Redox processes at the mineral-water interfaces under sub-surface conditions play a vital role in deciding the fate of reducible contaminants in groundwater. Oxidation of surfae-bound and/or structural Fe(II) species associated with iron bearing minerals may couples with reductive dechlorination of chlorinated hydrocarbons under anoxic conditions. The presence of other metal ions also may influence on this dechlorination reactions. This book provides some laboratory experimental evidences of enhanced degradation of chlorinated methanes by Fe(II) originated by chemical or microbiological mediated reactions, associated with various types of iron containing minerals in the presence of low concentrations of copper ions and possible formation of new reactive surface-sites.

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Influence of Cu ions on the dechlorination reactions and modification of mineral surfaces

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Degradation of Chlorinated Hydrocarbons by Fe(II) species associated with Iron minerals and Influence of Cu species

R.A.Maithreepala

Preface

Dechlorination of chlorinated aliphatic hydrocarbons including carbon tetrachloride (CCl_4), and byproduct of dechlorination of it, tetrachloroethane (C_2Cl_4) and trichloroethene (C_2HCl_3) using different types of Fe(II)/Fe(III) systems that produce suitable redox conditions leading abiotic dechlorination reactions and the effect of Cu(II) ions on these reactions were investigated using laboratory based experiments. This study provides evidence that there are possibilities of such chemical reactions occur under natural subsurface conditions and may cause natural attenuation of chlorinated hydrocarbons in groundwater in contaminated sites. Chapet 1 gives an introduction and basic background for the relevant topics and the main objectives of this study.

Results of dechlorination experiments using most abundant iron oxi(hydro)-xide in the nature, namely goethite (α-FeOOH), iron oxide, hematite (α-Fe_2O_3), magnetite (Fe_3O_4) and iron hydroxide, amorphous ferrihydrite ($Fe(OH)_3$) associated with dissolved Fe(II) are discussed in chapter 2. Surface-bound iron species were found to be reactive under anoxic conditions. X-ray photoelectron spectroscopy (XPS) and X-ray powder diffraction (XRPD) were used to characterize the chemical states and crystal phases of solid phases, respectively. Also, scanning electron microscopy (SEM) was employed to identify the surface morphology of the solid phases. CCl_4 was not dechlorinated by dissolved Fe(II) or iron oxides at neutral pH. However, significant dechlorination of $_{\text{CCL4}}$ was observed at near neutral pH when Fe(II) was associated with iron oxides and the experiments done for comparison of reactivity of dissolved Fe(II) and Fe(II) associated with iron minerals systems.

Chapter 3 illustrates the reactivity of CCl_4 degradation in the mixture, when Cu(II) was added into Fe(II) solution in the absence of iron oxide minerals, the oxidation of Fe(II) into Fe(III) coupling with Cu(II) reduction to form a new mineral phase was observed. XRD and XPS analysis suggested that this solid phase contained amorphous ferrihydrite and Cu_2O. When the initial Fe(II)/Cu(II) ratios in the solution varied from 1 to 10, the reaction rate for $_{\text{CCL4}}$ dechlorination (k_{obs}) increased 250-fold and the produced secondary minerals also changed from ferrihydrite to goethite and hematite and then again changed to ferrihydrite at higher ratios. Cu(II) ion also has the synergistic effect on the dechlorination of chlorinated hydrocarbons in the presence of green rust and biogenic iron oxides.

Chapter 4 discusses that the addition of Cu(II) in to the green rust which contained both Fe(II) and Fe(III) in suspension effectively dechlorinated chlorinated methanes Carbon Tetrachloride (CCl_4), Chloroform ($CHCl_3$) and chlorinated ethenes (C_2Cl_4, C_2HCl_3). The k_{obs} for dechlorination increased by 84 times for CCl_4, 4.7 times for C_2Cl_4 and 7 times for C_2HCl_3. The results of XRD and XPS analysis showed that the oxidation of green rust chloride (GR(Cl)) to magnetite resulted in the reduction of Cu(II) to Cu(0) and Cu(I).

Chapter 5 explains that in the presence of *Geobacter sulfurreducens*, Fe(III) reducing anaerobic bacteria, ferrihydrite can be biologically dissoluted to produce Fe(II) and magnetite, resulting in the formation of biogenic Fe(II)-Fe(III) system for the dechlorination of chlorinated hydrocarbons. Addition of Cu(II) also enhanced the dechlorination of $CCl_4$by biogenic Fe(II) under microbial Fe(III) reducing condition.

Chapter 6 explains that results obtained in this study give impetus that Cu(II) can increase the reductive dechlorination process led by natural Fe(II) systems in the subsurface conditions for the natural attenuation of highly chlorinated compounds or engineered systems that facilitate the *in-situ* cleanup of chlorinated hydrocarbons using Fe(II)/Fe(III) systems.

Acknoledgement

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Chapter 1

GENERAL INTRODUCTION

1.1 BACKGROUND AND THEORY

1.1.1 Introduction

The contamination of groundwater with chlorinated hydrocarbons is a well-recognized environmental problem. The most prevalent chlorinated hydrocarbons are polychlorinated aliphatic hydrocarbons such as chlorinated methanes and ethenes.[1-3] These compounds have been used for several decades in large quantities for different purposes as solvents, refrigerants, forming agents, pesticides, propellants and for household purposes such as dry-cleaning.[4-6] Due to the frequent release to the environment by accidental leakage from tanks, or from leaching of waste disposal sites, these compounds can be found in the contaminated environment.[7-9] Generally, many chlorinated hydrocarbons can exist for a significant long time in subsurface environments and gradually enter into human body and biosphere. Table 1-1 shows the physico-chemical properties and the suggested half-lives of chlorinated methanes and ethenes those are most frequently found in soil and ground water in contaminated sites.[10] Their toxic effects to human beings and environmental impact prompt the investigation of possible remedial technologies. Up to now, remediation of groundwater contaminated with chlorinated solvents is one of more complex technical challenges faced by environmental engineers and scientists.[1,2] Because of the limitation of oxygen concentration under subsurface conditions[11], reductive dechlorination is the most favorable pathway for the dechlorination of chlorinated hydrocarbons. Reductive dechlorination of chlorinated hydrocarbons in natural anoxic environments can undergo through both biotic and abiotic reactions.[12] Biological reductive dechlorination is driven by microorganisms using chlorinated compounds as terminal electron acceptors coupling with the oxidation of organic Compounds.[13-17] Abioti reductive dechlorination occurs due to the reduction by various types of natural reductants such as co-enzymes[18-20], and reduced natural organic matter (NOM) and quinone moieties.[21] Moreover, Fe(II)-bearing sulfide minerals and sheet silicates (biotite and vermiculite)[22-25], clay mineral and silica [26,27] have been used for the dechlorination of chlorinated aliphatic hydrocarbons. In the sulfur-containing Fe(II) mineral systems, sulfide ions play a major role in the dechlorination reaction.[22] However, in the Fe(II)-bearing and Fe(III) minerals (including oxides, hydroxides and oxihydroxides), Fe(II) ions have been identified as efficient natural reductants for the dechlorination of chlorinated compounds in subsurface environments. Since iron minerals are ubiquitous in the natural environment and being non-toxic, the abiotic dechlorination of chlorinated hydrocarbons by Fe(II) species has recently received highly attention.

1.1.2 Iron oxides

Iron is the fourth most abundant element and the second most abundant metal in the earth's crust and contains around 5.1% by mass.[28] Therefore, iron is an ubiquitous element in the surface and subsurface. Depending on the conditions, iron forms stable compounds in the state of ferrous Fe(II) or ferric Fe(III) such as hematite ($Fe^{III}_2O_3$), siderite ($Fe^{II}CO_3$), and magnetite ($Fe^{II}Fe^{III}_2O_4$). Fe(II) iron is one of the most abundant reductants typically present in aquatic and terrestrial environments under suboxic and anoxic conditions.[29-31] In these environments, Fe(II) may be available in different ways such as in soluble form complexing with organic and inorganic compounds, in structural form as Fe(II)-bearing mineral phases and clays and in surface-bound form complexing with solid Fe(III) or Fe(II) containing minerals. Fe(II)-bearing minerals in soils and sediments under anoxic conditions are commonly available with sulfur ions, carbonate ions or phosphate ions including FeS_2 (pyrite), FeS (troilite), $FeCO_3$ (siderite) and hydrated phosphates $Fe_3(PO_4)_2.(H_2O)_8$ (vivianite).[28,29] Fe(II) ions in those minerals can react with oxygen and be oxidized to Fe(III) in the case of exposing to air. Therefore, many types of Fe(II) minerals are only available under anoxic conditions or at low pH in the presence of oxygen.[28,29] Large fractions of iron in the subsurface also exist in the form of Fe(III) and are available as oxides, hydroxides and oxihydroxides those are commonly referred as iron oxides. Basically, Fe(III) ions have low solubility, and measurable concentrations of Fe(III) ions in natural water could be available only under strong acidic conditions.[32] Hence, almost all of the Fe(III) ion is available as insoluble oxides or hydroxides of which containing Fe(II) is structurally combined with Fe(III). To date 16 types of "iron oxide" minerals have been identified.[33] These iron oxides are summarized in Table 1-2 with their mineral names and molecular formula.[32] Certain iron oxides such as magnetite and green rusts contain both Fe(II) and Fe(III) . Basically iron oxides those do not contain Fe(II) are very stable and do not react as electron donors and exist even in surface soil or surface water that often expose to air. Depending on the pH, Eh, temperature, water-activity, and microbial activity in the local subsurface environment, the precipitation, dissolution and re-precipitation of iron oxides may occur in different ways and those activities result in various compositions of iron oxides. However, the most abundant and available iron oxides in the nature are goethite, hematite, ferrihydrite and magnetite.[32,34,35] Goethite, hematite and magnetite are well crystalline iron minerals, while ferrihydrite is a poorly crystalline mineral, which shows most amorphous characteristics with very small particle size compared to other crystalline iron minerals. In the natural environments, ferrihydrite is thought to be a precursor to form other crystal iron oxide minerals through different mechanisms, depending on the presence of Fe(II) ions[36], other transition metal ions, anions[37-39], pH and temperature.[32] During the transformations, the resultant minerals include hematite, goethite and in some cases, lepidocrocite or mixtures of those minerals.[37] Moreover, ferrihydrite also converted to crystal minerals under subsurface conditions through reductive dissolution of Fe(III) by microbial activities and the product is mainly mixed-valence iron oxides such as magnetite.

1.1.3 Microbial Fe(III) reduction

Until late 1980s, it was generally believed that Fe(II) available in the subsurface and anoxic sediment environment was only due to the abiotic reduction of Fe(III) minerals.[40] However, isolation and characterization of dissimilatory Fe(III)-reducing bacteria (DIRB) has indicated that complete oxidation of complexed organic matter into CO_2 using Fe(III) as the sole electron acceptor.[41]The schematic explanation of organic matter metabolism coupling to the Fe(III) reduction by DIRB is shown in Figure 1-1. Microbial Fe(III) reduction is believed to be the major reason for the formation of Fe(II) associated with Fe(III) oxides and dissolved Fe(II) under subsurface conditions.

Geochemical and microbiological evidences indicate that Fe(III) reduction occurred on early earth prior to other metabolisms such as sulphur-reduction.[42] Up to date numbers of bacteria that can reduce Fe(III) have been isolated form various natural environments such as soil, marine sediment, freshwater sediment, deep aquifers as well as hot springs. The metabolic pathways of iron reducing bacteria vary according to the phylogenic characteristics.

Table 1-1. Some physic=chemical properties and suggested half-lives of selected chlorinated methanes and ethanes

Compound	Molecular formular	Molecuar weight (g Mole^{-1})	Physio-chemical properties				half-life (Years)^{b}
			Boiling point (ºC)	Vapor pressure (Pa) at 25ºC	Density (g mL^{-1}) at 20ºC	Solubility (g mL-1)	
Methyl chloride	CH_3Cl	50.49	-23.73 ~-24.22	480000 ~ 574483	0.915~0.921	7400 ~5049	
Dichloromethane	CH_2Cl_2	84.94	39.64 ~ 41.00	54813 ~ 58400	1.32 ~1.33	1969 ~ 2000	704 ~
Chloroform	$CHCl_3$	119.38	61.00 ~ 62.00	25700 ~ 26344	1.483~1.490	7361 ~8000	3500
Carcon tetrachoride	CCl_4	153.82	76.50 ~ 77.00	4339 ~	1.584~1.591	770 ~800	7000
Vinyl chloride	CH_2=CHCl	62.50	-13.37 ~ 13.80	354578 ~ 35000	0.910	1100 ~2700	11
1-1-Dichloroethene	CCl_2=CH_2	96.94	31.56 ~ 31.70	79713 ~ 86433	1.218~1.220	1594 ~2250	-
Cis-1,2-Dicholorethene	CCl_2 =CH_2	96.94	60.00 ~ 60.44	2660 ~ 2761	1.280~1.283	3500 ~3520	-
Trans-1,2-Dicholorethene	CHCl=CHCl	96.94	47.5 ~ 48.00	43177 ~ 45300	1.255~1.260	6259 ~5300	-
Trichloroethene	CHCl = CCl_2	131.83	86.9 ~87.30	9224 ~ 10264	1.460~1.464	1100~1366	15
Tetrachlorothene	CCl_2=CCl_2	165.83	120.97 ~ 121.4	2462 ~ 2666	1.614~1.630	150 ~489	15

A; data from 10, b; half life data from reference 12

Table 1-2. molecular formula, chemical forms and mineralogical names of major ironoxi(hydro)xides minerals

Mineralogical name	Chemical form	Molecular formular
Goethite	Feric oxihydroxide	α-FeOOH
Akaganeite	Feric oxihydroxide	β-FeOOH
Lepidocrocite	Feric oxihydroxide	γ- FeOOH
Ferrihydrite	Ferric hydroxide	$Fe(OH)_3$ $Fe_5OH_8.4H_2O$
Feous hydroxide	Ferous hydroxide	$Fe(OH)_2$
Wȕsite	Ferous oxide	FeO
Hematite	Ferric oxide	α-Fe_2O_7 β-Fe_2O_3
Maghemite	Ferric oxide	γ-Fe_2O_3 ε-Fe_2O_3
Magnetite	Ferrosoferric oxide	Fe_3O_4 or $Fe^{II}Fe^{II}{}_2O_4$
Greeen rust chloride	Ferrous ferric hydroxide chloride	$[Fe^{II}{}_3Fe^{III}(OH)_8{}^+.[Cl.2H_2O]^-$
Green rust sulfate	Ferrous ferric hydroxide sulfate	$[Fe^{II}{}_4Fe^{III}{}_2(OH)_{12}{}^{2+}.[SO_4.2H_2O]^{2-}$
Green rust carbonate	Ferrous ferric hydroxide carbonate	$[Fe^{II}{}_4Fe^{III}{}_2(OH)_{12}{}^{2+}.[CO_3.2H_2O]^{2-}$

1.1.4 Electron shuttling compounds to facilitate microbial Fe(III) reduction

In the biological Fe(III) reduction, electrons are transformed from bacteria to Fe (III). Basically Fe(III) oxides are present as insoluble solids under near neutral or alkaline conditions. Although it is still unclear how the Fe(III) reducers access Fe(III) oxides, it is believed that Fe(III) reducer must directly contact with the Fe(III) oxide particle surfaces to transfer electrons to Fe(III).[63] In previous experiments using semi permeable membranes for the separation of bacteria and solid Fe(III) minerals, it was indicated that direct connection between bacterial cell membrane and solid iron oxide is needed.[64,65] Lovley et al [66] fortuitously found that humic acid could be reduced by bacteria serving as terminal electron acceptor coupling with the oxidation of organic compounds and hydrogen and then greatly enhanced the rate and efficiency of biological Fe(III) oxide reduction by *Geobacter metallireducens*.[66] Further studies found that anthraquinone disulfonate (AQDS) and quinone compounds can act as electron shuttling compounds between solid Fe(III) oxide minerals and DIRB to enhance the rate of microbial reduction of solid Fe(III) oxide.[66] The proposed mechanism for the electron shuttling process by quinone compounds between Fe(III) reducing bacteria and solid Fe(III)oxide mineral is shown in Figure 1-2, using 2, 6-anthraquinon disulfonate (AQDS) as the model compound. Both in the pristine and contaminated subsurface environments, humic substances are available and transformation of electrons from reduced humic substances to insoluble Fe(III) oxides is an abiotic process which can take place even in the absence of bacteria.[49]

However, the reduction of oxidized humic acid to the reduced form is a biological reaction that can contribute a vital role to continue the Fe(III) reduction. It has been studied that all the Fe(III) reducing bacteria also can reduce humic compounds[49], suggesting that biological reduction of solid Fe(III) occurs through electron shuttling with humic compounds.

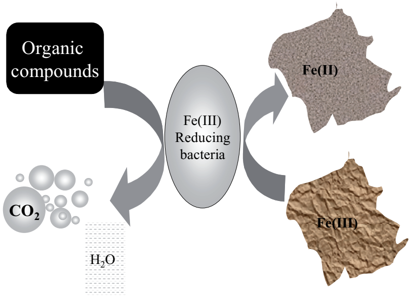

Figure 1-1. Schematic diagram showing microbial reduction of Fe(III) oxides by Fe(III)-reducing bacteria to respirate organic compounds.

Recent studies also proposed the probability of excreting electron shuttling compounds like *c-type* cytochrome by Fe(III)-reducing bacteria.[67] However, this hypothesis is still under critical arguments.[68] In addition to quinone compounds, cysteine at concentrations higher than 0.5 mM has been found to be an effective mediator to transfer electrons between solid Fe(III) and DIRB.[45] Reduction of chelated-Fe(III) and solid Fe(III) oxides by cysteine has been known for several years.[69-71] Doong and Schink[45] experimentally proved that dissimilatory iron reducing bacteria, *G. sulfurreducens* could reduce cystine to cysteine coupling with the oxidation of acetate to $CO_{2,}$ and subsequently enhanced the microbial Fe(III) reduction. The schematic mechanism for biotic abiotic processes for cysteine-mediated Fe(III) reduction is shown in Figure 1-3. Since cysteine is not available at outside of living cells in natural environments, the cysteine should be released or execrated from bacterial cells and the knowledge related to this point is still lacking.

Table 1-3. Examples of Fe(III)-reducing bacteria, their electron donors, acceptors with e shuttling compounds studied for Fe(III) oxide reduction.

Microbial Strain	Electron donor	ctron acceptor	Shuttling Compound	Ref.
Geobactor bremensis **Geobactor.pelophilus**	hydrogen, formate, acetate, pyruvate, succinate	ferrihydrite, Mn, fumarate	no data	43
Geobacter-sulfurreducens	acetate , hydrogen lactate	Fe(III)PP, Fe(III) citrateFe(III)oxihydroxide,ferrihydrite, S^0, Co-EDTA, fumarate, malate Tc(VII),Co(III)-EDTA,U(IV), Cr(IV), oxygen	cysteie AQDS,humics	44 45 46 47, 48,49
Geobacter-.metallireducens	acetate, short-chain fatty acids, alcohols, monoaromatic compounds	Ferrihydrite , V(V), Mn(IV), U(VI), nitrate	AQDS lawson naphthalene sulfonic humics	50, 51 49
Desulfomonas TT4B	acetate, pyruvate,	tetrachloroethylene terichloroethylene Fe(II)-nitirloacetate	not relevant	17
Shewanella.alga	hydrogen, lactate	Co-EDTA, fumarate, malate Tc(VII), Co(III)-EDTA, U(IV), Cr(IV), Mn(IV), U(VI), thiosulfate, or trimethylamine	not relevant	47,52
Shewanella.-putrefaciens (Shewanella.-oneidensis)	hydrogen, Lactate	Co-EDTA, Fumarate, malate, Tc(VII), Co(III)-EDTA, U(IV),Cr(IV), Fe(III)-citrate, Ferrihydrite, magnetite, Smectite-Fe(III) Smectite-Fe(III)	no data	47,53, 54 ,5 5,56
Rhodoferax-ferrireducens	acetate, lactate, malate, propionate, pyruvate, succinate benzoate	Fe(III)-oxides, Fe(III)-NTA Mn(IV) oxide, nitrate, fumarate, oxygen	no data	57
Geothermobacter-enrlichii	malate, sugar, starch, amino acid	Fe(III)oxide, nitrate	No data	58
Desulfitobacterium dehalogenans	acetate	ferrihydrite	AQDS,	49
Geothrix fermentens	acetate	ferrihydrite	AQDS,	49
Goespirillum barnesii	propionate, succinate, lactate, valerate	propionate, succinate, lactate, valerate	AQDS	49,59
Geothrix fermentens	acetate	ferrihydrite	AQDS,	49

The microbial reduction of Fe(III) oxide minerals to Fe(II) has several impacts on the environments. These include the depletion of Fe(III) in the sediment, the increase of dissolved Fe(II) concentration in groundwater and the formation of surface-bound Fe(II) species and structural Fe(II)-containing minerals in the subsurface environments.[72] The various forms of resulted Fe(II) is contributive to the reducing conditions in the relevant environments. Although the Fe(III) reduction is a biological reaction, the formation of Fe(II)-containing oxide or hydroxide minerals (mainly magnetite and green rust) that occurs during microbial Fe(III) reduction is considered to be an abiotic reaction due to the interaction of biogenic Fe(II) ions with Fe(III) oxides.

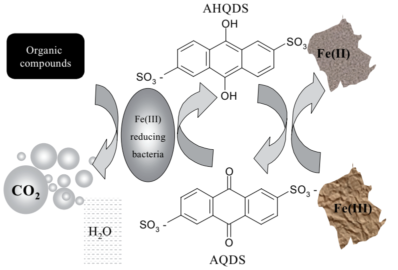

Figure 1-2. The schematic electron flow in microbial Fe(III) reduction coupling to organic compounds oxidation to carbon dioxide and water using quinone compounds (i.e Anthraquinone disulfonate (AQDS)) as electron shuttling compound between bacterial cell membrane and solid Fe(III) oxide.

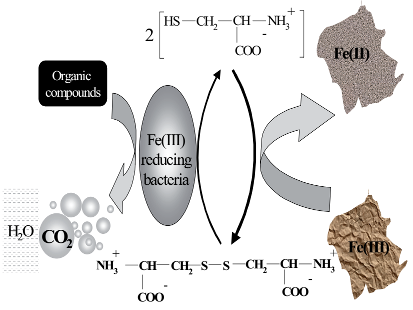

Figure 1-3. The schematic electron flow in microbial Fe(III) reduction coupling to organic compound oxidation to carbon dioxide and water using cysteine as electron shuttling compound between bacterial cell membrane and solid Fe(III) oxide.

1.1.5 Interaction of dissolved Fe(II) ions with iron minerals

Iron oxides can serve as efficient natural sorbents and play a vital role in the mobility and bioavailability of metal ions in the environment.[73]

Numerous studies have been carried out on the sorption of metal ions onto iron oxides.

$$\equiv Fe^{III}OH + Fe^{II} + H_2O \rightarrow \equiv Fe^{III}OFe^{II}OH + 2H^+ \tag{1-1}$$

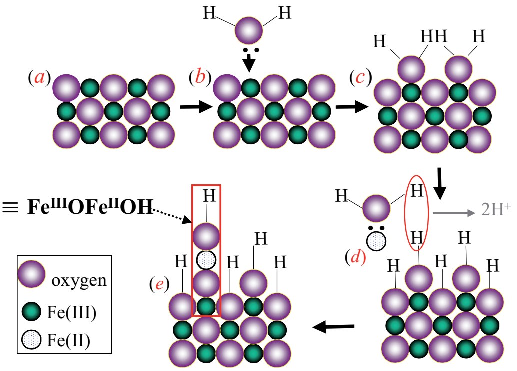

Figure 1-4. The schematic diagram of formation of surface hydroxyl groups on metal oxides (Fe(III) oxides) and replacement of hydrogen ion in the surface hydroxyl group by aqueous ferrous ion to form surface complexed ferresoferric hydroxyl ($\equiv Fe^{III}Fe^{II}OH$) groups.

However, only few studies have reported the interaction of Fe(II) ions on iron minerals.[74-77] Basically, Fe(II) ions are sorbed on iron oxides through surface complexation. Availability of hydroxyl groups on surface of iron oxide mineral are prerequisite for the surface complexation. Figure 1- 4 shows the schematic illustration of the formation of hydroxyl groups on the surface of Fe(III) oxides and replacement of hydrogen in the surface hydroxyl group by aqueous Fe(II) to form $\equiv Fe^{III}OFe^{II}OH$ groups. Consider a schematic cross section of metal oxide, the metal ions at the surface layer having reduced coordination number can act as Lewis acid to preferably accept electron pairs. Therefore, H_2O molecules are coordinated on the surface layer and then dissociated upon contacting with Fe(III) oxide surface, forming various types of surface hydroxyl groups to become thermodynamically more stable.[78,79] The aqueous Fe(II) ions in heterogeneous system can thus replace the hydrogen ions of surface-OH groups to form inner-sphere surface complexation, resulting in the formation of surface-bound ferresoferric oxihydroxide ($\equiv Fe^{III}OFe^{II}OH$) groups. Additionally, there is another possible reaction to form surface complex of ferrous ions with hydrogen of surface hydroxyl groups in the iron oxides as shown in equation 1-2 [74].

$$\equiv Fe^{III}OH + Fe^{II} \rightarrow \equiv Fe^{III}OFe^{I} + H^{+} \tag{1-2}$$

It is generally believed that equation 1-1 is the most possible reaction according to the results of potentiometric titrations.[74] The formation of inner-sphere surface complexes on the surface of Fe(III) oxide minerals can be explained by surface complexation model.[80] The surface complexation model is based on the hypothesis of monolayer sorption and hence can be expressed using well-known Langmuir isotherm, particularly in the range of low concentrations of dissolved Fe(II) to avoid precipitation on the mineral surfaces.[80] At high concentrations of dissolved Fe(II) and at high pH conditions, however Fe(II) species may precipitate over the monolayer and form multi-layers which can be explained by other sorption isotherm models such as surface precipitation model. The rate of surface complexation of Fe(II) on Fe(III) minerals may also depends on the affinity of Fe(III) minerals surface to Fe(II) ions. For instance, the sorption of Fe(II) onto solid surface depends on the coulombic interactions between surface and Fe(II) ion in the aqueous phase.[81] Therefore, surface charge is also an important factor to influence the rate of Fe(II) surface complexation.[81] Accumulation of positive charge on the surface can inhibit (or slow down) the uptake of metal ions.[78] Since the net charge on the hydrous oxide surface is established according to the proton balance at the solid-water interface, pH of the system is an important factor for Fe(II) surface complexation. The surface charge becomes more negative when pH increases, and thus enhance the sorption of Fe(II) towards the surface.[78] This phenomenon is not only valid for Fe(II) sorption but also for the other cation sorption on the surface of hydrous oxide surfaces.

