Dechlorination of Carbon Tetrachloride by Fe(II) Associated with Goethite

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Carbon tetrachloride (CT) was dechlorinated to chloroform (CF) under anoxic conditions by Fe(II) that was sorbed to the surface of goethite (α-FeOOH). No reaction occurred when Fe(II) was present and goethite was absent. Several abiotic experiments were conducted with goethite at 30 °C in which the total amount of Fe(II) in the system, the amount of sorbed Fe(II), the density of sorbed Fe(II), and the pH were varied. Regeneration of sorbed Fe(II) occurred when dissolved Fe^{2+} was available and maintained pseudo-first-order conditions with respect to CT. Analysis of the rates of CT loss for experiments with sorbed-Fe(II) regeneration showed the rate-determining-step to be first order with respect to CT, second order with respect to the *volumetric* concentration of sorbed Fe(II) (i.e., mmol sorbed Fe(II) L^{-1} suspension), and zero order with respect to H^+ for pH between 4.2 and 7.3. The absolute rate constant for the reaction was determined to be 42 ± 5 M^{-2} s^{-1}. Normalization of the observed rate constants to account for different goethite concentrations yielded reaction orders of one and zero, respectively, for CT and H^+, and a second-order reaction with respect to the *density* of sorbed Fe(II) (i.e., mmol sorbed Fe(II) g^{-1} goethite). On the basis of the kinetic data, the rate-determining step is proposed to be a termolecular two-electron-transfer reaction involving two Fe^{2+} ions sorbed to adjacent sites on the goethite surface and a CCl_4 molecule that approaches the surface. The primary role of the goethite surface, thus, is to catalyze the reaction by fixing the position of the two charged reactants in a geometry suitable for reaction with CT. In separate experiments, biogenic Fe(II) formed by the enzymatic reduction of goethite by the Fe(III)-reducing bacterium *Shewanella alga*, strain BrY, dechlorinated CT. Of the CT degraded by abiotic and biogenic Fe(II) on goethite, 83–90% was converted to chloroform (CF), which accumulated in the reaction vial. These results indicate that dechlorination reactions in Fe(III)-reducing environments may indirectly result from the enzymatic or chemical reduction of Fe(III)-bearing minerals such as goethite.

Introduction

Release of chlorinated aliphatic hydrocarbons to sediments and groundwater is a widespread environmental problem.

Reductive dehalogenation of carbon tetrachloride (CT) and other halogenated hydrocarbons has been reported in a number of anoxic sediments and groundwaters (1–4). Degradation of CT in these environments has been attributed both to biotic reactions involving the enzymatic activity of microorganisms (5–7) and to abiotic, geochemical reactions involving reduced, inorganic compounds such as sulfide or Fe(II) (8–10). It is likely that degradation occurs through a combination of both chemical and biological processes. Many of the reactive chemical species identified as potential chemical reductants, including sulfide and Fe(II), directly or indirectly result from the enzymatic activity of anaerobic bacteria.

Ferrous iron, a common component of anoxic environments, primarily results from the enzymatic activity of dissimilatory Fe-reducing bacteria (11). These bacteria can reduce a wide range of Fe minerals, including poorly crystalline Fe oxyhydroxides (12), crystalline goethite, hematite, and magnetite (13, 14), and Fe bound in layer silicates (15). Magnetite is a mixed-valence Fe mineral that can be produced by the enzymatic reduction of poorly crystalline Fe oxyhydroxides (12). Heijman et al. (16) demonstrated that biogenic magnetite reduced 4-chloronitrobenzene to 4-chloroaniline in a biologically active system. Subsequently, reduction of nitroaromatic compounds was demonstrated in an abiotic system containing Fe(II) sorbed to the magnetite surfaces (17). Recently, several mineral surfaces containing adsorbed Fe(II) were reported to reduce a variety of substituted nitroaromatic compounds to amines (18).

In this work, we examine the factors controlling the rates of CT dechlorination under abiotic conditions by Fe(II) sorbed to the surface of goethite. On the basis of the rate data we propose a termolecular, two-electron-transfer reaction, involving two adjacent sorbed-Fe(II) atoms and the CT molecule, as the rate-controlling step in the dechlorination of CT to chloroform (CF). We also demonstrate reductive dechlorination of CT by biogenically derived Fe(II) sorbed to the surface of goethite. This result provides strong evidence that nondechlorinating microorganisms can, through the production of reactive forms of Fe(II) as part of their natural metabolic activity, contribute to the transformation of CT in Fe-bearing sediments.

Materials and Methods

Chemicals and Stock Solutions. Tryptic soy broth without dextrose (TSB) served as a growth medium for aerobic cultures and was obtained from DIFCO Laboratories, Detroit, MI. The CT, CF, methylene chloride, and chloromethane were obtained as purified liquids (99.9%) from J. T. Baker Co., Phillipsburg, NJ. Stock solutions, nominally 100 mM in Fe(II), were prepared by adding $FeCl_2$ to anoxic 10 mM buffer solutions. The buffers and their nominal pH values were as follows: sodium acetate (pH 4 and 5), 4-morpholineethanesulfonic acid monohydrate (MES, pH 6), 4-morpholinepropanesulfonic acid (MOPS, pH 7), 1,4-piperizinebis(ethanesulfonic) acid monohydrate (PIPES, pH 7), 2-amino-2-(hydroxymethyl)-1,3-propanediol (Trizma base, pH 8), and 1,3-bis[tris(hydroxymethyl)methylamino]propane (Bis-tris, pH 9). At pH 7 and higher, a green precipitate formed during a 24 h incubation of the stock solutions. Geochemical modeling suggested that the dominant form of the precipitate was $Fe(OH)_2(s)$. The precipitate was removed by filtration (0.2 μm pore size), and no additional precipitate formed in the filtrates over a four-week period.

Bacteria and Culture Conditions. The facultative Fe(III)-reducing bacterium *Shewanella alga* strain BrY, hereafter

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referred to as BrY (*19*), was cultured aerobically in 100 mL of TSB and incubated for 16 h at 30 °C on a rotary shaker at 100 rpm. Cells in late-log phase of growth were aseptically harvested by centrifugation (6000×g, 15 min, 5 °C) and washed three times in sterile 10 mM PIPES buffer (pH 7), which was previously made anoxic with N_2 gas. The cells were suspended to a final density of about 10^9 cells mL^{-1}, sealed in a stoppered serum bottle under a headspace of O_2- and CO_2-free N_2 gas, and stored at 4 °C for no longer than 30 min before use. Cells prepared in this manner exhibited metal reductase activity with either lactate or H_2 as the electron donor (*20*) and were used as inocula for Fe(III)-reduction experiments described below.

