Environ. Sci. Technol. **1998**, *32,* 244−250

Microbial Reduction of Cobalt^{III}EDTA$^-$ in the Presence and Absence of Manganese(IV) Oxide

YURI A. GORBY,*,† FRANK CACCAVO, JR.,‡ AND HARVEY BOLTON, JR.†

Environmental Microbiology Group, Pacific Northwest National Laboratory, Richland, Washington 99352, and Department Microbiology, University of New Hampshire, Durham, New Hampshire 03824

Codisposal of $^{60}Co^{2+}$ and EDTA has promoted the transport of radioactive ^{60}Co in the environment as ^{60}CoEDTA complexes. Chemical oxidation of Co^{II}EDTA^{2-} to highly stable and mobile Co^{III}EDTA$^-$ by manganese(VI) oxide minerals can occur under aerobic and anaerobic conditions. Reduction of Co(III) to Co(II) decreases the stability of the radionuclide−chelate complex and can limit the transport of the ^{60}Co in subsurface environments. This study investigated the microbial reduction of Co^{III}EDTA$^-$ in the presence and absence of reactive manganese(IV) oxides. The metal-reducing bacterium *Shewanella alga* strain BrY enzymatically reduced Co^{III}EDTA$^-$ to Co^{II}EDTA^{2-} with a 1:1 stoichiometry. Reduction of Co^{III}EDTA$^-$ was not affected by radioactive ^{60}Co^{III}EDTA$^-$ at concentrations exceeding those recorded in contaminated environments. Bacterial reduction of Co^{III}EDTA$^-$ could be coupled to the chemical oxidation of Co^{II}EDTA^{2-} by the manganese(IV) oxide mineral pyrolusite, resulting in biotic-abiotic cycling between Co^{II}EDTA^{2-} and Co^{III}EDTA$^-$. Co^{III}EDTA$^-$ significantly increased the rate and extent of manganese(IV) oxide reduction in the presence of metal reducing bacteria, and the Co^{II}EDTA^{2-} complex did not dissociate in these anoxic studies. Direct reduction of Co^{III}EDTA$^-$ by microorganisms and geochemical oxidation of Co^{II}EDTA^{2-} by manganese-(IV) oxides are important components of a complex set of coupled microbial and geochemical reactions that may influence the fate and transport of ^{60}Co^{III}EDTA^{2-} in the environment.

Introduction

Radioactive ^{60}Co is a significant constituent of nuclear waste and has been transported in ground water at selected sites through intentional or accidental corelease with synthetic organic chelating agents, such as EDTA (*1*, *2*). Chelated forms of Co are generally more mobile than unchelated forms in saturated subsurface sediments (*3*), and the stability of CoEDTA complexes is directly related to the oxidation state of the metal. Co^{III}EDTA$^-$ is over 25 orders of magnitude more thermodynamically stable than Co^{II}EDTA^{2-} (*4*), Co^{III}EDTA^{2-} is kinetically inert to exchanging Co with other metals in solution (*5*), and Co^{III}EDTA$^-$ is highly mobile in saturated subsurface sediments (*6*). In comparison, Co^{II}EDTA^{2-} can dissociate in the presence of cationic metals (e.g., Al or Fe) at pH values below 6.5, and the displaced Co(II) can become immobilized in the presence of oxide minerals (*7*, *8*). Manganese(IV) and iron(III) oxide minerals can oxidize Co^{II}EDTA^{2-} to Co^{III}EDTA$^-$, thereby enhancing the solubility and mobility of ^{60}Co (*9*, *10*). Thus, the oxidation and reduction of Co in the EDTA complex directly influences the stability and mobility of ^{60}Co near waste disposal sites.

Most of the research concerning the fate of ^{60}Co has focused on geochemical processes affecting its migration (*2*, *6*, *7*, *9*−*12*). However, biological reactions influencing the fate and transport of this and other chelated radionuclides are receiving increased attention. Recently, the enzymatic reduction of Co^{III}EDTA$^-$ by the Fe(III)-reducing bacterium *Geobacter sulfurreducens* was reported (*13*). This bacterium coupled the oxidation of acetate to the reduction of Co^{III}EDTA$^-$ in an anoxic, aqueous medium without metal oxide minerals. The product of the reaction was not identified, although the authors assumed that Co^{III}EDTA$^-$ was reduced to Co^{II}EDTA^{2-}.

In the study presented here, we report the stoichiometric reduction of Co^{III}EDTA$^-$ to Co^{II}EDTA^{2-} by *Shewanella alga*. Microbial and abiotic oxidation-reduction reactions were investigated in systems containing this metal-reducing bacterium and pyrolusite, a reactive manganese(IV) oxide mineral that can oxidize Co^{II}EDTA^{2-} to Co^{III}EDTA$^-$ (*9*). This work expands our understanding of coupled microbial and geochemical reactions that may influence the fate and migration of ^{60}Co in the environment.

Materials and Methods

Growth Conditions. *S. alga* strain BrY was grown aerobically in tryptic soy broth for 16 h on a rotary shaker (100 rpm). Cells were harvested by centrifugation (6000*g*, 4 °C) and washed three times with 10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (pH, 7.0), which was previously made anoxic by bubbling with O_2-free N_2 gas. Washed cell suspensions were stored at 4 °C and used within 30 min.

Growth of *S. alga* with Co^{III}EDTA$^-$ as the terminal electron acceptor was demonstrated by transferring cultures five times in an anaerobic, chemically defined medium (*14*) in which 0.5 mM Co^{III}EDTA$^-$ replaced ferric citrate as the sole terminal electron acceptor. Co^{III}EDTA$^-$ was prepared by the method of Dwyer et al. (*15*). After the fifth transfer, the increase in cell numbers was determined by staining and counting cells as described by Lovley et al.(*14*). The concentration of Co^{III}EDTA$^-$ was monitored spectrophotometrically at 535 nm.

