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Effect of biogenic iron species and copper ions on the reduction of carbon tetrachloride under iron-reducing conditions

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Abstract

In this study, the cell-mediated and abiotic reduction of carbon tetrachloride (CCl_4) by biogenic iron species produced from the reductive dissolution of ferrihydrite in the presence of *Geobacter sulfurreducens* and copper ions (Cu(II)) were investigated. 9,10-Anthraquinone-2,6-disulfonate (AQDS), serving as a surrogate of natural organic matters and an electron shuttling compound, was added to enhance the efficiency of biological reduction of the solid Fe(III) minerals. *G. sulfurreducens* drove the reduction of CCl_4, primarily through the formation of biogenic surface-bound iron species produced from the reductive dissolution of ferrihydrite, in the presence of 10 μM AQDS. The pseudo-first-order rate constant (k_{obsCT}) for CCl_4 transformation in the presence of ferrihydrite was 3.0 times higher than that resulting from the use of *G. sulfurreducens* alone. Addition of 0.5 mM Cu(II) slightly inhibited both the growth of *G. sulfurreducens* and the production of biogenic Fe(II). However, the k_{obsCT} values for CCl_4 transformation in ferrihydrite suspensions containing *G. sulfurreducens* and 0.3–0.5 mM Cu(II) were 2.1–4.2 times higher than that observed in the absence of Cu(II). X-Ray powder diffraction analysis indicated that the added Cu(II) reacted with the biogenic Fe(II) ions to produce catalytic cuprous ions (Cu(I)) and secondary iron oxide minerals such as magnetite and goethite, resulting in accelerating the chemical transformation efficiency and rate of CCl_4 under iron-reducing conditions.
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1. Introduction

Chlorinated hydrocarbons are the most often found recalcitrant pollutants in the contaminated aquifers. It has been demonstrated that biotic and abiotic reactions both contribute to the reductive transformation of contaminants under anoxic conditions (Curtis and Reinhard, 1994; Lovley and Anderson, 2000; Doong et al., 2003; Cervantes et al., 2004; Doong and Chiang, 2005). Recently, the dechlorination of chlorinated hydrocarbons under iron-reducing conditions has received much attention (Kao et al., 2003; Cervantes et al., 2004). Minerals that contain

structural Fe(II) such as green rust and magnetite, are known to effectively reduce halogenated hydrocarbons (O'Loughlin et al., 2003; Maithreepala and Doong, 2005). In addition, the surface-bound iron species associated with crystalline ferric oxides have high reactivity towards chlorinated methanes under anoxic conditions (Amonette et al., 2000; Maithreepala and Doong, 2004a; McCormick and Adriaens, 2004). The high reactivity of the heterogeneous Fe(II)–Fe(III) systems in the contaminated subsurface environments can be maintained over a long period of time because such Fe(II) species may be regenerated constantly through the reduction of Fe(III) oxides by dissimilatory iron-reducing bacteria (DIRB) (Heijman et al., 1995; Pecher et al., 2002). Therefore, the investigation of the reduction of chlorinated hydrocarbons by biogenic iron species is important because the microbial

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reduction of Fe(III) is an important biogeochemical process in both natural and contaminated environments.

DIRB can utilize both amorphous and crystalline Fe(III) oxides as terminal electron acceptors for respiration to produce substantial amounts of Fe(II) in the contaminated aquifers (Lovley, 1991; Fredrickson et al., 1998; Roden and Urrutia, 2002). *Shewanella putrefaciens* and *Geobacter metallireducens* are the most often used DIRB to determine the relative contributions of the biotic and abiotic reactions of chlorinated hydrocarbons under iron-reducing conditions (Heijman et al., 1995; Kim and Picardal, 1999). Heijman et al. (1995) first addressed the contribution of DIRB to the abiotic reduction of nitroaromatic compounds under iron-reducing conditions in laboratory batch systems containing *G. metallireducens*. Kim and Picardal (1999) reported that the dechlorination of carbon tetrachloride (CCl$_4$) by *S. putrefaciens* could be enhanced in the presence of iron minerals and that the increased transformation rates are due to the production of biogenically surface-bound Fe(II). In addition, McCormick et al. (2002) concluded that the mineral-mediated abiotic reaction of CCl$_4$ occurring on the surfaces of biogenic magnetite particles was 60–260 times faster than the biotic reaction by *G. metallireducens*. However, the transformability of chlorinated hydrocarbons by biogenic iron species in the presence of *G. sulfurreducens* remains unclear.

DIRB can reduce heavy metal ions, such as U(VI), Mn(IV), Co(III), Cr(VI), Tc(VII) and Au(III), to their low-valence-state ions (Holmes et al., 2002; Liu et al., 2002; Jeon et al., 2005). However, the influence of copper ions (Cu(II)) on the enzymatic processes of Fe(III) reduction in DIRB is not well-understood. Our previous studies have recently demonstrated that the addition of Cu(II) to both structural Fe(II) (e.g., green rust) and surface-bound iron systems (e.g., goethite) can significantly enhance the dechlorination rate of chlorinated hydrocarbons (Maithreepala and Doong, 2004b,c). Markwiese and Colberg (2000) reported that Cu toxicity impeded the anaerobic carbon oxidation and the bacterial reduction of hydrous ferric oxide under anaerobic conditions. More recently, Lee et al. (2005) have depicted that the chemical and biological interactions may be important on the immobilization of U(VI) in an iron oxide system when trichloroethylene was present as a co-contaminant. However, the influence of Cu(II) on the biotic and abiotic transformation of chlorinated hydrocarbon in the presence of *G. sulfurreducens* under iron-reducing condition remains unclear.

In this study, the effect of Cu(II) on the biological and chemical transformations of CCl$_4$ mediated by the biogenic iron species resulting from the reductive dissolution of ferrihydrite by *G. sulfurreducens* was investigated. Anthraquinone-2,6-disulfonate (AQDS) was added into the system as the surrogate of natural organic matters to enhance the efficiency of biological reduction of the solid ferric oxide minerals (Scott et al., 1998; Nevin and Lovley, 2000). X-ray powder diffraction (XRPD) and scanning electron microscopy (SEM) were used to identify the secondary iron species formed on the ferric oxides. In addition, kinetics of ferrihydrite dissolution and CCl$_4$ transformation mediated by biogenic Fe(II) species were studied.