Goethite Synthesis. Goethite, which was prepared by the method of Goodman and Lewis (*21*), was used as a model mineral because it was well-characterized with regard to its crystallinity and surface area (*13*) and is a common mineral in soils and sediments. The goethite was lyophilized, passed through a 100-mesh (<150 μm pore size) sieve, suspended in anaerobic DI water, and incubated in an anoxic chamber for at least one week before use. The BET surface area of the goethite was 55 $m^2\ g^{-1}$, as reported by Roden and Zachara (*13*), and 87 $m^2\ g^{-1}$ as determined by gravimetric sorption of ethylene glycol monoethyl ether (EGME) as the adsorbate using the method of Cihacek and Bremner (*22*).

Preparation of Reduced Goethite. The surface of goethite was "reduced" abiotically by mixing an anoxic mineral suspension with a stock solution of $FeCl_2$. Suspensions of goethite with sorbed Fe(II) were prepared by adding varied amounts of the anoxic $FeCl_2$ stock solution to anoxic suspensions of goethite (5 mg goethite mL^{-1}) in 10 mM PIPES and stirring the mixture overnight in an anaerobic glovebox under an $Ar:H_2$ (95:5) atmosphere.

Biotically reduced goethite was prepared by inoculating anoxic suspensions of the mineral in 10 mM PIPES buffer (pH 7) with washed suspensions of BrY to give a final cell concentration of approximately 10^7 cells mL^{-1}. When appropriate, 20 mM lactate served as the electron donor and was added through the rubber stopper from anoxic stock solutions using a degassed needle and syringe. Negative controls consisted of vials without lactate. Suspensions of goethite (0.9 mg mL^{-1}) were reduced in 50 mL batches in sealed, 150 mL serum bottles. The suspensions were incubated at 30 °C on a rotary shaker at 150 rpm. Reduction of Fe(III) was monitored by the ferrozine method until reduction of Fe ceased. Five-mL aliquots from the 50 mL suspensions were transferred to 20 mL headspace vials in an anaerobic glovebox using a needle and syringe. Vials were sealed with thick butyl-rubber stoppers and crimp seals. Samples were heated to 60 °C following the two-week incubation to stop further metabolic activity.

Analytical Methods. The concentration of dissolved Fe(II) in suspensions of goethite was determined by first removing the mineral and sorbed Fe(II) from the aqueous phase using a 0.2-μm syringe filter and then assaying the filtrate using ferrozine as the colorimetric reagent (*23*). All manipulations were performed in an anaerobic glovebox to avoid oxidation of Fe(II). The total concentration of Fe(II), i.e., dissolved and sorbed Fe(II), in the suspensions was determined by extracting Fe(II) from the samples using 0.5 N HCl, as described previously (*13, 24*), and assaying the extract using ferrozine. The concentration of sorbed Fe(II) was calculated as the difference between the concentration of total and dissolved Fe(II). Anoxic solutions of ferrous ethylenediammonium sulfate served as standards for the ferrozine assay.

Transformation of CT and the formation of volatile, chlorinated degradation products were determined by headspace analysis (direct injection) using a Hewlett-Packard 5890 series II gas chromatograph equipped with an electron-capture detector. Operating conditions were as follows: 100 m Vocal fused capillary column (Supelco, Bellefonte, PA); injector temperature, 250 °C; detector temperature, 200 °C; initial oven temperature 50 °C for 1 min, initial ramp up rate, 7 °C min^{-1} to 140 °C, then 25 °C min^{-1} to 200 °C for 1 min. Helium was the carrier gas at 12 mL min^{-1}, and argon/methane was the makeup gas at 53 mL min^{-1}. Standard curves for CT and CF were generated by relating the known mass of each component added to 5 mL of PIPES buffer to the peak area obtained by GC analysis.

Transformation of CT. All CT transformation experiments were conducted using headspace vials maintained at 30 °C in a darkened rotating incubator oscillating at 150 rpm. Initial experiments focused on the dechlorination of CT in abiotic suspensions of goethite containing a range of $FeCl_2$ concentrations. Aliquots, 5 mL, of reaction mixture containing 5 mg goethite mL^{-1} and $FeCl_2$ ranging from 0 to 3 mM in 10 mM PIPES buffer were dispensed to 20 mL headspace vials under anaerobic conditions. The final pH of these preparations was recorded to be between 6.8 and 7.0. Stabilization of pH resulted from preparing concentrated stock solutions of $FeCl_2$ in pH 7 PIPES buffer. Reaction mixtures were spiked with 10 μL of a methanolic stock of CT, giving an initial concentration of 1.5 μmol CT per vial. The headspace vials were immediately sealed with Teflon-lined gray butyl-rubber stoppers and aluminum crimp caps. Transformation of CT was monitored in replicate samples by GC analysis. Reaction mixtures receiving 3 mM $FeCl_2$ and no goethite served as negative controls.

Abiotic dechlorination of CT and the amount of sorbed Fe(II) in suspensions of goethite were also evaluated over a range of pH values. Goethite, 5 mg mL^{-1}, was suspended in anoxic buffers with nominal pH values ranging from 4 to 9. Each suspension received 2 mM $FeCl_2$ from the appropriate buffered Fe(II) stock solution. The actual pH values of the mineral suspensions with $FeCl_2$ were 4.11, 5.22, 6.02, 6.88 (MOPS buffer), 7.32, and 8.22. CT was added to each preparation to a final concentration of 1.5 μmol/vial, and the vials were sealed with Teflon-coated butyl rubber stoppers and crimp seals. The amount of sorbed Fe(II) and the loss of CT as a function of pH were determined using the ferrozine method and GC analysis, respectively.

A third set of abiotic CT dechlorination experiments was conducted at pH 7 starting with either 0.3 mM or 3 mM $FeCl_2$ in solution and varying the amount of goethite in suspension from 1 to as high as 20 mg mL^{-1}. Sorbed Fe(II) and loss of CT over time were determined as before.

Transformation of CT using microbially reduced goethite was evaluated in batch systems. In an anaerobic chamber, 5-mL aliquots of the microbially reduced goethite (0.9 mg mL^{-1}), which was heated to 60 °C following enzymatic reduction of goethite, were dispensed into 20-mL headspace vials. The CT was added to each vial from a methanolic stock to give a final concentration of 0.15 μmol CT per vial. Vials were immediately sealed with Teflon-lined butyl-rubber stoppers and aluminum crimp caps, removed from the anaerobic glovebox, and placed in the incubator. Vials treated in this manner were used to determine whether microbially reduced goethite could serve as an electron donor for reductive dechlorination of CT following cessation of microbial activity. Vials serving as negative controls contained goethite that had not been subjected to microbial reduction.