Compared to the dissolved Fe(II), the Fe(II)-Fe(III) complexes, especially ≡$Fe^{III}OFe^{II}OH$-like complexes are strong reductants. The high reactivity of ≡$Fe^{III}OFe^{II}OH$ complexes can be attributed to (i) hydroxy ligands favour the oxidation of Fe(II)[82], and (ii) increased electron density at the Fe(II) center.[35,78] The low redox potential in most contaminated groundwaters and subsurface environments are thought to be mainly controlled by Fe(II)/Fe(III) couple resulted from surface-bound Fe(II) species.[83] The redox potentials of Fe(II) /Fe(III) oxide systems resulted from dissolved Fe(II) and Fe(II) associated with most abundant Fe(III) oxides are shown in Figure 1-5. This means that following by surface complexation of Fe(II), there is a possibility to form new secondary mineral layer on the surface. The composition of secondary mineral layer is resulted from the thermodynamically favourable redox couple. According to Figure 1-5, the redox couple shows the lowest potential is Fe(II)/ magnetite. The mixed valence magnetite ($Fe^{II}\,Fe^{III}{}_2O_4 = Fe_3O_4$) has been identified as the common product in reductive dissolution of Fe(III) minerals by DIRB.[84-87] Moreover, green rust which is a mixed-valence meta-stable mineral, also has been identified as a product in biological Fe(III) reduction with other iron minerals including magnetite and goethite.[84,88-91]

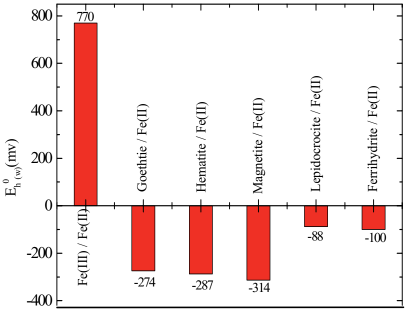

Figure 1-5. The oxidation-reduction potential for Fe(II) in dissolved form and associated with iron minerals. (at pH 7.0 and 25^{0}C) (the values were quoted from Stum and Morgen[78], and Straub et al.[33])

Green rust also can be formed abiotically by sorption of Fe(II) onto Fe(III) oxide surfaces.[74,92] Green rust is automatically formed in Fe(II)-containing solutions as a result of mineral phase transformation after the adsorption of Fe(II) on the ferrihydrite which generates as a result of oxygenation of Fe(II) species in the solution at the preliminary stage.[74] This is also the basic theory in the preparation of synthetic green rust in the laboratory by partial oxidation of Fe(II) solutions.[93,94]

It is known that green rust can be oxidized into magnetite or goethite depending on the oxidation rate and physico-chemical conditions. Field and laboratory experimental results show that sorption of Fe(II) onto Fe(III) minerals makes the environments more reducing and generates various types of iron minerals. The formation of green rust and magnetite after the sorption of Fe(II) onto ferric oxide (ferrihydrite) in the presence of anion A^{2-} can be explained as follows.[91]

$$2\,Fe(OH)_3 + 4\,Fe(II) + A^{2-} + 9H_2O \rightarrow [Fe_4^{II}Fe_2^{III}(OH)_{12}][A^{2-}.3H_2O] + 6H^+ \quad (1\text{-}3)$$

$$2\,Fe(OH)_3 + Fe(II) \rightarrow Fe_3O_4 + 2H_2O + 2H^+ \quad (1\text{-}4)$$

However, in the presence of trace concentrations of oxidants, under alkaline conditions, magnetite may also be formed form the oxidation of $Fe(OH)_2$ or green rust.[95]

$$3\,Fe(OH)_2 \rightarrow Fe_3O_4 + 2H^+ + 2H_2O + 2e^- \quad (1\text{-}5)$$

$$[Fe_4^{II}Fe_2^{III}(OH)_{12}][A^{2-}.3H_2O] \rightarrow 2\,Fe_3O_4 + 2e^- + A^{2-} + 4H^+ + 7H_2O \quad (1\text{-}6)$$

Depending on the environmental conditions, such as the presence of relatively high concentrations of oxidants, green rust can also be oxidized into goethite as shown in equation 1-7.

$$[Fe_4^{II}Fe_2^{III}(OH)_{12}][A^{2-}.3H_2O] \rightarrow 6\alpha\text{-}FeOOH + A^{2-} + 6\,H^+ + 3H_2O + 4e^- \quad (1\text{-}7)$$

Equations 1-3 ~ 1-7 depict that after sorption of Fe(II) ions on Fe(III) oxides, green rust is formed and then it could be transformed into different iron minerals. Therefore, Fe(II) ions are fixed on the Fe(III) oxides minerals and become non-desorbed because of the oxidation and transformation to the well-crystalline minerals. Several studies reported the incomplete recovery of Fe(II) after adsorption on Fe(III) oxides.[77,96,97] It suggests that electron transfer from adsorbed Fe(II) to structural Fe(III) (magnetite, hematite) could be occurred and such transformations of electron are referred to as interfacial electron transfer (IET).[98,99] Then the new Fe(III) on the surface may hydrolyze and act as a secondary Fe(III) mineral phase on the surface of primary mineral phase. Equation 1-8 demonstrates the interfacial electron transformation.[77] This means that not only the surface but the bulk of the Fe(III) mineral also could be reduced to Fe(II) due to the reactivity of surface-bound Fe(II) species.

$$\mathrm{Fe^{III}OFe^{II}OH} \quad \leftrightarrows \ \mathit{IET} \leftrightarrows \quad \equiv\mathrm{Fe^{II}OFe^{III}OH} \tag{1-8}$$

Iron oxide minerals are one of the most important and efficient natural sorbents in soils, sediments and water bodies.[100] The Fe(II) associated with iron oxides are also important in the biogeochemistry as well as in the environmental chemistry in relation to the fate and transport of environmental contaminants. The surface-bound Fe(II) species have strong reductive capacity to decompose priority pollutants under anoxic conditions and can be utilized for the remediation of groundwater and soil contaminated with reducible contaminants. However, this issue has been neglected over the past decade.

1.1.6 Reduction of contaminants by surface-bound Fe(II).

Owing to the anoxic conditions in the contaminated subsurface environments, the most favorable pathway for the transformation and/or detoxification is the reduction. It was until 1995 that the studies on the kinetics of the organic pollutants reduction in the aquatic environments has been focused on the coenzymes [101,102], sulfur containing minerals, and H_2S [22] and anaerobic microorganisms. Heijman et al.[103] first reported that

Fe(II) associated with Fe(III) surfaces could play a pivotal role in the Fe(II)-mediated reductive denitrification of nitro-aromatic compounds under microbial iron reducing condition.

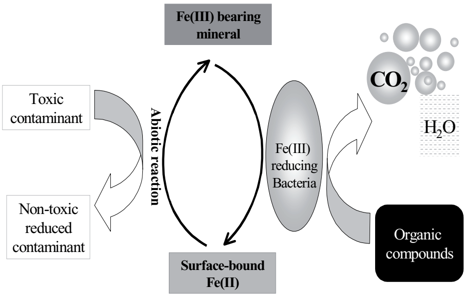

Figure 1-6. Schematic explanation of abiotic reductive transformation of toxic environmental contaminants by surface-bound Fe(II) that is regenerated by Fe(III)-reducing bacteria by organic compounds breathing

As it is shown in Figure 1-6, the reduction of contaminants coupling to Fe(II) oxidation can be continued by using the generated Fe(II) by microbial Fe(III) reduction. This investigation prompted the use of surface-bound Fe(II) associated with various Fe(III) minerals for reductive transformation of several groups of priority pollutants, such as nitro aromatic compounds [35,75,104-106], nitrites[107] polyhalogenated alkanes [108-112], inorganic metal ions including Cr(VI) [113], Tc(VI) [114] and U(VI) [115], carbamate pesticides [116], and disinfectant such as monochloroamine.[117] In certain cases dissolved Fe(II) also contributed to the reductive transformation of some target compounds such as niro-aromatics [35] and polychlorinated hydrocarbons [117,35,118] depending on the experimental conditions, the time scale for the reduction reaction and the formation of precipitation due to the possible oxygenation and also on the increase in pH.

However, the common conclusion is that Fe(II) is more reactive on the reduction of target compounds in the presence of solid Fe(III) mineral compared to the dissolved Fe(II) alone. Also, it has been reported that Fe(III) oxides could not reduce any compounds in the absence of $Fe(II)_{aq}$ in the heterogeneous anoxic systems.

The crystalline Fe(III) minerals including goethite and hematite have been used in laboratory studies in anoxic Fe(II) solutions for the reductive transformation of priority pollutants. Surface-bound Fe(II) on the goethite systems has received much attention for the reductive transformation of various target organics, probably due to the greater abundance in the nature.

Hematite also has been studied to reduce polyhalogenated aliphatic compounds [35,111] and U(VI).[75,115] Since these minerals do not contain Fe(II), surface-bound Fe(II) is responsible for the target compounds reduction. Pecher et al.[111] compared the non-desorbed surface-bound concentrations of Fe(II) due to surface uptake by hematite and goethite using similar surface area concentration and identical experimental conditions. The non-desorbed Fe(II) fixed on the surface of goethite was 2~3 times higher than that on hematite. Elsner et al.[35] compared the reactivity of goethite and hematite under identical experimental conditions for the reduction of two nitro-aromatic compounds including 4-chloronitro-benzene, 4-chlorophenyl hydroxylamine and hexachloroethane. The rate constant normalized to surface-bound Fe(II) concentration showed that goethite has much higher reactivity in the reduction of both nitro-aromatic compounds and nearly similar rate constant for hexachloroethane reduction. This means that goethite is more efficient than hematite for the reduction of contaminants.[35]

Large fraction of sediments in oceans and rivers contains amorphous ferrihydrite.[119] Compared to other Fe(III) oxides ferrihydrite has higher surface area. However, few studies used surface-bound F(II) associated with ferrihydrite to reductively transform contaminants. Kim and Picardal [120] observed the dehalogenation of polyhalogenated methanes using surface-bound Fe(II) onto ferrihydrite under bacterial Fe(III) reducing condition and noted that reductive transformation of target organic is due to the biogenic surface-bound Fe(II).

Magnetite (Fe_3O_4) is another iron mineral that has been used for the reductive transformation of environmental contaminants. Even though magnetite is a well crystalline iron oxide, it is mainly different form goethite and hematite having Fe(II) in the structure. It is not clear how the Fe(II) in the bulk of the magnetite structure contribute to the reductive transformation. Probably due to this reason, laboratory experiments results showed that magnetite in the absence of aqueous Fe(II) in the heterogeneous anoxic suspensions did not reductively transform nitroaromatic compounds.[108] Even though magnetite contained structural Fe(II), no reduction of nitroaromatic compounds have been observed in the absence of aqueous Fe(II) and it is suggested that the oxidative dissolution of such minerals by target organic is quite slow. Lee et al.[121] observed that the transformation of halogenated ethenes by magnetite in aqueous Fe(II) suspension, but the transformation of the target organic was insignificant in the absence of aqueous Fe(II). However, a recent study has observed the reduction of CCl_4 using nano-size magnetite particles, a product of bio-reduction of ferrihydrite [85] probably because nano-particles have high specific surface area that could expose more Fe(II) to oxidative dissolution coupling to the reduction of target organic compound.

Green rust, a meta stable mixed valence iron hydroxide, can reductively transform various types of priority pollutants such as nitrites[122], chlorinated ethenes[123], poly halogenated alkanes [124-126], Cr(VI)[127-129], Se(VI)[130] and U(VI).[131] In green rust systems, the reactive Fe(II) may be structural Fe(II) or adsorbed Fe(II) on the surface. Because of its instability, green rust is more sensitive to oxidants than other iron oxides. Also, green rust is a layered structure made by Fe(II), Fe(III) and anionic inter-layers, thus providing great specific surface area and amphoteric surface hydroxyl groups lead to both high sorption and reduction efficiency.[132] It means that green rust is an ideal mineral for reducing many types of environmental contaminants. However, the activity of green rust may decrease and change the kinetics of the reduction reaction during long time reactivity, because green rust is oxidized to magnetite during the reduction process.

1.1.7 Degradation kinetics of the contaminants by surface-bound Fe(II) systems

The degradation kinetics of the reduction of target contaminants by surface-bound Fe(II) has been reported to follow first-order rate equation. The first order kinetics can be explained using rate laws as follows

$$R = \frac{d[A]}{dt} = k[A]^1 \qquad (1\text{-}9)$$

where R is the rate of the reaction, $[A]$ is the concentration of substance A, k is the rate constant at temperature T. If the temperature is constant, it can be integrated from $[A]_0$, the concentration of A at the time of reaction start, $t = 0$, to the variable upper limit$[A]$, as a function of t,.

$$-\int_{[A]_0}^{[A]} \frac{1}{[A]} d[A] = t\int_0^t kdt \qquad (1\text{-}10)$$

$$\ln\frac{[A]_0}{[A]} = kt \quad \text{or} \quad \ln\frac{[A]}{[A]_0} = -kt \qquad (1\text{- }11)$$

Therefore, the first-order rate constant at the relevant temperature can be calculated by using the slope of the plot of $\ln[A]/[A]_0$ versus time t. However, in the reduction of target contaminants, by Fe(II)/Fe(III) systems, the relationship of $\ln[A]/[A]_0$ slightly deviates from the linearity with time.[104,108,111] Two possibilities are rationalized for such a deviation. First is the most reactive Fe(II) species are depleted and then less reactive species. The second is change in the properties of the system that control the reaction rate at latter stage of the reducing system.[108] Generally, the surface-mediated reactions include three major steps firstly contacting the reactant molecules with the reactive sites, secondly electron transfer between surface reactive sites and reactant molecules and thirdly release of the product molecule from the reactive surface sites. This means mainly reaction rate is controlled by electron transfer and/or mass transfer. Recently it was reported that the deviation from the linear relationship between $\ln[A]/[A]_0$ versus time t might be due to the slow mass transportation after the surface reaction was completed on the reactive Fe(II) because of the possible higher affinity of products (of reducing reaction) towards the surface sites on iron oxides.[35]

1.1.8 Factors controlling the reactivity of heterogeneous Fe(II)/Fe(III) aqueous systems

1.1.8.1 pH value.

In heterogeneous aqueous systems, pH is the most important factor controlling the reactivity of surface-bound Fe(II) species. In many types of target contaminants, the reduction rate constant increased with increasing pH.[108,109,111] Similar to the increase in the observed rate constant (k_{obs}) for the reduction of target compounds, the sorbed Fe(II) concentrations also increased with the elevated of value of pH of the suspension. This means that the major reason for the relationship between pH and rate constant is the increases in the surface reactive sites with pH.[108,109] The surface negative charge increases at high pH, resulting in the increase in the columbic repulsions to attract Fe(II) onto the surface.[78] The adsorbed Fe(II) ions can then form inner-sphere complexation. Usually, the sorption of Fe(II) on iron minerals starts at pH 5.5 and the adsorption is completed at around pH 7.5. Further increase in pH facilitates the precipitation of aqueous Fe(II) species on the surface.[75] Therefore, different types of reactive species may be formed at surface, depending on the pH and the amount of Fe(II) uptake. However, the knowledge on the types of species on the surface and their roles in the surface reactivity with respect to the reduction of contaminants is limited. Generally, the increase in Fe(II) concentration on the surface due to the increase in pH is considered as the major reason for enhancing of reduction rate. Moreover, the redox potentials of both aqueous Fe(II) and surface sites of iron mineral vary due to variable pH.[108]

The effect of pH on the reactivity of meta-stable mixed valence iron hydroxides (green rust) systems also has been observed.[123] Similar to that in surface-bound Fe(II) systems, the reactivity increases upon increasing pH in green rust systems. In neutral buffered mediums, some dissolved Fe(II) can be detected due to dissolution of green rust.[35] Therefore, the increase in pH may enhance the uptake of Fe(II) onto surface and form inner-sphere complexation from $\equiv Fe^{III}OH$ groups to form $\equiv Fe^{III}OFe^{II}OH$ species, as previously described in equation 1-1. The other possibility is the change in reactivity of Fe(II) due to the protonation and de-protonation of reactive ferrous-hydroxyl groups. Green rust is a layered ferrous and ferric hydroxide and between two layers, anions are available making legands. Thus each layers has $\equiv Fe^{II}OH$ and $\equiv Fe^{II}OH_2^+$ groups which are originally from the hydrolysis of partially un-coordinated surface iron.[123] Generally, $\equiv Fe^{II}OH$ groups are reactive to be oxidized but $\equiv Fe^{II}OH_2^+$ groups are not reactive due to the low electron density on the centred Fe(II) ion. When the pH increases, the protonated groups can be deprotonated, as shown in equation (1-12) and form more reactive sites. In contrast, by lowering pH the deprotonated groups accept protons, resulting in the decrease in reactivity. Moreover, decrease in the pH also stimulates the dissolution of green rust, which is easy to be dissolved in diluted acids.

$$\equiv Fe^{II}OH_2^+ \quad \textit{pH increase} \rightarrow \quad \equiv Fe^{II}OH \qquad (1\text{-}12)$$

$$\equiv Fe^{II}OH \quad \textit{pH decrease} \rightarrow \quad \equiv Fe^{II}OH_2^+ \qquad (1\text{-}13)$$

1.1.8.2 Remodeling time of Fe(II) at Fe(III) mineral surface

The remodeling of Fe(II) species sorbed on the surfaces of iron minerals is especially important factor in laboratory batch experiments. The reactivity of Fe(II) in the heterogeneous suspensions of Fe(III) oxides strongly depends on the duration of the contact of Fe(II) ions with the surface of Fe(III) mineral prior to the reduction of contaminants. The reactivity is higher if the contact time is longer independently on the type of target compound.[108] The remodeling time is thought to be important for the surface complexation of Fe(II) with the surface. With the increase in contact time the non-disorbed Fe(II) concentration increases. Schwarzenbach et al.[133] reported that the contact time should be at least 20 h to remodel the Fe(II) onto iron oxides. Therefore, in laboratory batch experiments it is necessary to keep the heterogeneous system of dissolved Fe(II) with solid Fe(III)-oxide minerals at least for 20 hours prior to introducing the target compound into the system.

1.1.8.3 Sorbed Fe(II) concentration

Although the dissolved Fe(II) has a little effect on the reductive transformation of many target compounds, the sorbed Fe(II) concentration plays an important role in the degradation reaction in the heterogeneous systems. Several studies demonstrated a linear relationship between the sorbed Fe(II) concentration and the rate constant of the reducing reaction (k_{obs}).[109,134] In order to increase the sorbed Fe(II) concentration, the available surface area of the Fe(III) minerals is also an important parameter. Surface density of Fe(II) calculated from the sorbed concentration and surface area of iron oxide measured by BET (Brenauer-Emmett-Teller) was also used to normalize the reduction rate of target compounds in the heterogeneous Fe(II)/Fe(III) suspensions.[109] However, the use of surface density of Fe(II) to compare the reactivity is an expedient approach because the same surface density can be obtained using different mineral-loadings and different concentrations of added Fe(II) in batch experiments, although the total numbers of reactive surface Fe(II) sites are different. Therefore, use of surface-bound Fe(II) is more meaningful to show the relationship between surface –bound Fe(II) and the rates of reductive transformation of target compounds using the concentration of Fe(II) bound to the mineral surface.

1.1.9 Reactivity of Fe(II)/Fe(III) systems towards dechlorination of chlorinated compounds

The study the reactivity and mechanism of heterogeneous Fe(II)/Fe(III) systems, nitro compounds and polyhalogenated aliphatic compounds have been used. Among polyhalogenated compounds, chlorobromomethanes, chlorinated methanes and ethanes have been used. to evaluate the reducing capacity of surface-bound Fe(II) systems. However, it is still difficult to compare the reactivity of different studies because the experimental conditions such as mineral concentration, pH, initial concentration of Fe(II), the pre-equilibrium time and specific surface area of the mineral are different. Table 1-4 summarizes the dehalogenation of halogenated compounds by various iron minerals in the presence and absence of Fe(II). According to Table 1-4, the major chlorinated products of CCl_4 dechlorination in surface-bound Fe(II) systems are $CHCl_3$ and CH_2Cl_2. The non chlorinated products found were $HCOO^-$ and CO.[35,111,112] However, further reduction of $CHCl_3$ seems uncertain in many studies due to slow reactivity. Other specialty is that chlorinated ethenes have not been reported to be reduced in the surface-bound Fe(II) systems. In mixed valence iron mineral systems (green rust), however, chlorinated ethenes such as C_2Cl_4 can be dechlorinated to some extent.[123] A recent study reported that the dechlorination of dichloroethene (C_2H_2Cl) and vinyl chloride (C_2H_3Cl) by magnetite was insignificant, but amendment of Fe(II) has contributed to dechlorinate those chlorinated ethenes within 3.5 months.[121] According to the standard oxidation-reduction potentials, all the chlorinated methanes and ethenes are possible to undergo reductive dechlorination in all the major Fe(II)/Fe(III) systems (Figure 1-7). Whereas dissolved Fe(II) has higher standard potential for the oxidative half reaction indicating that dissolved Fe(II) can not reduce many chlorinated compounds. However, if the thermodynamic possibility for the reduction of chlorinated compounds is predicted using the pE of whole reaction (coupling the oxidation of Fe(II) species and reduction of chlorinated compounds), Fe(II) in the dissolved form also can reduce many chlorinated compounds. Table 1-5 summarizes the thermodynamic prediction of major chlorinated methanes and ethenes considering the whole reaction. The reduction potential E values can be calculated using standard reduction potentials (E^0) of iron species shown in Figure 1-7 according to the relationship in equation 1-14.

$$E = E^0 + \frac{2.303RT}{nF}\log\frac{[Fe^{+3}]}{[Fe^{+2}]} \tag{1-14}$$

Using the similar relationship, the E values of chlorinated compounds also can be calculated by their E° values and their concentration initial and reduced species. Then the pE of the half reaction can be calculated by the E values using Nernst equation

$$pE = \frac{E}{\frac{2.303RT}{F}} \tag{1-15}$$

where E is the reduction potential, R is the gas constant and F represents faraday constant. The pE value of the whole reaction can be calculated from the pE of of both oxidative and reductive half-reactions. The positive pE values indicate that the reaction can go forward. However, Practically, the possibilities for the dechlorination of chlorinated hydrocarbons are different, depicting that the dechlorination of chlorinated compounds by Fe(II)/Fe(III) systems can not be predicted only by considering the redox potentials. The dechlorination of chlorinated hydrocarbons occurs through various pathways. The major suggested pathways for the dechlorination of chlorinated methanes and chlorinated ethens are shown in Figure 1-8 and Figure 1-9 using carbon tetrachloride (CCl_4) and tetrachloroethen (C_2Cl_4) representing these groups respectively.

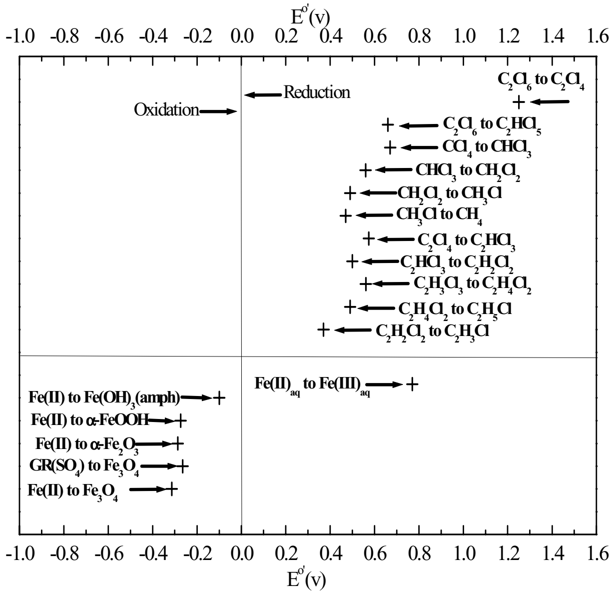

Figure 1-7. Representative half reduction potentials in the redox couples of Fe(II) / Fe(III) and some chlorinated hydrocarbons (at pH 7 and 25°C)

(the values for chlorinated compounds were quoted form Vogel et al [12]. The values in Figure 1-7 for redox couples of Fe(II) with iron oxides were obtained from Straub et al.[33] and redox potentials for aqueous iron species were quoted form Stumm and Morgan[78])

Table 1-4. Halogenated aliphatic hydrocarbons and chlorinated products formed during the dechlorination processes by Fe(II)/ Fe(III) systems.