Enzymatic Reduction of Co^{III}EDTA$^-$ and Mn(IV) by Cell Suspensions. The stoichiometric reduction of Co^{III}EDTA$^-$ to Co^{II}EDTA^{2-} was followed with washed cells of *S. alga* injected into sealed tubes containing 30 mM sodium bicarbonate, 20 mM sodium lactate, and 50 μM Co^{III}EDTA$^-$, which was made anoxic by bubbling with a mixture of N_2:CO_2 (80:20). Negative controls lacked either the electron donor or the cells. All suspensions were incubated at 30 °C. The concentration of Co^{III}EDTA$^-$ and Co^{II}EDTA^{2-} in filtered samples was determined by ion chromatography using a Dionex 500 fitted with a AS11 anion exchange column and a AD20 adsorption detector.

The enzymatic reduction of ^{60}Co^{III}EDTA$^-$ was determined to ensure that microbial activity can influence the oxidation state of ^{60}Co. A mixture of ^{59}Co^{III}- and ^{60}Co^{III}EDTA$^-$ was

* Corresponding author, Pacific Northwest National Laboratory, Mailstop P7-54, P.O. Box 999, Richland, WA 99352. Fax: (509) 375-6666; email: ya_gorby@ pnl.gov.
† Environmental Microbiology Group.
‡ University of New Hampshire.

S0013-936X(97)00516-6 CCC: $15.00
Published on Web 01/15/1998

synthesized by the following method. $Co^{II}EDTA^{2-}$ was prepared by adding 5 mL of 1 mM disodium EDTA to 5 mL of 1 mM $CoCl_2$ containing 0.2 MBq ^{60}Co mL^{-1}. The solutions were buffered to pH 7.0 with 10 mM PIPES. Manganese oxide, prepared as previously described (*16*), was added to the $Co^{II}EDTA^{2-}$ solution and served as an oxidizing agent at 1.5 mmol L^{-1}. The mixture was filtered after 24 h to remove any remaining manganese precipitate. The filtrate, containing 0.5 mM $Co^{III}EDTA^-$ [0. 1 MBq mL^{-1} $^{60}Co^{III}EDTA^-$], was made anoxic by bubbling for 5 min with O_2-free N_2 gas and sealed in Balch tubes fitted with thick butyl rubber stoppers and aluminum crimp seals. Washed cells of *S. alga* were added to the anaerobic filtrate and 10 mL of 100% H_2 gas, which served as the electron donor, was added to the tubes through the rubber stopper using a needle and syringe. Loss of $Co^{III}EDTA^-$ in filtered samples was measured spectrophotometrically at 535 nm.

Enzymatic reduction of the manganese(IV) oxide pyrolusite was investigated by injecting washed cells of *S. alga* into sealed vials containing an anoxic solution of 30 mM bicarbonate buffer, 20 mM sodium lactate, and 0.67 g of pyrolusite-coated sand prepared by the method of Jardine et al. (*9*). This amount of sand provided 10 mmol of Mn(IV) L^{-1} of medium. The final concentration of cells in these reaction vessels was 8.2×10^7 cells mL^{-1}. The concentration of Mn(II) was determined by first acidifying the contents of reaction vials with 0.4 mL of concentrated HCl and shaking the stoppered vials for 1 min. This procedure, which was slightly modified from one described by Lovley and Phillips (*14*), liberated adsorbed Mn(II) from the oxide surface. The preparation was then filtered, and 0.25 mL of the filtrate was added to 2.5 mL of 0.1 N HCl. The acidified filtrate received 0.25 mL of the colorimetric formaldoxamine-ammonium hydroxide reagent described by Armstrong et al. (*17*), and the absorbance was read at 450 nm. $MnCl_2$ dissolved in 0.5 N HCl served as the standard.

Coupled Geochemical/Microbiological Reactions. The hypothesis that CoEDTA could support growth of metal reducing bacteria by serving as an electron shuttle between cells and manganese(IV) oxide was tested with *S. alga*. Cells were added to three sets of tubes containing 0.5 mM $Co^{III}EDTA^-$ anaerobic growth medium. Two sets of tubes received lactate as the electron donor. One set of lactate tubes contained a sealed dialysis bag (MWCO 14 KD, Spectrum Medical Industries, Houston), which was filled with a Mn-oxide mineral (MnO_2) produced by the method of Lovley (*16*). The other lactate tubes contained no MnO_2. A third set of tubes served as negative controls and contained MnO_2 in dialysis tubing but no lactate. Growth of *S. alga* was monitored by direct cell count.

The relationship between coupled chemical and enzymatic transformation of Co and Mn was examined in anoxic, batch systems. A series of vials received 10 mL of a solution containing 30 mM sodium bicarbonate, 20 mM sodium lactate, and 50 μM $Co^{III}EDTA^-$. Each vial also received 0.67 g of pyrolusite-coated sand, which provided 10 mmol of Mn-(IV) L^{-1} of medium. Washed cells of strain BrY were added to each vial with a final concentration of 8.2×10^7 cells mL^{-1}. The concentrations of $Co^{II}EDTA^{2-}$ and $Co^{III}EDTA^-$ were monitored by ion chromatography and the concentration of Mn(II) was quantified as described earlier.