Results and Discussion

Sorbed Fe(II). The ability of Fe(II) to serve as a reductant in dechlorination reactions might depend on its association with solid-phase materials for two reasons. First, Fe(II) bound to an oxyhydroxide mineral surface is a more effective reductant than dissolved Fe(II) (*25*). Surface hydroxyl groups
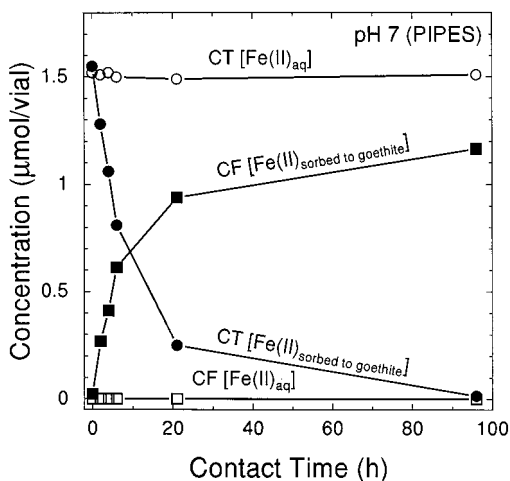
FIGURE 1. Dechlorination of CT by aqueous solutions containing 2 mM FeCl$_2$ at pH 7 in the absence and presence of 5 mg mL^{-1} goethite.

FIGURE 2. Pseudo-first-order rate plots for dechlorination of CT by aqueous suspensions of goethite (5 mg mL^{-1}) at various pHs, showing initial and secondary rates. All subsequent analysis of rate data focused on the initial rates.

act as ligands to form inner-sphere bonds that increase the density of electrons around the adsorbed Fe(II) centers (26). Second, sorption of multiple Fe(II) atoms in close proximity to each other may promote multiple-electron-transfer reactions typical of dechlorination reactions. In our experiments, Fe(II) sorbed to the surface of goethite was able to serve as an electron donor for the dechlorination of CT while dissolved Fe(II) was nonreactive (Figure 1). Our results are consistent with those of Klausen et al. (18) who demonstrated that Fe(II) sorbed to surfaces of a variety of oxide minerals transformed nitroaromatic compounds while dissolved Fe(II) was nonreactive.

The relationship between sorbed Fe(II) and rate of dechlorination of CT was examined by various approaches designed to evaluate the relative importance of volumetric *concentration* of sorbed Fe(II) (mmol L^{-1}) and the mass-based *density* of sorbed Fe(II) (mmol g^{-1}). At constant solid: solution ratios, no distinction between these parameters can be made. However, when total dissolved Fe(II) is in excess of the amount needed to achieve saturation of the goethite surface, a variation of the amount of goethite contacted by a fixed amount of Fe(II) will change the total amount of sorbed Fe(II) in the system [i.e., the volumetric concentration of sorbed Fe(II) (mol L^{-1})] with little change in the density of sorbed Fe(II). Conversely, when total Fe(II) is far below the saturation level, essentially all the Fe(II) will be sorbed, and changes in the amount of goethite contacted by a fixed concentration of Fe(II) will result in a change in density of sorbed Fe(II) with little change in the total amount of sorbed Fe(II).

We purposely refrain from evaluating the CT-dechlorination results in terms of an areal density of sorbed Fe(II) (i.e., mmol m^{-2}). As noted by others (27) and demonstrated in our results, interactions between suspended particles can result in only a fraction of the total surface area as determined by gas-phase sorption being available for sorption by dissolved species. Thus, reporting the density of sorbed Fe(II) in terms of total goethite surface area may suggest that the reactive surface area is known, when in fact it is not. The mass-based density, though less informative, is easily verified.

Our initial experiments were conducted at a fixed solid: solution ratio (5 mg goethite mL^{-1}) where the volumetric concentration and density of sorbed Fe(II) changed simultaneously. To determine the sorption characteristics of Fe(II) on goethite, anoxic suspensions of goethite were provided FeCl$_2$ at concentrations ranging from 0 to 6 mM. Sorption of Fe(II) onto goethite at pH 7 (Figure 2) was well-described by the Langmuir equation, from which a value of 0.144 mmol
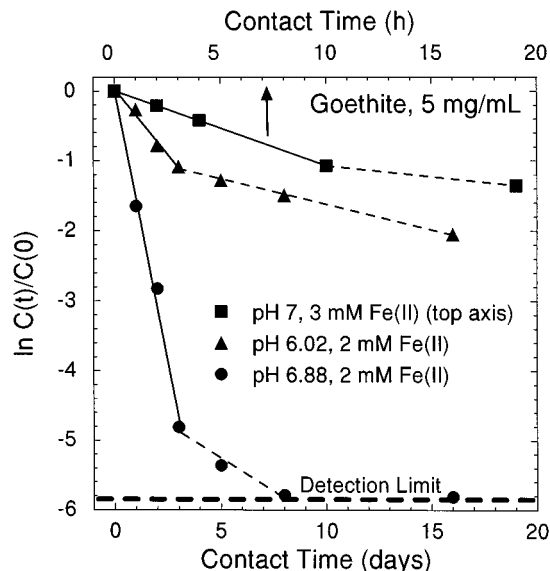
Fe(II) g^{-1} of goethite was calculated for maximum sorptive capacity. Using this result, and the specific surface of the goethite (55 m^2 g^{-1}), the Fe(II) reactive-site density at pH 7 and 5 mg mL^{-1} goethite was calculated to be 1.6 sites/nm^2. This value is an order of magnitude below the theoretical maximum *proton* site-density (15−16 sites/nm^2) as estimated by Rustad et al. (28) for a nonsolvated goethite surface and supported by tritium exchange studies of Yates et al. (29). The reactive site density was also 3.2 times smaller than the value of 5.1 sites/nm^2 obtained by Hayes (30) for Pb^{2+} adsorption on goethite at pH 7. In large part, the low Fe(II) reactive-site density at pH 7 stems from reluctance of the cation to hydrolyze (pK_{MOH} = 9.5), and therefore the sorption envelope occurs at higher pH than for more readily hydrolyzed cations such as Pb^{2+} (pK_{MOH} = 7.7) (31).