Iron mineral	Target compound	Fe(II) Y/N	Halogenated organics as byproducts	Ref. No.
Geothite	CBr_4	Y	$CHBr_3$	136
	$CHBrCl_2$	Y	no data available	111
	$CHBr_2Cl$	Y	CH_2BrCl	111,136
	$CHBr_3$	Y	CH_2Br_2	111
	$CH2Br_2$	Y	CH_3Br	136
	$CFBr_2Cl$	Y	$CHBrClF$	136
	$CHFBrCl$	Y	CH_2FCl	136
	$CFBr_3$	Y	$CHBr_2F$	111,136
	$CBrCl_3$	Y	$CHCl_3$	111,136
	CBr_2Cl_2	Y	$CHBrCl_2$	111,136
	CCl_4	Y	$CHCl_3$, $^{\bullet}CCl_3$	111
		Y	$CHCl_3$	109,136
		Y	$CHCl_3$, CH_2Cl_2	134
		Y	HCl3, $^{\bullet}CCl_3$	112
	$CHCl_3$	Y	no data available	136
	C_2Cl_6	Y	C_2Cl_4	34,35
Lepidocrocite	CBr_2Cl_2	Y	$CHBrCl_2$	111
	C_2Cl_6	Y	C_2Cl_4	34,35
Magnetite	CBr_2Cl_2	Y	$CHBrCl_2$	111
	C_2Cl_6	Y	C_2Cl_4	35
	C_2Cl_4	Y/N	-	121
	C_2HCl_3	Y/N	-	121
	$C_2H_2Cl_2$	Y/N	-	121
	C_2H_3Cl	Y/N	-	121
(Biogenic-nano)	CCl_4	Y	$CHCl_3$,C_2Cl_4,CH_3Cl, $^{\bullet}CCl_3$,	85
	CCl_4	N	$CHCl_3$	86
Hematite	CBr_2Cl_2	Y	$CHBrCl_2$	111
	C_2Cl_6	Y	C_2Cl_4	34,35
Ferrihydrite	CCl_4	biogenic	$CHCl_3$	120
Green rust (SO_4)	CCl_4	N	$CHCl_3$, C_2Cl_6	124
		N	$CHCl_3$, C_2Cl_4	125
	C_2Cl_4	N	-	123
	C_2HCl_3	N	-	123
	$C_2H_2Cl_2$	N	-	123
	C_2H_3Cl	N	-	123
	C_2H_5Cl	N	-	126
	$C_2H_4Cl_2$	N	-	126
	$C_2H_2Br_2$	N	-	126
	$C_2H_3Cl_3$	N	$C_2H_4Cl_2$, C_2H_5Cl, C_2HCl_3, $C_2H_2Cl_2$	126
	C^2HCl_5	N	C_2Cl_4, C_2HCl_3,	126
	C_2Cl_6	N	C_2Cl_4, C_2HCl_3	126

Note: Y= with the amendment of Fe(II)$_{aq}$. N= No Fe(II); blank means only non-chlorinated products were identified

Table 1-5. The pE values for the reductive dechlorination of major chlorinated methanes and ethenes by dissolved Fe(II), structural Fe(II) and Fe(II) associated with various Fe(III) minerals.

Oxidative half-Reaction → Reductive Half- reaction↓	$Fe_{aq}^{+2} \rightarrow Fe_{aq}^{+3}$	$Fe^{+2} \rightarrow Fe(OH)_3$	$Fe^{+2} \rightarrow \alpha FeOOH$	$Fe^{+2} \rightarrow \alpha\text{-}Fe_2O_3$	$Fe^{+2} \rightarrow \alpha Fe_3O_4$	$GR(SO_4) \rightarrow Fe_3O_4$
$CCl_4 \rightarrow CHCl_3$	10.249	12.781	15.193	15.413	15.868	16.070
$CHCl_3 \rightarrow CH_2Cl_2$	8.292	10.824	13.236	13.456	13.911	14.113
$CH_2Cl_2 \rightarrow CH_3Cl$	7.989	10.521	12.933	13.153	13.608	13.810
$CH_3Cl \rightarrow CH_4$	6.815	9.347	11.759	11.779	12.437	12.636
$C_2Cl_4 \rightarrow C_2HCl_3$	8.529	11.061	13.473	13.693	14.148	14.350
$C_2HCl_3 \rightarrow C_2H_2Cl_2$	7.281	9.813	12.225	12.445	12.900	13.102
$C_2H_2Cl_2 \rightarrow C_2H_3Cl$	5.088	7.620	10.031	10.251	10.700	10.908

Note: It is assumed that the experimental conditions are 25 ℃ and pH 7.0 and amended Fe(II) and Fe(III) mineral concentration in all the systems are 3mM and 10 mM, respectively. In the green rust systems it is assumed that 50% of GR(SO$_4$) has been oxidized to magnetite. The dissolved Fe(III) concentration is assumed to be the equilibrium concentration of $10^{-17.8}$ M at pH 7. It is also assumed that 50% of initial concentrations of all the chlorinated compounds have been reduced. E values were calculated according to equation (1-14) using E° mentioned in Figure 1-7. pE of the half-reactions were calculated by Nernst euation (equation 1-15).

In the reduction of CCl_4, hydrogenolysis is first occurred reaction. After the formation of trichloromethyl radical, it can follow several pathways such as formation of trichlorocarbene, coupling, or addition. If trichlorocarbene was formed, it can undergo reductive elimination reaction to form dichlorocarbene which can further undergo several reactions such as coupling, addition or hydrolysis. Formate ions can be formed during the hydrolysis reaction.[85,111,112]

In the dechlorination of tetrachloroethene three types of reactions including hydrogenolysis, reductive elimination and hydrogenation reactions may undergo.[121,135] Although the hydrogenolysis is thought to occur though radical formation, for the simplicity, Figure 1-9 shows only the final product of the hydrogenolysis. Hydrogenolysis and reductive elimination are the two major routes for the dechlorination of C_2Cl_4. In the hydrogenolysis rout less chlorinated homologs will sequentially form ethane as the product.

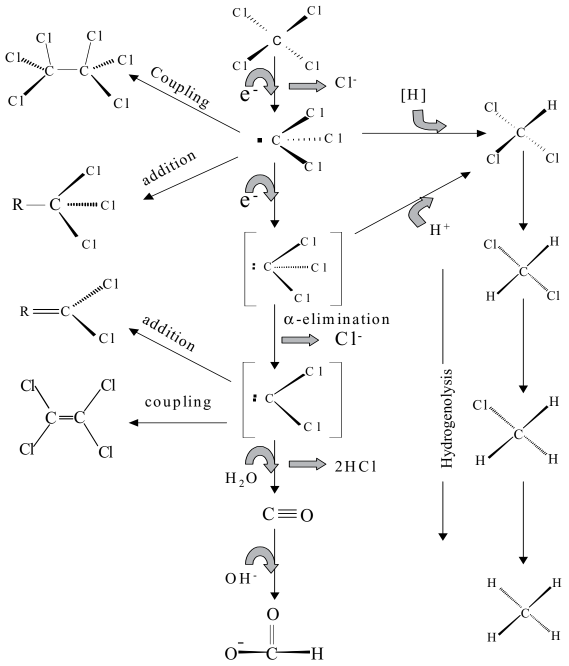

Figure 1-8. The suggested pathways for the reductive transformation of carbon tetrachloride under anoxic non-sulfidic environments (adapted from McCormick and Adriaens [85])

In the β-elimination pathway, the fist product is dichloroacetylene that can further follow hydrogenolysis reaction to chloroacetylene and then acetylene. Acetylene can be converted to ethylene by hydrogenation reaction. Finally ethane can follow hydrogenation reaction to form ethane. Reductive eliminations are the two major routes for the dechlorination of C_2Cl_4. In the hydrogenolysis rout less chlorinated homologs will sequentially form ethane as the end product. In the β-elimination pathway, the fist product is dichloroacetylene that can further follow hydrogenolysis reaction to chloroacetylene and then acetylene. Acetylene can be converted to ethylene by hydrogenation reaction. Finally, ethene can follow hydrogenation reaction to form ethane.

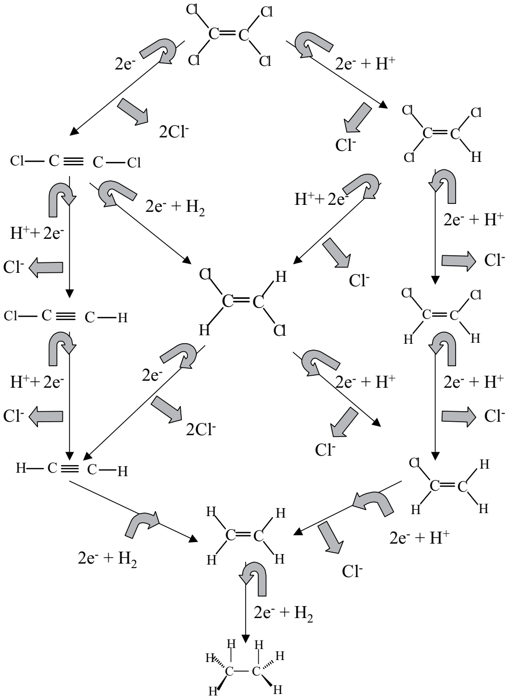

Figure 1-9. Schematic pathways for the dechlorination of tetrachloroethene under reducing conditions (adapted form Lee and Batchelor[121] and Arnold and Roberts [135]).

1.2 MOTIVATION

For the dechlorination of highly chlorinated compounds to non-chlorinated or environmental benign compounds, it is necessary to evaluate the efficiency of dechlorination using surface-bound Fe(II) systems in the presence of iron oxides most available in the environment. Moreover, a comparison of the dechlorination kinetics and efficiencies in the systems of surface-bound Fe(II) associated with most abundant natural iron oxides is important to understand the contribution of each types of iron oxide on the dechlorination. However, there is no such a comparison for dechlorination using Fe(II) associated with both crystalline and amorphous Fe(III) oxides. Although some laboratory studies have used iron oxides in separately for the reductively transformation of different types of target compounds, it is not easy to compare the results due to the different experimental conditions. Basically, the rates and efficiencies of the dechlorination of chlorinated methanes and ethenes by surface-bound Fe(II) system are quite low and need several days to months to be dechlorinated those compounds. Probably due to this reason the effects of environmental parameters on the reactivity of Fe(II) systems have been widely studied using more reducible nitro compounds and brominated hydrocarbons.[103,104,111] However, those data can not be manipulated with that for highly chlorinated hydrocarbons towards the evaluation of the reactivity of various Fe(II)/Fe(III) systems. Moreover, although the microbial iron reduction is known to play a major role in the re-generation of Fe(II) species, only few studies have used microbially reduced iron oxides (mainly magnetite) for the dechlorination of chlorinated hydrocarbons in the presence of iron reducing bacteria.

It is well recognized that transition metal ions are always available in the environment and these metal ions generally exist as dissolved forms or associated with iron oxide minerals under subsurface conditions. Certain metal ions including U(VI), Tc(VI), Cr(VI) have been reported to be reduced by structural Fe(II) or surface-bound Fe(II).[113-115] However, the effect of transition metal ions on the dechlorination of chlorinated hydrocarbons by Fe(II)/Fe(III) systems have not been focused. Transition metal ions such as Ni(II) and Cu(II), are ubiquitous in the environment, both in pristine and contaminated sites, and are also introduced into aqueous and solid environments by human activities. Some transition metal ions are reported to act as catalyst for mediating various types of reactions. A recent study depicted that the dechlorination of carbon tetrachloride by mixed valance iron hydroxide can be enhanced in the presence of Cu(II), Ag(I) and Au(II).[125] However, the effect of metal ions in green rust systems on the dechlorination of highly chlorinated ethenes is unknown. Similarly, the effect of metal ions on the dechlorination of chlorinated hydrocarbons by surface-bound Fe(II) associated with Fe(III) minerals has not been addressed. Moreover, the interaction of transition metal ions with dissolved aqueous Fe(II) ions also need to be investigated because groundwater may contain dissolved Fe(II) and transition metal ions simultaneously.[137]

Cu(II) is generally assumed to be the predominant oxidation state of copper and one of ubiquitous metal ion in soil and aquatic environments.[138] Cu(II) is introduced into the environments as a result of ore smelting, land application of biosolids, use as agrochemicals such as pesticides and other human activities.[139] Copper species have been used as catalyst for dechlorination reactions in laboratory studies as well as in the pilot plants.[140] Cu(II) also can form complexes with organic biomolecules such as bacterial co-enzymes [141] in the presence of other bulk reductants.[142] Recently Chien et al.[143] reported that CuO could dechlorinate polyhalogenated aliphatic in the absence of bulk reductant at relatively high temperature. The results show that copper species can catalyze the dechlorination reactions at high temperature. Generally, the catalytic effects depend on the environmental parameters. Because of the availability in the environment and the catalytic property, effect of Cu(II) species on the dechlorination of chlorinated compounds by Fe(II) species should be studied to elucidate, the interaction between transition metals and chlorinated hydrocarbons under iron reducing condition. It is known that copper species is an essential trace element for living cells, but at the high concentration copper shows toxic effects for the microbial activities.[139] Therefore, it is also important to evaluate the effect of Cu(II) on the Fe(III) reduction by DIRB prior to the study of the effect of Cu(II) on the dechlorination reactions mediated by Fe(II)/Fe(III) systems can be concluded.

1.3 OBJECTIVES

The main purpose of this study was to (comparatively) evaluate the reactivity of Fe(II) ions associated with most abundant crystalline iron minerals (goethite, hematite and magnetite) and amorphous Fe(III) oxides (ferrihydrite) under iron reducing conditions towards the dechlorination of highly chlorinated compounds using carbon tetrachloride(CCl_4) as model compound. The effect of Cu(II) ions on the dechlorination of CCl_4 by surface-bound Fe(II) was evaluated to understand the possible use of Cu(II) ion as a catalyst to enhance the dechlorination efficiency and rate of chlorinated compounds. Major environmental factors including pH, concentration of Fe(II), and concentration of Cu(II) on the dechlorination efficiency and rate were optimized. Investigation of the dechlorination of tetrachloroethene (C_2Cl_4) and trichloroethene (C_2HCl_3) by metastable mixed valence iron hydroxide (green rust) and the effect of Cu(II) ion on the rate and efficiency of dechlorination was another objective. In addition, the interaction of Cu(II) ions with dissolved Fe(II) species and the formation of heterogeneous Fe(II)/Fe(III) systems which stimulate the dechlorination of CCl_4 was elucidated. The changes in crystal phases and chemical species on the surface of iron oxide were characterized by X-ray diffractometer (XRD) and X-ray photoelectron spectroscopy (XPS). Moreover, the dechlorination of carbon tetrachloride in the absence and in the presence of Cu(II) ions under microbial Fe(III) reducing condition using dissimilatory Fe(III) reducing bacteria was studied to understand the possibility of simultaneously undergoing the processes of abiotic dechlorination of CCl_4 by Fe(II)/Fe(III) system and biological Fe(III) reduction by *Geobacter sulfurreducens.*

1.4 EXPERIMENTAL PLAN

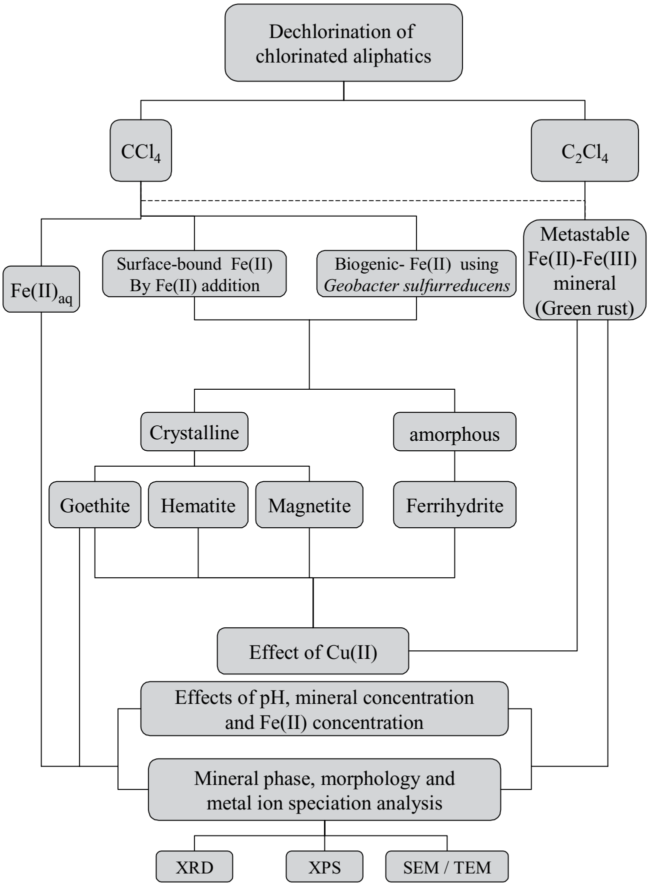

Figure 1-10. Schematic diagram of experimental design

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Chapter 2

DECHLORINATION OF CARBON TETRACHLORIDE BY FERROUS ION ASSOCIATED WITH IRON OXIDE MINERALS

The dechlorination of carbon tetrachloride (CCl_4) by Fe(II) ion in the suspensions of crystalline iron oxides minerals including goethite (α-FeOOH), hematite (α-Fe_2O_3), magnetite (Fe_3O_4), and amorphous ferrihydrite ($Fe(OH)_3$) was investigated. Experiments were performed using 10 mM iron oxides and 3 mM Fe(II) to form surface-bound iron suspensions at pH 7.2 under anoxic condition. The dechlorination followed pseudo first-order kinetics. The rate constants (k_{obs}) for CCl_4 dechlorination were 0.380 h^{-1} and 0.836 h^{-1} in goethite and hematite suspensions, respectively. Whereas the k_{obs} was 0.0609 h^{-1} for magnetite and 0.0144 h^{-1} for ferrihydrite, which were lower than those in the highly crystalline Fe(III) oxide suspensions. The major product of CCl_4 dechlorination was chloroform ($CHCl_3$) and the ratio of CCl_4 conversion to $CHCl_3$ was within the range of 14 – 57 %, depending on the type of iron oxides. The rate and efficiency of dechlorination were also dependent on the Fe(II) concentration and a Langmuir-type relationship between initial Fe(II) concentration and the k_{obs} was found in goethite system. The sorption of Fe(II) on goethite followed Langmuir sorption isotherm, indicating that surface-bound Fe(II) is mainly responsible for the dechlorination. A linear relationship between surface concentration and the k_{obs} was established. The pH of the surface-bound Fe(II) system strongly influenced the rate and efficiency of dechlorination and both k_{obs} and sorbed Fe(II) concentrations were exponentially increased with the increase in the pH within the range of 4 - 8.5 depicting that the increase in k_{obs} with the increase in pH is mainly attributed to the increase in the surface- bound Fe(II) concentration. The CCl_4 dechlorination was significantly enhanced by the amendment of 0.5 mM Cu(II) into the suspension of iron oxide and

Fe(II). The k_{obs} values for CCl_4 dechlorination were 119, 100, 30 and 3 times greater than those in the absence of Cu(II) in the magnetite, goethite, hematite and ferrihydrite suspensions, respectively. Moreover, $CHCl_3$ could be dechlorinated in the Cu(II)-amended iron suspensions, indicating that Cu(II) plays a positive role in enhancing the efficiency of Fe(II)/Fe(III) systems on the dechlorination of highly chlorinated compounds to form environmental benign compounds.

2.1 INTRODUCTION

The contamination of groundwaters with chlorinated hydrocarbons is a widespread environmental problem.[1-3] The environmental and health impacts of chlorinated hydrocarbons have prompted investigations regarding to their attenuations from natural environments.[4-6] A number of chlorinated solvents including carbon tetrachloride (CCl_4) and tetrachloroethene (C_2Cl_4) are rather persistent under aerobic conditions, but they may undergo reductive dehalogenation under reducing environments.[7-9] Basically, the oxygen concentration is limited in both pristine and contaminated subsurface environments.[10,11] Therefore, reductive transformation has become an important issue from the environmental engineering point of view with respect to the remediation of chlorinated hydrocarbons in contaminated groundwater or soils. Fe(II) is one of the natural reductants available in various forms including dissolved, mineral bound, and as hosts of Fe(II) bearing minerals.[12] Laboratory and field studies have focused on the significance of Fe(II) ions in the abiotic reductive transformation of halogenated compounds in soil and groundwaters.[13,14,15] Fe(II) ion complexes in dissolved form have shown to reduce different types of organic compounds such as polyhalogenated methanes, halogenated ethanes and nitroaromatic compounds under anoxic conditions.[6,16] However, several studies have pointed out the high reactivity of Fe(II) ion when they bound to Fe(III)-bearing minerals compared to the reactivity of aqueous Fe(II) ion.[13-15] The high reactivity of Fe(II) species attached to solid Fe(III) minerals is rationalized within the framework of surface complexation theory.[16] A previous study observed a linear relationship between the surface density of Fe(II) ions on the surface of Fe(III) minerals and the reactivity for the reductive transformation of polyhalogenated compounds.[14] The increase in the reactivity for the dehalogenation is apparently proportional to the total Fe(II) concentration when the Fe(II) ions in the Fe(III) mineral suspension reach the equilibrium.[17,18] It is generally believed that during the contact of Fe(II) ions with the surface of iron oxide minerals, the protons in the surface hydroxyl groups are replaced by $Fe(OH)_2$ to form $\equiv Fe^{III}OFe^{II}OH$, and the concentration of this species is proportional to the initial rates of reduction reaction.[19] Therefore, it is clear that the formation of surface reactive Fe(II) sites is the result of the interaction of Fe(II) ions with the surfaces of Fe(III) minerals and it is the key point for the higher reactivity. Under the subsurface conditions, the surface-bound Fe(II) species can be continuously generated by abiotic surface sorption of Fe(II) into Fe(III) minerals or by microbial Fe(III)-reduction.[1,20] Recent field studies demonstrated that even in geochemically complex polluted aquifers, surface-bound Fe(II) was the predominant reductant for nitroaromatic compounds.[21] Therefore, surface-bound Fe(II) systems play a pivotal role as natural mediators in the *in-situ* reduction of contaminants. Also, several environmental factors such as surface area of iron mineral, available Fe(II) concentration in the aqueous medium, and pH of the system can influence the reactivity of surface-bound Fe(II) species. The affinity of the surfaces of different Fe(III) minerals for the Fe(II) ions might be the most important factor to form reactive surface Fe(II) sites. According to the physicochemical properties of the surfaces of various Fe(III)-containing minerals, the different sorption behaviors of Fe(II) ion from the aqueous phase may reflect the reactivity of surface-bound Fe(II) species associated with various iron minerals. Iron oxides minerals are well-known strong sorbents for metal ions.[22-24] However, only limited information is available for the sorption behaviors of Fe(II) ion on iron oxide minerals compared to these of other metal ions.[25] A comparative study of Fe(II) sorption isotherms for various iron oxide minerals is thus essential in order to understand the reactivity of the surface-bound Fe(II) for the dechlorination in iron oxide mineral systems.

The effects of several environmental parameters such as pH, surface Fe(II) density and the contacting time of Fe(II) with iron minerals on the reductive dehalogenation of polyhalogenated alkanes by surface-bound Fe(II) systems have been previously investigated.[13,15-17] Since inorganic constituents such as transition metal ions often coexist with chlorinated solvents in wastewater[26], the understanding of the effect of transition metals on reductive dechlorination is important for the consideration of possible *in-situ* remediation. Depending on the crystalline properties and surface conditions, however, the transition metal ions also show different affinities on the surfaces of iron oxides minerals.[27]

Goethite (GT) (α-FeOOH), hematite (HM) (α-Fe_2O_3), amorphous ferrihydrite (FH) ($Fe(OH)_3$), and magnetite (MG) (Fe_3O_4) are ubiquitous natural iron oxides in the environments.[28,29,30] They have different crystalline properties, surface characteristics and mineralogical characteristics with respect to their originalities. Therefore, the Fe(II) ions associated with these Fe(III) mineral surfaces may show different reactivity in the reduction of chlorinated compounds. Cu(II) is a common environmental pollutant and predominant oxidation state of copper in natural water bodies as well as in wastewater.[31-33]. The objectives of this study was to investigate the dechlorination of carbon tetrachloride (CCl_4) by Fe(II) ions associated with different iron oxides including goethite, hematite, ferrihydrite and magnetite. The effect of Cu(II) ions on the dechlorination in each surface-bound Fe(II) systems was also studied. Moreover, the sorption experiments of Fe(II) and Cu(II) onto iron oxides were also performed to verify the reactivity of such systems for the reductive transformation of carbon tetrachloride.

2.2 MATERIALS AND METHODS

2.2.1 Chemicals

All chemicals were used as received without further treatment. Carbon tetrachloride (CCl_4, > 99.8%, GC grade), chloroform ($CHCl_3$, > 99.8%, GC grade), $CuCl_2{\cdot}2H_2O$ (99%), $FeSO_4{\cdot}7H_2O$(99%) were purchased from Merck Co. (Darmstadt, Germany). $FeCl_2{\cdot}4H_2O$ (99%), $FeCl_3{\cdot}6H_2O$ (99%), N-(2-hydroxyethyl)-piperazine-N'-(2-ethanosulfonic acid (HEPES) (99.5%), KOH (99%), NaOH (> 98%), KNO_3 (99%), $Fe(NO_3){\cdot}9H_2O$ (> 98%), HCl (37%), HNO_3 (65%), Ferrozine monosodium salt ($C_{20}H_{13}N_4O_6S_2Na$), and ammonium acetate (CH_3COONH_4) were purchased form Sigma-Aldrich Co. (Milwaukee, WI). Methylene chloride (DCM, > 99.8%, GC grade) and ethanol (HPLC grade) were obtained from J. T. Baker Co. (Phillipsburg, NJ). Bathocuproinedisulfonic acid disodium salt ($C_{26}H_{18}N_2Na_2O_6S_2$, 90%) was purchased from Fluka (Buchs, Switzerland).

2.2.2 Preparation of anoxic water and anoxic solutions.

In the preparation of all the aqueous solutions and mediums, O_2-free water was used. Anoxic water was prepared by repeatly vacuuming and N_2-purging.[34] Distilled deionized water (18.3 MΩ) (Millipore) was added to a 1-L serum bottle (Scott, Duran) and a magnetic stirring bar was placed in the bottle. The bottle was then sealed using a rubber septum (2.5 cm thickness) and a screw cap with an open hole in the center. The bottle was placed on a magnetic stirrer and the gas in the headspace was evacuvated by vacuuming while the water was vigorously stirred. After 30 min of vacuuming, N_2 with high purity (99.999%) was flushed into the bottle untill the pressure in the headspace reached 1 PSI and then kept stirring for 10 min. This procedure (vacuuming and purging) was repeated for 5 times. The oxygen concentration of this water detected by oxygen electrode in an anaerobic glovebox was below 0.3 ppm.

