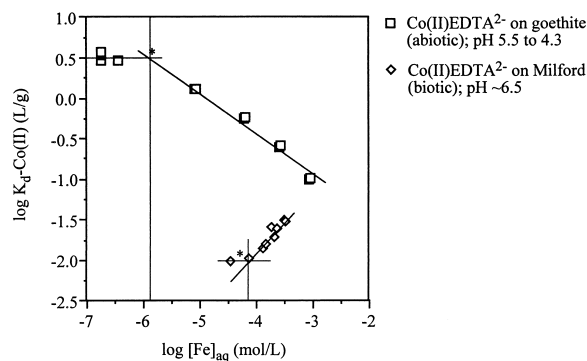


Fig. 11. Influence of  $\text{Fe}_{(\text{aq})}^{2+}$  on the  $K_d$  of Co(II) associated with  $\text{Co(II)EDTA}^{2-}$ . Selected data from an abiotic experiment with goethite (Fig. 7a) and a biotic experiment (Fig. 5a) with the Milford sediment. For the abiotic experiment, the  $\text{Fe}_{(\text{aq})}^{2+}$  is the final concentration remaining after spiking with a larger initial value. The noted responses for both data sets are less than the anticipated 1:1 relationship.

1. pH differences between the isotherm and bioreduction experiments;
2. Biosorption of Fe(II) to microorganism surfaces or cell fragments (e.g., Urrutia et al., 1998);
3. An increase in effective surface area accompanying dissolution; or
4. Deep structural bioreduction.

Regardless of mechanism, the sorbing surface presented to  $\text{Co(II)EDTA}^{2-}$  in the intermediate to latter stages of the bioreduction experiments appears to be saturated with Fe(II).

#### 4.2. Impacts of Fe(III) Reduction on $\text{Co(II)EDTA}^{2-}$ Chemistry

The impacts of biologic reduction on the solid-liquid distribution of  $\text{Co(II)EDTA}^{2-}$  may be explained by separately considering the effects of aqueous and sorbed Fe(II).

##### 4.2.1. Effects of $\text{Fe}_{(\text{aq})}^{2+}$

The most important impact of DIRB-Fe(III) oxide reduction on the sorption and stability  $\text{Co(II)EDTA}^{2-}$  was through the liberation of  $\text{Fe}_{(\text{aq})}^{2+}$ . Although Fe(II) was generated in mM concentration, most of this remained in a sorbed state and had little discernable impact on  $\text{Co(II)EDTA}^{2-}$  speciation or sorption. Goethite, in particular, strongly held Fe(II); and for this reason the impacts of bioreduction on  $\text{Co(II)EDTA}^{2-}$  sorption were minimal. However, increasing amounts of  $\text{Fe}_{(\text{aq})}^{2+}$  were observed with time during bioreduction as the solids approached sorption saturation. The effect of  $\text{Fe}_{(\text{aq})}^{2+}$  was to induce dissociation of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  via reactions 7 and 1.

Significant dissociation was only noted when  $\text{Fe}_{(\text{aq})}^{2+} > \text{Co(II)EDTA}_{(\text{aq})}^{2-}$  because of the difference in the stability constants of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  and  $\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  (Table 2). However, because the sorption of  $\text{Co(II)EDTA}^{2-}$  and  $\text{Co}^{2+}$  varied with both pH and the sorbent (Figs. 1 and 2), a common threshold  $\text{Fe}_{(\text{aq})}^{2+}$  concentration that induced dissociation was not defined. This is shown in Figure 11 where the apparent dissociation of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  was implied from the relationship of  $K_d$ -Co(II) to  $\text{Fe}_{(\text{aq})}^{2+}$ . Dissociation commenced at approximately

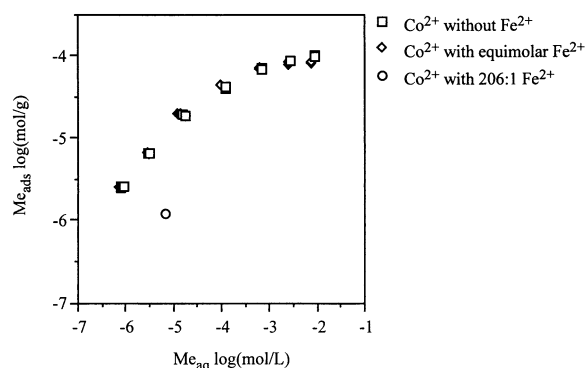


Fig. 12. Competitive sorption isotherm of  $\text{Co}_{(\text{aq})}^{2+}$  and  $\text{Fe}_{(\text{aq})}^{2+}$  on goethite at pH 6.5 in 0.003 mol/L  $\text{Ca(ClO}_4)_2$ . Conditions were as noted. At the Fe(II): Co ratio of 206:1, only a single measurement was obtained.