When CT was added to similarly prepared vials containing 0−3 mM Fe(II) and goethite, plots of ln [CT] vs contact time consistently yielded straight lines during the initial part of the experiments (Figure 2), whereas plots of [CT] vs contact time were nonlinear (data not shown). In the latter part of most experiments, a change in the slope of the lines was observed in the ln [CT] plots, often yielding a second linear region (Figure 2). As several explanations for this behavior are possible, we focused our analysis on the initial rates shown by the solid lines in Figure 2. Least-squares regression analyses were performed on the linear portions of the data and yielded fits with minimum and average r^2 values of 0.936 and 0.988, respectively. The slopes of these fitted lines then were assumed to be pseudo-first-order rate constants, k_{obs}, as given by

$$\ln\left(\frac{[CT]_t}{[CT]_0}\right) = -k_{obs}t \qquad (1)$$

where $[CT]_0$ is the initial concentration of CT, and $[CT]_t$ is the concentration at the time of sampling, t.

Comparison of the values for k_{obs} obtained at different loadings of Fe(II) on the goethite surface at pH 7 (Figure 3) showed that k_{obs} increased with increasing amounts of dissolved, as well as sorbed, Fe(II). As dissolved Fe(II) increased, both the density of sorbed Fe(II) and the k_{obs} increased by similar proportions. These results show that
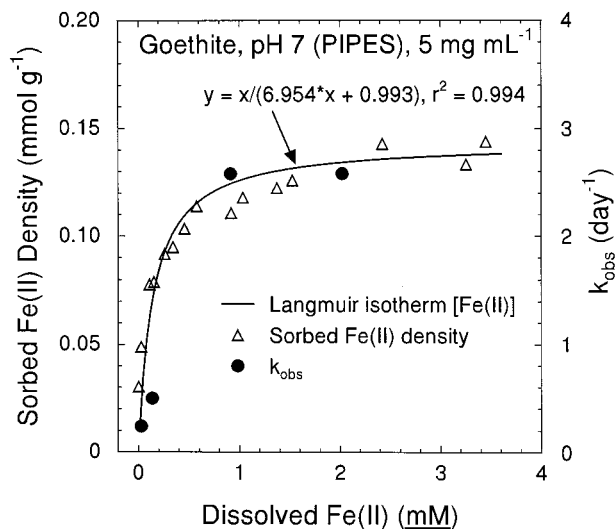
FIGURE 3. Sorption of Fe(II) onto goethite (left axis) and observed initial rate constants for degradation of CT (k_{obs}, right axis) at various equilibrium concentrations of dissolved Fe(II). All data for 5 mg mL^{-1} suspensions of goethite at pH 7.

k_{obs} is linearly correlated with the density of sorbed Fe(II) and, by inference, the volumetric concentration of sorbed Fe(II).

Effect of pH. The correlation between k_{obs} and the volumetric concentration and density of sorbed Fe(II) also was observed in solutions where the degree of Fe(II) sorption was modified by changing the solution pH. Oxide surfaces, such as goethite, exhibit changes in surface-charge in response to the solution activities of potential-determining ions such as H$^+$. The effect is most pronounced within a pH region that extends about two pH units above and below the point of zero charge (PZC) of the oxide surface. As the pH increases in this region, the surface becomes more negatively charged and sorption of cations, such as the Fe(II) species Fe^{2+} and FeOH$^+$, increases. The PZC for goethite is about 6.1 (*32*), and our experiments were conducted in a pH range from 4.2 to 8.2. As expected, the amount of sorbed Fe(II) increased with increasing pH when pH-buffered suspensions of goethite (5 mg mL^{-1}) were spiked to an initial concentration of 2 mM FeCl$_2$ (Figure 4a). An exception was noted at pH 7.32 where the sorbed Fe(II) concentration was lower than the concentration at pH 6.88. At pH 8.22, the amount of "sorbed" Fe(II) exceeded the maximum sorption capacity measured at pH 7 (0.144 mmol g^{-1}) by about 50%. Although an increase in the number of negatively charged surface sites with pH may account for the increased sorption, some of the excess "sorption" likely stemmed from precipitation of Fe(OH)$_2$(s). When the dissolved Fe concentrations were speciated for Fe^{2+} activity and these values plotted as a function of pH (Figure 4b), the pH 8.22 datum lies on the stability line for Fe(OH)$_2$(s) calculated assuming a pK_{sp} of 14.7 (*33*). When the pK_{sp} of 12.85 recommended by Baes and Mesmer (*34*) is used, no precipitation is predicted (Figure 4b).

CT was degraded in the goethite suspensions at all pH values. The rate of degradation, as shown by k_{obs}, corresponded directly with the volumetric concentration of sorbed Fe(II) (Figure 4a). Because the source of Fe(II) in the pH 8.22 solution could have included Fe(II) from Fe(OH)$_2$(s), the corresponding k_{obs} datum was omitted from further comparisons with the other goethite experiments.

Goethite Concentration. In the variable-pH experiments, as well as those where the total concentration of Fe(II) was varied, the amounts of goethite in suspension remained constant, and so no distinction could be made between the effects of the concentration of sorbed Fe(II) and the density
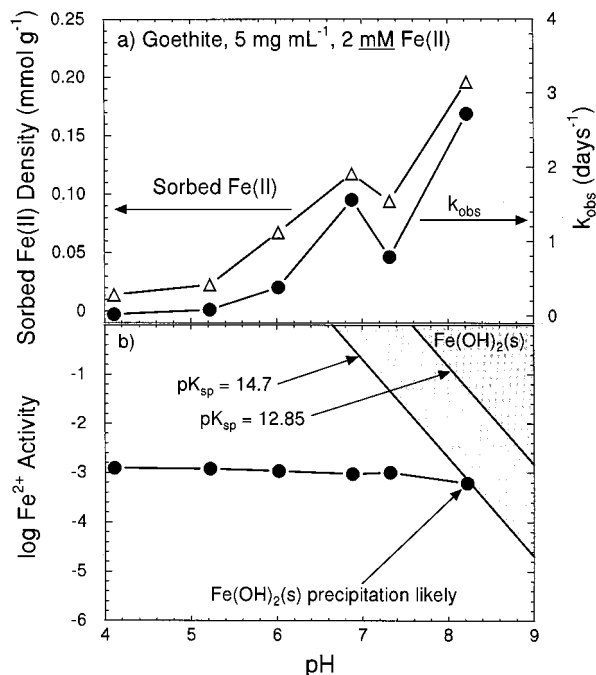

FIGURE 4. (a) Sorption of Fe(II) onto goethite (left axis) and observed initial rate constants for degradation of CT (k_{obs}, right axis) at goethite concentrations of 5 mg mL^{-1} and various pH values. (b) Activity of Fe^{2+}(aq) species calculated from total soluble-Fe(II) data for goethite sorption experiments at different pHs and the stability of Fe(OH)$_2$(s). Shaded regions show solution conditions for which Fe(OH)$_2$(s) is thermodynamically stable according to two widely reported solubility values.

of sorbed Fe(II). To separate the effects of these two variables, two experiments were conducted at pH 7 in which the total amount of Fe(II) was held constant and the amount of goethite in suspension was varied.