The solutions of oxygen-sensitive chemicals were prepared using the similar procedure. Solid chemicals were first delivereded into the N_2-purged serum bottles. After capping with rubber septum and aluminium crip caps, required volume of anoxic water was injected into the serum bottles using plastic syringe with continueous N_2-purging to dissolve the chemicals. The headspaces of the bottles were maintained under positive pressure to avoid possible O_2 contamination.

In this study, all the dechlorination and sorption experiments were carried out at near neutral condition (pH 7.2). HEPES buffer was used to control pH at 7.2. For the preparation of 50 mM buffer solution, 11.96 g of HEPES buffer was dissolved in 900 mL of distilled deionized water in a 1-L bottle and the pH was adjusted to 7.2 by adding 1M NaOH soluton. After adjusting the volume, the buffer solution was de-oxygenated following the similar procedure that was used for the preparation of anoxic water.

2.2.3 Synthesis and characterization of iron oxide minerals.

Iron oxides including goethite, hematite, magnetite and ferrihydrite were synthesized following the well-established methods by previous workers.[35] Goethite was synthesized using ferric ion solution method. Briefly, 1M $FeCl_3$ solution was prepared by dissolving 27.03 g of dessicated $FeCl_3 \cdot 6H_2O$ in 100 mL of water in a 2-L plastic flask and 180 mL of 5M KOH solution was rapidly added with vigorous stirring. Then, the brown color suspension was immediately diluted to 2 L with DI water under ambient condition and was placed in an oven at 70 °C for 60 hours. The supernatant and the bright yellow precipitate were separated by centrifugation at 8,000 × g for 10min followed by washing with deoxygenated DI water repeatedly for five times. The solid pellet was decanted in sealed serum bottle with N_2-stream and vacuuming. For the quantification of goethite suspension, 1 mL of the suspension was transferred into a ceramic crucible with a known weight. The crucible was palced in a vacuumed dessicator under N_2 atmosphere until the wieght became constant. The concentration of the suspension could be calculaed by the dry weight of goethite.

Hematite was synthesized by a hydrolysis of Fe(III) salt solution. One liter of 0.002M HNO_3 was prepared and the acid solution was heated to 98 °C in an oven. When the solution temperature reached 98 °C, the bottle was taken out and 8.4 g of desiccated $Fe(NO_3)_3 \cdot 9H_2O$ was added into the acid solution with vigorous stiring. After screw capping, the bottle was returned to the oven immediately and hold at 98 °C for seven days to form hematite. The bright red suspension (hematite) was then washed and quantified following the method that was used for goethite.

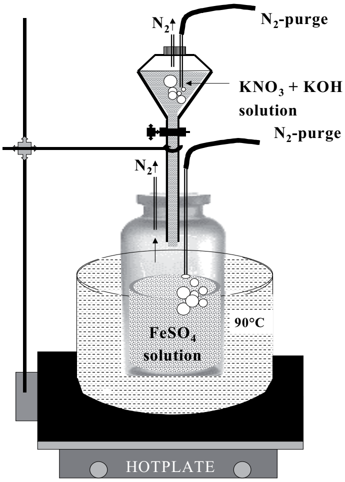


Figure 2-1 Experimental setup used for Magnetite synthesis.

Synthesis of magnetite was conducted the experimental setup as shown in Figure 2-1. Eighty gram of desiccated $FeSO_4 \cdot 7H_2O$ was added into 560 mL of O_2-free water in a 1-L bottle with a magnetic stirring bar in a 90°C water bath. A 240 mL of O_2-free solution containing 6. 46 g KNO_3 and 44.9 g KOH was drop wise added within 5 ~ 6 min using a drop funnel while the mixture in the bottle was stirred continuously. Then the temperature was maintained at 90 °C for 1 h and allowed to cool down with continuous N_2-purge. The bottle was opened after 12 h and the black precipitate and the supernatant were separated by centrifugation and the precipitate was washed 5 times using O_2-free water. The washed precipitate was stored and quantified as previously described.

For ferihydrite synthesis, 500 mL of 1M $FeCl_3$ solution was prepared in a 2-L beaker and the solution was titrated with 1M NaOH in a drop funnel with vigorous stirring until the pH of the mixture reach 7.0 ~ 7.2. The precipitate was allowed to settle down and the supernatant was removed. Precipitate was then washed 5 times and the ferrihydrite was stored as a suspension under N_2-atmosphere.

In order to prepare dry samples of iron oxide for the mineralogical characterization and surface morphology studies, dry powder samples were prepared in a sealed vials by continuous purging with $N_{2.}$ A 50-mL serum bottle was first sealed using rubber septum and aluminium crimp cap. Then the bottle was purged with N_2 using syringe needles. A 1 mL of the stock suspension of iron oxide was withdrawn using N_2-purged 1-mL plastic syringe and was injected into the serum bottle. The continuous N_2-purge was maintained until the suspension transformed to dry powder.

2.2.4 Dechlorination Experiments

Batch experiments were conducted using 70 mL serum bottles those were filled with 50 mL of deoxygenated buffer solution. The buffer solutions were delivered to serum bottles using 60 mL air-tight plastic syringe under gentle flow of N_2 (42 L min^{-1}).[34] Iron oxide minerals including goethite (GT), hematite (HM), ferrihydrite (FH) and magnetite (MG) were withdrawn from anoxic slurry in stock bottles using N_2-purged syringe and were delivered into serum bottles to get the final concentration of 10 mM. In order to study the effect of iron oxide concentration on the dechlorination, various volumes of iron oxide suspensions were injected into serum bottles to obtain the final concentrations of 5 ~ 25 mM. The required volume of stock solution of Fe(II) was introduced into the serum bottles to obtain a concentration of 3 mM. Stock solutions of 150 mM Fe(II) were prepared by dissolving 1.491 g of vacuumed desiccated $FeCl_2{\cdot}4H_2O$ in deoxygenated buffer solution and the solution was filtered through 0.2 μM filter cartridge (PTTE) and the concentration of the filtered Fe(II) stock solution was quantified by ferrozene method.[34,36] A 50 mM stock solution of $CuCl_2{\cdot}2H_2O$ was prepared using deoxygenated water in sealed bottles. Appropriate amounts of stock solution were introduced into the serum bottles to obtain a final concentration of 0.5 mM using N_2-purged plastic syringes. Bottles were then sealed with Teflon-lined rubber septa and aluminium crimp caps, and were incubated in an orbital shaker at

150 rpm (25 ± 1 ^{o}C) in the dark. After 20 h of equilibrium, an aliquot of CCl_4 (CT) stock solution dissolved in degassed methanol was delivered into the serum bottles by a N_2-purged gastight glass syringe to obtain the final concentration of 20 µM. In every bottles the total liquid volume was maintained at 50 mL, and a 20-mL headspace was left for headspace analysis. Parallel experiments were also carried out without the addition of Fe(II). In order to study the combined effect of Fe(II) and Cu(II) on the dechlorination of CCl_4, experiments were carried out according to the procedures described above without addition of iron oxides. The concentrations of Cu(II) and Fe(II) in the systems were within the range of 0 - 3 mM.

2.2.5 Fe(II) and Cu(II) sorption experiments

In order to understand the sorption behaviours of Fe(II) or Cu(II) on the iron oxide minerals, the experimental procudures as in the dechlorination studies were followed with idendical conditions with the exception of CCl_4 addition. To study the Fe(II) sorption isotherms on iron oxides, various volumes of the stock solution of Fe(II) was added to iron oxide suspensions (10 mM) to get the final concentraions of 0.05 - 5 mM. After the addition of Fe(II), bottles were incubated in an orbital shaker at 25 °C and at 150 rpm in the dark. After 20 h of equilibrium, 1 mL aliquote was withdrawn using N_2-purged gastight plastic syringe and 0.5 mL volume was filtered into 1 mL of 0.5 M HCl solution through acidified 0.2 µM filter cartrige (1 cm in diameter). Then, the Fe(II) concentrations in the filtrate was determined.

For Cu(II) sorption experiments, various volumes of stock of $CuCl_2$ solution was injected into iron oxide suspensions (10 mM) using N_2-purged gastight plastic syringe to get the final concentration of Cu(II) ranging between 0.01 and 0.5mM. The bottles were incubated for 20 h and 1 mL of suspension was withdrawn by plastic syringe and was filtered through a 0.2 µM filter cartrige into 1 mL of 1 M HCl. The dissolved concentrations of copper ions were determined by atomic absorption spectrometry (AAS).

2.2.6 Analytical Methods

The headspace analytical technique was used in this study for the determination of chlorinated hydrocarbons. The concentrations of chlorinated hydrocarbons and their dechlorination products in the headspace of the test bottles were monitored by withdrawing 50 μL of gas in the headspace using a 100 μL gas-tight syringe. The mixture was immediately injected 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 fused-silica megabore capillary column (0.545 mm × 3.0 μm, Supelco Co.) was used to separate chlorinated compounds. The temperature of the GC-column was maintained at 90 ºC isothermally with the nitrogen (N_2) as the carrier gas.

The concentrations of Fe(II) in the serum bottles were monitored using Ferrozine method.[34,36] Ferrozine solution was prepared by dissolving 0.1g of ferrozine in 100 mL of ammonium actate solution (50%). A 0.5 mL of ferrozine solution was addd into a 1 mL of acidified sample solution in a 2-mL vial and after 10 min the mixture was analyzied by UV-visible spectrometer (Hitachi, U3010) at 562 nm. The concentrations were calculated using a calibration curve that was prepared by using the absorption values obtained for the standard Fe(II) solutions following the similar procedure.

Copper ion concentrations in acidified samples were determined by flame atomic absorption spectrophotometer (Perkin Elmer) using a Cu hollow cathod lamp. The wavelength used was 324.8 nm. Acetylene and air were used as fuel and oxidant, respectively. The concentration was calculated by the standard calibration obtained using standard copper solutions. The limit of detection for the Cu was 2 μM.

Samples for specific surface area analyses were stored in a vacuumed desicator until they were introduced into a surface area analyser. The specific surface areas of the dried powder samples of iron oxide samples were measured by a BET surface area analyzer (Micrometrics, ASAP 2020) using N_2 as the adsorbate. Single point surface area was determined following the standard procedure.

For XRPD analysis, iron oxide powder samples were stored in a vacuumed desicator untill they were subjected to analyze. Powder samples were mounted on a glass sample holder using small amounts of grease. XRPD was performed using an X-ray diffractometer (Regaku D/max-II B) and a Cu Kα-radiation source (λ = 1.54056 Å) operated at 30 kV voltage and 20 mA current. The peak pattern for relevant iron oxides was compared with the standard peak pattern of pure iron oxide minerals available in the database.

The dry powdered samples prepared and stored in the same manner were also used for the surface morphology studies by scanning electron microscopy (SEM-Topcon ABT-150S).

2.3 RESULTS AND DISCUSSION

2.3.1 Characterization of iron oxide minerals

The XRPD patterns of iron oxides are simple but provide strong evidence to elucidate the charateristic of crystal phases of minerals.[35] Moreover, electron microscopy also provides the information about the morphology and particle shpae of solid minerals. Therefore, in this study, these two techniques were used to characterize the synthesized iron oxides including goethite, hematite, magnetite and ferrihydrite.

Goethite. As shown in Figure 2-2, the synthesized bright yellow colored iron oxide showed peaks at 21.22°, 36.72°, 33.34° and 53.24° 2θ with intensities of 100%, 26.4%, 18.0% and 17.2%, respectively. This peak pattern is in a good agreement with that of goethite in the database (Appendix). The needle shape of the synthesized mineral shown in the scanning electron microscopy (SEM) image is a characteristic of goethite (Figure 2-3). Morover, the bright yellow color of this iron oxide also depicts that the mineral is goethite.

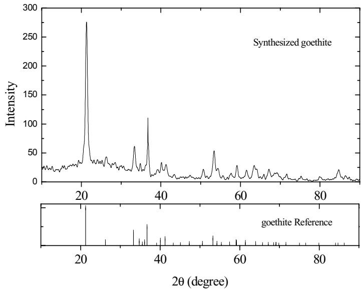


Figure 2-2. XRPD patterns of the synthesized goethite (α-FeOOH)

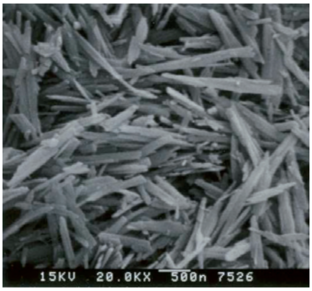


Figure 2-3. SEM the synthesized goethite (α-FeOOH)

Hematite. As shown in Figure 2-4, the iron oxide that was synthesized by following the hematite preparation procedure had dominant peaks at 33.11°, 35.61°, 54.0° and 24.12° 2θ with intensities of 100%, 70%, 36% and 24.12% respectively, which were well-fitted with the XRD patern of standard hematite in the database (Appendix). The SEM image shown in Figure 2-5 indicates that the synthesized hematite is a well-crystallized fine particles with particle sizes of 30 ~ 50 nm, which is similar to that of the reported data[35]. However, the cubic shape of the hematite crystals is not clearly observed in the SEM image, presumably due to the low magnification of SEM (Figure 2-5).

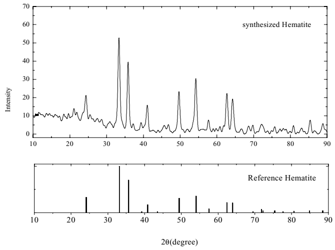


Figure 2-4. XRD pattern of synthesised hematite

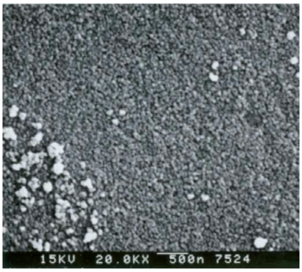
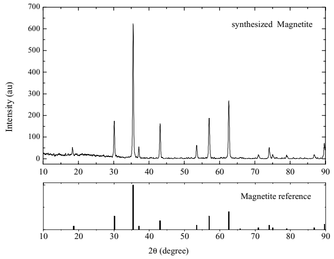


Figure 2-5. SEM image of synthesised hematite

Magnetite. The XRPD pattern of the black colored minerals synthesized by using the procedure for preparation of magnetite is shown in Figure 2-6. The black color of the mineral provided a priliminery evidence that the synthesized material could be magnetite. Moreover, the Cu Kα x-ray diffractogram shows dominant peakes at 35.42°, 62.51°, 30.09°, 56.94° and 43.05° 2θ with intensity ratios of 100%, 40%, 30%, 30% and 20%, respectively, which can be assigned as magnetite. Figure 2-7 shows the SEM image of the synthesized magnetite. The cubic shapes with variable particle sizes in the range of 50 ~ 200 nm were clearly observed.



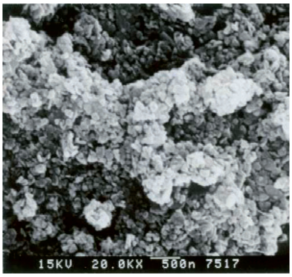
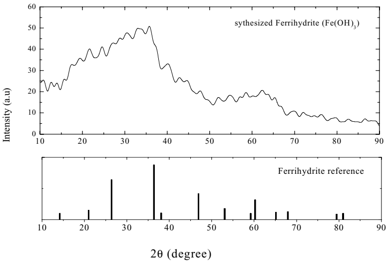


Figure 2-6. XRPD patterns for synthesized magnetite

Figure 2-7 SEM image of synthesized magnetite

Ferrihydrite. In contrast to other crystalline iron oxide minerals, ferrihydrite, also known as amorphous ferric oxide, is a poorly crystalline mineral. Since XRPD only shows peaks for crystalline materials, the XRD pattern of ferryhydrite does not show sharp peaks. As shown in Figure 2-8, the synthesized ferrihydrite has two broad peaks at around 36.4° and 60.2° 2θ. This sort of ferrihydrite is referred to as two-line ferrihydrite. The poor crystallinity and small particle size of the synthesized ferrihydrite are shown in the SEM image (Figure 2-9).



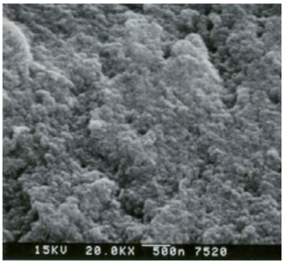


Figure 2-8. XRPD patterns for the synthesized ferrihydrite ($Fe(OH)_3$) and reference peak patterns for ferrihydrite

Figure 2-9. SEM image of the synthesized ferrihydrite ($Fe(OH)_3$)

Specific surface area. The specific surface areas of iron oxides were determined by BET single point measurements using N_2 as adsorbate. The determined values of specific surface areas were 28.80 ± 0.11, 39.4 ± 0.21 , 222.00 ± 0.30 and 11.67 ± 0.08 m^2g^{-1} for goethite, hematite, ferrihydrite and magnetite, respectively. These specific surface areas are generally in good agreement with the reported values.[35]

2.3.2 Sorption of Fe(II) onto iron oxide minerals

The sorption of heavy metal ions onto iron minerals has been carried out by numerous studies with the objective of possible removal of those metal ions from the aqueous solutions.[37-39] However, only few studies have focused on the sorption of Fe(II) species onto iron minerals, probably because Fe(II) ions are easy to be oxidized to Fe(III) in the presence of trace amounts of oxygen. Since sorbed Fe(II) ions on the Fe(III) oxides form Fe(II)/Fe(III) oxide redox couple which often buffers the oxidation-reduction potential of anoxic systems[40], the sorption behavior of Fe(II) onto Fe(III) minerals may be compatible with the dechlorination behaviours of chlorinated hydrocarbons by surface-bound Fe(II) systems. The Fe(II) adsorption on the goethite was studied using 10 mM of goethite (0.89 gL^{-1}) at various concentration of Fe(II) ranging between 0.05 and 5 mM at 25°C. The Fe(II) sorption on goethite followed Langmuir-type isotherm, which gives the relationship of the sorbed concentration in the surface of goethite and the equilibrium concentration:

$$\Gamma = \Gamma_{\max} \frac{K_{ads}[A]}{1 + K_{ads}[A]} \tag{2-1}$$

where Γ is the density of sorbate on the surface, $\Gamma_{\max}$ is the maximum density of the sorbate, and [A] represents the aqueous concentration of adsorbate at equilibrium. As shown in Figure 2-10, the sorption of Fe(II) on goethite can be fitted with the equation (2-1), sugesting that Fe(II) sorption follows Lagngmuir isotherm. A previous study reported that the Fe(II) sorptionon on the goethite followed Langmuir isotherm at Fe(II) concentrations of 0 ~ 3 mM.[14] However, continuous increase in the sorption of Fe(II) on goethite with increasing Fe(II) concentration has been observed when Fe(II) concentrations ranged from 0.005 to 0.5 mM after 24 h of equilibrium.[17,28] Those observations are consistent with the sorption isotherm of Fe(II) on goethite obtained in this study.

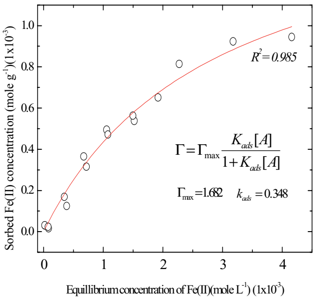


Figure 2-10. The sorption isotherm of Fe(II) onto goethite at 25 °C. Symbol and line represent experimental data and fitted results, respectively

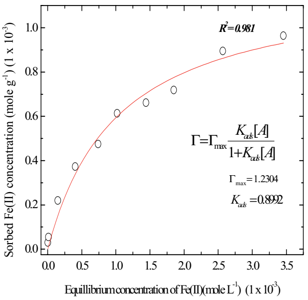


Figure 2-11. The sorption isotherm of Fe(II) on hematite at 25 °C. Symbol and line represent experimental data and fitted results, respectively

Ferrihydrite is a poorly crystalline Fe(III) mineral which has high specific surface area and amorphous characteristics. Therefore, ferrihydrite has been proven as an efficient sorbent for the removal of dissolved toxic metal ions.[44-46] The surface complexation of Fe(II) ions onto frrihydrite has been rationalized for the reductive immobilization of several metal ions such as U(VI) and As(V).[43 47] The sorption capacity of ferryhydrite also has been found to be infuenced by the type of the medium. For instance, the bicarbonate ions in the buffered medium decreased the Fe(II) sorption capability.[47] Hence, the real sorption capacity of ferrihydtire may be different from the results obtained from laboratory experiments. In this study, the sorption isotherm was obtained at 25 °C in HEPES buffer(pH 7.2) solution containing 10 mM ferrihydrite (1.07 g L^{-1}). The sorption behaviour was different form those in goethite and hematite systems. As shown in Figure 2-12, an exponential increase in sorbed Fe(II) density with the increasing Fe(II) concentration in the solution was observed, which can be assigned as Freundlich isotherm

$$\Gamma = K[C]^{n} \tag{2-2}$$

where Γ is the density of the sorbate at surface, C is the equilibrium concentration of sorbate, K is the Freundlich constant and n is the measure of the non-linearity. This equation has been applied to explain the adsorptive capacity of ordinary soil which contained iron oxides.[48] As shown in Figure 2-12, the Freundlich constant (K) was 0.176 and the n value was 4.586.

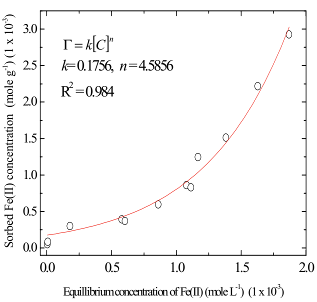


Figure 2-12. The sorption isotherm of Fe(II) on ferrihydrite at 25°C. Symbol and line represent experimental data and fitted results, respectively

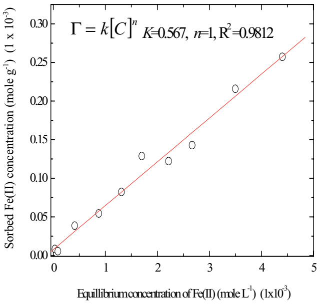


Figure2-13. Sorption isotherm of Fe(II) on magnetite at 25 °C. Symbol and line represent experimental data and fitted results, respectively

The sorption isotherm of Fe(II) onto hematite also followed Langmuir isotherm (Figure 2-11). The surface saturation level for hematite is lower than that for goethite, while the Fe(II) adsorption constant (k_{ads}) for hematite is larger. A recent study depicted that the sorption of Fe(II) onto hematite followed a two-phase stage, a first rapid sorption and followed by a slow sorption phase.[41] The rapid sorption occurs within 1 ~ 2 d, while slow sorption continues even after 45 d.[25,41,42] Therefore, the long-term sorption of Fe(II) may be somewhat higher than the observed value obtained in this study. However, the formation of ferreso-ferrichydroxo surface complexes ($\equiv Fe^{III}OFe^{II}OH$) within several hours has also been reported.[43] Previous studies showed that a contact time of 20 h for dissolved Fe(II) ion and Fe(III) minerals is necessary before the onset of pollutant reduction to get the better degradation efficiency of contaminants.[13,17] Therefore, an equilibrium time of 20 h was used in this study.

Ferrihydrite is a poorly crystalline Fe(III) mineral which has high specific surface area and amorphous characteristics. Therefore, ferrihydrite has been proven as an efficient sorbent for the removal of dissolved toxic metal ions.[44-46] The surface complexation of Fe(II) ions onto frrihydrite has been rationalized for the reductive immobilization of several metal ions such as U(VI) and As(V).[43 47] The sorption capacity of ferryhydrite also has been found to be influenced by the type of the medium. For instance, the bicarbonate ions in the buffered medium decreased the Fe(II) sorption capability.[47] Hence, the real sorption capacity of ferrihydtire may be different from the results obtained from laboratory experiments. In this study, the sorption isotherm was obtained at 25 °C in HEPES buffer(pH 7.2) solution containing 10 mM ferrihydrite (1.07 g L^{-1}). The sorption behaviour was different form those in goethite and hematite systems. As shown in Figure 2-12, an exponential increase in sorbed Fe(II) density with the increasing Fe(II) concentration in the solution was observed, which can be assigned as Freundlich sotherm

$$\Gamma = K[C]^{n} \tag{2-2}$$

where Γ is the density of the sorbate at surface, C is the equilibrium concentration of sorbate, K is the Freundlich constant and n is the measure of the non-linearity. This equation has been applied to explain the adsorptive capacity of ordinary soil which contained iron oxides.[48] As shown in Figure 2-12, the Freundlich constant (K) was 0.176 and the n value was 4.586.

Sorption of Fe(II) on the magnetite was studied at the concentration of 2.32 g L^{-1}. As illustrated in Figure 2-13, the sorption isotherm of Fe(II) on magnetite also seems to follow Freundlich isotherm with a good linearity, which gives the n value equal to unity and the Freundlich constant (K) is 0.0567. Probably the used concentration range of Fe(II) may not enough to deveate the n value from unity.

By summarizing the results of Fe(II) sorption on iron oxides, it is clear that, under the condition at neutral pH and 20 h of equilibrium, goethite and hematite show Langmuirian isotherms, while Freundlich isotherms are obtained in ferrihydrite and magnetite systems.