Chemical Oxidation of $Co^{II}EDTA^{2-}$ by Manganese(IV) Oxide. The rate of chemical oxidation of $Co^{II}EDTA^{2-}$ by manganese(IV) oxide was determined by adding 10 mL of an anoxic solution of 50 μM $Co^{II}EDTA^{2-}$, 20 mM lactate, and 30 mM sodium bicarbonate buffer (pH 7.0) through the stoppers of vials that contained 0.67 grams of pyrolusite-coated sand under a N_2:CO_2 atmosphere. Vials were sacrificed at specified times by passing the liquid phase through a 0.2 syringe filter to remove the reactive mineral and the concentration of $Co^{II}EDTA^{2-}$ and $Co^{III}EDTA^-$ in the filtrate was monitored by ion chromatography.

Experiments designed to determine the impact of Mn(II) on the oxidation of $Co^{II}EDTA^{2-}$ by Mn(IV) used 5 mL of an anoxic solution of 2 mM $MnCl_2$, 30 mM sodium bicarbonate, and 20 mM sodium lactate added to headspace vials containing 0.67 g of pyrolusite-coated sand under a N_2:CO_2 atmosphere. The stoppered vials were shaken for 12 h to allow for equilibration of Mn(II) onto the mineral surface. Each vial then received 5 mL of an anoxic solution of 100 μM $Co^{II}EDTA^{2-}$, 30 mM sodium bicarbonate, and 20 mM sodium lactate. A similar system was used to determine the influence of Mn(II) on the fate of CoEDTA complexes in the presence of metal-reducing bacteria.

Results and Discussion

$Co^{III}EDTA^-$ Reduction by Cell Suspensions. The dissimilatory metal-reducing bacterium *S. alga* reduced $Co^{III}EDTA^-$ to $Co^{II}EDTA^{2-}$ in aqueous suspensions with nearly 1:1 stoichiometry (Figure 1). This zero order enzymatic reaction proceeded at a rate of 4.3 μmol L^{-1} min^{-1}, with a specific activity of 89 μmol min^{-1} mg^{-1} protein. Enzymatic reduction of $Co^{III}EDTA^-$ by *S. alga* was previously reported (*18*), but the products of the reduction were not determined. The results presented here clearly demonstrate that $Co^{III}EDTA^-$ was quantitatively reduced to $Co^{II}EDTA^{2-}$ and that the reduced Co(II) did not dissociate from the EDTA in aqueous solutions within the time frame of these studies (hours).

The hypothesis that microbial activity can influence the oxidation state of ^{60}Co was evaluated by measuring $Co^{III}EDTA^-$ reduction in a mixture of $^{59}Co^{III}EDTA^-$ and $^{60}Co^{III}EDTA^-$. Anaerobic cell suspensions containing 10^7 cells mL^{-1} of *S. alga* reduced 0.5 mM ^{59}Co/$^{60}Co^{III}EDTA^-$ (0.1 MBq/mL) to below detection limits (10^{-4} M) in less than 200 min (Figure 2). This concentration of ^{60}Co is over 10-fold higher than the concentration detected in any contaminated groundwater to date (*2*). The results indicate that short-term exposure of metal-reducing bacteria to γ-emitting ^{60}Co does not inhibit their ability to reduce $^{60}Co^{III}EDTA^-$ and that this metabolism could catalyze valence transformation of radioactive metal–ligand complexes in the environment.

Growth and $Co^{III}EDTA^-$ Reduction. *S. alga* gained energy to support anaerobic growth with $Co^{III}EDTA^-$ as the sole terminal electron acceptor. Cell numbers increased with a concomitant decrease in the concentration of $Co^{III}EDTA^-$ (Figure 3). Cell growth ceased after all the $Co^{III}EDTA^-$ had been reduced. Growth of cells or reduction of $Co^{III}EDTA^-$ was not observed in controls that lacked an electron donor. These results are consistent with electrochemical data generated from cyclic voltammetry experiments (*4*) showing that the redox potential for $Co^{III}EDTA/Co^{II}EDTA^{2-}$ ($E^{o\prime} = 129$ mV) is great enough to provide energy to support microbial growth with acetate, lactate, or H_2 as an electron donor. Growth of the dissimilatory Fe(III)-reducing bacterium *Geobacter sulfurreducens* with $Co^{III}EDTA^-$ as the sole terminal electron acceptor has also been reported (*13*). The results presented here suggest that this metabolism may be common among Fe(III)-reducing bacteria.

Concentrations of $Co^{III}EDTA^-$ in contaminated environments are typically 3 orders of magnitude lower than those used in these experiments and are almost certainly too low to support significant increases of microbial biomass except, possibly, near points of disposal. However, some subsurface environments contain highly reactive manganese(IV) oxide minerals that could resupply $Co^{III}EDTA^-$ to existing microbial populations through surface-catalyzed oxidation of $Co^{II}EDTA^{2-}$ (*9*). To determine whether geochemical regeneration of $Co^{III}EDTA^-$ by manganese(IV) oxide could support growth of bacteria, cells of *S. alga* were suspended in a buffered $Co^{III}EDTA^-$ growth medium and were separated