2. Materials and methods

2.1. Chemicals

Carbon tetrachloride (CCl$_4$, >99.8%, GC grade), chloroform (CHCl$_3$, >99.8%, GC grade), CuCl$_2 \cdot$ 2 H$_2$O (99%), FeSO$_4 \cdot$ 7H$_2$O (99%), and sodium acetate (CH$_3$COONa) (>99%) were purchased from Merck Co. (Darmstadt, Germany). Fumarate disodium salt (C$_4$H$_2$O$_4$Na$_2$, 99%), *N*-(2-hydroxyethyl)-piperazine-*N'*-(2-ethanosulfonic acid (HEPES, 99.5%), ferrozine monosodium salt (C$_{20}$H$_{13}$N$_4$O$_6$-S$_2$Na), 9,10-anthraquinone-2,6-disulfonic acid disodium salt (AQDS, C$_{14}$H$_6$O$_8$S$_2$Na$_2$, >98%), and ammonium acetate (CH$_3$COONH$_4$) were purchased form Sigma–Aldrich Co. (Milwaukee, WI). Bathocuproine disulfonic acid disodium salt (C$_{26}$H$_{18}$N$_2$Na$_2$O$_6$S$_2$, 90%) was purchased from Fluka (Buchs, Switzerland). All the other chemicals were of analytical grade and were used as received without further purification. All chemical solutions were prepared with high-purity deoxygenated deionized water (Millipore, 18.3 MΩ cm) using a vacuum and N$_2$ (>99.99%) purging system (Maithreepala and Doong, 2005).

Ferrihydrite was synthesized according to the method of Schwertman and Cornell (1991) and was characterized using XRPD. After the microwave digestion, the total Fe concentration of ferrihydrite was determined by inductively coupled plasma-optical emission spectrometer. The surface area of ferrihydrite, determined by a BET N$_2$ adsorption surface area analyzer (Micromeritics, ASAP 2020), was 325 m^2/g.

2.2. Microorganism and cultivation

G. sulfurreducens was cultivated in bicarbonate-buffered mineral medium (pH 7.1–7.3) as previously reported (Doong and Schink, 2002). The compositions of mineral media used for growth and reduction studies contained the following mineral salts (g l^{-1}): NH$_4$Cl, 0.25; NaCl, 1; MgCl$_2 \cdot$ 6H$_2$O, 0.4; KCl, 0.5; CaCl$_2 \cdot$ 2H$_2$O, 0.15; KH$_2$PO$_4$, 0.2. After autoclaving and cooling under an atmosphere of N$_2$/CO$_2$ (80/20, v/v), 30 mM sodium bicarbonate buffer solution, 1 ml of trace mineral solution and 1 ml of selenite–tungstate solution were added per liter. The trace elements solution contained (mg l^{-1}): Fe(NH$_4$)$_2$(SO$_4$) $\cdot$ 7H$_2$O, 800; CoCl$_2 \cdot$ 6H$_2$O, 200; ZnSO$_4 \cdot$ 7H$_2$O, 200; H$_3$BO$_3$, 50; NiCl$_2 \cdot$ 6H$_2$O, 20; CuCl$_2 \cdot$ 2H$_2$O, 20; Na$_2$MoO$_4 \cdot$ 2H$_2$O, 20. 20 mM acetate and 40 mM fumarate solutions were added as electron donors and acceptors, respectively. All cultures were incubated at 25 ± 1 °C in the dark and the purity was checked by microscopy at regular intervals. In addition, the optical density at 660 nm (OD$_{660}$) was used to monitor the growth of *G. sulfurreducens* during the incubation period.

2.3. Fe(III) reduction studies

A previous study has shown that DIRB can grow both in HEPES and bicarbonate buffer solutions (Fredrickson et al., 1998). However, the produced Fe(II) could react with bicarbonate to form siderite, which may influence the dissolution rate of ferrihydrite and dechlorination efficiency of CCl_4. Therefore, HEPES buffer was selected in this study for Fe(III) reduction and CCl_4 dechlorination experiments to minimize the precipitation.

Serum bottles (70 ml) capped with Teflon-lined rubber septa and aluminum crimp caps were used in the Fe(III) reduction experiments. Anoxic HEPES-buffered mineral solutions (50 mM) were used to maintain the solution pH at 6.9–7.1. Ferrihydrite solution was introduced into serum bottles using N_2-purged sterilized syringes to obtain a final concentration of 10 mM. In addition, 30 mM acetate and 10 μM AQDS were added as electron donor and shuttle, respectively. One ml of *G. sulfurreducens* at OD_{660} value of 0.5–0.6 was then injected into the serum bottles. In sterilized control experiments, medium of the bacterial culture was introduced into the serum bottle through a 0.2-μm sterilized membrane filter to normalize all of the possible effects of the trace elements on the reduction of iron oxides.

2.4. CCl₄ transformation experiments

Batch experiments of CCl_4 transformation were conducted under anoxic conditions using 70-ml serum bottles filled with deoxygenated buffered mineral solutions. High-purity N_2 was introduced at a flow rate of 15-20 l min^{-1} continuously to maintain the anoxic conditions during the preparation periods. Ferrihydrite and acetate were anoxically transferred into serum bottles to get final concentrations of 10 and 30 mM, respectively (Maithreepala and Doong, 2005). HEPES (50 mM) buffer solutions were used to control the pH at 6.9–7.1. Appropriate amounts of Cu(II) stock solutions were introduced into the serum bottles up to a final concentration of 0.5 mM. After the removal of nitrogen purge, bottles were then sealed with Teflon-lined rubber septa and aluminum crimp caps. An appropriate amount of the CCl_4 stock solution dissolved in deoxygenated methanol was delivered into the serum bottle through a gas-tight glass syringe to obtain a final concentration of 3.5 μM. The total volume of the liquid phase in the serum bottle was maintained at 50 ml, resulting in a 20-ml volume available for headspace analysis. Parallel experiments were also performed without adding ferrihydrite and/or bacteria (sterilized control). All serum bottles were incubated in the dark using an orbital shaker at 150 rpm and at 25 ± 1 °C.

To determine the concentration effect of Cu(II) on the microbial reduction of Fe(III) oxides, 0.1–0.5 mM Cu(II) were injected into the bacterial systems incorporating ferrihydrite as the electron acceptor. A solution of CCl_4 dissolved in deoxygenated methanol was also added into the serum bottles containing Cu(II) to investigate the effect of Cu(II) on the transformation of CCl_4 in the presence of *G. sulfurreducens*. The volume of the total liquid phase in the serum bottles was 50 ml.

2.5. Analytical methods

The headspace analytical technique was used for the determination of chlorinated hydrocarbons. The concentrations of CCl_4 and byproducts in the headspace of the test bottles were monitored by withdrawing 50 μl of gas from the headspace and then injecting into a gas chromatograph (GC) (Perkin–Elmer, Autosystem) equipped with a flame ionization detector (FID) and an electron capture detector (ECD). A 60-m VOCOL megabore capillary column (0.545 mM × 3.0 μM, Supelco Co.) was used to separate the chlorinated compounds. The column temperature was isothermally maintained at 90 °C using N_2 as the carrier gas. The relative standard deviation for GC analysis was controlled within 10%. The limits of detection for CCl_4 and chloroform ($CHCl_3$) were 0.04 and 0.1 μM, respectively.