2.3.3 Dechlorination of CCl_4 by surface-bound Fe system

In order to compare the reductive capacities of dissolved and surface-bound Fe(II), CCl_4 was incubated with 3 mM Fe(II) at pH 7.2 in the absence and presence of iron oxide minerals. Figure 2-14 illustrates the dechlorination of 20 μM CCl_4 and the production of chloroform ($CHCl_3$) as the major chlorinated product in Fe(II)-amended solution with and without the addition of iron oxides. No obvious change in CCl_4 concentration was observed during the experimental course in solutions containing Fe(II) alone. Several studies showed that CCl_4 cannot be dechlorinated by dissolved Fe(II) at neutral pH during a relative short time.[14,49] Addition of

Fe(II) into the iron oxide suspensions increased the rate and efficiency of CCl_4 dechlorination. A nearly complete dechlorination of CCl_4 was observed within 48 h in the suspention containing 10 mM hematite and 3 mM Fe(II). Good removal efficiencies of CCl_4 by Fe(II) were also observed in suspensions of ferrihydrite and goethite. However, the mixed-valence iron mineral, magnetite, showed a relative low reductive capacity and only 43% of the initial CCl_4 was dechlorinated within 48 h. Chloroform ($CHCl_3$) was found to be the major product in all the surface-bound Fe(II) systems, which agreed with the previous studies on the dechlorination of CCl_4 by different types of Fe(II)/Fe(III) systems such as surface-bound Fe(II) associated with goethite[14,50] and biogenic magnetite.[51] Because of the formation of chloroform, it is clear that reductive dechlorination is the prominent pathway for CCl_4 transformation. The maximum concentrations of $CHCl_3$ in all the iron oxide systems except the magnetite system were in the range of 7.2 – 12 μM. The production of $CHCl_3$ concentration in magnetite system was only 1.15 μM. Table 2-1 shows the concentrations of $CHCl_3$ after 48 h of incubation in iron oxide systems. It is clear that the mechanism in the reductive transformation of CCl_4 in each surface-bound system is not as the same. The carbon mass balance with respect to chloroform formation in surface-bound Fe(II) systems is 73.5% in ferrihydrite, 57.4% in goethite, 54.3% in hematite and 13.7% in magnetite systems, clearly showing that there may exist another mechanism other than reductive dehalogenation for the CCl_4dechlorination.

Previous studies showed that Fe(II)-goethite suspension transformed 58% ~ 80% of CCl_4 to $CHCl_3$ at neutral pH.[14,50] However, low conversion ratio (0 ~ 24%) was also observed in magnetite system.[52] In this study, a trace amount of dichloromethane (DCM) and tetrachloroethene (C_2Cl_4) were also determined by GC-ECD after10 d of incubation. However, the concentration of $CHCl_3$ did not show a significant decrease during the experimental period. Resent study revealed that the product distribution in transformation of target organics by surface-bound Fe(II) and Fe(II)-bearing minerals may be mainly dependent on the types of iron oxide mineral and target organic compound.[16] According to the result obtained this study, a difference in product formation ratio was observed when CCl_4 was transformed by Fe(II) associated with different iron oxide systems**.** The dechlorination of CCl_4 by the surface-bound Fe(II) species followed pseudo first-order kinetics:

$$\ln\left[\frac{C_t}{C_0}\right] = k_{obs}t \qquad (2\text{-}3)$$

where C_0 and C_t are the concentrations of carbon tetrachloride at the initial and at time t, respectively and k_{obs} is the observed pseudo first-order rate constant for carbon tetrachloride dechlorination. A good linearity was found in the plot of $\ln(C_t/C_0)$ vs time during the first 10~20 hours and then the slope decreased slightly. Such a deviation in the linearity has been reported to be a common phenomenon in reduction of various compounds such as nitroaromatic compounds and polyhalogenated alkanes by surface-bound Fe(II) species.[17,53]

The decrease in reactivity of surface-bound Fe(II) in the latter part of the experimental time is presumably attributed to the possible consumption of more reactive Fe(II) species at the first stage and the high affinity of surface reactive sites towards the produced compounds.[17,50] Table 2-2 shows the apparent pseudo first-order rate constant (k_{obs}) and the normalized surface area rate constant (k_{sa}) for CCl_4 dechlorination in systems contained various types of iron oxides and 3 mM Fe(II) at pH 7.2. The k_{obs} for the dechlorination in different iron oxide systems followed the descending order of hematite > ferrihydrite > goethite > magnetite, according to the magnitude. When the normalized surface area rate constant (k_{sa}) was used by dividing the k_{obs} by available surface area of iron oxide minerals in the serum bottles, the k_{sa} followed the order of goethite > hematite > magnetite > ferrihydrite. Since similar molar concentration (10 mM) of iron oxides were amended into the bottles and the dechlorination in Fe(II)-Fe(III) suspension is a surface mediated reaction, it is reasonable to use k_{sa} to compare the kinetics of CCl_4 dechlorination by surface-bound Fe(II) associated with various iron oxide.

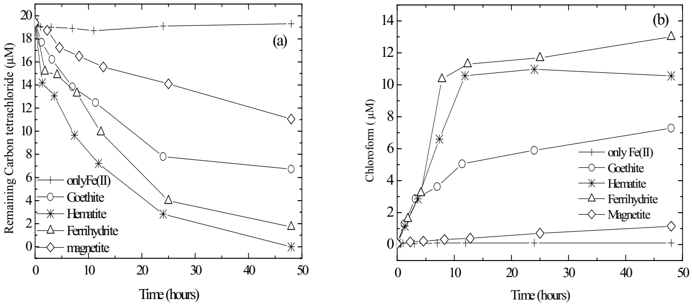


Figure 2-14. Reductive dechlorination of 20 μM CCl_4 by 3 mM Fe(II) in the presence and absence of 10 mM iron oxides under anoxic condition at pH 7.2. (a) Dechlorination of CCl_4, (b) Formation of $CHCl_3$ as the major product during CCl_4 dechlorination.

Table 2-1. Concentrations changes in carbon tetrachloride and chloroform and the percentage of CHCl3 to CCl_4 during the dechlorination of CCl_4 by surface-bound Fe(II) associated with different iron oxide system at pH 7.2.

Iron oxide system	Dechlorinated CCl_4 (μM)	Produced $CHCl_3$ (μM)	$[CHCl_3]$ / [dechlorinated CCl_4] (%)
Goethite	12.68	7.28	57.41
Hematite	19.40	10.54	54.32
Ferrihydrite	17.68	12.99	73.47
Magnetite	8.35	1.14	13.65

It is clear that goethite, the most abundant crystalline iron oxide, has the highest k_{sa} value, indicating the positive role of natural iron oxides in the abiotic dechlorination of chlorinated solvents. Hematite is also a well crystalline and the most stable iron oxide and has a high k_{sa} value. Magnetite is a relatively low stable crystalline mineral which bears structural Fe(II) and frequently generated by bacterial Fe(III) reduction.[51,54] Its dechlorination capability with respect to k_{sa} is in the third place of the descending order. Ferrihydrite, the amorphous iron mineral with high specific surface area, has the lowest k_{sa} value obtained in this study. This means that crystal property of the iron mineral may be one of the important factors influencing the efficiency of CCl_4 dechlorination. Since goethite suspension has the highest capability for CCl_4 dechlorination regarding k_{sa}, it was selected as the model iron oxide to examine the effect of environmental parameters on the dechlorination of CCl_4 in the further experiments.

Table 2-2. The pseudo first-order rate constant (k_{obs}) and the normalized surface area rate constant (k_{sa}) for the dechlorination of CCl_4 by 3 mM Fe(II) amended with 10 mM iron oxides.

Mineral Type	Abbreviation	Specific surface area (m^2g^{-1})	Rate constant for CCl_4 dechlorination	
			k_{obs} (h^{-1})	k_{sa} ($h^{-1}m^{-2}$)*
Goethite	GT	28.80 ± 0.11	0.0380	0.0296
Hematite	HM	39.40 ± 0.21	0.0836	0.0265
Ferrihydrite	FH	222.00 ± 0.30	0.0609	0.0051
Magnetite	MG	11.67 ± 0.08	0.0144	0.0106

* k_{sa}= (k_{obs}/surface area of added iron oxides)

2.3.4 Effect of pH on the dechlorination of CCl_4 by goethite

The pH value plays a pivotal role in determining the reducing power of the surface-bound Fe(II) species. Figure 2-15 illustrates the effect of pH on the rate constants (k_{obs}) for CCl_4 dechlorination in Fe(II)-goethite system. A 22.5% of the initial CCl_4 was dechlorinated after 350 h of incubation at pH 4, depicting that the reactivity of Fe(II)-goethite system is quite low at low pH. However, the dechlorination of CCl_4 increased up to 63% when the pH increased to 6.5. The rapid increase in the dechlorination efficiency may be due to the change in pH from acid to neutral and then to basic. At pH 7.2, the CCl_4concentration decreased from 20 M to 6 µM during 36 h of incubation. Nearly complete dechlorination of CCl_4 was occurred within 9 h when pH was 8.5. The observed rate constant (k_{obs}) increased exponentially with the increase in pH (Figure 2-16). A parallel series of serum bottles was prepared by following the similar procedures with the exception of CCl_4 amendments to determine the concentrations of surface-bound Fe(II). The surface-bound Fe(II) onto goethite surface also increased in the similar trend as the increase in the k_{obs}. This relationship clearly evidences that the increase in pH enhanced the rate and efficiency of CCl_4 dechlorination due to increase in the surface-bound Fe(II) concentration. Similar trend in the relationship between k_{obs} and pH with surface-bound Fe(II) concentration has been observed in the reduction of fluorotribromomethane ($CFBr_3$) by Fe(II)-goethite suspension.[17] Several studies also depicted that the increase in k_{obs} at high pH and it may be because the pH controls some factors directly responsible for the reactivity of the system.[13,17] For example, the uptake of dissolved Fe(II) ions onto the surface of iron mineral significantly depends on the pH. The precipitation of dissolved Fe(II), mainly as hydroxide, can actively participate in the reducing reaction at high pH.[17,25] Also, the oxidation-reduction potentials (Redox) of reactions may depend on the pH and may serve as another factor influencing the dechlorination of chlorinated compounds.

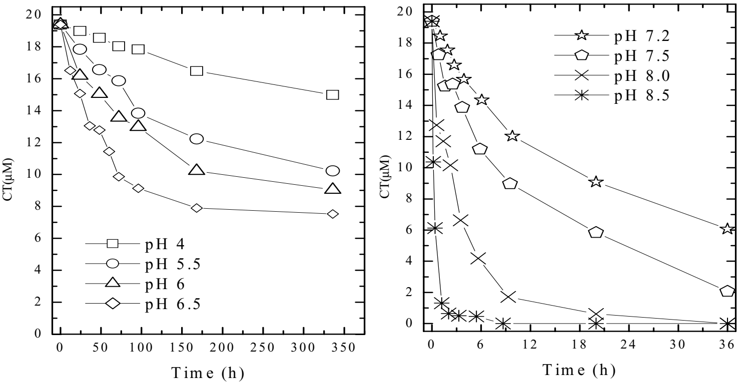


Figure 2-15. Effect of pH on the dechlorination of CCl_4 by 3 mM Fe(II) in the suspension of 10 mM goethite. 50 mM of MES, HEPES and TRIS buffers were used to maintain the system at pH 4.0 ~ 6.5, pH7.0 ~ 8.0 and pH 8.5, respectively

2.3.5 Effect of Fe(II) on the dechlorination of CCl_4 in goethite system

Figure 2-17 shows the dechlorination of CCl_4 by Fe(II) at various concentrations within the range of 0.5 ~ 4.0 mM in 10 mM goethite suspension. The initial concentration of Fe(II) has a good relationship with the dechlorination efficiency at pH 7.2. No significant amount of CCl_4 was dechlorinated by goethite in the absence of Fe(II) ions. When the initial Fe(II) concentration increased from 0.5 to 1.5 mM, the efficiency of CCl_4 dechlorination increased from 25% to 57% within 35 h of incubation. However, the increase in the dechlorination rate became lower when the Fe(II) concentration increased further. As shown in Figure 2-17, further increase in initial Fe(II) concentration to 2 ~ 4 mM did not enhance the dechlorination efficiency and only 61 ~ 68% of the initial CCl_4 was dechlorinated. Therefore, the relationship between the initial Fe(II) concentration and k_{obs} was studied. Figure 2-18 shows the k_{obs} as a function of Fe(II) concentration. The relationship followed Langmuir-Hinshelwood kinetics and can be expressed as:

$$k_{obs} = k_{app} \frac{K_{Fe} C_{Fe}}{1 + K_{Fe} C_{Fe}} \qquad (2\text{-}4)$$

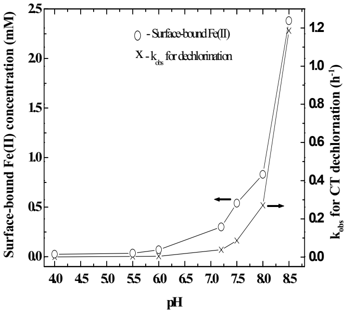


Figure 2-16. The k_{obs} for CCl_4 dechlorination as a function of pH.

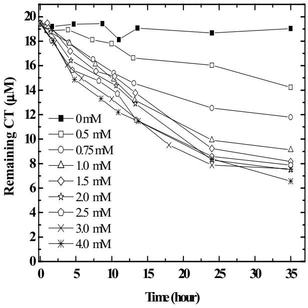


Figure 2-17. Effect of Fe(II) concentration on the dechlorination of CCl_4 in 10mM goethite suspension at pH 7.2

where k_{obs} is the pseudo-first-order rate constant for the dechlorination of CCl_4, k_{app} is the apparent reaction rate constant, K_{Fe} is the equilibrium constant for the adsorption of Fe(II) to goethite and C_{Fe} is the initial aqueous concentration of Fe(II). Equation (2-4) suggests that the CCl_4 dechlorination is a surface-mediated reaction and the observed reaction rate constant (k_{obs}) is dependent on the reactivity of the surface-bound Fe(II) species in relation to the apparent reaction rate constant k_{app}, sorption of Fe(II) on the iron oxide minerals and the initial concentration of Fe(II). In Figure 2-18, the reaction rate constant approaches to the plateau very slowly, indicating that a significant increase in Fe(II) concentration results in only moderate increase in CCl_4 dechlorination rate. It may be because the available surface of goethite becomes a rate-limiting factor after the surface saturation. Therefore, the effect of goethite concentration on CCl_4 dechlorination was evaluated.

2.3.6 Effect of goethite concentration

The effect of goethite concentration on the dechlorination of CCl_4 by 3 mM Fe(II) was studied at pH 7.2. As shown in Figure 2-19, the rate and efficiency of CCl_4 dechlorination increased from 35.7% to 87.6% when the goethite concentration increased from 5 mM (2.81 m^2L^{-1}) to 25 mM (64.08 m^2 L^{-1}). The calculated k_{obs} values for CCl_4 dechlorination also increased linearly with increasing goethite concentration (Figure 2-20). This results depict that goethite surface could be saturated with surface-bound Fe(II) at neutral pH and the limited surface area is one of the rate-limiting factors for the dechlorination reaction.

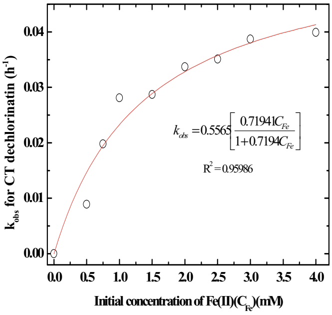


Figure 2-18. The k_{obs} for CCl_4 dechlorination as a function of initial Fe(II) concentration in 10mM goethite suspension at pH 7.2.

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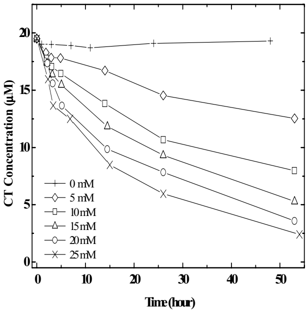

Figure 2-19. The concentration effect of goethite on the dechlorination of CCl_4 in the presence of 3 mM Fe(II) at neutral pH.

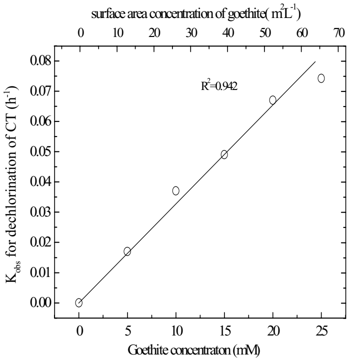

Figure 2-20. The rate constant (k_{obs}) for CCl_4 dechlorination as a function of goethite concentration at pH 7.2

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2.3.7 Dechlorination of CCl_4 by surface-bound Fe(II) species in the presence of Cu(II)

The effect of catalytic inorganic ions on the reactivity of surface-bound Fe(II) systems is not well-known. Cu(II) ions complexed with bulk reductants have shown to catalyze reduction reactions.[55] Therefore, the effect of Cu(II) ions on the dechlorination of CCl_4 by Fe(II) with various iron oxide was further studied. The concentrations of Fe(II) and iron oxide were 3mM and 10 mM respectively. As shown in Figure 2-21, addition of Cu(II) enhanced the rate and efficiency of CCl_4 dechlorination. Also, the amendment of 0.5 mM Cu(II) had different enhancement effects on the dechlorination of CCl_4 in various Fe(II)-iron oxide systems. The Fe(II)-goethite suspensions showed the highest efficiency for CCl_4 dechlorination and a nearly complete CCl_4dechlorination was observed during 1.5 h. The dechlorination efficiency with 0.5 mM Cu(II) is 90 times greater than that without Cu(II) in Fe(II)-goethite suspension. Similar degradation patterns were observed in magnetite-Fe(II) and hematite-Fe(II) systems and the degradation efficiency of CCl_4 were enhanced nearly 25 and 30 times, respectively, in comparison with Cu(II) un-amended suspensions. The CCl_4 degradation pattern in amorphous ferrihydrite-Fe(II) system was different from those in crystalline Fe(III) oxide systems and only 50% of the initial CCl_4 was dechlorinated within 2.8 h. This proves again that Fe(II) bound to crystal surfaces may be more effective than amorphous Fe(III)oxide in the presence of Cu(II).

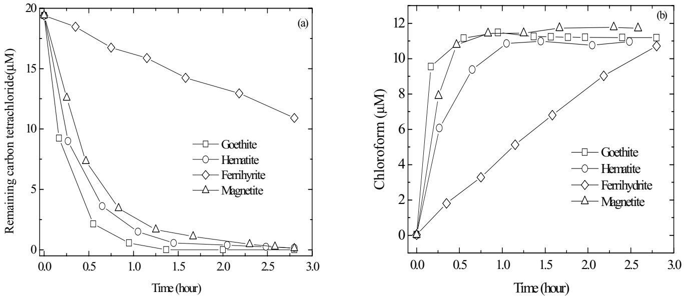

Figure 2-21. Reductive dechlorination of 20 μM CCl_4 by 3 mM Fe(II) and 0.5 mMCu(II) with different iron oxides at pH 7.2. (a) dechlorination of CCl_4, (b) formation of $CHCl_3$

Chloroform ($CHCl_3$) was found as the major chlorinated byproduct (Figure 2-21b). The production of $CHCl_3$ increased with the decrease in CCl_4 concentrations in Cu(II)-amended systems, clearly showing that reductive dechlorination is still the major pathway for CCl_4 dechlorination in the presence of Cu(II). The maximum concentrations of $CHCl_3$ were in range of 10.7 - 11.7 μM, which accounted for 55 – 60% of the CCl_4 concentration. It is noted that the maximum concentration of $CHCl_3$ in the ferrihydrite-Fe(II)-Cu(II) system corresponded to around 95 % of the dechlorinated CCl_4. This value is quite higher than that in the other iron oxide-Fe(II)-Cu(II) suspensions, further suggesting that the dechlorination mechanisms in amorphous iron oxide system could be different from those in crystalline iron oxide systems. The dechlorination of CCl_4 by Fe(II)-iron oxides in the presence of Cu(II) also followed first-order reaction kinetics, depicting that the addition of Cu(II) into the surface-bound Fe(II) systems did not change the order of the dechlorination reaction. Table 2-3 shows the observed pseudo first-order rate constant (k_{obs}) for CCl_4 dechlorination by the surface-bound iron species with 0.5 mM Cu(II). According to the magnitude of k_{obs} for CCl_4 dechlorination surface-bound Fe(II) systems were in the order of goethite > hematite > magnetite > ferrihydrite, which is a little different from that for the Fe(II)-iron oxide suspensions without Cu(II). Addition of Cu(II) ion enhanced the k_{obs} values up to 100, 29.6, 3.2 and 120 times in goethite, hematite, ferrihydrite and magnetite suspensions, respectively. The highest enhancement effect on the CCl_4

dechlorination was observed in magnetite suspension, while low enhancement effect was observed in the amorphous ferrihydrite system.

Table 2-3. The pseudo first-order rate constants (k_{obs}) for CCl_4 dechlorination by surface-bound iron species in the presence of 0.5 mM Cu(II) at pH 7.2.

Mineral type	Rate constant for CCl_4 dechlorination k_{obs} (h^{-1})
Goethite	3.807
Hematite	2.476
Ferrihydrite	0.196
Magnetite	1.722

Since rapid dechlorination of CCl_4 by surface-bound Fe(II) species was observed in the presence of Cu(II), the system might have enough reducing power to decompose more target organics. In order to study such a possibility for CCl_4 reduction, CCl_4 was re-spiked into the batches containing 10 mM iron oxides, 3 mM Fe(II) and 0.5 mM Cu(II). Figure 2-22 shows the concentration profiles of CCl_4 in each iron oxide minerals. Nearly complete dechlorination of CCl_4 was observed within 24 hours in the goethite and hematite systems when 20 µM CCl_4 was re-spiked four times. Magnetite suspension showed complete dechlorination only for three times of spike within the experimental course. In the ferrihydrite system, complete dechlorination of CCl_4 could be obtained in the first spike during 16 hours and only 70% of CCl_4 was dechlorinated for the second spike within 24h. This indicates that surface-bound Fe(II) systems have the capability in dechlorinating relatively large amount of chlorinated compounds in the presence of Cu(II). With the increase in the number of re-spikes, however, a decrease in the efficiency and rate of CCl_4 dechlorination in each iron oxide systems was observed. This decrease also depended on the types of iron oxides. Figure 2-23 shows the decreases in k_{obs} for CCl_4 dechlorination with the increasing number of spikes. It is clear that higher reactive iron oxide systems, such as goethite, deactivate faster than the low reactive systems. The decrease in reactivity could be due to the deactivation of highly reactive surface-bound Fe(II) sites on the iron oxides at the first stage of the incubation period[17]. However, the reactive sites of the surface-bound Fe(II) species could be regenerated in the *in-situ* subsurface environments by the biological Fe(III) reduction process of dissimilatory iron reducing bacteria (DIRB).

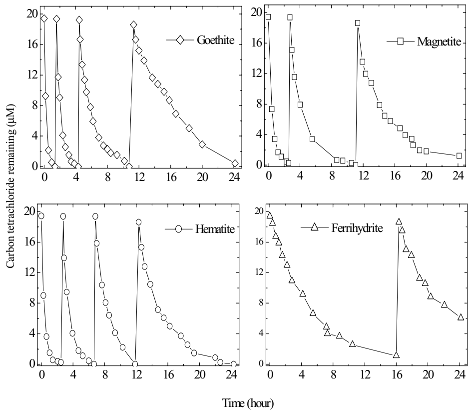

Figure 2-22. Dechlorination of re-spiked CCl_4 by 3 mM Fe(II) added 10 mM iron oxides (goethite, hematite, magnetite and ferrihydrite) in the presence of 0.5 mM Cu(II) at pH 7.2.

2.3.8 Reduction of chloroform by Cu(II) catalyzed surface bound Fe(II) systems

Since chloroform ($CHCl_3$) was accumulated with the rapid reduction of carbon tetrachloride, further experiment was carried out to understand the possible dechlorination of $CHCl_3$. Generally, the dechlorination of 20 μM CCl_4 can produce about 10 μM of $CHCl_3$. Therefore, similar concentration of $CHCl_3$ was used as the initial concentration. The $CHCl_3$ concentration was detected periodically during 90 days. A decrease in $CHCl_3$ concentration by 3 mM Fe(II) in various iron oxide suspensions was observed in the presence of 0.5 mM Cu(II) (Figure 2-24). A two-phase dechlorination behavior with a rapid dechlorination during first 15 days followed by a slow dechlorination phase within 90 days was observed in all the iron oxide suspensions. The $CHCl_3$ removal ratios were 53%, 49%, 39% and 35% in goethite, hematite, magnetite and ferrihydrite suspensions, respectively.

The initial rates for the dechlorination of $CHCl_3$ in iron oxide suspension were 0.27 μM d^{-1} for ferrihydrite, 0.29 μM d^{-1} for hematite and 0.31 μM d^{-1} for goethite. However, only a trace amount of dichloromethane (DCM) in the headspace of bottles was detected by GC-ECD. To understand whether other chlorinated products existed in the liquid phase, solid phase microextraction (SPME) method, a very sensitive analytical technique, using 75 μm Carboxen-PDMS fiber was employed.

Only DCM peak was observed and no additional peak was appeared in the chromatograph after the SPME analysis. This result suggests that chloroform was produced as the major byproduct of CCl_4 dechlorination and can be further dechlorinated into less chlorinated products after a relative long incubation time.

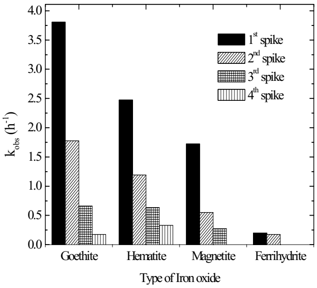

Figure 2-23. Rate constant (k_{obs}) for CCl_4 dechlorination when 20 μM CCl_4 was repeatedly spiked into the suspension containing 3 mM Fe(II) and 10 mM iron oxides in the presence of 0.5 mM cu(II) at pH 7.2.

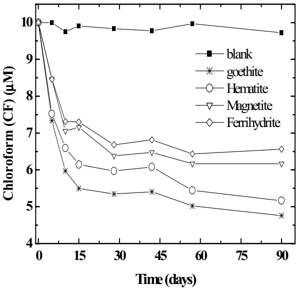

Figure 2-24. Dechlorination of chloroform ($CHCl_3$) by 3mM Fe(II) in 10 mM iron oxides (goethite, hematite, Magnetite and ferri-hydrite) in the presence of 0.5 mM Cu(II) at pH 7.2 and 25°C.