$10^{-6}$  mol/L  $\text{Fe}_{(\text{aq})}^{2+}$  in goethite suspension and  $10^{-4}$  mol/L  $\text{Fe}_{(\text{aq})}^{2+}$  in Milford suspension (see asterisks in Fig. 11). The net effect of dissociation was to change the  $K_d$ -Co(II), which either increased or decreased depending on pH (Fig. 11) and the sorbate preference of the sorbent. Generally, a linear or curvilinear (if pH varied) dependence of  $K_d$ -Co(II) on  $\text{Fe}_{(\text{aq})}^{2+}$  was observed above the threshold  $\text{Fe}_{(\text{aq})}^{2+}$  concentration (Fig. 11). In contrast, the  $K_d$ - $\text{EDTA}^{2-}$  was little changed by dissociation because the sorptivity of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  and  $\text{Fe(II)EDTA}_{(\text{aq})}^{2-}$  were similar at intermediate pH.

Although this study was not designed to evaluate the kinetics of Co(II)-Fe(II) exchange in the  $\text{EDTA}^{4-}$  complex, the experimental data suggests that the exchange rate was rapid, on the scale of hours at least. Consistent with our data, Xue et al. (1995) observed that the exchange rate of Fe(III)EDTA<sup>-</sup> was slow ( $t_{1/2} \approx 20$  d), but the rate for divalent metals (i.e.,  $\text{Ca}^{2+}$  and  $\text{Zn}^{2+}$ ) was far more rapid. They further speculated that the exchange rate of Fe(II) in/with  $\text{EDTA}^{4-}$  complexes under anoxic conditions would also be rapid and comparable to other divalent metals.

##### 4.2.2. Effects of surface Fe(II)

There was surprisingly little discernable impact of sorbed Fe(II) on the chemistry of  $\text{Co(II)EDTA}^{2-}$ . It was noted in several instances that the measured  $K_d$ -Co(II) [for  $\text{Co(II)EDTA}^{2-}$ ] in biotic or abiotic systems where full dissociation of  $\text{Co(II)EDTA}^{2-}$  by  $\text{Fe}_{(\text{aq})}^{2+}$  was computed, were lower by factors of 1.5 to 3 than  $K_d$ 's measured for  $\text{Co}^{2+}$  at comparable pH on non-reduced sediments. While it may be surmised that bioreduction may have reduced the sorption of  $\text{Co(II)EDTA}_{(\text{aq})}^{2-}$  by sorbent depletion or transformation, Figure 6 refutes that possibility. More likely is that  $\text{Fe}_{(\text{aq})}^{2+}$  and  $\text{Co}_{(\text{aq})}^{2+}$  compete for cation sorption sites on the oxide, and that the high surface saturation of Fe(II) blocks and reduces  $\text{Co}_{(\text{aq})}^{2+}$  sorption. A limited competitive isotherm was measured for  $\text{Co}_{(\text{aq})}^{2+}$  in presence of sorbed  $\text{Fe}_{(\text{aq})}^{2+}$  on goethite (Fig. 12) to test this hypothesis. Experimental procedures were used that were identical to those used for the isotherms in Figure 1, except that  $\text{Fe}_{(\text{aq})}^{2+}$  was equilibrated with the goethite for 2 h prior to  $\text{Co}_{(\text{aq})}^{2+}$  addition. At equimolar concentration  $\text{Fe}_{(\text{aq})}^{2+}$  had no impact on