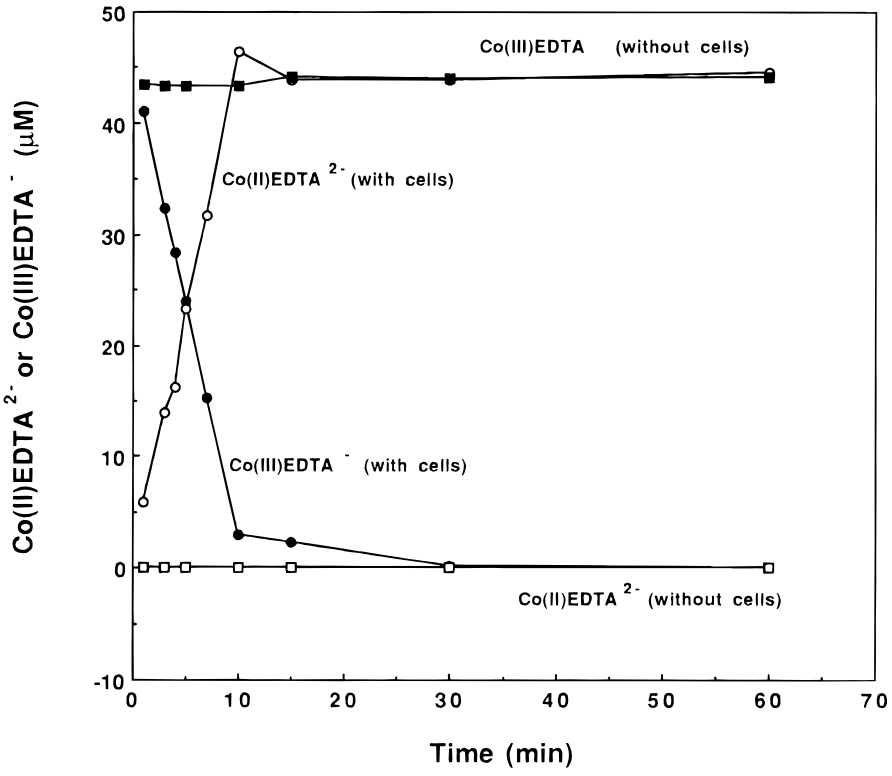

FIGURE 1. Reduction of 50 μM $Co^{III}EDTA^-$ by *S. alga.* No $Co^{III}EDTA^-$ was reduced in the absence of electron donors.

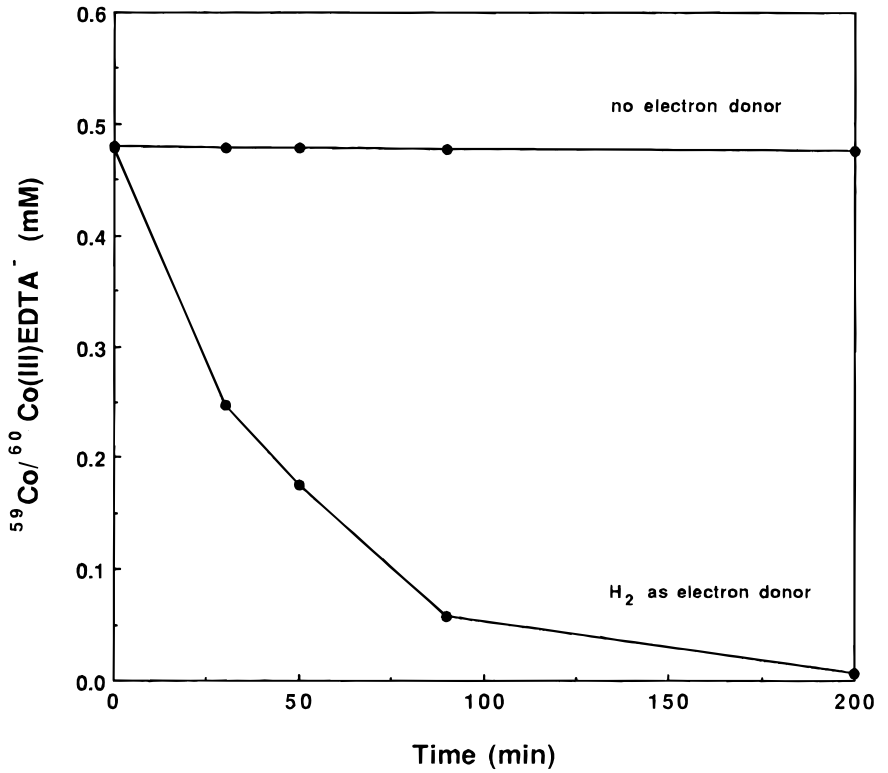

FIGURE 2. Reduction of 0.5 mM $^{59}Co/^{60}Co^{III}EDTA^-$ by *S. alga.* No $Co^{III}EDTA^-$ was reduced in uninoculated controls or inoculated controls lacking H_2.

from a poorly crystalline manganese(IV) oxide mineral by a semipermeable dialysis membrane. Soluble $Co^{III}EDTA^-$ could thus serve as a transmembrane electron shuttle between the bacteria and the insoluble manganese(IV) oxide. As shown in Figure 4, *S. alga* grew in the $Co^{III}EDTA^-$ medium with no manganese and exhibited a growth curve typical of batch cultures described in Figure 3. Growth ceased, and the number of cells declined after $Co^{III}EDTA^-$ was no longer detectable. In tubes containing MnO_2 in dialysis tubing, the number of cells outside of the tubing increased to values greater than those from cultures without MnO_2. As cell numbers increased, the black MnO_2 was reduced to a white precipitate, presumably the Mn carbonate mineral rhodochrosite, that remained within the dialysis tubing. No growth

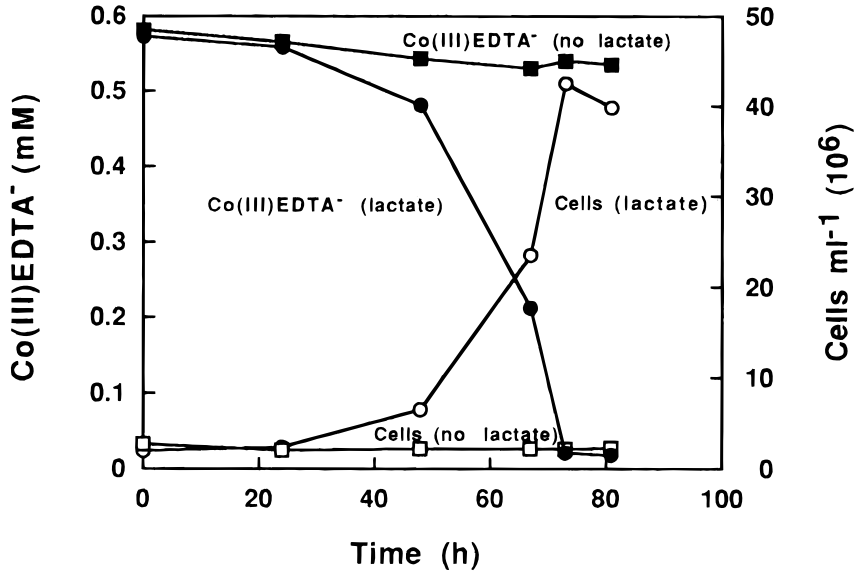

FIGURE 3. Growth of *S. alga* with 0.5 mM $Co^{III}EDTA^{-}$ as sole electron acceptor in an anaerobic, chemically defined medium.