2.3.9 The sorption of Cu(II) on iron minerals

Although the addition of 0.5 mM Cu(II) concentration could enhance the rate and efficiency of CCl_4 dechlorination, the solubility of Cu(II) ion at neutral pH is quite low compared to the added concentration. To verify the precipitated concentration of Cu(II) at pH 7.2 (50 mM HEPES buffer), the soluble Cu(II) concentration was determined by filtering the solution through a 0.2 μm PTFE filter after the equilibrium of 20 h. The precipitated concentration was calculated by subtracting the determined concentration from the added concentration. Figure 2-25 illustrates the relationship between the added Cu(II) concentration and the precipitated Cu(II) concentration. No obvious precipitation of Cu(II) was observed up to 0.1 mM Cu(II). Further increase in Cu(II) concentration up to 0.5 mM produced a precipitate, and a linear relationship between the added Cu(II) concentration and the amount of precipitated Cu(II) was clearly observed.

Cu(II) ion also can be sorbed onto the surface of iron oxides in the surface-bound iron systems. The sorption isotherms of Cu(II) on iron oxide surfaces are shown in Figure 2-26. The sorption of Cu(II) onto goethite followed the Langmuir-type isotherm. The mechanism for the sorption of Cu(II) on goethite has been studied extensively and found that Cu(II) has very high affinity on iron oxide surfaces and can form irreversible chemical bonds by inner-sphere surface complexation with goethite surfaces.[56] It is noted that the affinity of Cu(II) onto goethite is higher than that for other divalent metal ions on goethite.[57-59] This can be evidenced by the result obtained in this study. The adsorption constant (K_{ads}) for Cu(II) is several hundreds times higher than that for Fe(II) on all iron oxides minerals under the experimental conditions, showing that Cu(II) has higher sorption affinity than Fe(II) onto iron oxides.

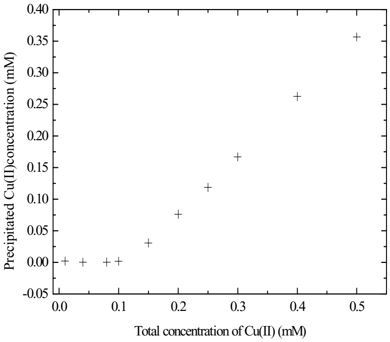

Figure 2-25. The relationship between total added Cu(II) and precipitated concentration of Cu(II) at pH 7.2 and at 25°C after 20 h of equilibrium

The sorption of Cu(II) on hematite also obeyed Langmuir-type isotherm. As shown in Figure 2-26 b, the adsorption constant (K_{ads}) for hematite is higher than that for goethite. However, studies related to Cu(II) sorption onto hematite are limited compared to that for goethite. The adsorption capability of Cu(II) by ferrihydrite has been studied by several researches.[44,60,61] The general conclusion is that ferrihydrite has very high sorption capacity for Cu(II) ions. However, this capability is mainly influenced by pH and the existence of other metal ions. At low pH, large fraction of sorbed Cu(II) was desorbed.[44] It also has been reported that the sorption of Cu(II) onto ferrihydrite occurs simultaneously with chemisorption in which Cu(II) ions infiltrated into the lattice of the ferrihydrite structure.[62] Also, coincidental sorption and precipitation of Cu

(II) on ferrihydrite was observed at a concentration of 0.06 mM.[60] In this study, however, only dissolved Cu(II) existed in the solution because the equilibrium concentration of Cu(II) were all lower than 0.1mM, the thresholds value for the dissolved Cu(II) in solution (Figure 2-25), and the sorption of Cu(II) onto ferrihydrite could be fitted with Langmuir isotherm, which is consistent with the reported result.[61]

The sorption behavior of Cu(II) onto magnetite is different from those onto other iron oxides. As depicted in Figure 2-25d, a linear relationship between equilibrium concentration and sorbed concentration was exhibited, which can be assigned to be a Freundlich-type isotherm with *n* equal to unity. It is worth mentioning that both sorption of Fe(II) and Cu(II) onto magnetite shows linear relationship that is different from the other iron oxide minerals. The chemical state of magnetite also different from other iron oxides having both Fe(II) and Fe(III) in the structure. The reduction of transition metal ions including Cu(II) by Fe(II) in magnetite has been observed [63]. Therefore, the different sorption behavior could be attributed to the changes in the mineral surface during the later stage of the incubation due to redox reaction. Other possibility is reduced Cu specie on the magnetite may have more attraction on the Cu(II) in the aqueous phase.

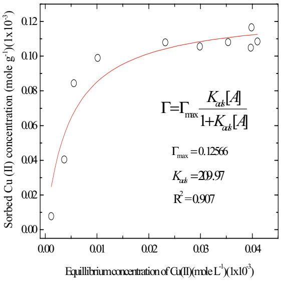

Figure 2-26a. The sorption isotherm of Cu(II) onto goethite at 25 °C and pH 7.2. Symbol and line represent experimental data and fitted results, respectively

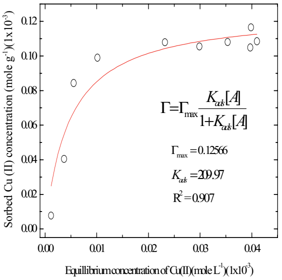

Figure 2-26b. The sorption isotherm of Cu(II) onto hematite at 25 °C and pH 7.2. Symbol and line represent experimental data and fitted results, respectively

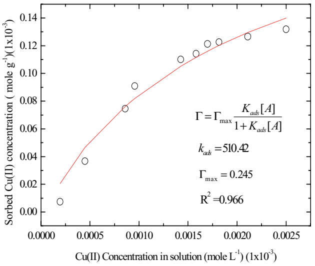

Figure 2-26c. The sorption isotherm of Cu(II) onto ferrihydrite at 25 °C and pH 7.2. Symbol and line represent experimental data and fitted results, respectively

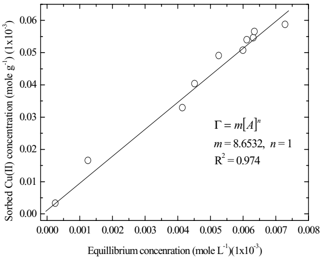

Figure 2-26d. The sorption isotherm of Cu(II) onto Magnetite at 25 °C and pH 7.2. Symbol and line represent experimental data and fitted results, respectively

2.4 ENVIRONMENTAL SIGNIFICANCE

In this study, Fe(II) associated with iron oxides were used to dechlorinate carbon tetrachloride(CCl_4). The reactivity of surface-bound Fe(II) species on the dechlorination was Fe(III) oxide-dependent. Comparison of the CCl_4 dechlorination by Fe(II) associated with iron oxides using normalized surface area rate constant (k_{sa}) shows that crystalline iron oxides are more reactive than amorphous ferrihydrite. Goethite has the highest reductive capability in dechlorinating chlorinated methanes, while ferrihydrite shows the lowest dechlorination efficiency. The chlorinated product generated during reductive transformation of CCl_4 shows that the formation ratio of $CHCl_3$ varies form 14% to 57%, which is dependent on the iron oxide minerals. This depicts that the natural attenuation of chlorinated contaminants by abiotic transformation may be varied in the subsurface environments, which contain various iron oxides. In addition, the availability of Cu(II) ions can significantly enhance the dechlorination processes, which is important to facilitate the engineered systems for the coupled treatment of inorganic and organochloride. In the presence of copper ions, not only the CCl_4 but also the major chlorinated product, chloroform ($CHCl_3$), could be dechlorinated into non-chlorinated or less chlorinated products. Chloroform has been known to be resistant in surface-bound Fe(II) systems according to previous studies.[14,17,18] This result shows the positive impact on natural attenuation when using surface-bound iron species as the natural reductant. The decrease in the reactivity observed during the re-spike of CCl_4 in this study is mainly due to the deactivation of most reactive surface-bound Fe(II) species during the first part of the dechlorination process[17]. However, the reactive Fe(II) species are regenerated continuously by dissimilatory iron reducing bacteria[18,53]. However, the effects of major environmental parameters on the dechlorination by surface-Fe(II) species in the presence of Cu(II) is required to be evaluated.

2.5 SUMMARY

The Fe(II) ion associated with iron oxides dechlorinated carbon tetrachloride(CCl_4) following pseudo-first order kinetics whereas dissolved Fe(II) did not dechlorinate CCl_4 under near neutral pH conditions. The rate constants (k_{obs}) for CCl_4 degradation were 0.380 h^{-1} and 0.836 h^{-1} in goethite and hematite suspensions respectively, in the presence of 3mM Fe(II). The k_{obs} value in the magnetite system was 0.0609 h^{-1} and it was 0.0144 h^{-1} in amorphous ferrihydrite system. Comparing the reactivity of Fe(II)-iron oxide suspensions using normalized surface area rate constant (k_{sa}), goethite-Fe(II) suspension was the most efficient in CCl_4 dechlorination which shows k_{sa} of 0.0296 $h^{-1}m^{-2}$. Amorphous ferrihydrite system showed the lowest reactivity and the k_{sa} of 0.005 $h^{-1}m^{-2}$. The ratio of reductive dechlorination of CCl_4 to $CHCl_3$ with the total loss of CCl_4 also varied in each iron oxide-Fe(II) systems. This results clearly shows that the reactivity of surface-bound Fe(II) associate with different iron oxides depends on the types of iron oxide. There was a similar trend in the increase in both the k_{obs} for dechlorination and bound Fe(II) concentration due to the increase in the pH of iron oxide-Fe(II) suspension indicating that high pH conditions are much favorable for the dechlorination of CCl_4 by surface-bound Fe(II) systems. The initial Fe(II) concentration in the goethite suspension had a Langmuir-Hinshelwood relationship with k_{obs} depicting that surface-bound Fe(II) is mainly responsible for the dechlorination reaction. Langmuir-type isotherm was also observed for the sorption of Fe(II) on the goethite. Whereas sorption of Fe(II) on ferrihydrite and magnetite obeyed Freundlich isotherms. The reactivity of surface- bound Fe(II) also depended on the surface area concentration of solid Fe(III) oxides. A linear relationship between the goethite surface area concentration and k_{obs} for the dechlorination of CCl_4 was observed in the presence of 3mM Fe(II) using various concentrations of goethite (5 ~ 25 mM). Addition of 0.5 mM Cu(II) in to 10 mM iron oxide increase the k_{obs}

for 119, 100, 30 and 3 times in the magnetite, goethite, hematite and ferrihydrite suspensions, respectively.

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Chapter 3

REDUCTIVE DECHLORINATION OF CARBON TETRACHLORIDE BY SURFACE-BOUND FERROUS IONS ASSOCIATED WITH GOETHITE

The dechlorination of carbon tetrachloride (CCl_4) by Fe(II) associated with goethite in the presence of transition metal ions including Cu(II) was investigated. X-ray photoelectron spectroscopy (XPS) and X-ray powder diffraction (XRPD) were used to characterize the chemical states and crystal phases of transition metals on solid phases, respectively. CCl_4 was dechlorinated to chloroform ($CHCl_3$) by 3 mM Fe(II) in 10 mM goethite (25.6 m^2 L^{-1}) suspension. The dechlorination followed pseudo-first order kinetics and a rate constant (k_{obs}) of 0.036 h^{-1} was observed at pH 7.0. Transition metals have different effects on CCl_4 dechlorination. Addition of Ni(II), Co(II), and Zn(II) lowered the k_{obs} of dechlorination of CCl_4, whereas the amendment of 0.5 mM Cu(II) into Fe(II)-Fe(III) system significantly enhanced the efficiency and the rate of CCl_4 dechlorination. The k_{obs} for CCl_4 dechlorination with 0.5 mM Cu(II) was 1.175 h^{-1} which was 33 times greater than that without Cu(II). Also the dechlorination of CCl_4 by surface-bound iron species is pH dependent and the rate constant increased from 0.008 h^{-1} at pH 4.0 to 1.175 h^{-1} at pH 7.0. When the solution contained Cu(II) and Fe(II) without goethite, a reddish-yellow precipitate was formed and the concentration of Fe(II) decreased with the increase in Cu(II) concentration. XPS and XRPD analyses suggested the possible presence of Cu_2O and ferrihydrite in the precipitate. A small amount of aqueous Cu(I) was also detected, reflecting the fact that Cu(II) was reduced to Cu(I) by Fe(II). A linear relationship between k_{obs} for CCl_4 dechlorination and the concentration of Cu(II) was observed when the amended Cu(II) concentration was lower than 0.5 mM. Moreover, the k_{obs} for CCl_4 dechlorination was dependent on the Fe (II) concentration in the 0.5 mM Cu(II)-amended goethite system and k_{obs} had a Langmuir-Hinshelwood relationship with Fe(II) concentration. These results clearly indicate that Fe(II) serves as the bulk reductant to reduce both CCl_4 a Cu(II). The resulting Cu(I) can further act as a catalyst to enhance the dechlorination rate of chlorinated hydrocarbons in the iron-reducing environments

3.1 INTRODUCTION

Chlorinated hydrocarbons are one of the major contaminants in soils and groundwaters.[1, 2] The dechlorination of chlorinated hydrocarbons by iron species has recently received much attention. In many of natural environments, the oxygen supply is limited, but iron oxides are present as an alternative electron acceptor. The use of iron species to reduce chlorinated hydrocarbons is of great advantage because iron oxides bind with aqueous and solid-phase Fe(II) species are natural reductants and are abundant in many hydromorphic soils and subsurface environments.[3]

Laboratory and field studies have demonstrated the importance of iron species in the reductive transformation of organic and inorganic compounds.[4-14] Minerals containing structural Fe(II) such as green rust and magnetite were found to reduce several classes of priority pollutants including halogenated hydrocarbons,[5-10] nitroaromatic compounds,[11, 12] and inorganic ions.[13,14] Moreover, the systems containing dissolved Fe(II) and various iron oxides have also shown high reactivity with respect to chlorinated hydrocarbons under anoxic conditions.[4, 15-17] It is generally believed that ferrous iron associated with iron oxides is much more reactive than the dissolved Fe(II).[4,11,12,15] The high reactivity of heterogeneous Fe(II)/Fe(III) systems can be maintained over a long period of time because of the continuous generation of surface-bound Fe(II) species by the uptake of Fe(II) from the aqueous phase.[4] Several factors including pH value, surface density of Fe(II), available surface area of iron minerals, and contact time of Fe(II) with minerals have been shown to influence the reactivity of Fe(II) species bound with iron oxides.[4,16,17] Amonette et al.[17] dechlorinated CCl_4 in Fe(II)-amended goethite system and found that the density of sorbed Fe(II) over goethite surface was the major factor on which the rate of dechlorination was dependent. More recently, Hofstetter et al.[18] showed that the various types of Fe(II) species on clays minerals influenced the reducing efficiency and the rate of nitroaromatic compounds. Both structural Fe(II) and Fe(II) complexed by surface hydroxyl groups of clay minerals reduced nitroaromatics to aniline effectively, while Fe(II) bound by ion exchange did not contribute to the reductive transformation.

Iron minerals are also potentially strong sorbents and are often used to remove a wide variety of metal ions from aqueous solutions and soils.[19,20] The sorption of metal ions including Fe(II), Zn(II), Cu(II), Ni(II) and Co(II), from aqueous solutions to goethite surface have been observed.[21-24] Metal ions are sorbed to the active adsorption sites on the surface of iron oxides at the first stage and then complex with surface hydroxyl groups to form reactive sites leading to the occurrence of redox reactions on the solid phase.[25] Moreover, the heterogeneous redox reactions involving electron transfer between Fe(II) and Fe-containing minerals have been demonstrated.[14,26] White and Peterson[14] systematically investigated the reduction of the first row of transition metal species on the surface of magnetite and illmenite and found that the surfaces of structural Fe(II) minerals could reduce Fe(III), Cu(II), Cr(VI), and V(V) in solution. This implies that the redox reactions controlled by solid state Fe(II), either structural or surface associated, might be useful reducing steps for transition metal ions in aqueous solution.

Transition metals are often found with chlorinated organic compounds in the contaminated groundwaters.[28] Although the reactivity of various surface-bound iron species are commonly considered in the dechlorination of chlorinated hydrocarbons, much less emphasize has been placed on the synergistic effect of transition metal ions with Fe(III)-containing minerals on the dechlorination. Certain transition metals such as nickel and copper, also can act as mediators to enhance the dechlorination efficiency of chlorinated hydrocarbons.[28-30] Therefore, the objective of the preset study was to investigate the synergistic effect of transition metal ions with surface-bound Fe(II) species on the dechlorination of CCl_4. Goethite was selected as the model iron oxide because it is one of the abundant crystalline iron oxyhydroxide minerals in the natural environment and is widely used to remove trace organics and metal ions.[22,31] Moreover, in a comparison with other iron oxides including hematite, magnetite and amorphous ferrihydrite, goethite showed higher reactivity with Fe(II) ions in the dechlorination of CCl_4 (chaper-1). Transition metal ions including Co(II), Ni(II), Cu(II) and Zn(II) were selected due to their availability and abundance in both natural and contaminated environments. The chemical states of transition metals and crystal phase of precipitates on iron oxides were characterized using x-ray photoelectron spectroscopy (XPS) and X-ray powder diffraction (XRPD) techniques. The, effects of pH and concentrations of Fe(II) and Cu(II) on the dechlorination efficiency were also examined to understand the role of transition metal ions in the dechlorination reaction under iron-reducing condition.

3.2 MATERIALS AND METHODS

3.2.1 Chemicals. All chemicals were used as received without further treatment. Carbon tetrachloride (CCl_4) (> 99.8%, GC grade), chloroform ($CHCl_3$) (> 99.8%, GC grade), $CuCl_2{\cdot}2H_2O$ (99%), $NiCl_2{\cdot}6H_2O$ (98%), $CoCl_2.2H_2O$ (99%) and $ZnCl_2$ (98%), were purchased from Merck Co. (Darmstadt, Germany). $FeCl_2{\cdot}4H_2O$ (99%), $FeCl_3{\cdot}6H_2O$ (99%), N-(2-hydroxyethyl) piperazine-N'-(2-ethanosulfonic acid (HEPES) (99.5%), and 2-(N-Morpholino) ethanesulfonic acid (MES buffer) (> 99.5%) were purchased form Sigma-Aldrich Co. (Milwaukee, WI). Methylene chloride (DCM) (> 99.8%, GC grade) and ethanol (HPLC grade) were obtained from J. T. Baker Co. (Phillipsburg, NJ). Bathocuproinedisulfonic acid disodium salt ($C_{26}H_{18}N_2Na_2O_6S_2$) (90%) was purchased from Fluka (Buchs, Switzerland). Goethite was synthesized according to the method of Schwertmann and Cornell[32] and was characterized by XRPD. The XRPD pattern showed major peaks at 21.25°, 33.3°, 36.65° and 53.3° 2θ which proved that the synthesized iron oxide was goethite. The surface area of goethite was determined by a BET N_2 adsorption surface area analyzer (Micromeritics. ASAP 2000). The powder sample of goethite was degassed with N_2 (99.9995%) and vacuumed (5×10^{-3} mmHg) repeatedly in a sealed vacuumed desiccator. The surface area was then determined by single-point measurement, and a value of $28.80 \pm 0.11 m^2 g^{-1}$ was obtained.

3.2.2 Dechlorination Experiments

Batch experiments were conducted using 70-mL serum bottles filled with 50 mL of deoxygenated buffer solution under anoxic conditions.[33,34] High purity of N_2 (> 99.9995%) at a flow rate of 42 L min^{-1} was used to maintain the anoxic conditions during the experimental processes. Goethite was withdrawn using a N_2-purged syringe and was delivered into serum bottles to get the final concentration of 10 mM (25.6 $m^2 L^{-1}$). Fe(II) solutions were prepared in deoxygenated buffer solutions in sealed bottles and were introduced into the serum bottles to get a concentration of 3 mM. HEPES (50 mM) buffer solutions were used to control pH at 7.0 ± 0.1. The stock solutions of divalent ions of transition metals (Cu, Zn, Co, Ni) were prepared using deoxygenated water in sealed bottles. Appropriate amounts of stock solutions were introduced into the serum bottles to obtain a final concentration of 0.5 mM using N_2-purged plastic syringes. Bottles were then sealed with Teflon-lined rubber septa and aluminum crimp caps and were incubated in an orbital shaker at 150 rpm and at 25 ± 1 °C in the dark. After 20 h of equilibrium, an aliquot of the CCl_4 stock solution dissolved in degassed methanol was delivered into the serum bottle by a gas-tight glass syringe to obtain the final concentration of 20 μM. The total volume of the liquid phase in the serum bottle was maintained at 50 mL, resulting in a 20 mL headspace left for headspace analysis. Parallel experiments were also carried out without the addition of Fe(II). All the experiments were run in duplicate or triplicate.

The combined effect of Fe(II) and Cu(II) on the dechlorination of CCl_4 without the addition of goethite was also performed according to the above procedures to understand the role of Cu(II) in the dechlorination of CCl_4. The concentrations of Cu(II) and Fe(II) in the aqueous solutions were in the range of 0.1 - 4 mM and 0.2 - 3 mM, respectively. After the addition of Cu(II) into Fe(II) containing solution, a precipitate was observed and it was harvested for species identification. The suspension was transferred into a 50-mL centrifuge tube using N_2-purged syringe, and was centrifuged at 8,000 × g for 10 min. After the supernatants were removed, the residue was dried with nitrogen gas and transferred to a 4-ml glass vial anaerobically. The glass vial was stored under nitrogen atmosphere in a tightly sealed vacuum desiccator until analysis was performed.

3.2.3 Analytical Methods

The headspace analytical technique was used to determine the chlorinated hydrocarbons. The concentrations of CCl_4 and the byproducts in the gas phase of the test bottles were monitored withdrawing 50 μL of headspace using a 100 μL gas-tight glass syringe. The mixture was then immediately injected into a gas chromatograph (GC) equipped with a flame ionization detector (FID) and an electron capture detector (ECD) (Perkin-Elmer, Autosystem, Norwalk, CCl_4). A 60-m VOCOL fused-silica megabore capillary column (0.545 mm × 3.0 μm, Supelco Co.) was used for separating the chlorinated compounds. The column was connected to FID and ECD simultaneously by a Y-splitter with 40% of the flow (1.85 mL min^{-1}) to ECD for better identifying and quantifying the chlorinated hydrocarbons. The column temperature was maintained at 50 ºC isothermally with nitrogen (N_2) as the carrier gas. The relative standard deviation (RSD) for GC analysis was controlled within 10%. The serum bottles were opened after the headspace analysis and pH was measured using a microprocessor pH meter.

The non-chlorinated compounds (methane) resulting from CCl_4 reduction were monitored by using a Varian 3800 GC equipped with a mass spectrometry (MS) (Saturn 2000) and an FID. Two capillary columns, a PLOT (Chrompack, 50 m × 0.32 mm × 5.0 μm) and a DB-1 (J&W, 60 m × 0.32 mm × 1.0 μm), were connected in parallel by a Y-splitter with approximately 1/3 of the flow going to the PLOT column. The PLOT column was coupled to an FID and the DB-1 column was connected to the MS for separation and detection of hydrocarbons.XRPD and XPS were used to identify the crystal phase and chemical states of transition metals on the solid residues. XRPD was performed using X-ray diffractometer (Regaku D/max-II B) and Cu Kα-radiation source with 30 kV voltage and 20 mA current. Samples were mounted on sample holder using small amount of grease. A drop of glycerol was immediately added on the mounted powder layer to minimize the reaction with oxygen. The scan range for all samples was from 10° to 90° (2θ), at a scanning speed of 4 °/min. The XPS measurements were performed by an ESCA PHI 1600 photoelectron spectrometer (Physical Electronics, Eden Prairie, MN) using Mg Kα X-ray source (1253.6 eV photon energy). The spherical capacitor analyzer with a multi-channel detector has a takeoff angle of 70° related to the horizontal of the sample plane. The binding energies of the photoelectrons were determined by assuming that the carbon 1s electron has a binding energy of 284.8 eV. The data were recorded digitally, and all peak scans were signal-averaged until an acceptable signal-to-noise ratio was obtained. During the data acquisition, the pressure in the sample chamber did not exceed 2.5×10^{-9} Torr. The binding energy of Fe and Cu were calibrated with reference to the binding energy of adventitious carbon 1s electron (284.8). Although, some probable errors may occur during the identification of chemical species, this technique is generally accepted for insulating samples.[36]

Concentrations of total 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. After mixing vigorously, the acidified samples were centrifuged at 8,000 g for 10 min to remove particles and the Fe(II) contents were determined with ferrozine at 562 nm.[34] The dissolved fraction of Fe(II) was determined in the filtrates (0.2-μm cellulose acetate filter) acidified with 0.5 ml of 1 M HCl.

Concentration of Cu(I) was determined by using bathocuproinedisulfonic acid method with minor modifications.[37] The aliquot was withdrawn by 1 mL N_2-purged plastic syringe and filtered through 0.2 μm membrane filter. A 0.2 mL of supernatants was then added into a 1.5 mL vial containing 0.5 mL of 1% bathocuproinedisulfonic acid solution and 1mL of 10% tartrate solution. The total volume was adjusted to 1 ml and centrifuged at 8,000 g for 5 min. The aqueous Cu(I) concentration was then determined at 483 nm. Standard solutions of Cu(I) were prepared by using 10% hydroxylammonium chloride as a reductant to reduce $CuCl_2$ solutions. Dissolved and total concentrations of copper species were determined using inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin Elmer, Optima 3000XL). The concentrations of copper ions attached to the solid-phase were calculated from the difference between total and dissolved concentrations.

3.3 RESULTS AND DISCUSION

3.3.1 Effect of transition metal ions on CCl_4 degradation.

Figure 3-1 illustrates the dechlorination of CCl_4 and the production of chloroform ($CHCl_3$) by surface-bound iron species in the presence of 0.5 mM metal ions. Ferrous iron species attached to solid surfaces have been reported to be more reactive in dechlorination reactions than that in dissolved form.[4,11,12,15-17] The results of the present study showed the similar trend. Although Fe(II) ion is thermodynamically capable of dechlorinating CCl_4 [38], no obvious degradation of CCl_4 by 3 mM of Fe(II) was observed within 10 days at neutral pH. In the presence of goethite (25.6 $m^2 L^{-1}$) and 3 mM Fe(II), however, 92% of CCl_4 degradation with the concomitant increase in $CHCl_3$ concentration was observed. Chloroform was identified as the major product. Trace amounts of dichloromethane (DCM) and methane were also identified after the experimental course of 30 days.