Mineral phases of the precipitates were characterized by XRPD using an X-ray diffractometer (Regaku D/max-II B) with a Cu Kα-radiation source ($\lambda = 1.54056$ Å) operated at 30 kV voltage and 20 mA current. After bacterial reduction in the ferrihydrite suspensions, the contents in the bottles were anaerobically withdrawn by a 50-ml syringe and injected into centrifuge tubes under nitrogen atmosphere. The mixtures were separated through centrifugation at 7000g for 10 min, and then the supernatants were carefully removed from the sealed bottles using N_2-purged syringes. After drying the precipitates under a gentle stream of N_2, the samples were mounted onto a glass sample holder using small amounts of grease. A drop of glycerol was added immediately to the mounted powder layer to minimize the exposure to air. The scan range for all samples was between 5 and 90° (2θ) at a scanning speed of 4° min^{-1}. In addition, scanning electron microscopy (SEM) (Topcon ABT-150s) was used to identify the morphology of the solids.

Concentrations of HCl-extractable Fe(II) in the serum bottles were monitored by withdrawing 0.5 ml of suspension using N_2-purged syringes, and were immediately acidified with 1 M HCl (Doong and Schink, 2002). The acidified samples were centrifuged at 14,000g for 10 min to remove ferric oxide minerals and then the acid-extractable Fe(II) contents were analyzed using the ferrozine method at 562 nm. The recovery of Fe(II) onto ferrihydrite extracted by 1 N HCl was found to be 100.3 ± 1.5% ($n = 3$), which indicates that 1 N HCl has excellent selectivity and reproducibility to extract Fe(II) onto ferrihydrite. The concentration of extractable cuprous ion (Cu(I)) was determined using the bathocuproine disulfonic acid method (Maithreepala and Doong, 2004b). The aliquot was withdrawn by a 1-ml N_2-purged plastic syringe and then immediately added into the mixture containing 1 ml of 10% tartarate solution and 0.5 ml of 1% bathocuproine

disulfonic acid solution. The Cu(I) concentrations in the supernatants were then determined by UV–Vis at 483 nm.

3. Results and discussion

3.1. Transformation of ferrihydrite by G. sulfurreducens in the absence of Cu(II) ions

The reductive dissolution of ferrihydrite to Fe(II) by *G. sulfurreducens* was examined first to understand the possible capability of forming surface-bound iron species. Fig. 1 shows the reduction of ferrihydrite mediated by *G. sulfurreducens* in the presence and absence of CCl$_4$. The Fe(II) concentration increased rapidly within the first 4 d and reached a maximum concentration of 5.1 mM after incubation of 15 d. In the absence of bacteria (blank control), the produced Fe(II) concentration was quite low, indicating that the increased Fe(II) concentration in the presence of *G. sulfurreducens* was due mainly to microbial Fe(III) reduction. In addition, the dissolved Fe(II) concentrations were 0.57 mM after 15 d, showing that the Fe(II) produced from the reductive dissolution of ferrihydrite was mainly in the sorbed form. The bulk Fe(II) production can be described by a pseudo-first-order rate equation in which Fe(III) reduction depends on the free reduction sites on the Fe(III) oxide surfaces (Doong and Schink, 2002). When plotting $\ln(C_t/C_0)$ vs time, a linear relationship was observed. The pseudo-first-order rate constants for Fe(II) formation (k_{obsFe}) from ferrihydrite in the presence and absence of CCl$_4$ were 0.16 and 0.15 d^{-1}, respectively.

The reduction of ferrihydrite by *G. sulfurreducens* in the presence of CCl$_4$ was further examined to understand the effect of chlorinated compounds on Fe(II) production. After incubation of 15 d, the maximum concentrations of total and dissolved Fe(II) produced by *G. sulfurreducens* from ferrihydrite in the presence of CCl$_4$ and AQDS were 4.6 and 0.48 mM, respectively. These Fe(II) concentrations are comparable with those obtained in the absence of CCl$_4$,
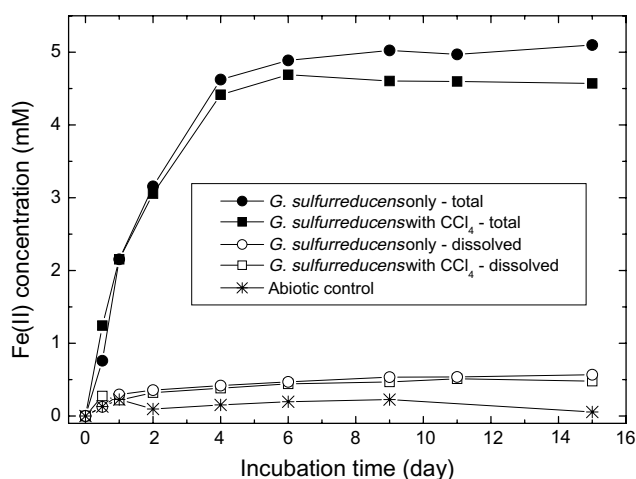
depicting that the presence of CCl$_4$ has little inhibition effect on the bioreduction efficiency of ferrihydrite mediated by *G. sulfurreducens*.

To confirm the mineral phase and the morphology of the products, XRPD and SEM were used to characterize the biologically produced solids. Fig. 2 shows the XRPD patterns and SEM image of the solid phases before and after the reductive dissolution of ferrihydrite by *G. sulfurreducens*. The XRPD patterns showed peaks at 35.46°, 43.31°, 56.96°, and 62.79° (2θ), which are consistent with both magnetite (Fe$_3$O$_4$) and maghemite (γ-Fe$_2$O$_3$). After the bioreduction of ferrihydrite, the original dark-brown color of ferrihydrite gradually transformed into a black-colored product and could be collected magnetically, suggesting that the produced solids could be magnetite. SEM image showed that the particle sizes of biogenic iron oxides ranged between 15 and 30 nm, which are similar to those obtained by McCormick et al. (2002) and McCormick and Adriaens (2004) (5-30 nm).

3.2. Transformation of CCl$_4$ by biogenic iron species in the absence of Cu(II) ions

The ability of *G. sulfurreducens* to reduce CCl$_4$ in the presence of ferrihydrite was further examined. Fig. 3 shows the reduction of 3.5 μM CCl$_4$ and the produced chloroform concentration at pH 6.9–7.1 in the presence of *G. sulfurreducens* under iron-reducing conditions. 10 μM AQDS were added to all the solutions to accelerate the dissolution efficiency of ferrihydrite. No significant decrease in CCl$_4$ concentration was observed in the sterilized control (in the absence of *G. sulfurreducens*, but contained AQDS). However, CCl$_4$ was transformed effectively in the presence of *G. sulfurreducens* and AQDS under iron-reducing conditions (Fig. 3a). Addition of ferrihydrite accelerated the biotransformation of CCl$_4$ mediated by *G. sulfurreducens*. The removal ratio of CCl$_4$ in the presence of ferrihydrite was 59% after incubation of 16 d. It is noted that 31% of the CCl$_4$ was biotransformed by *G. sulfurreducens* in the absence of ferric oxide. Previous studies showed that high concentration of reduced AQDS is likely serving an electron shuttle to abiotically reduce CCl$_4$ (Curtis and Reinhard, 1994; Cervantes et al., 2004). Cervantes et al. (2004) reported that about 50% of the initial CCl$_4$ was chemically transformed by 5 mM reduced form of AQDS (AHQDS) after 12 d of incubation. In this study, however, no obvious degradation of CCl$_4$ was observed when 10 μM AQDS was added to the solution in the absence of *G. sulfurreducens* and ferric oxide, depicting that *G. sulfurreducens* could convert AQDS to its reduced form and then effectively reduce CCl$_4$ under iron-reducing conditions.