In the first variable-goethite experiment, suspensions of goethite ranged from 1 to 10 mg mL^{-1}. The Fe(II) was provided at 3 mM (sufficient to saturate the goethite surface). The dechlorination rates and volumetric concentration of sorbed Fe(II) increased linearly with increasing concentration of goethite (Figure 5). In contrast, the density of sorbed Fe(II) on the surface of the goethite exhibited the opposite trend. As goethite concentration decreased from 10 mg mL^{-1}, the density of sorbed Fe(II) increased. At 1 mg mL^{-1}, a sorbed Fe(II) density of 0.325 mmol g^{-1} was obtained (Figure 5), which is roughly 2.5 times greater than the *maximum* sorption density measured at 5 mg mL^{-1} (Figure 3). As covered in more detail below, this change in sorption density likely stems from a decrease on particle aggregation at lower goethite concentrations.

Regeneration of Sorbed Fe(II). Using similar sorbed-Fe(II) systems, Klausen et al. (*18*) suggested that regeneration of sorbed Fe(II) occurred and, in some instances, was the rate-limiting step in the reduction of nitroaromatic compounds. With some minerals, they also noted considerable visual evidence for precipitation of "iron(III) (hydr)oxide coatings" during the reaction. Although the rates of CT dechlorination are much slower than nitroaromatic reduction and thus unlikely to be controlled by regeneration, there seems little doubt that regeneration of sorbed Fe(II) occurred in our experiments and helped to maintain a pseudo-first-order kinetic regime with respect to CT. As a result of reaction with CT, the sorbed Fe(II) is oxidized to Fe(III), which likely remains in the sorbed state for at least a short time. Three conceivable regeneration mechanisms are (1) ion exchange of sorbed Fe(III) by dissolved Fe^{2+}, (2) reduction of the sorbed Fe(III) by dissolved Fe^{2+}, and (3) hydrolysis and retention of sorbed
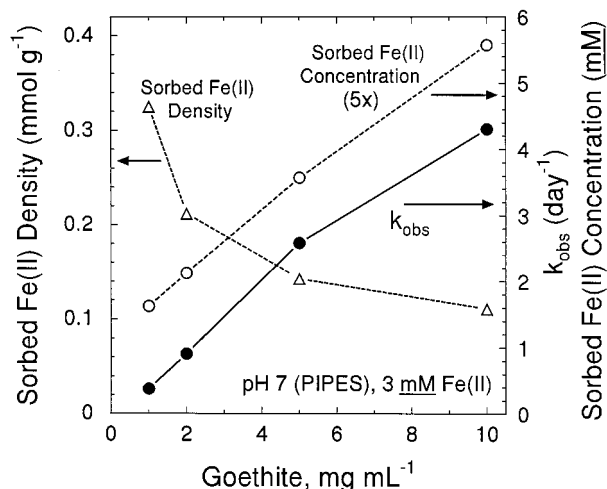
FIGURE 5. Density (left axis) and volumetric concentration (right axis) of sorbed Fe(II) onto goethite, and the observed initial rate constants for degradation of CT (k_{obs}, right axis), as functions of increasing suspension concentrations of goethite under conditions where sorbed Fe(II) regeneration occurred. All data for goethite in 3 mM FeCl$_2$ at pH 7.

Fe(III) to create a new sorption site (or sites) for Fe(II) from solution.

Because of the large $Fe^{2+}/Fe(OH)_2^+$ ratio in solution, one would expect Fe^{2+} to compete effectively for ion-exchange sites with Fe(III) species. The exchange process may be aided by rapid hydrolysis of the sorbed Fe(III) to yield a sorbed monovalent $Fe(OH)_2^+$ ion that would be even more easily desorbed by divalent Fe^{2+}. Although the rate of sorbed-Fe(III) hydrolysis is unknown, the half-life of Fe(III) hydrolysis in homogeneous solution at pH 7 (assuming an outer-sphere ion pair equilibrium constant of 10) can be estimated as being on the order of 1 h (33). The range in half-lives for CT dechlorination we have observed is 4−720 h, with the mean half-life being 14 h. Thus, it seems likely that both simple ion exchange of sorbed Fe(III) by Fe^{2+} and a combination of sorbed-Fe(III) hydrolysis and ion exchange by Fe^{2+} would be faster than the CT dechlorination reaction.

At first glance, a regeneration mechanism involving reduction by dissolved Fe(II) seems unlikely because sorbed Fe(III) has a lower standard reduction potential than dissolved Fe(III) (35−37). However, when *formal* reduction potentials are considered, aqueous Fe(II) becomes a very strong reductant because of the extremely high $Fe^{2+}/Fe(OH)_2^+$ ratio in solution. Assuming initial concentrations of 3×10^{-3} M Fe^{2+} and 10^{-14} M $Fe(OH)_2^+$ [i.e., Fe(III)(aq) in equilibrium with goethite] at pH 7, a formal potential of −0.38 V can be calculated for the aqueous couple, well below the standard potential of +0.36 V estimated for the sorbed-Fe couple by Wehrli (36). Even if only 1% of the sorbed Fe is oxidized by reaction with CT, the formal potential of the sorbed-Fe couple is +0.24 V, and a substantial driving force (−0.62 V) is present for reduction of the sorbed Fe(III) by aqueous Fe(II).