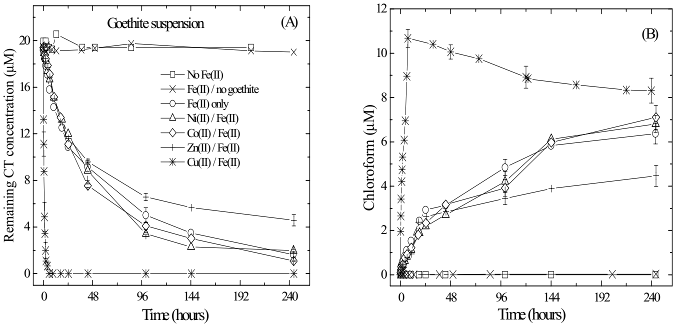


Figure 3-1. Dechlorination of 20 µM CCl_4 in the anoxic suspension of 10 mM goethite (25.6 $m^2 L^{-1}$) and 3 mM Fe(II) in the presence of transition metal ions. 50 mM HEPES buffer was used to control pH at 7.0 ± 0.1. (A) concentration profile of CCl_4 and (B) formation of chloroform. The error bars indicate standard deviation (n = 3)

Different effects of transition metals on the dechlorination of CCl_4 by Fe(II)-bound goethite were observed. An obvious difference between CCl_4 reduction by Fe(II) in the presence and absence of metal ions was observed after 48 h when the *F-test* was applied ($p < 0.05$). As depicted in Figure 3-1, addition of 0.5 mM Zn(II) into the heterogeneous Fe(II)/Fe(III) system decreased the dechlorination efficiency of CCl_4 and only 76% of CCl_4 was removed within 10 days. The maximum concentration of $CHCl_3$ was 4.5 μM. The addition of Ni(II) and Co(II) decreased the dechlorination of CCl_4 at first stage, but the dechlorination efficiency and the production of $CHCl_3$ were slightly enhanced after 96 h. Of interest, CCl_4 was rapidly dechlorinated to $CHCl_3$ in the presence of Cu(II).

Nearly complete removal of CCl_4 with a rapid accumulation of $CHCl_3$ was observed within 6 h. The maximum concentration of $CHCl_3$ was up to 10.8 μM and then slightly decreased to 8.5 μM followed by the increase of DCM and methane in trace amounts. The concentration of DCM increased very slowly and a maximum concentration of 1.35 μM was obtained during the incubation of 10 days. The low carbon recovery may be due to the formation of non-chlorinated compounds that cannot be detected by GC-ECD. When GC-MS was employed to study the intermediate products using high concentration of CCl_4 (1mM), the identified compounds were methane, acetylene, ethane, ethylene, dichloromethane and tetrachloro-ethene. A recent study[39] also showed that carbon monoxide, methane, ethane, ethylene were the final products in CCl_4 dechlorination by green rust amended with Cu(II). This implies that processes other than reductive dechlorination are also involved in CCl_4 dechlorination in the Cu(II)-amended Fe(II)/Fe(III) systems. Moreover, no obvious degradation of CCl_4 was observed when the goethite suspension contained only Cu(II), showing that Cu(II) associated goethite without Fe(II) ions has little effect on the dechlorination of CCl_4.

Table 3-1. The rate constants (k_{obs}, h^{-1}) for CT dechlorination in the anoxic suspension of goethite (25.6 $m^2 L^{-1}$), 3 mM Fe(II) and 0.5 mM transition metal ions. 50 mM HEPES buffer was used to control pH at 7.0 ± 0.1.

Experimental system	k_{obs} (h^{-1})	Number of replicates
Goethite + Fe(II)	0.036 ± 0.005	4
Goethite + Fe(II) + Ni(II)	0.026 ± 0.004	4
Goethite + Fe(II) + Co(II)	0.020 ± 0.007	4
Goethite + Fe(II) + Zn(II)	0.023 ± 0.004	4
Goethite + Fe(II) + Cu(II)	1.175 ± 0.189	5
only Fe(II) + Cu(II)	0.252 ± 0.011	3

The dechlorination of CCl_4 by surface-bound Fe(II) oxidation can be explained by a pseudo-first-order reaction kinetics.[4, 15-17]

$$\ln\frac{[C_t]}{[C_0]} = -k_{obs}t \tag{3-1}$$

where C_0 and C_t are the concentrations of at initial and at time t, respectively, and k_{obs} is the first-order rate constant for CCl_4 dechlorination. A good linear relationship between $\ln(C_t/C_0)$ and time was observed within the first 10 h. Table 3-1 shows the k_{obs} for CCl_4 dechlorination by surface-bound iron species in the presence of different metal ions. The k_{obs} for CCl_4 dechlorination in the absence of transition metal ions was 0.036 h^{-1} and decreased to 0.020 ~ 0.026 h^{-1} upon amending Ni(II), Co(II) and Zn(II). On the contrary, a 33-fold increase in k_{obs} relative to the un-amended system was obtained in the 0.5 mM Cu(II)-amended suspension, which shows that the addition of Cu(II) can significantly enhance the dechlorination rate of CCl_4 .

The effect of metal ions complexed with macromolecules acting as reactive electron transfer mediators in the reduction of contaminants in the presence of various bulk reductants have been recently studied in both heterogeneous and homogeneous systems.[31,40-42] A common result shows that the degradation rate and efficiency of the chlorinated compounds increase with the addition of certain transition metal ions into systems containing bulk reductants and macromolecules. Recent studies observed that the dechlorination rate of hexachloroethane in heterogeneous systems of sulfur-containing iron mineral (mackinawite) increased in the presence of Cu (II).[43, 44] White and Peterson[14] reported that the structural Fe(II) in Fe(II)-bearing minerals could be oxidized by soluble Cu(II) species. However, the role of Cu(II) in the dechlorination of chlorinated hydrocarbon under iron-reducing conditions is not well-understood. In this study, the efficiency and the rate of CCl_4 dechlorination by surface mediated goethite system increased greatly when Cu(II) was present. To further elucidate the possible mechanisms of Cu(II) on CCl_4 dechlorination, the effect of pH and surface characterization of solid phase were studied.

3.3.2 Effect of pH on CCl_4 dechlorination

The pH value plays an important role in the dechlorination of chlorinated compounds in heterogeneous Fe(II)/Fe(III) systems. Figure 3-2 illustrates the effect of pH on the dechlorination of CCl_4 in the presence of surface-bound iron species with 0.5 mM Cu(II). The pH values were in the range of 4.0 ~8.5. MES buffer was selected to maintain the suspension at pH 5.5 ~6.5, and HEPES buffer was used at pH 7.0 ~ 8.0 and TRIS buffer was used to control pH at 8.5. In the un-buffered system, the initial pH was 4.0 and the rate constant (k_{obs}) for CCl_4 degradation was 0.008 h^{-1}. The increase in pH value enhanced both the efficiency and the rate of CCl_4 dechlorination. The k_{obs} for CCl_4 dechlorination increased from 0.031 to 1.175 h^{-1} when the pH increased from 5.5 to 7.0, which is 4~146 times higher than that in the un-buffered system. Further increase in pH exponentially increased the k_{obs} value. When the pH value was 8.5, k_{obs} was 15.143 h^{-1}. This relationship is similar to that in the goethite- Fe(II) system in the absence of Cu(II) which was discussed in chapter 2. Also previous reports of dehalogenation of halogenated methanes by surface bound Fe(II) systems without the amendment of Cu(II) have shown similar relationship between pH and k_{obs} for the reductive transformation of halogenated methanes.[16,17] The increase in the dechlorination rate of chlorinated compounds at high pH values may be attributed to the increase in the sorbed Fe(II) density on the surface of iron oxyhydroxide particles. The formation of precipitates with different types of surface active sites at the solid surface is another possibility. When Cu(II) was added into the heterogeneous Fe(II)/goethite buffered suspensions at pH 7, the color changed from bright yellow to yellowish blue-green. However, the color remained in its original bright yellow at low pH in the un-buffered suspensions (pH 4). Similar results were observed when 0.5 mM Cu(II) was added into 3 mM Fe(II) solution without goethite. Nearly complete removal of CCl_4 was observed within 10 h and the k_{obs} for CT dechlorination was 0.252 h^{-1} in the solution at neutral pH. However, a small quantity of precipitation with little CCl_4 dechlorination was observed in the homogeneous solution at pH 4. When the precipitate of 0.5 mM Cu(II) and 3 mM Fe(II) ions were filtered through 0.2 μm membrane under N_2 atmosphere and the filtrate was re-injected into another sealed serum bottle, no CCl_4 was dechlorinated within 10 h. This implies that the reactivity of Cu(II) amended Fe(II)/Fe(III) system might be mainly from the surface-bound species on solids.

To further understand the characteristics of the precipitate generated in the absence of goethite in the buffered system at pH 7, XRD and XPS were used to identify the crystal phase and chemical structures of the precipitate. As shown in Figure 3-3, the XRD pattern showed clear peaks at 36.42°, 42.22°, 61.30° 2θ and XPS spectra also showed a peak at 932.0 eV (Cu $2p_{3/2}$) . These results clearly show that cuprous oxide (Cu_2O) was formed in the precipitate. The Fe $2P_{3/2}$ spectrum showed a peak at 710.8 eV, indicating that Fe(III)OFe(III) is substantial. However, no XRD peak of iron oxide was observed, which suggests that ferrihydrite ($Fe(OH)_3$) a poorly crystalline ferric oxide could be formed. This means that the aqueous $CuCl_2$ reacts with $FeCl_2$ to form Cu_2O and ferrihydrite at neutral pH according to the following relationship:

$$2\,Fe^{2+} + 2\,Cu^{2+} + 7\,H_2O \rightarrow Cu_2O + 2\,Fe(OH)_3 + 8\,H^+ \qquad (3\text{-}2)$$

According to equation (3-2), 1 mol of Cu(II) can react with 1 mol of Fe(II) to form catalytic Cu(I) ion. Also, 4 mol of proton are released into the solution. At high concentration of Cu(II), the release of large amount of proton lowers the pH value of the solution, subsequently decreases the extent and the rate of CCl_4 dechlorination.

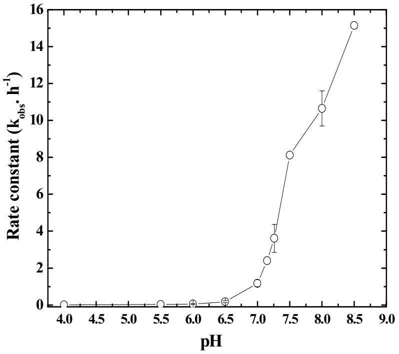


Figure 3-2 The rate constant (k_{obs}) for dechlorination of CCl_4 as a function of pH value in 0.5 mM Cu(II) and 3 mM Fe(II) added 10 mM goethite suspension. The error bars indicate the standard deviation (n=3). The error bar shown at pH 6 is not observable being smaller than the size of the legend.

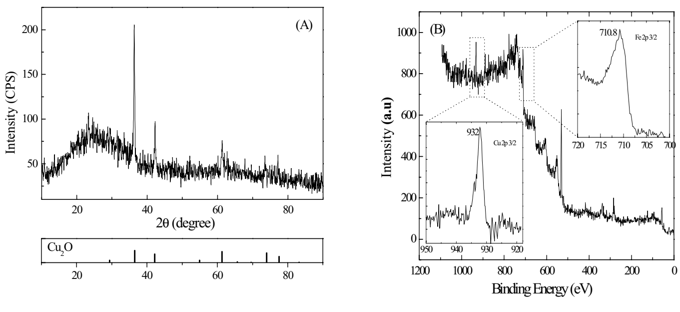


Figure 3-3. (A) XRD pattern and (B) XPS spectra of the precipitation in buffer solutions containing Fe(II) and Cu(II) at neutral pH.

3.3.3 Effect of Cu (II) concentration on CCl_4 dechlorination

In order to further understand the effect of Cu(II) concentration on the efficiency and the rate of CCl_4 dechlorination and the change in the pH of solution, various concentrations of Cu(II) ranging from 0.1 to 4 mM were added into solutions that contained 3 mM of Fe(II) and 10 mM goethite at pH 7. Figure 3-4 illustrates the dechlorination efficiency and the rate constants of CCl_4 by surface-bound Fe(II) species in the presence of various concentrations of Cu(II). At the low concentrations of Cu(II) ranging between 0.1 ~0.5 mM greatly enhanced the efficiency and the rate of CCl_4 dechlorination. The k_{obs} increased rapidly from 0.363 h^{-1} at 0.1 mM Cu(II) to 1.294 h^{-1} at 0.4 mM, and then slightly decreased to 1.143 h^{-1} when Cu(II) was at 1 mM. This shows that the addition of relatively low concentrations of Cu(II) enhances the dechlorination of CCl_4 effectively in Fe(II)-amended goethite suspensions. Further increasing the Cu(II) concentration, however, lowered the dechlorination efficiency and only 20% of CCl_4 was dechlorinated when Cu(II) concentrations were up to 3 mM. Little degradation of CCl_4 was observed as Cu(II) concentration increased to 4 mM. This decrease in dechlorination efficiency at high Cu(II) concentrations may be attributed to the change in the pH value of the solution. As shown in Figure 3-4 b, the pH value was maintained at 7.0 ± 0.1 when the Cu(II) concentrations were in the range of 0.1 ~ 0.5 mM.

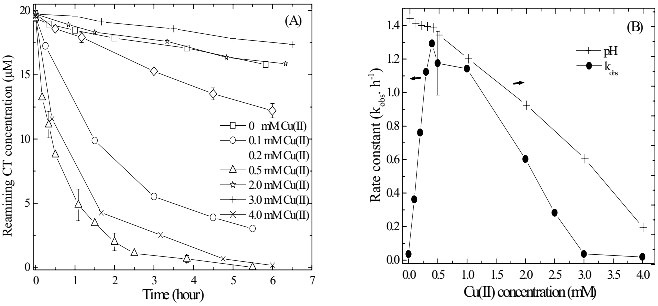


Figure 3-4. Effect of the Cu(II) concentration in 3 mM Fe(II) amended goethite (25.6 m^2 L^{-1}) suspension in 50 mM HEPES buffer solution. (A) concentration profile of remaining CCl_4, (B) first-order rate constant (k_{obs}) for CCl_4 dechlorination and pH of the system.

However, the pH value of the solution decreased from 7.0 to 5.3 when the initial Cu(II) concentration was 0.5 mM. The decrease in pH may be due to the hydrolysis of $CuCl_2$ in water or the release of protons during the reduction of Cu(II) by Fe(II) (eq. 2). Only 0.2 unit of pH decreased when 4 mM Cu(II) was added into the goethite suspension in the absence of Fe(II) which demonstrates that the release of protons by hydrolysis of $CuCl_2$ is not the main reason for the decrease in pH.

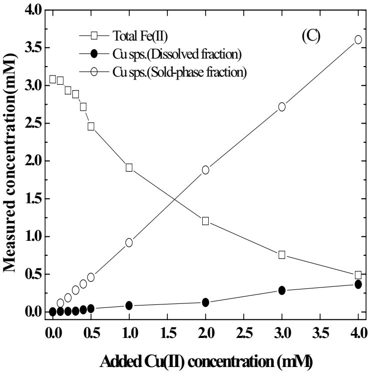


Figure 3-4. Effect of the Cu(II) concentration in 3 mM Fe(II) amended goethite (25.6 $m^2 L^{-1}$) suspension in 50 mM HEPES buffer solution. (C) total Fe(II) and Cu concentrations in aqueous and solid-phases

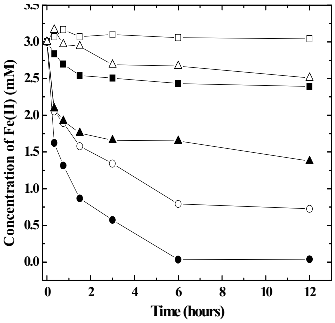


Figure 3-5. The Fe(II) concentration profile during the first 12 hours when various concentrations(0 mM, 0.5 mM and 3.0 mM) of Cu(II) were added into the 3 mM Fe(II) amended goethite (25.6 m^2L^{-1}) suspension. (□) Total (1M HCl extractable) Fe(II) in the absence of Cu(II), (■) Dissolved Fe(II) in the absence of Cu(II), (Δ) Total (1M HCl -extractable) Fe(II) in 0.5 mM Cu(II) added suspension, (▲) Dissolved Fe(II) in 0.5 mM Cu(II) added suspension, (○) Total (1M HCl extractable) Fe(II) in 3 mM Cu(II) added suspension, and (●) Dissolved Fe(II) in 3mM Cu(II) added suspension.

coupled with the reduction of Cu(II) to Cu(I) could be another possible reason for the decrease in the rate constant of dechlorination at high concentrations of Cu(II). The Fe(II) concentration at various Cu(II) concentrations in Fe(II)-amended goethite systems were measured after the termination of the dechlorination experiment. The total concentration of Fe(II) decreased when the Cu(II) concentration increased, which indicates that Fe(II) was oxidized by Cu(II) (Figure3- 4(c)). The oxidation reduction potential (ORP) in the Goethite-Fe(II) suspension increased from –334 mV in the absence of Cu(II) to –50 mV when the Cu(II) concentration was increased to 3mM. Also, the fraction of solid-phase Cu species increased with increasing Cu(II) concentration and was much higher than that of dissolved species, suggesting that Cu(II) was mainly sorbed onto the surface of goethite or was converted to Cu_2O. Moreover, the sorbed Cu(II) concentration was higher than the consumed Fe(II) concentrations when the added concentration of Cu(II) was higher than 2mM, presumably because of the precipitation of Cu(II) onto the surface of goethite.

To further study the oxidation of Fe(II) by Cu(II), total and dissolved Fe(II) concentrations in Fe(II)/Fe(III) suspensions amended with various concentrations of Cu(II) were measured. As depicted in Figure 3-5, the concentration of dissolved Fe(II) decreased from 3 mM to 1.5 mM within 12 h with the addition of 0.5 mM Cu(II), whereas only 0.03 mM of Fe(II) was detected in 3 mM Cu(II) amended suspension. This decrease in Fe(II) is due to both the sorption of Fe(II) onto the surfaces of goethite, and the oxidation of Fe(II) by Cu(II). In the absence of Cu(II), the dissolved Fe(II) concentration decreased from 3 mM to 2.4 mM within 12 h, while the total Fe(II) concentration was maintained a nearly constant value (3 mM). This suggests that about 20% of Fe(II) was sorbed onto the surface of goethite, which is in agreement with the results of previous reports of the dechlorination of polyhalogenated methanes by surface-bound Fe(II) in goethite suspensions without Cu(II).[16] In contrast, the total concentration of Fe(II) decreased from 3 mM to 2.5 mM and to 0.7 mM within 12 h in the presence of 0.5 and 3 mM Cu(II), respectively. This results show that a large fraction of Fe(II) was oxidized by Cu(II). The differences between the total and dissolved Fe(II) concentrations in suspensions with 0.5 mM and 3mM Cu(II) added were 1.0 mM and 0.7 mM respectively which are higher than that of sorbed Fe(II) in blank controls (without Cu(II)). This indicates that the sorbed amount of Fe(II) onto goethite increased slightly in the presence of Cu(II). The increase in the surface density of F(II) may also enhance the dechlorination efficiency and the rate of CCl_4.

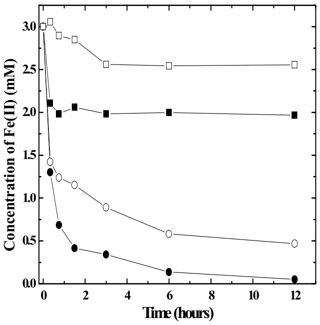


Figure 3-6. The Fe(II) concentration profile during first 12 hours when various concentrations (0.5 mM and 3.0 mM) of Cu(II) were added into the 3 mM Fe(II) in 50mM HEPES (pH 7) buffer solution. (□) Total (1M HCl extractable) Fe(II) in 0.5 mM Cu(II) added solution, (■) Dissolved Fe(II) in 0.5 mM Cu(II) added solution,(○) Total Fe(II) in 3mM Cu(II) added solution, and (●) Dissolved Fe(II) in 3 mM Cu(II) added solution

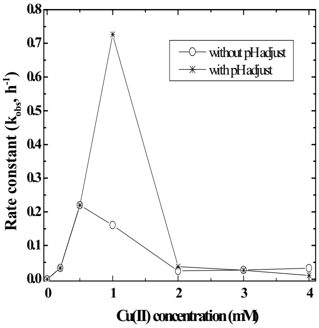


Figure 3-7. The change in the rate constant (k_{obs}) for the CCl_4 dechlorination in the solution of 3mM Fe(II) with various concentrations of Cu(II) at pH 7. At high concentrations of Cu(II) the pH was decreased and in one experimental set pH was re-adjusted to its initial value.

The oxidation capability of Fe(II) by Cu(II) was further evaluated in the absence of goethite. Figure 3-6 illustrates the aqueous Fe(II) concentration at various concentrations of Cu(II) without goethite. A reddish-yellow precipitate was formed when Cu(II) was mixed with 3 mM Fe(II). Therefore, the total and dissolved Fe(II) concentrations were determined. Similar to the concentration profile of Fe(II) in the goethite-amended system, the dissolved Fe(II) concentration decreased rapidly during the first 2 h and then decreased slowly within 12 hours. This decrease was more rapid than that in the presence of goethite, suggesting that the oxidation of Fe(II) by Cu(II) in the absence of goethite is more rapid than that its presence. This may be due to the sorption of both Cu(II) and Fe(II) onto goethite surface. Cu(II) has a higher sorption affinity onto goethite.[45] The added Cu(II) would sorb over goethite surface leading to a decrease in the dissolved concentration and oxidation capability of Cu(II). To elucidate the relationship of Cu(II) and Fe(II) concentrations on CCl_4 dechlorination, another independent experiment was performed. Various concentration of Cu(II) ranging from 0.1 to 4.0 mM were added to homogeneous solution containing 3 mM Fe(II) without goethite. PH values of the solutions were re-adjusted to pH 7 by using anoxic solution of NaOH under N_2 atmosphere. After 20h of equilibrium, 20 μM of CCl_4 was injected to start the dechlorination reaction. Results showed that the k_{obs} for CCl_4 dechlorination increased linearly with increasing Cu(II) concentration. However, the k_{obs} decreased rapidly when the Cu(II) concentration was higher than 2mM (Figure 3-7).

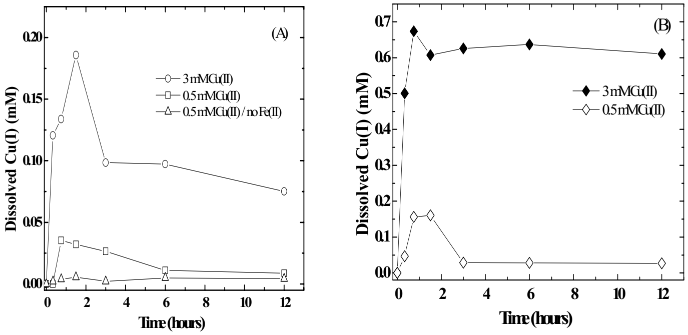


Figure 3-8. Concentration profile of dissolved Cu(I) at various concentrations of Cu (II) (**A**) in 3 mM Fe(II) amended goethite (25.6 $m^2 L^{-1}$) suspension and, (**B**) in 3 mM Fe(II) solution.

Also the measured redox potentials increased from –320 to +100 mV when the Cu(II) concentrations increased from 0 to 4 mM. These results depict that Cu(II) has great effect on the dechlorination of CCl_4 when solutions contain large excess of Fe(II) and the low reactivity at high Cu(II) concentration is due to the consumption of Fe(II) reductant and the decease in pH.

Figure 3-8 shows the dissolved aqueous Cu(I) concentration in the presence and in the absence of goethite. The Cu(I) concentration in the presence of goethite was lower than that without the addition of goethite, which is in a good agreement with the concentration profile of Fe(II). To further understand the possibility of freshly formed Cu(I) precipitate acting as an additional reductant, 3mM Cu_2O was amended into the Fe(II)-goethite suspensions at pH 7. A nearly complete dechlorination of CCl_4 within 10 h was observed, indicating that Cu_2O is having catalytic activity for the dechlorination of CCl_4 . The k_{obs} for CCl_4 dechlorination was five times higher than that without Cu(I), proving that Cu(I) is an effective species for the dechlorination of CCl_4.

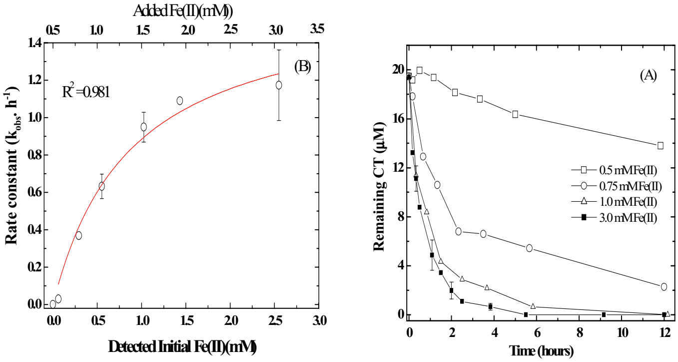
3.3.4 Effect of Fe(II) concentration on CCl_4 dechlorination

The dechlorination rates of CCl_4 in Cu(II)-amended Fe(II)-Fe(III) suspension was found to be influenced both by Cu(II) and Fe(II) concentrations. Therefore, various concentrations of Fe(II) were added into the goethite system containing 0.5 mM Cu(II) to understand the influence of Fe(II) concentration on CCl_4 dechlorination. Figure 3-9 shows the degradation efficiency and the rate constant of CCl_4 with the amendment of various concentrations of Fe(II). The dechlorination efficiency of CCl_4 increased with increasing Fe(II) concentration. Only 27% of CCl_4 was dechlorinated within 12 h in the 0.5 mM Fe(II)-amended system, whereas a nearly complete degradation of CCl_4 was observed when the Fe(II) concentration was higher than 1 mM. Also, the k_{obs} for CCl_4 dechlorination increased from 0.029 h^{-1} at 0.5 mM to 0.949h^{-1} at 1.5 mM and then leveled off to 1.175 h^{-1} at 3.0 mM, reflecting that the addition of 1.5 mM of Fe(II) is sufficient for CCl_4 dechlorination in Fe(II)-goethite suspension with 0.5 mM Cu(II).