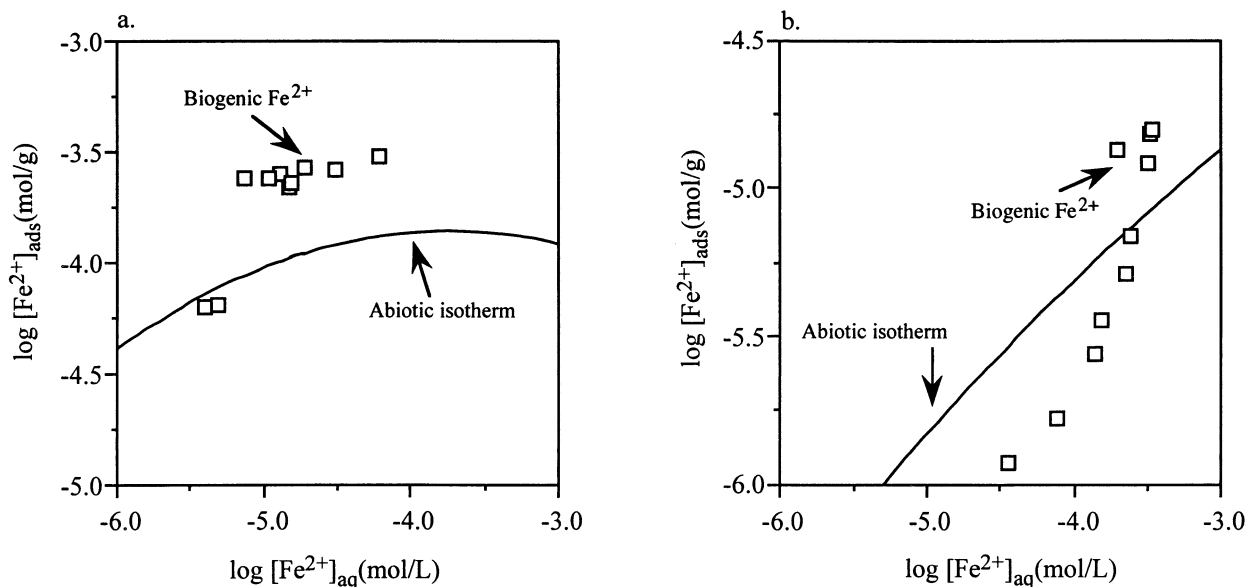


Fig. 13. A comparison of the measured abiotic  $Fe^{2+}$  adsorption isotherms with the noted Fe(II) solid-liquid distribution in the biotic reduction experiments with BrY. a.) goethite. b.) Milford sediment.

$Co^{2+}_{(aq)}$  sorption implying independence in adsorption sites. However,  $Fe^{2+}_{(aq)}$  had a strong competitive effect on  $Co^{2+}_{(aq)}$  sorption (one replicate measurement only) at a Fe:Co ratio (206:1) and an initial  $Co^{2+}_{(aq)}$  concentration ( $10^{-5.2}$  mol/L) that was similar to the biotic experiments (Fig. 12). Under these specific conditions,  $Co^{2+}_{(aq)}$  sorption was reduced by a factor of nine, indicating that competitive interactions may have been important in our experiments with high surface concentrations of Fe(II). Our data was insufficient to establish whether the apparent competitive effect resulted from mass action or surface modification.

#### 4.3. Biotic Effects

The abiotic  $Fe^{2+}_{(aq)}$  titration experiments (Figs. 7, 8) were performed to determine whether the microbial effects could be attributed solely to Fe(II) generation. Indeed, there were differences between the abiotic and biotic experiments, but generalizing the results was difficult because of experimental pH variations and differences in sorption of the goethite and the sediment. The addition of microorganisms to goethite/ $Co(II)EDTA^{2-}$  suspensions tended to reduce the overall sorption of both Co(II) and  $EDTA^{2-}$  (Fig. 4), possibly because the negatively charged cells adhered to sorbing particle surfaces. Comparable effects were not evident for the Milford sediment (Fig. 5). Another observed difference was that the  $K_d$ -Co(II) in the abiotic experiment tended to decrease (relative to  $EDTA^{4-}$ ) after  $Fe^{2+}_{(aq)}$  induced displacement from  $Co(II)EDTA^{2-}$  (except at high pH in Fig. 8a), while it increased in the biotic experiment. We attribute these differences to variations in suspension pH. In the abiotic experiments, a significant decrease was noted in solution pH (Table 8 and 9) with increased sorption density of Fe(II) and the appearance of  $Fe^{2+}_{(aq)}$ . The proton evolution was consistent with reaction (1). Thus, the build-up of  $Fe^{2+}_{(aq)}$ , which destabilized  $Co(II)EDTA^{2-}$ , was associated with both a

decrease in solution pH and a decrease in sorption affinity of the solid for  $Co^{2+}$ , consistent with the pH trend in Figure 1a. In contrast, the bioreduction increased pH [reaction (8), Table 4], which, in turn, lead to an increase in sorption affinity of  $Co^{2+}$  [e.g.,  $K_d$ -Co(II)].