Although ferrihydrite generated a relatively high concentration of surface-bound Fe(II) species by *G. sulfurreducens*, its capability in the reduction of CCl$_4$ is only moderate. Our previous study showed that crystallization of the iron minerals is one of the factors influencing the

Fig. 1. Reduction of ferric oxides mediated by *G. sulfurreducens* at pH 7.0 ± 0.1.
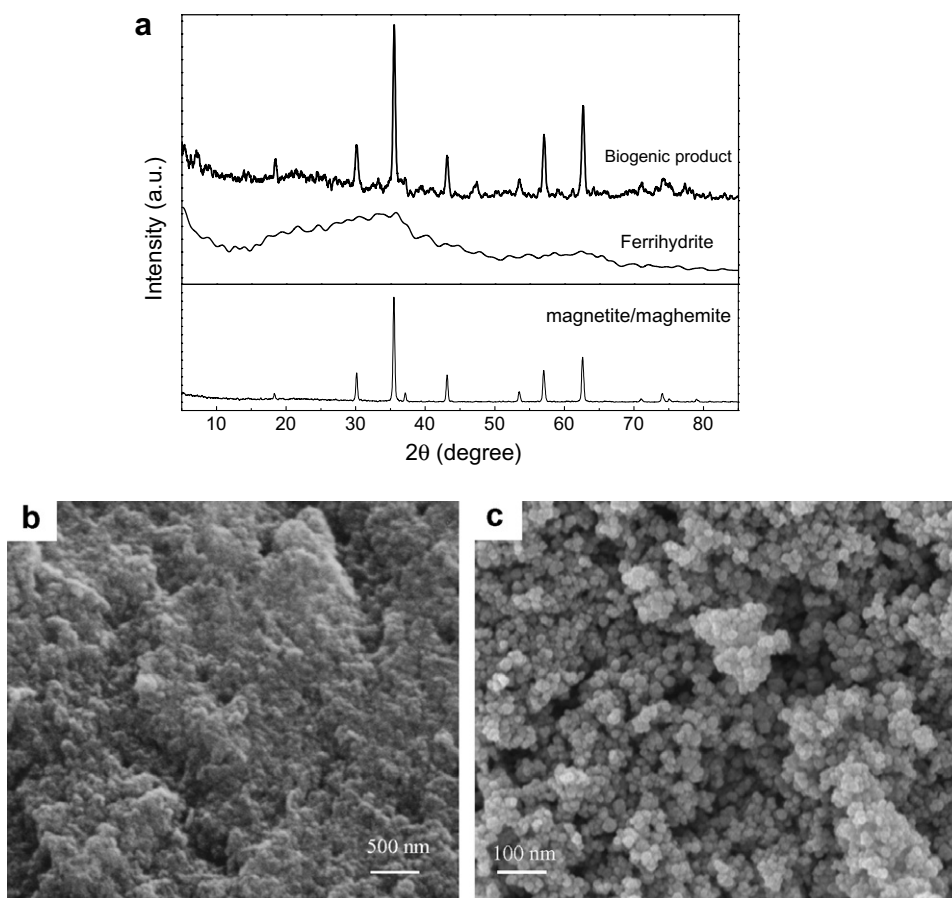
Fig. 2. (a) XRPD patterns and SEM images of (b) ferrihydrite and (c) biogenic solids produced from the reductive dissolution of ferrihydrite mediated by *G. sulfurreducens* in the presence of 10 μM AQDS.

dechlorination efficiency of CCl_4 (Maithreepala and Doong, 2004a). Several studies have depicted that the reactivity of Fe(II) could be greatly enhanced when associated with the crystalline Fe(III)-bearing minerals, such as goethite and magnetite (Haderlein and Pecher, 1998; Amonette et al., 2000). However, the reactivity of surface-bound iron species would be low when associated with ferrihydrite. Maithreepala and Doong (2004a) reported that the degradation rate of CCl_4 can be enhanced when 3 mM Fe(II) was added to the different iron oxide suspensions, and the rate constant for CCl_4 dechlorination followed the order hematite, goethite and ferrihydrite, which is in good agreement with the result obtained in this study. In addition, chloroform ($CHCl_3$) was found to be the major identified chlorinated product in the *G. sulfurreducens* suspensions. Formation of $CHCl_3$ accounted for 60% of the consumed CCl_4, which is consistent with previously reported results in the presence of *G. metallireducens* or *S. putrefaciens* (Kim and Picardal, 1999; McCormick et al., 2002). The incomplete carbon mass balance through biotic pathways in the *G. sulfurreducens* suspensions may be the result of the formation of dichloromethane (CH_2Cl_2), methane (CH_4), cell-bound products, and other unidentified products. Several intermediates including CH_4, CH_2Cl_2 and tetrachloroethylene (C_2Cl_4) were

detected in abiotic batches when 3 mM Fe(II) associated with goethite were used to dechlorinate 1 mM CCl_4 under anaerobic conditions (Maithreepala and Doong, 2004b). However, no dichloromethane and methane was detected both by GC-ECD-FID and GC–mass spectrometer (MS), presumably due to the low concentration of CCl_4 (3.5 μM) used in this study. In addition, the use of Fourier transformation infrared spectroscopy to identify the functional groups of intermediates adsorbed onto the surface of biogenic solids may be conducive to elucidate the possible product distribution and reaction mechanisms for CCl_4 dechlorination in the presence of *G. sulfurreducens*.