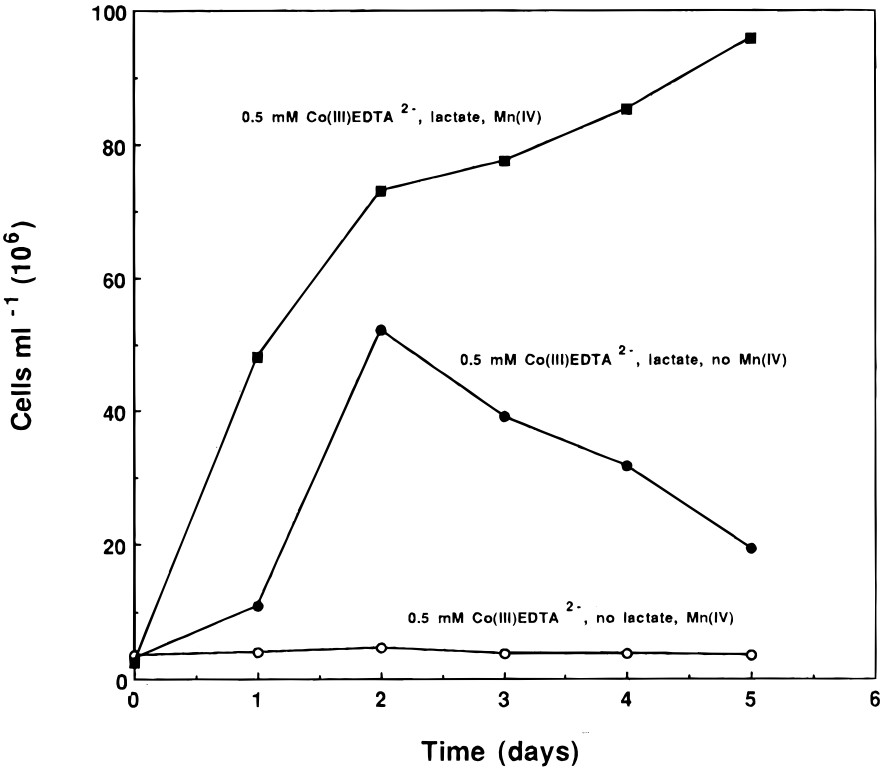

FIGURE 4. Growth of *S. alga* with 50 μM CoEDTA serving as an electron shuttle between cells and 10 mmol L^{-1} manganese(IV) oxide.

was detected and no MnO_2 was reduced in control tubes that contained no electron donor.

Dissimilatory metal-reducing bacteria can gain energy for growth using manganese(IV) oxide minerals as the sole terminal electron acceptors (*19*−*21*), but this metabolism is believed to require direct cell contact with the mineral surface. The results presented here suggest that soluble CoEDTA complexes can serve as electron shuttles between metal reducing bacteria and Mn(IV) minerals. Because such a system may be important for the reduction of Mn(IV) and for the fate of ^{60}Co in anoxic environments contaminated with ^{60}CoEDTA, these coupled geochemical/microbiological reactions were examined in greater detail.

Coupled Geochemical/Microbiological Reactions. Manganese(IV) oxides and, to a lesser extent, iron(III)hydroxide minerals can catalyze the oxidation of $Co^{II}EDTA^{2-}$ to $Co^{III}EDTA^{-}$ under anoxic conditions (*9, 10*). Such reactions would theoretically compete with microbial reduction reactions and serve to maintain the CoEDTA complex in the relatively stable Co(III) form under metal-reducing conditions, assuming no dissolution and formation of FeEDTA or MnEDTA. The effect of pyrolusite on the fate of CoEDTA complexes in the presence and absence of metal-reducing bacteria is presented in Figure 5. Pyrolusite oxidized $Co^{II}EDTA^{2-}$ to $Co^{III}EDTA^{-}$ in the absence of metal-reducing bacteria. The initial rate of this reaction, 22 μmol L^{-1} min^{-1}, was significantly faster than the rate of enzymatic reduction of $Co^{III}EDTA^{-}$ (4.3 μmol L^{-1} min^{-1}) in the absence of pyrolusite. When both bacteria and pyrolusite were present, less than 10% of the initial concentration of $Co^{III}EDTA^{-}$ was