It has been assumed, herein, that sorbed biogenic Fe(II) (e.g., Fig. 3) was chemically equivalent to sorbed Fe(II) resulting from abiotic spiking with  $Fe^{2+}_{(aq)}$  (e.g., Fig. 1 or Fig. 2). Clearly, there is no basis to assume that a ferrous iron surface complex is chemically equivalent to biotically generated lattice Fe(II). Nonetheless, we assumed that the solid-liquid distribution of biogenic Fe(II) would follow the abiotically determined  $Fe^{2+}_{(aq)}$  sorption isotherm, and that the abiotic experiment was a relevant chemical model of the biotic one. It was difficult in practice to demonstrate this presumed chemical equivalence because of the inability to distinguish Fe(II) species associated with the solids; and lack of control on the bacterial reduction reaction (e.g.,  $Fe(II)_{TOT}$ ) and the final pH, in spite of the buffer. For the limited cases where sufficient data was available for comparison, the results were ambiguous (Fig. 13). The biotic systems seemed to reach higher maximum sorption densities of Fe(II) for both goethite and the Milford sediment. The pH issue as noted in the preceding paragraph was crucial here as well. The measured tendency for pH to decrease with increasing sorption density in the abiotic experiment lead to a reduction in isotherm slope with increasing  $[Fe^{2+}]_{TOT}$ , while the increase in pH that accompanied biotic Fe(II) generation lead to a sharply contrasting effect when microorganisms were present (Fig. 13b, note the pH for the biotic experiment in Table 6).

Urrutia et al. (1998) found that *S. alga* adsorbed significant quantities of  $Fe^{2+}_{(aq)}$ , implying that enhanced Fe(II) sorption noted here in the biotic experiments could result from binding to cells and cell fragments. Using their data, however, and the organism concentrations in our experiments, we calculated that