As the ion-exchange and reduction mechanisms proceed, however, the [$Fe(OH)_2^+$(aq)] would be expected to increase because of the slow kinetics of the goethite-precipitation reaction. When [$Fe(OH)_2^+$(aq)] exceeds 10^{-8} M, nucleation and precipitation of an Fe(III)-bearing hydroxide could occur at pH 7. Precipitation would result in a new surface, either as a coating on the goethite surface or as a discrete phase. Under the conditions of this study, reduction of only 1% of the CT would generate enough $Fe(OH)_2^+$(aq) by ion exchange or reduction to exceed the solubility of Fe(III) hydroxide by at least 2 orders of magnitude. It seems quite certain then, that Fe(III) hydroxide starts to precipitate (either as a surface

coating or a discrete phase) very early in the CT reaction and must also be considered as a sorbent for Fe(II). The effect of this new surface on the overall reaction rate is probably minimal, however, because the total additional surface created by transformation of all the CT is at most 10−15% of the goethite surface [assuming ∼600 m^2 g^{-1} and 96 g mol^{-1} Fe(III) for the hydroxide phase, and the creation of 3×10^{-6} mol Fe(III) by reaction with CT]. Furthermore, the sorption site density for Fe(II) on the hydroxide surface is not very different from that for goethite. With emergence of the Fe(III) hydroxide phase as the reactive Fe(III) solid, the formal reduction potential of the aqueous Fe couple at pH 7 would increase to −0.02 V, which is still sufficient to reduce sorbed Fe(III), albeit more slowly.

The third mechanism proposed for regeneration of sorbed Fe(II) involves hydrolysis of the sorbed Fe(III) and its retention by the surface to create a new site for sorption by Fe(II). As in the ion-exchange mechanism, this approach assumes rapid hydrolysis of the sorbed Fe(III) relative to the reduction of CT. A new surface is created as a monolayer-thick coating on the goethite and aqueous Fe(II) is consumed by sorption onto this surface. Although the new surface is probably quite similar to that of pristine goethite, it may not have Fe(II)-sorption properties identical to the pristine goethite surface. On the other hand, with this mechanism at pH 7 and maximum Fe(II) sorption, transformation of all the CT [i.e., generation of 3×10^{-6} mol Fe(III)] will result in as much as 83% of the pristine goethite surface being converted to the new surface.

It seems that all three conceivable regeneration mechanisms eventually change the properties of the Fe(II)-sorbing surface during the course of the reaction. The overall effect of this change is probably small, either because the extent of change is small or the properties of the new surface are similar to those of pristine goethite. The ion-exchange and reduction mechanisms can be distinguished from the hydrolysis/retention mechanism by the possible precipitation of a discrete Fe(III)-hydroxide phase. Absence of a discrete Fe(III)-hydroxide phase, however, is not diagnostic, as all three mechanisms can form coatings on the goethite surface.

Experimental evidence for regeneration of sorbed Fe(II) comes from a comparison of the Fe(II) reaction stoichiometry with measured CT loss. Reductive dechlorination involves the transfer of two electrons, while reduction of Fe(III) to Fe(II) is a one-electron-transfer reaction. Hence, reductive dechlorination of one mole of CT to CF would require the oxidation of two moles of Fe(II) according to the following overall reaction:

$$CCl_4 + 2Fe(II) + H^+ \rightarrow CHCl_3 + Cl^- + 2Fe(III) \quad (2)$$

Using this approach, consumption of as much as 124% of the Fe(II) initially present in the sorbed state was estimated for several experiments. We conclude that replenishment of sorbed Fe(II) was a significant factor in the experiments where dissolved Fe(II) was available and likely maintained sorbed Fe(II) concentrations close to their initial values (i.e., pseudo-first-order conditions with respect to CT) during these experiments.

Density of Sorbed Fe(II). To further evaluate the impact of regeneration and to assess the impact of sorbed Fe(II) density independently of the volumetric concentration of sorbed Fe(II), we conducted a second variable-goethite experiment in which increasing amounts of goethite were added to anaerobic buffered solutions of 0.3 mM FeCl$_2$. At each goethite concentration (5, 10, and 20 mg mL^{-1}), enough surface area was available to maintain the equilibrium concentration of dissolved Fe(II) at 4 uM or less, thus preventing significant amounts of regeneration from occurring. In this experiment, the initial *density* of sorbed Fe(II)
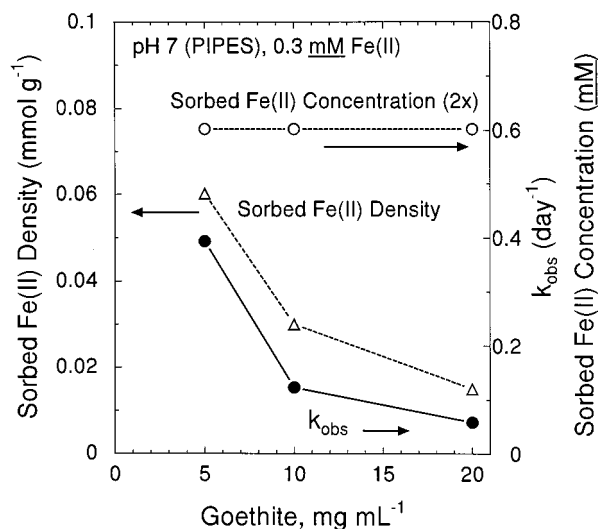
FIGURE 6. Density (left axis) and volumetric concentration (right axis) of sorbed Fe(II) onto goethite, and the observed initial rate constants for degradation of CT (k_{obs}, right axis), as functions of increasing suspension concentrations of goethite in the absence of sorbed Fe(II) regeneration. All data for goethite reacted with 0.3 mM $FeCl_2$ at pH 7.
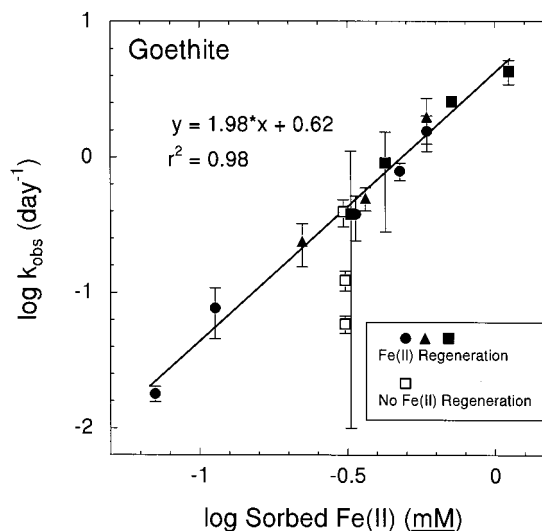

FIGURE 7. Reaction-order plot for volumetric concentration of sorbed Fe(II) using data from twelve kinetic experiments with goethite under conditions where sorbed Fe(II) regeneration occurred (filled symbols), and three experiments where regeneration was absent (open symbols). The slope of the line through the filled symbols indicates the order of the reaction. Triangles are varied-Fe(II) data at pH 7, squares are varied-goethite data at pH 7, and circles are varied-pH data. Error bars correspond to 95% confidence limits for the k_{obs} values.

decreased with increasing amounts of goethite, whereas the initial *concentration* of sorbed Fe(II) remained constant. The results (Figure 6) showed that as the goethite concentration increased and the density of sorbed Fe(II) decreased, the value of k_{obs} for the dechlorination of CT decreased. Under nonregenerative conditions and constant sorbed Fe(II) concentration, dechlorination rates seemed to be directly correlated with initial sorbed-Fe(II) density.