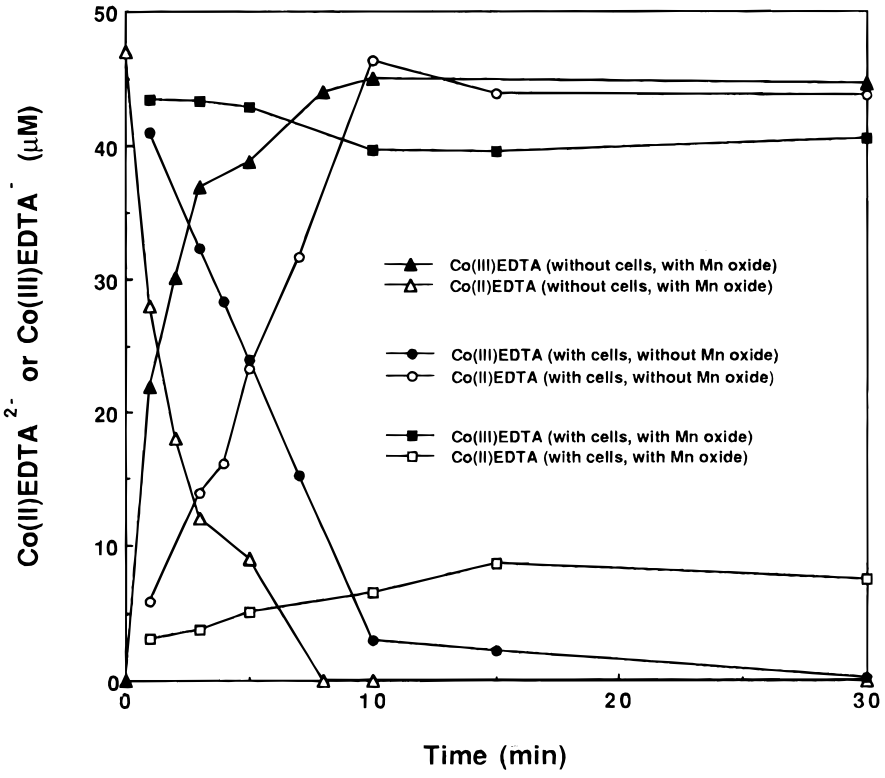

FIGURE 5. Chemical oxidation of $Co^{II}EDTA^{2-}$ to $Co^{III}EDTA^{-}$ by pyrolusute and enzymatic reduction of 50 μM $Co^{III}EDTA^{-}$ by *S. alga* in the presence and absence of pyrolusite.

reduced to $Co^{II}EDTA^{2-}$. These results are consistent with the hypothesis that chemical oxidation of $Co^{II}EDTA^{2-}$ by Mn oxide can compete with microbial reduction of $Co^{III}EDTA^{-}$.

The following set of reactions are pertinent to the present study.

Microbiological

$$Co^{III}EDTA^{-} + e^{-} \rightarrow Co^{II}EDTA^{2-} \quad (1)$$

$$Mn(IV) + e^{-} \rightarrow Mn(III) \quad (2)$$

$$Mn(IV) + 2e^{-} \rightarrow Mn(II) \quad (3)$$

$$Mn(III) + e^{-} \rightarrow Mn(II) \quad (4)$$

Chemical

$$Mn(IV) + 2Co^{II}EDTA^{2-} \rightarrow Mn(II)/Mn(III) + 2Co^{III}EDTA^{-} \quad (5)$$

$$Mn(III) + Co^{II}EDTA^{2-} \rightarrow Mn(II) + Co^{III}EDTA^{-} \quad (6)$$

$$Mn(III) + Co^{III}EDTA^{-} \rightarrow Mn^{III}EDTA^{-} + Co(III) \quad (7)$$

The rates and products of reduction reactions 1−6 are important for interpreting the results of coupled geochemical-microbiological reactions involving CoEDTA and manganese-(IV) oxides. Exchange reaction 7 is not believed to because the log *K* for complex formation with Co(III) is much higher than for Mn(III), and $Co^{II}EDTA^{2-}$ and $Co^{III}EDTA^{-}$ exhibited mass balance in our experiments.

As discussed above, *S. alga* reduced $Co^{III}EDTA^{-}$ to $Co^{II}EDTA^{2-}$ with an initial rate of 4.3 μmol L^{-1} min^{-1}, in the absence of pyrolusite, and pyrolusite oxidized $Co^{II}EDTA^{2-}$ to $Co^{III}EDTA^{-}$ with a rate of 22 μmol L^{-1} min^{-1}. *S. alga* reduced the manganese(IV) oxide mineral pyrolusite to Mn(II) at an initial rate of 0.19 μmol L^{-1} min^{-1} in the absence of $Co^{III}EDTA^{-}$ (Figure 6). Reduction of Mn(IV) was significantly faster (0.98 μmol L^{-1} min^{-1}) when $Co^{III}EDTA^{-}$ was included in the reaction mixture (Figure 6). Presumably, this rate increase was directly related to the chemical reduction of manganese-(IV) oxide by $Co^{II}EDTA^{2-}$, which resulted from the microbial reduction of $Co^{III}EDTA^{-}$. This biotic−abiotic cycle is conceptualized in Figure 7 and is consistent with the results of the growth experiment reported in Figure 4.

The presence of $Co^{III}EDTA^{-}$ increased not only the rate but the extent of reduction of the Mn(VI) oxide (Figure 6). Theoretically, coupled geochemical and microbiological reactions would continue until all of the Mn(IV) was reduced. However, only about 17% of the 10 mmol L^{-1} Mn(IV) was reduced to Mn(II). Of this amount, no more than 0.3 mM was soluble, with the remaining Mn(II) either adsorbing to the surface of the oxide or cell surfaces or precipitating as the Mn carbonate mineral rhodochrosite. Mn(II) may play an important role in limiting the amount of Mn(IV) that can be reduced by the biotic-abiotic cycling.