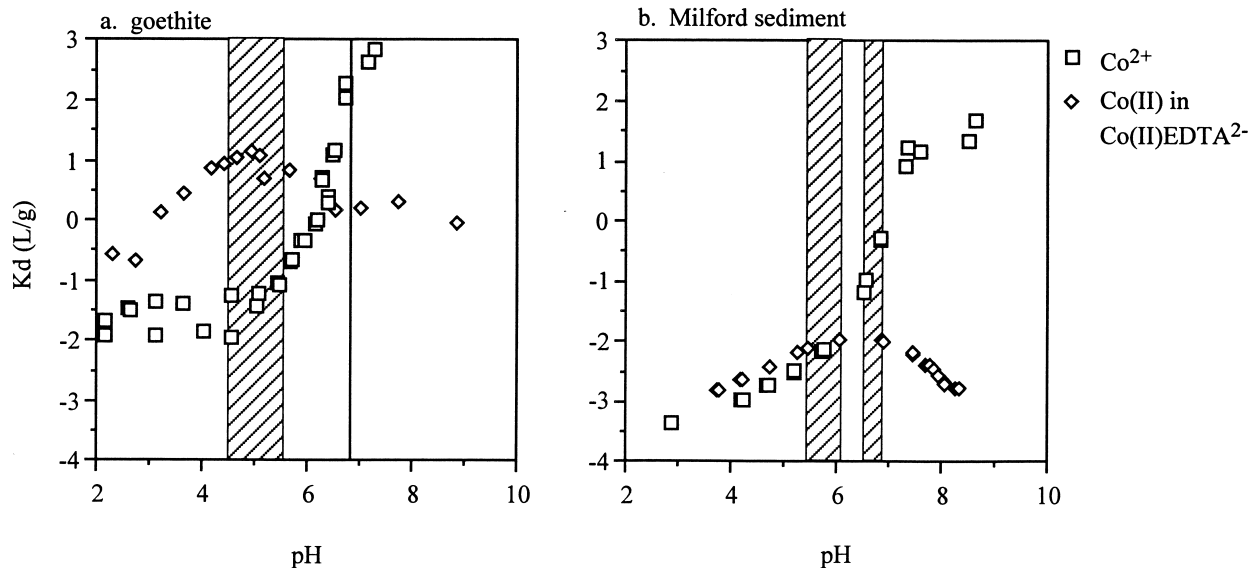


Fig. 14. A comparison of the sorption of  $\text{Co}^{2+}_{(\text{aq})}$  and  $\text{Co(II)EDTA}^{2-}_{(\text{aq})}$  (as  $\log K_d$ ) on goethite and Milford sediment. Data for panel a.) was taken from Figures 1a,c. Data for panel b.) was taken from Figures 2a and 2c.

the amount of Fe(II) sorbed to the organisms should be insignificant compared to the mineral solids.

#### 4.4. Differences Between Goethite and the Subsurface Sediment

Significant differences were noted in the behavior of  $\text{Co(II)EDTA}^{2-}$  in the biotic experiments with goethite and the Milford sediment (Figs. 4 and 5). In both experiments there was a tendency for the  $K_d$ - $\text{Co(II)}$  to increase, relative to  $\text{EDTA}^{4-}$ , as bacterial reduction liberated Fe(II). However, the relative extent of  $K_d$  increase was far greater in the Milford sediment. This difference resulted from two related factors. First, the Milford sediment was a less effective sorbent of Fe(II) in both strength and capacity than was goethite. Consequently,  $\text{Fe}^{2+}_{(\text{aq})}$  in the microbially-reduced Milford suspensions evolved to higher concentrations (Fig. 3) which facilitated dissociation of  $\text{Co(II)EDTA}^{2-}$ . Secondly,  $\text{Me(n)EDTA}^{(4-n)-}$  complexes were more weakly sorbed in the Milford sediment than on goethite (Fig. 14). Organic coatings on the oxides or co- or surface-precipitated silica may be responsible for the reduced sorptivity of the anionic complexes (Zachara et al., 1995a,b). The differences between  $K_d$ - $\text{Co(II)}$  and  $K_d$ - $\text{EDTA}^{4-}$  at maximum were approximately 2 orders of magnitude on goethite, but were close to four orders for the Milford sediment (Fig. 14). Thus, the initial  $\text{Co(II)EDTA}^{2-}$  complex was weakly sorbed by the Milford sediment, and  $\text{Co}^{2+}$  displaced from the complex by biogenic  $\text{Fe}^{2+}_{(\text{aq})}$  experienced a greater relative enhancement in sorption [e.g.,  $K_d$ - $\text{Co(II)}$ ] than on goethite.

The goethite and Milford sediment were mineralogically different. The Milford sediment contained fine-grained kaolinite in mass concentration comparable to, or exceeding that of goethite. We speculated that the speciation of biogenic Fe(II), would be different from goethite in the Milford sediment, with significant concentrations of Fe(II) adsorbed by kaolinite and other accessory phases. We expected that these differences in

Fe(II) speciation would impact  $\text{Co(II)EDTA}^{2-}$  behavior, but did not observe such effects.

#### 5. IMPLICATIONS TO SUBSURFACE ENVIRONMENTS

Weakly-sorbing metal-ligand complexes [e.g.,  $\text{Co(II)EDTA}^{2-}$ ] of intermediate stability (e.g.,  $\log K > 15$ ) may be destabilized in ferrogenic groundwaters. Competition with  $\text{Fe}^{2+}_{(\text{aq})}$  displaces the metal ion from the complex. The degree of displacement is controlled by the concentration ratio of  $\text{Fe}^{2+}_{(\text{aq})}$  to the metal in the complex [e.g.,  $\text{Co(II)}$  in this case], their respective stability constants for the ligand, and the adsorption strength of the dissociation products for mineral surfaces. The displacement reactions were rapid, occurring within the time frame of hours to days. Sorbed Fe(II) did not appear to participate in the dissociation reaction. Complicated geochemical behavior can result for contaminant metals such as  $\text{Co}^{2+}$  that are complex-bound because of the multi-species distribution of products. Different chemical species of the metal [e.g.,  $\text{Co(II)EDTA}^{2-}$  and  $\text{Co}^{2+}$ ] may exhibit vastly different retardation behavior. In ferrogenic groundwaters promoted by dissimilatory bacterial iron reduction, the metal-ligand complexes may show progressive down-gradient dissociation as a result of the changing Me/Fe ratio promoted by chemical reaction and advection.