The transformation of CCl_4 by surface-bound Fe(II) oxidation also can be described using pseudo-first-order reaction kinetics (Amonette et al., 2000; Pecher et al., 2002; Maithreepala and Doong, 2004b). The pseudo-first-order rate constants for CCl_4 biotransformation (k_{obsCT}) mediated by *G. sulfurreducens* in the absence of ferrihydrite and AQDS was $0.055 \, d^{-1}$ ($n = 5$, $r^2 = 0.974$). In addition, the k_{obsCT} values for CCl_4 transformation in the ferrihydrite-containing solutions was $0.16 \, d^{-1}$ ($n = 5$; $r^2 = 0.982$). Cervantes et al. (2004) investigated the effect of AQDS on the dechlorination of CCl_4 by sludge under anaerobic conditions. The pseudo-first-order rate constant for CCl_4 dechlorination increased from 0.17 to $0.52 \, d^{-1}$
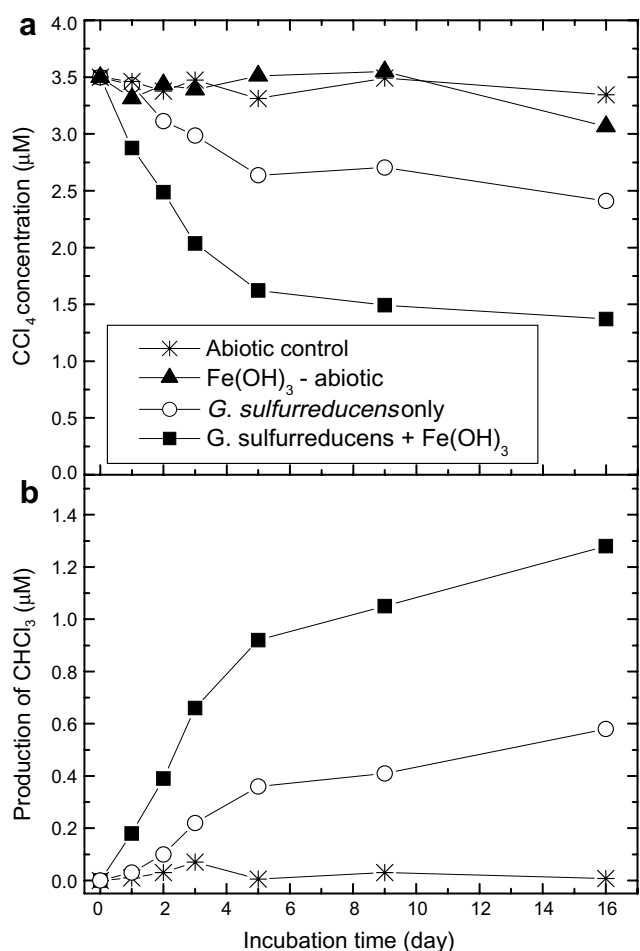
Fig. 3. Transformation of 3.5 μM CCl₄ mediated by *G. sulfurreducens* at neutral pH under iron-reducing conditions. 10 μM AQDS were added as electron shuttles to accelerate the dissolution efficiency of iron oxide: (a) concentration profile for CCl₄ transformation and (b) the produced chloroform (CHCl₃) concentration.

upon increasing AQDS concentration from 5 to 50 μM, which is consistent with the results obtained in this study. Similar to the profile for Fe(II) formation, the CCl₄ concentration decreased rapidly within the first 5 d and then leveled off to a plateau. A similar trend has been observed previously for RDX and CCl₄ reduction by adsorbed Fe(II) (Amonette et al., 2000; Maithreepala and Doong, 2004b; Gregory et al., 2004). Recent work depicted that regeneration of the adsorbed Fe(II) was necessary to balance the number of electrons needed to maintain pseudo-first-order kinetics for CCl₄ dechlorination by surface-bound iron species (Amonette et al., 2000; Gregory et al., 2004), suggesting that this deviation is presumably due to the limited Fe(II) sorbed on the surface of ferrihydrite. In addition, the transformation efficiencies of CCl₄ in the presence of Fe(III) oxides were 3.0 times higher than that mediated by *G. sulfurreducens* alone. This finding indicates that abiotic transformation may play a crucial role in the transformation of CCl₄ in the presence of *G. sulfurreducens* under Fe(III)-reducing conditions.

3.3. Transformation of CCl₄ by biogenic iron species in the presence of Cu(II) ions

Copper is an essential trace element for living cells, but it is detrimental to the enzymatic metabolic activities at elevated concentrations. In this study, the effect of Cu(II) on the growth of *G. sulfurreducens* and on the removal of CCl₄ was examined in the anoxic cultural media. Previous studies showed that 0.5 mM Cu(II) is the optimal concentration to enhance the dechlorination efficiency of CCl₄ by surface-bound Fe(II) species (Maithreepala and Doong, 2004b,c). Fig. 4 shows the growth curves of *G. sulfurreducens* under anaerobic conditions in the presence and absence of 0.5 mM Cu(II). 20 mM acetate and 40 mM fumarate were added as electron donor and acceptor, respectively. A rapid growth after a lag phase of 4 d for *G. sulfurreducens* was observed in the absence of 0.5 mM Cu(II). Addition of Cu(II) slightly inhibited the growth of *G. sulfurreducens*, and the growth rate decreased from $0.15 \pm 0.01 \, d^{-1}$ in the absence of Cu(II) to $0.12 \pm 0.01 \, d^{-1}$ in the presence of 0.5 mM Cu(II).

Fig. 5 shows the effects of Cu(II) concentrations on the production of Fe(II) and Cu(I) concentrations in the ferrihydrite suspensions containing *G. sulfurreducens* and 10 μM AQDS. The maximum Fe(II) concentration produced from the reductive dissolution of ferrihydrite by *G. sulfurreducens* decreased from 5.2 mM in the absence of Cu(II) to 2.3 mM in the presence of 0.5 mM Cu(II) after incubation of 46 d. This result clearly shows that the dissolution efficiency of ferrihydrite decreased upon increasing Cu(II) concentration. The low concentration of Fe(II) produced in the presence of Cu(II) may partially be attributed to the growth inhibition of *G. sulfurreducens* by Cu(II). It is noted that Fe(II) concentrations for the experiments with 0.3 mM Cu(II) increased rapidly after 12 d of incubation and approached to those in experiments with 0.1 mM Cu(II). This depicts that low concentration of Cu(II) may only slightly inhibit the growth of *G. sulfurreducens*, which
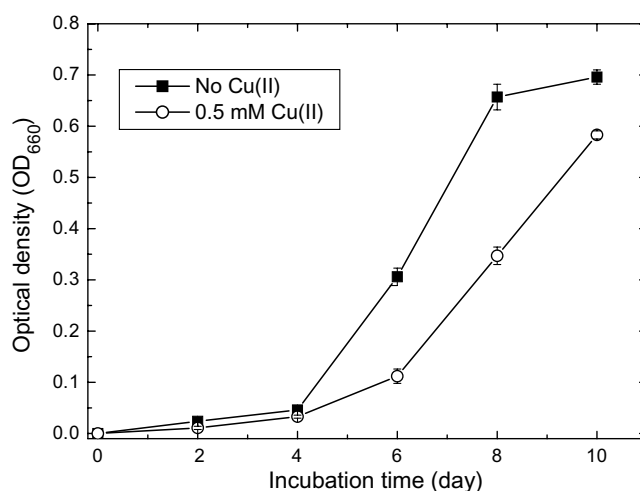