Effects of Mn(II). Roden et al. (*22*) recently demonstrated that adsorbed Fe(II)-limited enzymatic reduction of Fe(III) minerals, presumably by blocking reactive surface sites on the Fe(III) minerals. To determine whether a similar phenomenon could limit the amount of manganese(IV) oxide that was reduced in the present study, Mn(II) was preadsorbed to manganese oxide before adding $Co^{III}EDTA^{-}$, *S. alga*, and sodium lactate as the electron donor. Of the 1 mM $MnCl_2$ that was added, about 0.25 mM Mn(II) associated with the solid phase during a preincubation period of 48 h. The remaining 0.75 mM remained in solution and could be separated from the solid phase by filtration before the addition of cells. At 1 mM, Mn(II) completely inhibited the reduction of Mn oxide by *S. alga* with $Co^{III}EDTA^{-}$ (data not shown for brevity). This concentration of Mn(II) also eliminated the effect of pyrolusite on the enzymatic reduction of $Co^{III}EDTA^{-}$ described in Figure 5. That is to say, bacteria completely reduced $Co^{III}EDTA^{-}$ to $Co^{II}EDTA^{2-}$ in the presence of Mn(II)-coated pyrolusite with a rate that was identical to that obtained from cells in the absence of pyrolusite. Jardine

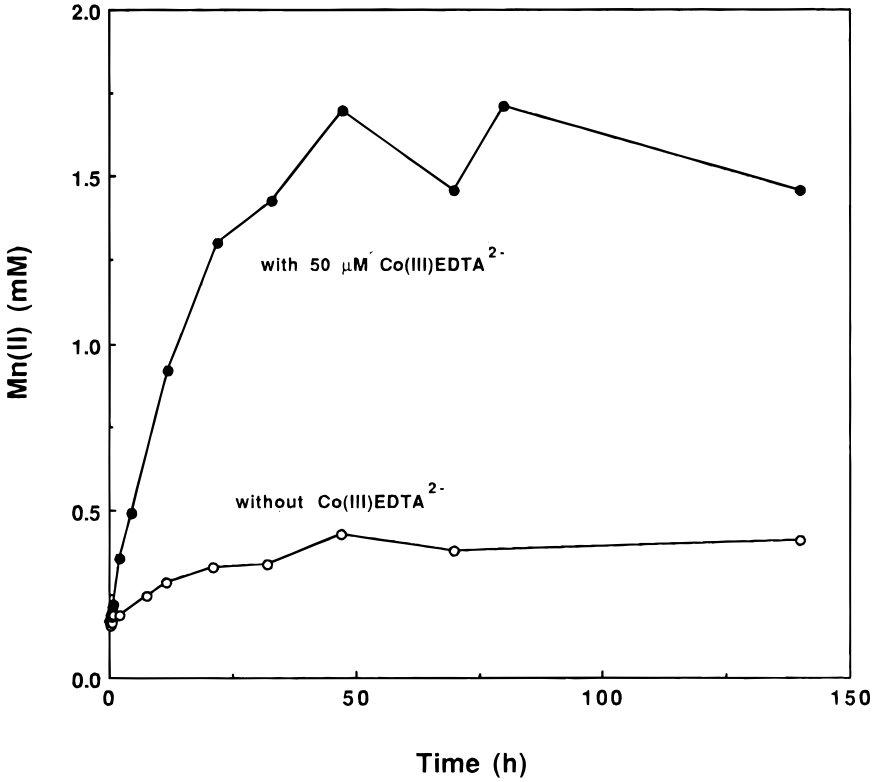

FIGURE 6. Reduction of 10 mmol L^{-1} manganese(IV) oxide to Mn(II) by *S. alga* in the presence and absence of 50 μM $Co^{III}EDTA^{-}$.

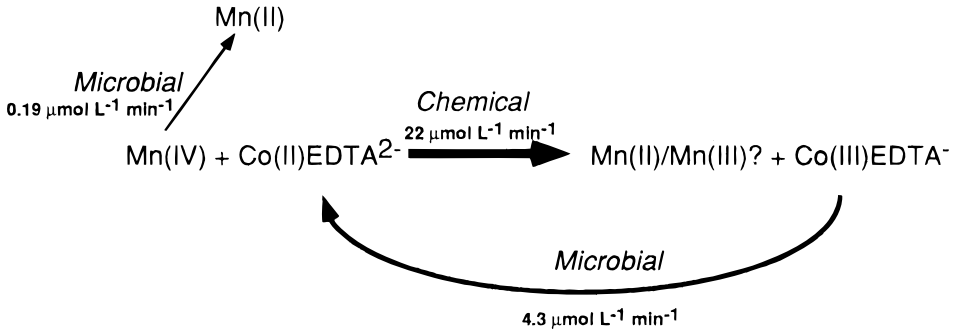

FIGURE 7. Illustration of coupled geochemical/microbiological reactions. Relative rates of reactions are depicted by thickness of the arrows and experimentally derived rates of the reactions are presented next to each arrow.

and Taylor demonstrated that pyrolusite lost the capacity to chemically oxidize $Co^{II}EDTA^{2-}$ with extended incubation (*9*). The authors proposed that a nonreactive manganese(III) oxide mineral formed on the surface of the pyrolusite in their unbuffered system, although this hypothesis has not been critically evaluated. In our system, redox reactions between sorbed Mn(II) and manganese(VI) oxide may have generated Mn(III), thus forming a "reworked" surface that was chemically inert. Alternatively, rhodochrosite may have precipitated from the bicarbonate buffered solution and coated the mineral surface.

Environmental Implications. Understanding coupled geochemical and microbiological processes in relatively simple systems is the first step toward being able to predict the fate of multivalent metal and radionuclide contaminants in the environment. The results of this study illustrate that microbial reduction of $Co^{III}EDTA^{-}$ may have important implications for the fate of ^{60}Co in the environment. This reaction converts highly stable $^{60}Co^{III}EDTA^{-}$ ($\log K = 44$), to relatively unstable $Co^{II}EDTA^{2-}$ ($\log K = 17$) (*4*). $^{60}Co^{III}EDTA^{-}$ is mobile in most subsurface sediments and is significantly less likely to undergo exchange reactions with solid phase Fe(III). However, exchange reactions between relatively unstable $^{60}Co^{II}EDTA^{2-}$ and solid phase Fe(III) can form soluble Fe(III)EDTA ($\log K = 27.6$) (*12*) and free ^{60}Co(II) that interacts with negatively charged minerals and would be immobile in sediments containing these minerals (*23*).