In the circumneutral pH range with Fe(III) and Al(III) oxide containing sediments, small differences in pH may be important in controlling whether the displaced metal (e.g.,  $\text{Co}^{2+}$ ) or the ligand (as  $\text{Fe(II)L}^{2-}$ ) is more mobile, because their adsorption edges tend to cross in that pH region. In the biotic experiments performed here, the evolution of  $\text{Fe}^{2+}_{(\text{aq})}$  invariably led to an increase in the  $K_d$  of the displaced metal relative to the complexing ligand, which, in groundwater, would reduce the mobility of the originally complexed metal. This resulted from the net increase in system pH that accompanied bacterial Fe(III) oxide reduction. Ferrogenic conditions would, for example, lead to the immobilization of  $^{60}\text{Co}$  or  $^{239/240}\text{Pu}$ , which

are reportedly mobilized by complexation with EDTA<sup>4-</sup> (Means et al., 1978; Means and Alexander, 1981; Olsen et al., 1986). The magnitude of adsorption of the displaced metal, however, would be less than in comparable oxidized sediments due to sorptive competition with surface and aqueous Fe<sup>2+</sup>. Sorbing mineral surfaces in circumneutral ferrogenic groundwaters are likely to be near saturation with Fe(II), as shown here and by Heron et al., (1994). Such saturation may or may not influence the sorptive behavior of incoming contaminant anions or cations. Surprisingly the bacterial reduction of a sizable portion, i.e., 20%, of the Fe(III) oxide fraction of a subsurface material need not influence the intrinsic sorptivity of the sediment as shown for the sorption of Co(II)EDTA<sup>2-</sup> by the bioreduced [washed free of sorbed Fe(II)] Milford sediment.

*Acknowledgments*—This research was supported by the Natural and Accelerated Bioremediation Research Program (NABIR), Office of Biological and Environmental Research (OBER), and U.S. Department of Energy (DOE). Geological materials used in this paper were taken from the NABIR subsurface sediment collection. Critical reviews of this manuscript by Dr. D. C. Girvin of Pacific Northwest National Laboratory and two anonymous reviewers are appreciated.