Fig. 4. The growth curves of *Geobacter sulfurreducens* in the absence and presence of 0.5 mM Cu(II) under anoxic conditions at pH 7.0 ± 0.1.
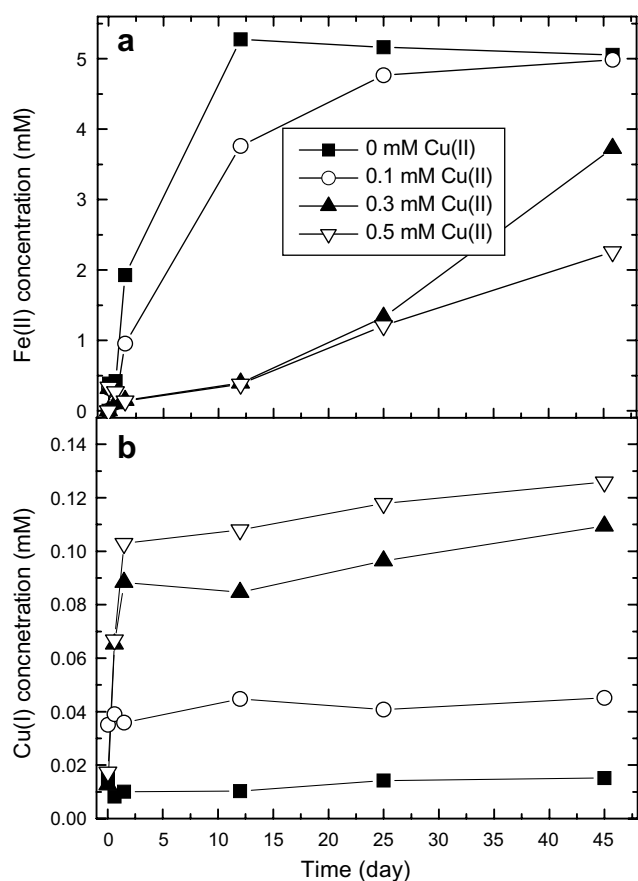
Fig. 5. Effect of Cu(II) concentrations on the concentration of (a) Fe(II) and (b) Cu(I) produced in the ferrihydrite suspensions at pH 7.0 ± 0.1.

Fig. 6. Effect of Cu(II) on the transformation of 3.5 μM CCl_4 under microbial Fe(III)-reducing conditions at pH 7.0 ± 0.1.

is consistent with the results obtained in the microbial growth experiment. In addition, the oxidation of Fe(II) by Cu(II) to form secondary Fe(III) oxide minerals may also explain this phenomenon. Our previous study (Maithreepala and Doong, 2004b) found that Fe(II) can serve as a reductant that reacts with the available Cu(II) under anaerobic conditions to form ferrihydrite and Cu(I). The aqueous Cu(I) concentration after filtration increased from 0.045 mM at 0.1 mM Cu(II) to 0.126 mM at 0.5 mM Cu(II) after incubation of 45 d, which corresponds to 26–45% conversion of the added Cu(II). This result is in good agreement with the results obtained in our previous study in which 0.5 mM Cu(II) was added into a 3 mM aqueous Fe(II) solution (Maithreepala and Doong, 2004b). This means that biogenic Fe(II) may react with Cu(II) to form Cu(I) and other ferric oxides at neutral pH under microbial Fe(III)-reducing conditions.

Because the addition of Cu(II) into the ferric oxide suspensions containing Fe(II) has a synergistic effect on the abiotic dechlorination of CCl_4 under anoxic conditions (Maithreepala and Doong, 2004b,c), the impact of Cu(II) on the biotransformation of CCl_4 under the microbial iron-reducing conditions was also studied. Fig. 6 shows the effect of Cu(II) on the transformation of 3.5 μM CCl_4 in the ferrihydrite suspensions containing *G. sulfurreducens* and AQDS at neutral pH. The CCl_4 concentration
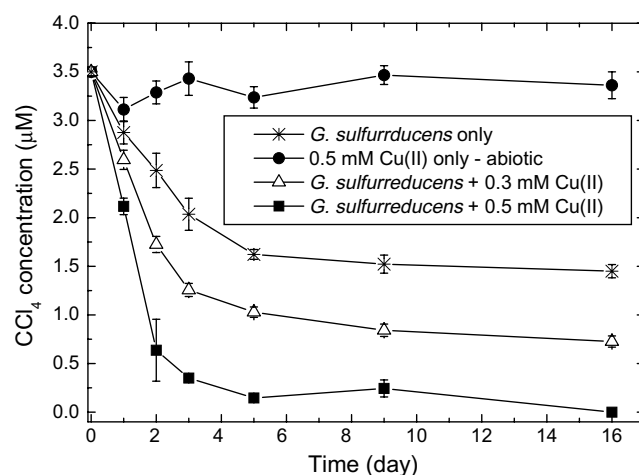
decreased rapidly in the bacteria-inoculated solutions, while no obvious decrease in CCl_4 concentrations occurred in the sterilized control. Nearly 80% and 99% of the initial CCl_4 was transformed after incubation of 16 d when solutions containing 0.3 and 0.5 mM Cu(II), respectively. The rate constant for CCl_4 transformation in the presence of 0.3 and 0.5 mM Cu(II) were 0.34 and 0.69 d^{-1}, respectively, which are 2.1–4.2 times higher than that obtained in the absence of Cu(II) (0.16 d^{-1}).

The enhanced efficiency and rate of CCl_4 transformation in the presence of Cu(II) ions is attributed to the formation of Cu(I) and secondary iron minerals (e.g., goethite). Dissolved Fe(II) can react with Cu(II) chemically to produce different compositions of Fe(III) oxides that accelerate the dechlorination of CCl_4 (Maithreepala and Doong, 2004c). Fig. 7 shows the XRPD patterns of the biologically produced solids after incubation of 16 d in the absence and presence of 0.5 mM Cu(II). In the absence of Cu(II) ion, peaks at 30.10°, 35.42°, 56.94°, and 62.51° (2θ) were clearly
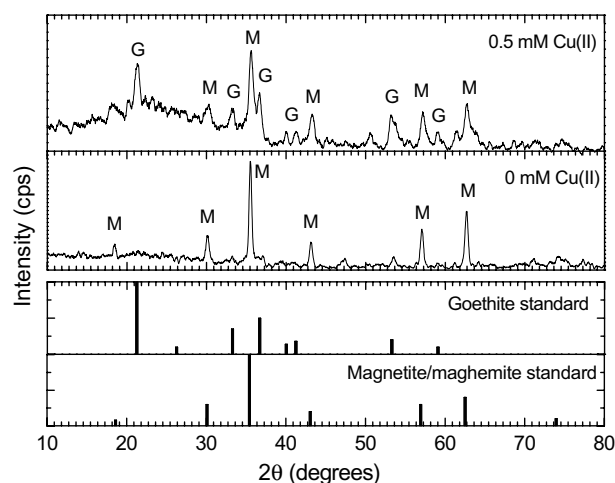