Aggregation. Physical phenomena, such as particle aggregation, might affect the dechlorination rates. Aggregation of individual goethite particles increases with goethite concentration and can have two effects on surface reactions. First, the fraction of the surface available for sorption by dissolved species decreases with aggregation. Other goethite particles effectively compete with dissolved species for the fixed number of sorption sites. As shown in both Figures 5 and 6, the net result is a decrease in the calculated density of sorbed Fe(II) as the concentration of goethite increases. Lesser degrees of aggregation in the 1 and 2 mg mL^{-1} goethite samples, thus, can explain the densities of sorbed Fe(II) in these samples that are significantly higher than the "maximum" density of sorbed Fe(II) calculated at 5 mg mL^{-1}. Second, the accessibility of sorbed Fe(II) to dissolved species decreases with aggregation. Dissolved species must diffuse through a network of small pores to get to the sorbed Fe(II) in the interior of aggregates. The net effect would be to decrease the rate of reaction below that expected from sorbed Fe(II) concentration alone. Although some data in Figure 6 are consistent with an effect of aggregation on accessibility (e.g., the rate decreases about 70% when goethite was increased from from 5 mg mL^{-1} to 10 mg mL^{-1}), a similar rate decrease would be expected for the 10 mg mL^{-1} datum in Figure 5 (after allowing for the increase in sorbed Fe(II) concentration). Such a decrease was not observed. Because the accessibility effect would be analogous to an activity coefficient, it would be observed regardless of whether regeneration of sorbed Fe(II) was occurring. We conclude that, in contrast to its effect on sorption-site density, the effect of aggregation on accessibility of sorbed Fe(II) is insignificant under the conditions of our experiments.

Reaction Mechanism. Differences in CT dechlorination rates among the 5, 10, and 20 mg mL^{-1} samples would seem to stem from differences in the density of the sorbed Fe(II),

given that (1) the initial sorbed-Fe(II) concentrations for all samples in the second variable-goethite experiment were identical, (2) Fe(II) regeneration did not occur, and (3) the effect of aggregation on sorbed-Fe(II) accessibility was considered negligible. For the density of sorbed-Fe(II) to be the main factor, however, either both electrons in the dechlorination reaction must be donated simultaneously (i.e., a termolecular rate-determining step), or the first electron-transfer step in the dechlorination reaction must be reversible with the second electron-transfer being rate-determining. Distinction between these two possible mechanisms can be made on the basis of reaction order with respect to Fe(II). The first mechanism requires a reaction order of two, whereas the second requires a reaction order of one. Thus, determination of the rate equation for the elementary rate-determining step is needed.

The overall rate equation can be written as

$$\text{rate} = k_{obs}[\text{CT}] \qquad (3)$$

where it is assumed that the reaction order with respect to CT is 1. From the reaction stoichiometry given in eq 2 and the experimental results, we can postulate the following rate equation for the elementary rate-determining step

$$\text{rate} = k[\text{H}^+]^n[\text{Fe(II)}]^m[\text{CT}] \qquad (4)$$

where k is the absolute rate constant, [H$^+$] and [Fe(II)] refer to the volumetric concentrations of H$^+$ and sorbed Fe(II), respectively, and n and m are coefficients to be determined. Thus, a plot of log k_{obs} vs log [Fe(II)] for all the experiments for which pseudo-first-order conditions hold and pH remains constant will yield a line whose slope corresponds to the order of the reaction with respect to the volumetric concentration of sorbed Fe(II). When the results of the first three experiments (i.e., those in which regeneration of sorbed Fe(II) occurs) are plotted in this way, a line with a slope near 2 is obtained (Figure 7). The data from the variable-pH experiment yield the same slope as the data from the pH 7 experiments, suggesting that pH does not play a role in the rate-determining step (i.e., $n = 0$) at pH between 4.2 and 7.3.
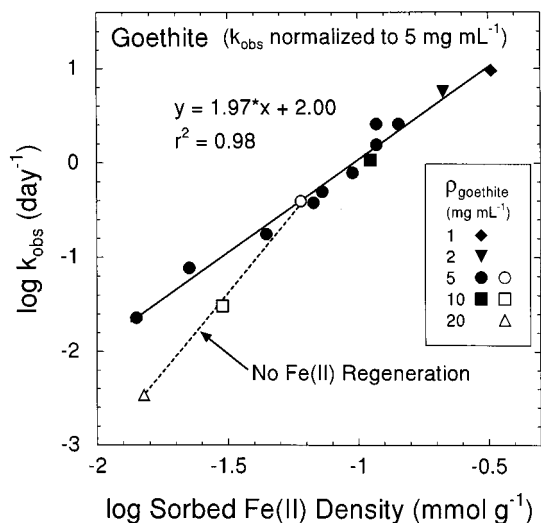
FIGURE 8. Reaction-order plot for density of sorbed Fe(II) using data from twelve kinetic experiments with goethite under conditions where sorbed Fe(II) regeneration occurred (filled symbols), and three experiments where regeneration was absent (open symbols). The slope of the line through the filled symbols indicates the order of the reaction.

On the other hand, $m = 1.98 \pm 0.20$ (95% confidence limit), showing that the reaction is second-order with respect to sorbed Fe(II). The elementary rate equation for the reduction of CT by sorbed Fe(II) then is

$$\text{rate} = k[\text{Fe(II)}]^2[\text{CT}] \qquad (5)$$

When the data from the second variable-goethite experiment are plotted, a slope of infinity is seen because the total concentration of sorbed Fe(II) is the same for all three samples (Figure 7). This result confirms that pseudo-first-order conditions do not hold for this experiment, with the possible exception of the highest sorbed-Fe(II) density. Because

$$\log k_{\text{obs}} = \log k + m \log [\text{Fe(II)}] \qquad (6)$$

the y intercept of the regression line in Figure 7 yields a value for the absolute rate constant, k, of 42 ± 5 M^{-2} s^{-1} (95% confidence limit).