## REFERENCES

- Abrechtsen H.-J. and Christensen T. H. (1994) Evidence for microbial iron reduction in a landfill leachate-polluted aquifer (Vejen, Denmark). *Appl. Environ. Microbiol.* **60**(11), 3920–3925.
- Arnold R. G., DeChristina T. J., and Hoffman M. R. (1988) Reductive dissolution of Fe(III) oxides by *Pseudomonas* sp. 200. *Biotechnol. Bioengineer.* **32**, 1081–1096.
- Bedsworth W. W. and Sedlak D. L. (1999) Sources and environmental fate of strongly complexed nickel in estuarine waters: The role of ethylenediaminetetraacetate. *Environ. Sci. Technol.* **33**, 926–931.
- Bowers A. R. and Huang C. P. (1986) Adsorption characteristics of metal-EDTA complexes on hydrous oxides. *J. Coll. Interf. Sci.* **110**, 575–590.
- Brooks S. C. and Jardine P. M. (1996) Reactive transport of EDTA-complexed cobalt in the presence of ferrihydrite. *Geochim. Cosmochim. Acta* **60**(11), 1899–1908.
- Caccavo F., Blakemore R. P., and Lovley D. R. (1992) A hydrogen-oxidizing, Fe(III)-reducing microorganism from the Great Bay Estuary, New Hampshire. *Appl. Environ. Microbiol.* **58**(10), 3211–3216.
- Chapelle F. H. (1993) *Ground-Water Microbiology and Geochemistry*. John Wiley & Sons, Inc.
- Davis J. A. and Kent D. B. (1990) Surface complexation modeling in aqueous geochemistry. In *Mineral-Water Interface Geochemistry* (ed. A. White and M. Hochella); *Rev. Mineral.* **23**, 177–260.
- Evanko C. R. and Dzombak D. A. (1998) Influence of structural features on sorption of NOM-analogue organic acids to goethite. *Environ. Sci. Technol.* **32**, 2846–2855.
- Fredrickson J. K., Zachara J. M., Kennedy D. W., Dong H., Onstott T. C., Hinman N. W., and Li S.-M. (1998) Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. *Geochim. Cosmochim. Acta* (in press).
- Girvin D. C., Gassman P. L., and Bolton H. J. (1993) Adsorption of aqueous cobalt ethylenediaminetetraacetate by  $\delta$ -Al<sub>2</sub>O<sub>3</sub>. *Soil Sci. Soc. Am. J.* **85**, 1–11.
- Girvin D. C., Gassman P. L., and Bolton H. J. (1996) Adsorption of nitrilotriacetate (NTA), Co, and CoNTA by gibbsite. *Clays Clay Miner.* **44**, 757–768.
- Hayes K. F. and Katz L. E. (1996) Application of X-ray absorption spectroscopy for surface complexation modeling of metal ion sorption. In *Physics and Chemistry of Mineral Surfaces* (ed. P. V. Brady), pp. 147–223. CRC Press, Boca Raton.
- Heron G. and Christensen T. H. (1995) Impact of sediment-bound iron on redox buffering in a landfill leachate polluted aquifer (Vejen, Denmark). *Environ. Sci. Technol.* **29**, 187–192.
- Heron G., Crouzet C., Boug A. C. M., and Christensen T. H. (1994) Speciation of Fe(II) and Fe(III) in contaminated aquifer sediments using chemical extraction techniques. *Environ. Sci. Technol.* **28**, 1698–1705.
- Killey D. R. W., McHugh J. O., Champ D. R., Cooper E. L., and Young J. L. (1984) Subsurface cobalt-60 migration from a low-level waste disposal site. *Environ. Sci. Technol.* **18**, 148–157.
- Lovley D. R. (1993) Dissimilatory metal reduction. *Ann. Rev. Microbiol.* **47**, 263–290.
- Lovley D. R. and Chapelle F. H. (1995) Deep subsurface microbial processes. *Rev. Geophys.* **33**, 365–381.
- Lovley D. R., Chapelle F. H., and Phillips E. J. P. (1990) Fe(III)-reducing bacteria in deeply buried sediments of the Atlantic Coastal Plain. *Geology* **16**, 954–957.
- Lovley D. R., Phillips E. J. P., Gorby Y. A., and Landa E. R. (1991) Microbial reduction of uranium. *Nature* **350**, 413–416.
- Lyngkilde J. and Christensen T. H. (1992a) Fate of organic contaminants in the redox zones of a landfill leachate pollution plume (Vejen, Denmark). *J. Contam. Hydrol.* **10**, 291.
- Lyngkilde J. and Christensen T. J. (1992b) Redox zones of a landfill leachate pollution plume (Vejen, Denmark). *J. Contam. Hydrol.* **10**, 273.
- Means J. L. and Alexander C. A. (1981) The environmental biogeochemistry of chelating agents and recommendations for the disposal of chelated radioactive wastes. *Nucl. Chem. Waste Manage.* **2**, 183–196.
- Means J. L., Crerar D. A., and Duguid J. O. (1978) Migration of radionuclide wastes: Radionuclide mobilization by complexing agents. *Sci.* **200**, 1477–1486.
- Myers C. R. and Nealson K. H. (1988) Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. *Sci.* **240**, 1319–1321.
- Nowack B. and Sigg L. (1996a) Adsorption of EDTA and metal-EDTA complexes onto goethite. *J. Coll. Interf. Sci.* **177**, 106–121.
- Nowack B., Lutzenkirchen J., Behra P., and Sigg L. (1996b) Modeling the adsorption of metal-EDTA complexes onto oxides. *Environ. Sci. Technol.* **30**, 2397–2405.
- Olsen C. R., Lowry P. D., Lee S. Y., Larsen I. L., and Cutshall N. H. (1986) Geochemical and environmental processes affecting radionuclide migration from a formerly used seepage trench. *Geochim. Cosmochim. Acta* **50**, 593–607.
- Phillips E. J. P., Lovley D. R., and Roden E. E. (1993) Composition of non-microbially reducible Fe(III) in aquatic sediments. *Appl. Environ. Microbiol.* **59**, 2727–2729.
- Roden E. E. and Zachara J. M. (1996) Microbial reduction of crystalline Fe(III) oxides: Influence of oxide surface area and potential for cell growth. *Environ. Sci. Technol.* **30**, 1618–1628.
- Rosselló-Mora R. A., Caccavo F. J., Osterlechner K., Springer N., Spring S., Schler D., Ludwig W., Amann R., Vanncanneyt M., and Schleifer K. H. (1994) Isolation and taxonomic characterization of a halotolerant, facultatively iron-reducing bacterium. *System. Appl. Microbiol.* **17**, 569–573.
- Schwertmann U., Cambier P., and Murad E. (1985) Properties of goethite of varying crystallinity. *Clays Clay Miner.* **33**, 369–378.
- Sposito G. (1984) *The Chemistry of Soils*. Oxford University Press, New York.
- Szecsody J. E., Zachara J. M., and Bruckhart P. L. (1994) Adsorption-dissolution reactions affecting the distribution and stability of Co(II)EDTA in Fe oxide-coated sand. *Environ. Sci. Technol.* **28**, 1706–1716.
- Szecsody J. E., Zachara J. M., Chilakapati A., Jardine P. M., and Ferency A. S. (1998a) Importance of flow and particle-scale heterogeneity on Co<sup>II/III</sup>EDTA reactive transport. *J. of Hydrol.* **209**, 112–136.
- Szecsody J. E., Chilakapati A., Zachara J. M., and Garvin A. L. (1998b) Influence of iron oxide inclusion shape on Co<sup>II/III</sup>EDTA reactive transport through spatially heterogeneous sediment. *Water Resour. Res.* **34**(10), 2501–2514.
- Urrutia M. M., Roden E. E., Fredrickson J. K., and Zachara J. M. (1998) Microbial and surface chemistry controls on reduction of