Fig. 7. XRPD patterns of the solids produced from microbial reduction of Fe(III) oxides mediated by *Geobacter sulfurreducens* in the absence and presence of 0.5 mM Cu(II). M: magnetite/maghematite, G: goethite.

observed, which may be assigned as magnetite or maghemite. After the addition of 0.5 mM Cu(II), additional peaks appeared at 33.26°, 36.68° and 41.22°, which were assigned as goethite. Goethite is a highly crystalline ferric oxide that has been demonstrated to effectively enhance the dechlorination efficiency and rate of chlorinated compounds by Fe(II) under anoxic conditions (Amonette et al., 2000; Maithreepala and Doong, 2004b). Maithreepala and Doong (2004c) depicted that different stoichiometric ratios of Fe(II) and Cu(II) can be used to produce ferric oxides in different morphologies and to accelerate the dechlorination rate of CCl₄. Previous studies (Haderlein and Pecher, 1998; Maithreepala and Doong, 2004b) also reported that Fe(II) could be fixed to ferric oxide mineral surfaces, resulting in the formation of different Fe(III) oxide morphologies after relatively long contact times. The addition of Cu(II) may destabilize the ferrihydrite and accelerate the evolution of ferrihydrite to crystalline form. Although the addition of Cu(II) lowered the Fe(II) concentration produced by *G. sulfurreducens*, in this study, the formation of Cu(I) and goethite could significantly accelerate the transformation efficiency and rate of CCl₄ under iron-reducing conditions. In addition, the chemically produced AHQDS was found to be capable of converting Cu(II) to Cu(I) with high efficiency (>85%). This means that AQDS could be reduced by *G. sulfurreducens* under iron-reducing conditions, and then reacted with Cu(II) to form Cu(I). The Cu(I) ion may be re-oxidized to Cu(II) by providing electrons to CCl₄, and subsequently acts as an electron mediator to enhance the dechlorination rate of CCl₄.

To further understand the kinetics of CCl₄ reduction by biogenic Fe(II) in the presence of Cu(II) ions, experiments at a high concentration of CCl₄ (40 μM) using the biogenic ferric oxides was performed under anoxic conditions. Biogenic ferric oxides were produced from the conversion of 0.56 mg-Fe ml⁻¹ of ferrihydrite mediated by *G. sulfurreducens* at pH 6.9–7.1 in the presence of 10 μM AQDS. After incubation of 2 d, the color changed to black and the measured concentration of Fe(II) in cultures were 2.65 ± 0.10 mM ($n = 4$). Subsequently, Cu(II) and CCl₄ were added to the batch systems to obtain concentrations of 0.5 mM and 40 μM, respectively. Fig. 8 shows the transformation of CCl₄ by the biogenic iron species in the absence and presence of 0.5 mM Cu(II) ions. In the absence of Cu(II) ions, the concentration of CCl₄ decreased slightly from 40 μM to 32.3 μM within 72 h. Addition of 0.5 mM Cu(II) significantly enhanced the efficiency and rate of CCl₄ transformation and 94% of the initial CCl₄ was transformed within 72 h. The transformation of CCl₄ continued to follow the pseudo-first-order kinetics and the k_{obsCT} for CCl₄ transformation in the absence and presence of 0.5 mM Cu(II) were 0.085 ± 0.009 and 1.40 ± 0.21 d⁻¹, respectively. It is noted that, even at high concentration of CCl₄, the rate constant for 40 μM CCl₄ transformation in the presence of 0.5 mM Cu(II) was 2.1 times higher than that for 3.5 μM CCl₄ (0.69 d⁻¹). This result clearly shows that the addition of Cu(II) significantly enhances the chem-
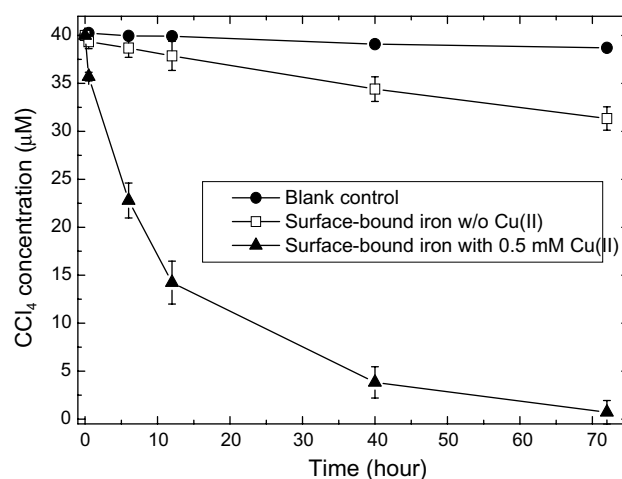

Fig. 8. Transformation of 40 μM CCl₄ by biogenic surface-bound iron species in the absence and presence of 0.5 mM Cu(II) at pH 7.0 ± 0.1.

ical transformation rate of CCl₄ under iron-reducing conditions.

4. Conclusions

In this study, we demonstrate the biological and chemical reactions of CCl₄ by the biogenic iron species produced from the reductive dissolution of ferrihydrite in the presence of AQDS and Cu(II) ions. Ferrihydrite was reductively dissolved to Fe(II) by *G. sulfurreducens* in the presence of low concentration of AQDS. The produced Fe(II) could sorb onto the surface of iron oxides to effectively facilitate the chemical transformation of CCl₄ under iron-reducing conditions. Addition of Cu(II) ions has different effects on the bioreduction of ferric oxides and the transformation of CCl₄. The microbial activity of *G. sulfurreducens* is slightly inhibited by Cu(II), which decreases the production of Fe(II). However, the produced Fe(II) species serves as reductants for Cu(II) to form Cu(I) and goethite, which enhances the abiotic transformation efficiency and rate of CCl₄. *Geobacter sulfurreducens* also can reduce AQDS to its reduced form, and then reacts with Cu(II) to produce Cu(I). Therefore, copper species may serve as the possible catalyst in the surface-bound Fe(II) species to significantly enhance the abiotic transformation of CCl₄ in the presence of DIRB under iron-reducing conditions. Results obtained in this study clearly show that addition of Cu(II) may destabilize the ferrihydrite and accelerate the evolution of ferrihydrite to crystalline iron oxide minerals, resulting in the formation of catalytic Cu(I) and secondary iron oxide minerals which may further maintain the chemical reactivity of surface-bound iron species over a long period of time.

References

Amonette, J.E., Workman, D.J., Kennedy, D.W., Fruchter, J.S., Gorby, Y.A., 2000. Dechlorination of carbon tetrachloride by Fe(II) associated with goethite. Environ. Sci. Technol. 34, 4606–4613.

Cervantes, F.J., Vu-Thi-Thu, L., Lettinga, G., Field, J.A., 2004. Quinone-respiration improves dechlorination of carbon tetrachloride by anaerobic sludge. Appl. Microbiol. Biotechnol. 64, 702–711.