The reaction can also be modeled in terms of the sorbed Fe(II) density. Since

$$[\text{Fe(II)}] = \rho_{\text{Fe(II)}}\rho_{\text{Goethite}} \qquad (7)$$

where $\rho_{\text{Fe(II)}}$ is the density of sorbed Fe(II) on the goethite (mmol g^{-1}) and ρ_{Goethite} is the density of goethite in suspension (mg mL^{-1}), the rate equation can be written as

$$\text{rate} = k(\rho_{\text{Fe(II)}})^2(\rho_{\text{Goethite}})^2[\text{CT}] \qquad (8)$$

At constant ρ_{Goethite}, a plot of $\log k_{\text{obs}}$ vs $\log \rho_{\text{Fe(II)}}$ will yield a line having a slope equal to the reaction order with respect to sorbed Fe(II) density. By assuming no effect of aggregation on rate, the k_{obs} values for the variable goethite experiments can be normalized to a constant ρ_{Goethite} of 5 mg mL^{-1} by

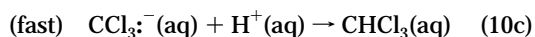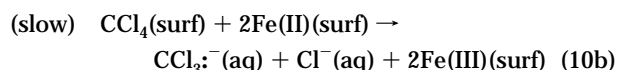$$k_{\text{obs}(\rho=5)} = k_{\text{obs}}(5/\rho_{\text{Goethite}})^2 \qquad (9)$$

and plotted together with the data for the 5 mg mL^{-1} experiments. The resulting plot (Figure 8) yields a line with slope near 2. The consistency of the normalized and unaltered 5 mg mL^{-1} data further demonstrates that particle aggregation has essentially no effect on reaction rate aside from its effect on sorbed Fe(II) density. As expected, the value for the

absolute rate constant ($39 \pm 7\,\text{M}^{-2}\,\text{s}^{-1}$) is statistically identical to that obtained when sorbed Fe(II) concentration is used.

The data from the second variable-goethite experiment also yield a straight line in Figure 8. Comparison of this line with that for the experiments where Fe(II) regeneration occurs clearly shows the increasing importance of Fe(II) regeneration at low sorbed Fe(II) densities. Under the conditions of our experiments with CT at pH 7, a sorbed Fe(II) density of 0.06 mmol g^{-1} or lower requires regeneration of Fe(II) to achieve the maximum CT reduction rate. Presumably, changes in initial concentration of CT would shift this regeneration initiation point to other sorbed-Fe(II) densities.

The reaction-order data for Fe(II) in Figures 7 and 8 is remarkably consistent across a wide range in pH and sorbed Fe(II) density/concentration. It seems, therefore, that the possible differences in the reactivity of sorbed Fe(II) stemming from different binding-site energies and geometries can be ignored, at least for this reaction. That is, the sorbed Fe(II) can be treated as if it were a soluble constituent in homogeneous solution.

Based on this kinetic analysis, the mechanism of CT reduction by sorbed Fe(II) occurs in three steps:

$$(\text{fast}) \quad \text{CCl}_4(\text{aq}) = \text{CCl}_4(\text{surf}) \qquad (10\text{a})$$

$$(\text{slow}) \quad \text{CCl}_4(\text{surf}) + 2\text{Fe(II)}(\text{surf}) \rightarrow$$
$$\text{CCl}_3\text{:}^-(\text{aq}) + \text{Cl}^-(\text{aq}) + 2\text{Fe(III)}(\text{surf}) \quad (10\text{b})$$

$$(\text{fast}) \quad \text{CCl}_3\text{:}^-(\text{aq}) + \text{H}^+(\text{aq}) \rightarrow \text{CHCl}_3(\text{aq}) \qquad (10\text{c})$$

The occurrence of a termolecular elementary reaction, while rare in homogeneous solution, is more likely in heterogeneous systems because two of the reactants can be fixed in space by the solid surface awaiting collision with the third. The goethite surface, therefore, acts as a catalyst in the dechlorination of CT by Fe(II).

Biogenic Fe(II). Soils and sediments can contain a variety of Fe-bearing minerals, including goethite, that can be enzymatically reduced by bacteria. The Fe-reducing bacterium strain BrY reduced about 5% of the 10 mM Fe(III) initially present in 0.9 mg mL^{-1} suspensions of goethite to yield a total Fe(II) concentration (dissolved and sorbed) of about 4.4×10^{-7} M. Although adsorption data for Fe(II) were not obtained under these conditions, a direct comparison of the rates of CT transformation by the biotically reduced goethite and an abiotically reduced goethite sample prepared to the same total Fe(II) concentration yielded similar k_{obs} values of 0.15 and 0.19 day^{-1}.

Picardal and co-workers first reported that the Fe(III)-reducing bacterium *S. putrefaciens* strain 200 dechlorinated CT in growing cultures or in washed cell suspensions (*38*). Evidence suggested that a respiratory cytochrome-*c* was involved in electron transfer to CT, although cells were not shown to gain energy by this process. Strain BrY, which contains a *c*-type cytochrome similar to that found in *S. putrefaciens* strain 200, did not directly dechlorinate CT under the conditions of experiments presented here (data not shown). However, the cell density of BrY (10^7 cells mL^{-1}) was nearly 2 orders of magnitude lower than cell densities used by Picardal and may have been too low to catalyze detectable dechlorination. Estimating the population density of Fe(III)-reducing bacteria in natural environments is difficult. However, the total biomass of anaerobic sediments and aquifers generally falls between 10^5 and 10^7 cells mL^{-1} (*39, 40*). The population of Fe(III)-reducing bacteria is expected to be somewhat less. Hence, Fe(III)-reducing bacteria probably do not contribute to the dechlorination of CT by direct enzymatic activity in contaminated, Fe(III)-reducing environments. It is more likely that biogenic Fe(II) produced from

anaerobic metabolism of Fe(III)-reducing bacteria provides the electrons needed for reductive dechlorination.

Reaction Products. Between 80 and 90% of the CT that was transformed by microbially reduced goethite and abiotic suspensions of goethite with sorbed Fe(II) accumulated as CF. No methylene chloride or chloromethane were found during the headspace analysis. Several possible pathways have been presented that may account for the remainder of the reaction products (*8, 9, 41*). The primary electron-transfer reaction yields an intermediate trichloromethane radical. Further reduction of this radical can result in the formation of dichlorocarbene that upon hydrolysis converts to either CO or formate. We think it likely, therefore, that either formate or CO comprise the other reaction products in the sorbed-Fe(II)/goethite system.

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