Microbial reduction of $Co^{III}EDTA^{-}$ could also influence the geochemistry of subsurface and sedimentary environments. In soil pore water, manganese(IV) oxides can be reduced and mobilized by $Co^{II}EDTA^{2-}$ (*24*). As demonstrated in the dialysis experiment, microbial reduction of $Co^{III}EDTA^{-}$ can enzymatically resupply $Co^{II}EDTA^{2-}$ and perpetuate this reaction. Of course, geochemical reactions involving Co^{III}/$Co^{II}EDTA^{2-}$ become increasingly complex in subsurface sediments containing mixtures of iron and manganese oxides and possibly other reactive minerals.

Acknowledgments

This research was supported by the Subsurface Science Program, Office of Health and Environmental Research, U.S. Department of Health and Environmental Research, U.S. Department of Energy (DOE). The continued support of Dr. Frank Wobber is greatly appreciated. We thank Jim Fredrickson and four anonymous reviewers for their insightful

and contructive comments. Pacific Northwest National Laboratory is operated for the DOE by Battelle Memorial Institute under contract DE-AC06-76RLO 1830.

Literature Cited

(1) Riley, R. G.; Zachara, J. M. *Chemical Contaminants on DOE Lands and Selection of Contaminant Mixtures for Subsurface Science Research;* U.S. Department of Energy, 1992.
(2) Killey, R. W. D.; McHugh, J. O.; Champ, D. R.; Cooper, E. L.; Young, J. L. *Environ. Sci. Technol.* **1984**, *18*, 148−157.
(3) Means, J. L.; Crerar, D. A.; Borcsik, M. P.; Duguid, M. P. *Nature* **1978**, *274*, 44−47.
(4) Ogino, H.; Ogino, K. *Inorg. Chem*. **1983**, *22*, 2208−2211.
(5) Margerum, D. W.; Cayley, G. R.; Weatherburn, D. C.; Pagenkopf, G. K. In *Coordination Chemistry*; Martell, A. E., Ed.; American Chemical Society: Washington, DC, 1978.
(6) Olsen, C. R.; Lowry, P. D.; Lee, S. Y.; Larsen, I. L.; Cutshall, N. H. *Geochim. Cosmochim. Acta* **1986**, *50*, 593−607.
(7) Girvin, D. C.; Gassman, P. L.; Bolton, H. *Soil Sci. Soc. Am. J.* **1993**, *57* (1), 47−57.
(8) Szecsody, J. E.; Zachara, J. M.; Bruckhart, P. L. *Environ. Sci. Technol.* **1994**, *28*, 1706−1716.
(9) Jardine, P. M.; Taylor, D. L. *Geochim. Cosmochim. Acta* **1995**, *59*, 4193−4203.
(10) Brooks, S. C.; Taylor, D. L.; Jardine, P. M. *Geochim. Cosmochim. Acta* **1996**, *60* (11), 1899−1908.
(11) Means, J. L.; Crerar, D. A.; Borcsik, M. P.; Duguid, J. O. *Geochim. Cosmochim. Acta* **1978**, *42*, 1763−1773.
(12) Zachara, J. M.; Smith, S. C.; Kuzel, L. S. *Geochim. Cosmochem. Acta* **1995**, *59* (23), 4825−4844.
(13) Caccavo, J. F.; Lonergan, D. J.; Lovley, D. R.; Davis, M.; Stolz, J. F.; McInerney, M. J. *Appl. Environ. Microbiol.* **1994**, *60*, 3752−3759.
(14) Lovley, D. R.; Phillips, E. J. P. *Appl. Environ. Microbiol.* **1988**, *54*, 1472−1480.
(15) Dwyer, F. P.; Gyarfas, E. C.; Mellor, D. P. *J. Phys. Chem*. **1955**, *59*, 296−297.
(16) Lovley, D. R.; Phillips, E. J. P. *Geomicrobiol. J.* **1988**, *6*, 145−155.
(17) Armstrong, P. A.; Lyons, W. B.; Gaudette, H. E. *Estuaries* **1970**, *2*, 198−201.
(18) Rossello-Mora, R.; Caccavo, J. F.; Springer, N.; Spring, S.; Osterlehner, K.; Shuler, W.; Ludwig, R.; Amann, R.; Schleifer, K. H. *Syst. Appl. Microbiol.* **1994**, *18*, 569−573.
(19) Myers, C. R.; Nealson, K. H. *Science* **1988**, *240*, 1319−1321.
(20) Lovley, D. R.; Phillips, E. J. P.; Lonergan, D. J. *Appl. Environ. Microbiol.* **1989**, *55* (3), 700−706.
(21) Nealson, K. H.; Myers, C. R. *Appl. Environ. Microbiol.* **1992**, *58* (2), 439−443.
(22) Roden, E. E.; Zachara, J. M. *Environ. Sci. Tech*. **1996**, *30*, 1618−1628.
(23) Swanson, J. L. Technical Report PNL-3927, UC-70; Pacific Northwest Laboratories: Richland, Washington, 1982.
(24) Jardine, P. M.; Jacobs, G. K.; Wilson, G. V. *Soil Sci. Soc. Am. J.* **1993**, *57*, 954−962.

Received for review June 11, 1997. Revised manuscript received October 17, 1997. Accepted October 20, 1997.[⊗]

ES970516R

[⊗] Abstract published in *Advance ACS Abstracts,* December 1, 1997.