Curtis, G.P., Reinhard, M., 1994. Reductive dehalogenation of hexachloroethane, carbon tetrachloride, and bromoform by anthrahydroquinone disulfonate and humic-acid. Environ. Sci. Technol. 28, 2393–2401.

Doong, R.A., Chiang, H.C., 2005. Dechlorination of carbon tetrachloride by thiol compounds in the presence of quinone compounds. Environ. Sci. Technol. 39, 7460–7568.

Doong, R.A., Schink, B., 2002. Cysteine-mediated reductive dissolution of poorly crystalline iron(III) oxides by *Geobacter sulfurreducens*. Environ. Sci. Technol. 36, 2939–2945.

Doong, R.A., Chen, K.T., Tsai, H.C., 2003. Reductive dechlorination of carbon tetrachloride and tetrachloroethylene by zerovalent silicon–iron reductants. Environ. Sci. Technol. 37, 2575–2581.

Fredrickson, J.K., Zachara, J.M., Kennedy, D.W., Dong, H.L., Onstott, T.C., Hinman, N.W., Li, S.M., 1998. Biogenic iron mineralization accompanying the dissimilatory reduction of hydrous ferric oxide by a groundwater bacterium. Geochim. Cosmochim. Acta 62, 3239–3257.

Gregory, K.B., Larese-Casanova, P., Parkin, G., Scherer, M.M., 2004. Abiotic transformation of hexahydro-1,3,5-trinitro-1,3,5-triazine by Fe^{II} bound to magnetite. Environ. Sci. Technol. 38, 1408–1414.

Haderlein, S.B., Pecher, K., 1998. Pollutant reduction in heterogeneous Fe(II)–Fe(III) systems. In: Sparks, D.L., Grundl, T.J. (Eds.), Kinetics and Mechanisms of Reactions at the Mineral/Water Interface, In: ACS Symposium Series. American Chemical Society, Washington, DC, pp. 342–357.

Heijman, C.G., Grieder, E., Holliger, C., Schwarzenbach, R.P., 1995. Reduction of nitroaromatic compounds coupled to microbial iron reduction in laboratory aquifer columns. Environ. Sci. Technol. 29, 775–783.

Holmes, D.E., Finneran, K.T., O'Neil, R.A., Lovley, D.R., 2002. Enrichment of members of the family *Geobacteraceae* associated with stimulation of dissimilatory metal reduction in uranium-contaminated aquifer sediments. Appl. Environ. Microbiol. 68, 2300–2306.

Jeon, B.H., Kelly, S.D., Kemner, K.M., Barnett, M.O., Burgos, W.D., Dempsey, B.A., Roden, E.E., 2005. Microbial reduction of U(VI) at the solid–water interface. Environ. Sci. Technol. 38, 5649–5655.

Kao, C.M., Chen, Y.L., Chen, S.C., Yeh, T.Y., Wu, W.S., 2003. Enhanced PCE dechlorination by biobarrier systems under different redox conditions. Water Res. 37, 4885–4894.

Kim, S., Picardal, F.W., 1999. Enhanced anaerobic biotransformation of carbon tetrachloride in the presence of reduced iron oxides. Environ. Toxicol. Chem. 18, 2142–2150.

Lee, B.D., Walton, M.R., Megio, J.L., 2005. Biological and chemical interaction with U(VI) during anaerobic enrichment in the presence of iron oxide coated quartz. Water Res. 39, 4363–4374.

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

Lovley, D.R., Anderson, R.T., 2000. Influence of dissimilatory metal reduction on fate of organic and metal contaminants in the subsurface. Hydrogeol. J. 8, 77–88.

Liu, C.X., Gorby, Y.A., Zachara, J.M., Fredrickson, J.K., Brown, C.F., 2002. Reduction kinetics of Fe(III), Co(III), U(VI) Cr(VI) and Tc(VII) in cultures of dissimilatory metal-reducing bacteria. Biotechnol. Bioeng. 80, 637–649.

Maithreepala, R.A., Doong, R.A., 2004a. Enhanced remediation of carbon tetrachloride by Fe(II)–Fe(III) systems in the presence of copper ions. Water Sci. Technol. 50 (8), 161–168.

Maithreepala, R.A., Doong, R.A., 2004b. Synergistic effect of copper ion on the reductive dechlorination of carbon tetrachloride by surface-bound Fe(II) associated with goethite. Environ. Sci. Technol. 38, 260–268.

Maithreepala, R.A., Doong, R.A., 2004c. Reductive dechlorination of carbon tetrachloride in aqueous solutions containing ferrous and copper ions. Environ. Sci. Technol. 38, 6676–6684.

Maithreepala, R.A., Doong, R.A., 2005. Enhanced dechlorination of chlorinated methanes and ethenes by chloride green rust in the presence of Cu(II). Environ. Sci. Technol. 39, 4082–4090.

Markwiese, J.T., Colberg, P.J.S., 2000. Bacterial reduction of copper-contaminated ferric oxide: copper toxicity and the interaction between fermentative and iron-reducing bacteria. Arch. Environ. Contam. Toxicol. 38, 139–146.

McCormick, M., Bouwer, E.J., Adriaens, P., 2002. Carbon tetrachloride transformation in a model iron-reducing culture: relative kinetics of biotic and abiotic reactions. Enviorn. Sci. Technol. 36, 403–410.

McCormick, M.L., Adriaens, P., 2004. Carbon tetrachloride transformation on the surface of nanoscale biogenic magnetite particles. Environ. Sci. Technol. 38, 1045–1053.

Nevin, K.P., Lovley, D.R., 2000. Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. Environ. Sci. Technol. 34, 2472–2478.

O'Loughlin, E.J., Kemner, K.M., Burris, D.R., 2003. Effects of Ag^{I}, Au^{III}, and Cu^{II} on the reductive dechlorination of carbon tetrachloride by green rust. Environ. Sci. Technol. 37, 2905–2912.

Pecher, K., Haderlein, S.B., Schwarzenbach, R.P., 2002. Reduction of polyhalogenated methanes by surface-bound Fe(II) in aqueous suspensions of iron oxides. Environ. Sci. Technol. 36, 1734–1741.

Roden, E.E., Urrutia, M.M., 2002. Influence of biogenic Fe(II) on bacterial crystalline Fe(III) oxide reduction. Geomicrobiol. J. 19, 209–251.

Schwertman, U., Cornell, R.M., 1991. Iron Oxides in the Laboratory: Preparation and Characterization. VCH Verlagsgsellschaft mbH, Weinheim D-6940, Germany.

Scott, D.T., McKnight, D.M., Blunt-Harris, E.L., Kolesar, S.E., Lovley, D.R., 1998. Quinone moieties act as electron acceptors in the reduction of humic substances by humics-reducing microorganisms. Environ. Sci. Technol. 32, 2984–2989.