- synthetic Fe(III)-oxide minerals by the dissimilatory iron-reducing bacterium *Shewanella alga*. *Geomicrobio.* **15**, 269–291.
- Urrutia M. M., Roden E. E., and Zachara J. M. (1999) Influence of aqueous and solid-phase Fe(II) complexants on microbial reduction of crystalline Fe(III) oxides. *Environ. Sci. Technol.* **33**, 4022–4028.
- Xue H., Sigg L., and Kari F. G. (1995) Speciation of EDTA in natural waters: Exchange kinetics of Fe-EDTA in river water. *Environ. Sci. Technol.* **29**, 59–68.
- Zachara J. M., Resch C. T., and Smith S. C. (1994) Influence of humic substances on  $\text{Co}^{2+}$  sorption by a subsurface mineral separate and its mineralogic components. *Geochim. Cosmochim. Acta* **58(2)**, 553–566.
- Zachara J. M., Smith S. C., Resch C. T., and Cowan C. E. (1992) Cadmium sorption to soil separates containing layer silicates and iron and aluminum oxides. *Soil Sci. Soc. Am. J.* **56**, 1074–1084.
- Zachara J. M., Gassman P. L., Smith S. C., and Taylor D. (1995a) Oxidation and adsorption of Co(II) EDTA<sup>2-</sup> complexes in a subsurface materials with iron and manganese oxides. *Geochim. Cosmochim. Acta* **59(21)**, 4449–4463.
- Zachara J. M., Smith S. C., and Kuzel L. S. (1995b) Adsorption and dissociation of Co-EDTA complexes in iron oxide-containing subsurface sands. *Geochim. Cosmochim. Acta* **59**, 4825–4844.
- Zachara J. M., Fredrickson J. K., Li S.-M., Smith S. C., and Gassman P. L. (1998) Bacterial reduction of crystalline Fe(III) oxides in single phase suspensions and subsurface materials. *Am. Mineral.* **83**, 1426–1443.
- Zachara J. M., Smith S. C., and Fredrickson, J. K. (1999) Reductive solubilization of  $\text{Co}^{2+}$  from FeCoOOH by a dissimilatory iron reducing bacterium. *Geochim. Cosmochim. Acta* (Submitted).