Because the dechlorination of CCl_4 on surface bound Fe(II) system is a surface-mediated reaction, a Langmuir-Hinshelwood kinetics can be used to describe the relationship between the Fe(II) concentration and the reaction rate

$$\Gamma = k_{rxn} \frac{K_{Fe} C_{Fe}}{1 + K_{Fe} C_{Fe}} \tag{3-3}$$

where k_{obs} is the rate constant of dechlorination reaction, k_{rxn} is the apparent reaction rate constant, K_{Fe} is the equilibrium constant for adsorption of Fe (II) to goethite, and C_{Fe} is the initial aqueous concentration of Fe (II). In this study, the added Fe(II) reacted with Cu(II) and the initial concentration of Fe(II) decreased before the injection of CCl_4. Therefore, the measured Fe(II) concentrations were used as the initial aqueous concentration of Fe(II). A good fit between the Fe(II) concentration and the k_{obs} with k_{app} and K_{Fe} of 1.141 h^{-1} and 1.66mM^{-1}, respectively, was obtained (r^2 = 0.981, n = 6), which indicates that Fe(II) serves as the bulk reductant for CCl_4 dechlorination (Figure 3-8b). Moreover, the rate constant (k_{obs}) for CCl_4 dechlorination was linearly correlated with the concentration of surface-bound Fe(II), showing that the sorption density of Fe(II) at the surface of iron oxide is a critical factor when evaluating the transformation rate of CCl_4 by surface-bound Fe(II) species (Figure 3-9c).



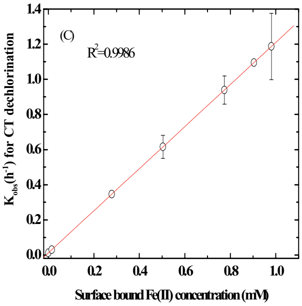


Figure 3-9. (A)The effect of total Fe(II) for the dechlorination of CCl_4 in 0.5 mM Cu(II) added goethite system at neutral pH. The concentration profile of CCl_4. (B)The effect of total Fe(II) for the dechlorination of CT in 0.5 mM Cu(II) added goethite system at neutral pH. (C) The relationship between the pseudo first-order rate constant (k_{obs}) and surface-bound Fe(II) concentration with the pseudo first-order rate constant (k_{obs}) for CCl_4 dechlorination.

3.4 ENVIRONMENTAL SIGNIFICANCE

Although the reductive dechlorination of chlorinated methanes by the surface mediated Fe(II) species under anoxic conditions has been investigated for several years, the influence of transition elements on the dechlorination reaction under natural iron reducing conditions is not well-understood. Copper species have been used as catalysts for dechlorination reactions in experiments of laboratory scale as well as the pilot plant scale.[46] Previous studies[39,47, 48] have shown that the addition of Cu^{2+} greatly enhanced the dechlorination rate of CCl_4 and $CHCl_3$ by green rust. Transformation of dichloroacetylene to chloroacetylene and then to acetylene in solutions containing cuprous chlorides (CuCl) was also observed at temperature of 67 – 87 ^{o}C.[30] Recently, Chien et al[49] observed that CCl_4 degradation was catalyzed by Cu(II) at temperatures higher than 300 ^{o}C. In this study, it was demonstrated that the addition Cu(II) within the concentration range of 0.1 ~ 0.5 mM can significantly enhance the dechlorination efficiency and the rate of CCl_4 by surface-bound Fe(II) species in goethite suspensions at pH7. Fe(II) serves as a reductant and reacts with the available Cu(II) to form Cu(I). Moreover, Cu(I) may also be re-oxidized to Cu(II) by providing electrons to CCl_4. Therefore, copper species may act as the electron mediator in the surface-bound iron system.

$$\mathrm{Cu(II) + Fe(II) \rightarrow Cu(I) + Fe(III)} \qquad (3\text{-}4)$$

$$\mathrm{2Cu(I) + CCl_4 + H^+ \rightarrow 2\,Cu(II) + CHCl_3 + Cl^-} \qquad (3\text{-}5)$$

Unlike the structural Fe(II) present in certain minerals such as green rust and magnetite, the high reactivity of Fe(II) bound to iron oxide surface can be maintained over long period of time because such Fe(II) species may constantly be regenerated, either by sorption of Fe(II) from aqueous solution or by the microbial iron(III) oxide reduction [12,16,34]. Therefore, surface-bound Fe(II) species are thought to be one of the promising materials that can be used for long-term application of remediation of groundwater contaminated with chlorinated hydrocarbons.[16] Cu(II) is a common environmental pollutant in contaminated aquifers and wastewater. In the contaminated aquifer where metal ions and chlorinated solvents coexist, where the surface-bound iron species can sorb Cu(II) first and then convert to Cu(I) by oxidizing Fe(II) to Fe(III). This process significantly accelerates the dechlorination rate of chlorinated hydrocarbons and gives great impetus on the coupled degradation of heavy metals and chlorinated hydrocarbons under anoxic conditions. In conclusion, the results of this study clearly shows the influence of Cu(II) on the dechlorination of chlorinated methanes by surface-bound Fe(II) species in the goethite suspensions. Although, the concentrations of Cu(II) used in this study was higher than naturally available Cu(II), results shown in this study it will be helpful in facilitating the development of process that could be useful for the coupled degradation of mixed contaminants and the detoxification of chlorinated solvents and metal ions.

3.5 SUMMARY

CCl_4 was dechlorinated to $CHCl_3$ by 3 mM Fe(II) in 10 mM goethite (25.6 m^2L^{-1}) suspension with the rate constant (k_{obs}) of 0.036 h^{-1} at pH 7.0. Transition metals have different effects on CCl_4 dechlorination. Addition of Ni(II), Co(II), and Zn(II) lowered the k_{obs} of dechlorination of CCl_4, whereas the addition of 0.5 mM Cu(II) into 3 mM Fe(II) amended 10 mM goethite suspension significantly enhanced the efficiency and the rate of CCl_4 dechlorination. The k_{obs} for CCl_4 dechlorination with 0.5 mM Cu(II) was 1.175 h^{-1} which was 33 times greater than that without Cu(II). It was notice that the addition of Cu(II) into Fe(II)-goethite suspension formed a precipitate which is a secondary solid phase in the heterogeneous system. The precipitate with reddish-yellow in color was formed even in the absence of goethite and the concentration of Fe(II) decreased with the increase in Cu(II) concentration. XPS and XRPD analyses suggested the possible presence of Cu_2O and ferrihydrite in the precipitate. Small amount of aqueous Cu(I) were also detected, reflecting the fact that Cu(II) was reduced to Cu(I) by Fe(II). Addition of Cu_2O into the Fe(II)- goethite suspension increased the k_{obs} for dechlorination 10 times indicating that Cu(I) in Fe(II)-Goethite system has catalytic effect. Also the dechlorination of CCl_4 by Cu(II) amended surface-bound iron species is pH dependent and the rate constant increased from 0.008 h^{-1} at pH 4.0 to 1.175 h^{-1} at pH 7.0. The relationship between k_{obs} for CCl_4 dechlorination and pH in Cu(II) amended Fe(II)-goethite system was in similar trend with that of Fe(II)-Goethite system in the absence of Cu (II). A linear relationship between k_{obs} for CCl_4 dechlorination and the concentration of Cu(II) was observed when the amended Cu(II) concentration was lower than 0.5 mM. At the high concentrations of Cu(II) the k_{obs} decreased due to decrease in pH and oxidation of a large fraction of Fe(II). Moreover, the k_{obs} for CT dechlorination was dependent on the Fe (II) concentration in the 0.5 mM Cu(II)-amended goethite system and followed the Langmuir-Hinshelwood relationship. Also the surface-bound Fe(II) concentration demonstrated a linear relationship with k_{obs} for CT dechlorination These results clearly indicate that Fe(II) serves as the bulk reductant to reduce both CT and Cu(II). The resulting Cu(I) can further act as a catalyst to enhance the dechlorination rate of chlorinated hydrocarbons in the iron-reducing environments.

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Chapter 4

REDUCTIVE DECHLORINATION OF CARBON TETRACHLORIDE IN AQUEOUS SOLUTIONS CONTAINING FEROUS AND COPPER IONS

While it is known that the surface-bound iron species has the capacity to dechlorinate various chlorinated compounds, the role of transition metals to act as catalysts with these iron species is of importance. It was previously observed that the reduction of Cu(II) by Fe(II) associated with goethite enhanced the dechlorination efficiency of chlorinated compounds. In this study, the reductive dechlorination of CCl_4 by dissolved Fe(II) with Cu(II) ions was investigated to understand the synergistic effect of Fe(II) and Cu(II) on the dechlorination processes in homogenous aqueous solutions. The dechlorination efficiency of CCl_4 by Fe(II) increased with increasing Cu(II) concentrations over the range 0.2 to 0.5 mM and then decreased at high Cu(II) concentrations. The efficiency and the rate of CCl_4 dechlorination also increased with increasing dissolved Fe(II) concentration in the presence of 0.5 mM Cu(II) at neutral pH. When the Fe(II)/Cu(II) ratio varied between 1 and 10, the pseudo first-order rate constant (k_{obs}) increased 250-fold from 0.007 h^{-1} at 0.5 mM Fe(II) to 1.754 h^{-1} at 5 mM Fe(II). X-ray powder diffraction (XRPD) and scanning electron microscopy (SEM) analyses of the precipitates shows that Cu(II) can react with Fe(II) to produce different ferric oxides, and subsequently accelerates the dechlorination rate of CCl_4 at a high Fe(II) concentration. Amorphous ferrihydrite was observed when the stoichiometric Fe(II)/Cu(II) ratio was 1, while green rust, goethite and magnetite were formed when the molar ratios of Fe(II)/Cu(II) reached 4 ~ 6. In addition, the dechlorination of CCl_4 by dissolved Fe(II) is pH dependent. CCl_4 can be dechlorinated by Fe(II) over a wide range of pH values in the Cu(II)-amended solutions, and the k_{obs} increased from 0.0057 h^{-1} at pH 4.3 to 0.856 h^{-1} at pH 8.5, which was 9 ~ 25 times greater than that in the absence of Cu(II) at pH 7-8.5. The high reactivity of dissolved Fe(II) on the dechlorination of CCl_4 in the presence of Cu(II) under anoxic conditions may enhance our understanding of the role of Fe(II) and the long-term reactivity of the zerovalent iron system in the dechlorination processes for chlorinated organic contaminants.

4.1 INTRODUCTION

Chlorinated hydrocarbons such as carbon tetrachloride (CCl_4), chloroform ($CHCl_3$) and trichloroethylene (C_2HCl_3) are the most frequently found contaminants in soil and groundwater.[1,2] Because they are prevalent in contaminated sites and are highly toxic to human beings and ecosystems, studies have been conducted to elucidate the kinetics and mechanisms of dechlorination of such compounds in contaminated sites.[3,4] The rapid dechlorination of chlorinated hydrocarbons in the presence of Fe(II) species as environmental reductants under anoxic conditions has recently been reported.[5-10] Fe(II) species can be present in subsurface environment in a wide variety of forms including dissolved, soluble complexes, surface-bound and as a structural component in Fe(II)-bearing minerals.[11] Studies showed that ferrous ion associated with Fe(III)-containing minerals can significantly reduce several pollutants, including nitroaromatics polyhalogenated alkanes, chloroamine compounds and carbamate pesticides.[5-10, 12-17] The degradation rate and efficiency are dependent on environmental conditions such as pH value, surface density of Fe(II), available surface area of ferric oxides, and the presence of transition metal ions.

Besides the surface-bound iron species, the dissolved ferrous species is also a ubiquitous component in contaminated groundwater and plays an important role in redox processes under reducing environments.[18,19] Aqueous Fe(II) is also known as a reducing agent that can effectively reduce inorganic ions such as Tc (VII), Cr(VI) as well as organic pollutants.[20,21] Doong and Wu[22] showed that it is thermodynamically possible for Fe(II) to dechlorinate CCl_4 with a relatively long incubation time of 33 days. Elsner et al.[23] reported that hexachloroethane (HCA) can be dechlorinated by dissolved Fe(II) under anoxic conditions at pH 7.2.[10] However, the dechlorination capability of aqueous Fe(II) is usually orders of magnitude lower than that of surface-bound Fe(II) species.[10] More recently, studies demonstrated the catalytic activity of transition metals species in the reduction of a range of contaminants by a number of bulk reductants.[9,11,24] Previous work[9] showed (as explained in chapter 3) that the reduction of Cu(II) to Cu(I) by Fe(II) associated with goethite (α-FeOOH) could produce cuprous oxide (Cu_2O) and amorphous ferrihydrite ($Fe(OH)_3$), resulting in an increase in the dechlorination rate and efficiency of CCl_4. This observation suggests that Fe(II) could be oxidized to ferric oxides and oxyhydroxides by Cu(II) to form surface-bound iron species, and thus gives impetus to use Cu(II) to enhance the reactivity of dissolved Fe(II) in aqueous solutions for the dechlorination of the chlorinated hydrocarbons in Fe(II)-rich environments.

In natural subsurface environments, high concentrations of dissolved Fe(II) can be primarily generated from the chemical and physical weathering, oxidation of pyrites and other iron-bearing minerals and biological processes of reductive dissolution of ferric oxides and oxyhydroxides by dissimilatory iron-reducing bacteria (DIRB). Moreover, dissolved Fe(II) can be generated and released into subsurface environment from the oxidation of metallic iron in permeable reactive barrier (PRB) systems. However, the role of Fe(II) in the reduction of chlorinated compounds is still unclear, especially when chlorinated hydrocarbons and transition metal ions coexist in the contaminated groundwater. Therefore, the objective of this study was to investigate the influence of Cu(II) ions on the reductive dechlorination of CCl_4 by aqueous Fe(II) species under anoxic condition. The effects of pH and concentrations of Fe(II) and Cu(II) on the dechlorination of CCl_4 were also examined. Moreover, X-ray powder diffraction (XRPD) and scanning electron microscopy (SEM) were used to identify the crystal phases and the morphologies of the precipitates generated from the reaction of Fe(II) and Cu(II) in aqueous solutions.

4.2 MATERIALS AND METHODS

4.2.1 Chemicals

All chemicals were used as received without further treatment. Carbon tetrachloride (CCl_4) (>99.8%, GC grade), chloroform ($CHCl_3$) (>99.8%, GC grade), tris (hydroxymethyl) aminomethane (TRIS buffer)(>99.8%), $CuCl_2{\cdot}2H_2O$(99%) were purchased from Merck Co. (Darmstadt, Germany). $FeCl_2{\cdot}4H_2O$ (99%), $FeCl_3{\cdot}6H_2O$ (99%), N-(2-hydroxyethyl)- piperazine-N'-2-ethanosulfonic acid (HEPES buffer) (99.5%), 2-(N-Morpholino)- ethanosulfonic acid (MES) (>99.5%) and sodium tatrate-2-hydrate ($C_4H_4Na_2O_6{\cdot}2H_2O$) (>99.5%) were purchased from Sigma-Aldrich Co. (Milwaukee, WI). Methylene chloride (DCM) (> 99.8%, GC grade) and ethanol (HPLC grade) were obtained from J. T. Baker Co. (Philipsburg, NJ). Bathocuproinedisulfonic acid disodium salt ($C_{26}H_{18}N_2Na_2O_6S_2$) (90%) was purchased from Fluka (Buchs, Switzerland). All the solutions were prepared using distilled deionized water (Millipore, 18.3 mΩ) and were deoxygenated in vacuum sealed bottles under an N_2 atmosphere.[9, 25, 26]

4.2.2 Dechlorination Experiments

Batch experiments were conducted using 70mL serum bottles filled with 50 mL of deoxygenated buffer solutions under anoxic conditions. Anoxic HEPES (50 mM) buffer solutions were used to control pH at 7.0 ± 0.1. Anoxic solutions were prepared by purging N_2 (99.9995%) with a flow rate of 42 L min^{-1} in vacuum-sealed bottles. This process was repeated 4-5 times to remove trace amounts of oxygen in solutions.[9,25,26] Stock solutions of Fe(II) were prepared in 50 mL of deoxygenated buffer solutions and injected into vacuum- sealed serum bottles after filtration through 0.2 μm PTFE filter cartridge. The Fe(II) concentration in the filtrate was quantified by ferrozine method at 562 nm[26,9] using UV-visible spectrophotometer (U-3010. Hitachi, Japan). The stock solution of Cu(II) was prepared by dissolving $CuCl_2$ in deoxygenated deionized water in a sealed and vacuumed serum bottles. Appropriate amounts of Fe(II) and Cu(II) stock solutions were introduced into the deoxygenated buffer solutions using N_2-purged plastic syringes to obtain final concentrations of 3 mM and 0.5 mM, respectively. For the pH effect experiment, 50 mM MES buffer solutions were used for pH 5.5, 6.0 and 6.5. HEPES buffer was selected to maintain pH at 7.0, 7.5 and 8.0, while TRIS buffer was used for pH 9. The concentration effects of Fe(II) and Cu(II) were also conducted by introducing various volumes of stock solutions into deoxygenated buffer solutions to get final concentrations ranging between 0.5 and 5.0 mM. All the reactor bottles were sealed with Teflon-lined rubber septa and aluminum crimp caps. After 20 h of equilibrium, an aliquot (50 μL) of the CCl_4 stock solution dissolved in degassed methanol was delivered into serum bottles by a gas-tight glass syringe to obtain the final concentration of 20 μM CCl_4. Serum bottles were then incubated in an orbital shaker at 150 rpm maintained at 25 ± 1 C in the dark. The total liquid volumes were maintained at 50 mL, resulting in a 20-mL headspace for headspace analysis. The headspace of the bottles was maintained at anoxic condition by filling N_2 and Ar (80/20, v/v). Control experiments were also carried out without the addition of Fe(II) or Cu(II). All experiments were run in duplicates or triplicates.

4.2.3 Analytical Methods

The headspace analytical technique was used for the determination of chlorinated hydrocarbons.[9,25] The concentrations of CCl_4 and the byproducts in the headspace of the test bottles were monitored by withdrawing 50 μL of gas in the headspace using a 100 μL gas-tight syringe. The mixture was then injected 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 fused-silica megabore capillary column (0.545 mm × 3.0 μm, Supelco Co.) was used to separate the chlorinated compounds. The column was connected to FID and ECD simultaneously by a Y-splitter with 40 % of the flow (1.85 ml/min) to ECD for better identification and quantification of chlorinated hydrocarbons. The column temperature was isothermally maintained at 90ºC with nitrogen (>99.9995%) as the carrier gas. The relative standard deviation (RSD) for GC analysis was controlled within 10% in ECD and 5% in FID. The limits of detection for CCl_4 and $CHCl_3$ by GC for the used method were 0.04 μM and 0.1μM respectively. After headspace analyses, serum bottles were opened under N_2 atmosphere, and pH and redox potential (ORP) were measured using a microprocessor pH meter equipped with pH electrode and ORP combination electrode.

XRPD was performed using an X-ray diffractometer (Regaku D/max-II B) and a Cu Kα-radiation source (λ = 1.54056 Å) with 30 kV voltage and 20 mA current to identify the crystal phases of the precipitates. The precipitates were allowed to settle down after the termination of dechlorination experiment and the supernatant was carefully removed from the sealed bottles using N_2–purged syringe. After drying the precipitates using a gentle stream of N_2, samples were mounted on a glass sample holder using small amounts of grease. A drop of glycerol was immediately added on the mounted powder layer to minimize the exposure in air. The scan range for all samples was between 5 and 90° (2θ) at a scanning speed of 4°/min. Moreover, scanning electron microscopy (SEM) (Topcon ABT-150s) was used to identify the morphology of the precipitates.

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 1M HCl.[26] The acidified samples were centrifuged at 14,000 × g for 10 min to remove particles and the acid extractable-Fe(II) contents were determined with ferrozine at 562 nm.[9,26] To determine the dissolved fraction of Fe(II) in serum bottles, aliquots were withdrawn using N_2-purged 1 mL-plastic syringe, and immediately filtered through acidified filter cartridge (0.2-μm cellulose acetate filter) into a solution containing 0.5 mL of 1M HCl solution. The concentration of dissolved Fe(II) in the filtrate was then determined using ferrozine method. The sorbed Fe(II) concentration was calculated from the difference between total and dissolved concentrations. Also, the total concentrations of iron species were determined using inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer, Optima 3000XL).

The concentration of extractable Cu(I) was determined using the bathocuproinedisulfonic acid method with minor modifications (27). The aliquot was withdrawn by a 1 mL N_2-purged plastic syringe and immediately added into the mixture containing 1 mL of 10% tatrate solution and 0.5 mL of 1% bathocuproinedisulfonic acid solution. After 30 min of reaction, the mixture was centrifuged at 14,000 × g for 5 min. The Cu(I) concentration in the supernatant was then determined at 483 nm. Standard solutions of Cu(I) were prepared using 10% hydroxylammonium chloride as a reductant to reduce $CuCl_2$ solution. The added Cu(II) concentration in the serum bottles was based on the gravimetrical concentration of $CuCl_2$ in the anoxic stock solution and was confirmed by using ICP-OES.

4.3 RESULTS AND DISCUSSION

4.3.1 Concentration effect of Cu(II) on CCl_4 dechlorination in the presence of 3 mM Fe(II)

To understand the synergistic effect of aqueous Cu(II) and Fe(II) on the dechlorination of CCl_4 under anoxic conditions, the concentration effect of these divalent ions was evaluated. Figure 4-1 shows the dechlorination of CCl_4 as well as the changes in pH and ORP of the solutions in the presence of various concentrations of Cu(II) at a constant Fe(II) concentration of 3 mM. No obvious dechlorination of CCl_4 by 3 mM Fe(II) was observed in the absence of Cu(II). Addition of low concentrations of Cu(II) significantly enhanced the dechlorination of CCl_4. The dechlorination efficiency of CCl_4 by Fe(II) increased with increasing Cu(II) concentrations ranging from 0.2 to 0.5 mM. Further increase in Cu(II) concentration lowered the dechlorination efficiency of CCl_4, and only 35% of initial CCl_4 was removed within 12 h when Cu(II) concentration was increased to 4 mM. This decrease in dechlorination efficiency at high Cu(II) concentrations may be attributed to the decrease in pH values and the increase in redox potentials. As depicted in Figure 4-1b, the pH value was maintained at 7.0 ± 0.2 after 12 h in the Cu(II) concentration range of 0 ~ 0.5 mM, while the pH value decreased to 5.5 when the concentration was up to 4 mM. In addition, the redox potentials of the solutions increased from – 350 mV in the absence of Cu(II) to + 100 mV at 4 mM Cu(II). Maithreepala and Doong[9] reported that 4 mol of protons could be released into the solution when 1 mol of Cu(II) reacted with 1 mol of Fe(II).

Moreover, the hydrolysis of Cu(II) ions can also release protons into the solution to lower the buffer capacity. Since Fe(II) was oxidized to Fe(III) by Cu(II), the pH of the system decreased and the redox potential increased when a high concentration of Cu(II) was added to the solution.[9]

4.3.2 Concentration effect of Fe(II) on CCl_4 dechlorination in the presence of 0.5 mM Cu(II)

Figure 4-2 shows the dechlorination of CCl_4 and the production of chloroform ($CHCl_3$) in the presence of various concentrations of Fe(II) with 0.5 mM Cu(II). The dechlorination efficiency of CCl_4 increased from 15% at 0.5 mM to 93% at 2 mM within 24 h. A nearly complete dechlorination of CCl_4 was observed when the Fe(II) concentration was higher than 2.5 mM, clearly showing that Fe(II) species is the crucial factor controlling the rate and efficiency of CCl_4 dechlorination in aqueous solution. In order to evaluate the change in reductive condition of the mixture due to the increase in Fe(II) concentration. The oxidation reduction potential (ORP) in the serum bottles decreased form +106 mV at 0.5 mM Fe(II) to –219 mV at 2mM Fe(II) and then slowly to –257mV when the Fe(II) concentration was increased to 5 mM. Chloroform was identified as the major product of CCl_4 via hydrogenolysis and the maximum concentrations of 13 ~ 14 M were obtained after the termination of experiment. This corresponds to 60 ~ 70 % of CCl_4 dechlorination, which is in a good agreement the reported results.[7,27] Moreover, small peaks of dichloromethane (DCM) and tetrachloroethene (C_2Cl_4) appeared in the GC-ECD chromatograms. A trace amount of methane in the headspace was detected by GC-MS when a high concentration of CCl_4 (3 mM) was used in dechlorination experiment, implying the further dechlorination to the less chlorinated byproducts or non-chlorinated final products occurred. In addition, several studies showed that CCl_4 cold be transformed to carbon monoxide (CO) and formate ($CHCOO^-$) via reductive hydrolysis[1,8,23] suggesting that the 30-40% loss of CCl_4 in the present study may be due to formation of carbon monoxide and formate in the liquid phase.

$$\ln\left[\frac{C_t}{C_0}\right] = -k_{obs}t \tag{4-1}$$

The dechlorination of CCl_4 by Fe(II) in Cu(II)-amended solutions followed pseudo-first order reaction kinetics[9] which can be explained by the where C_0 and C_t are the concentrations of CCl_4 at the initial time and at time t, respectively, and k_{obs} is the first-order rate constant for CCl_4 dechlorination. The dechlorination of CCl_4 in Figure 4-2A shows a clear first-order behaviour at the concentration levels of Fe(II) concentrations of 3 ~ 5 mM.

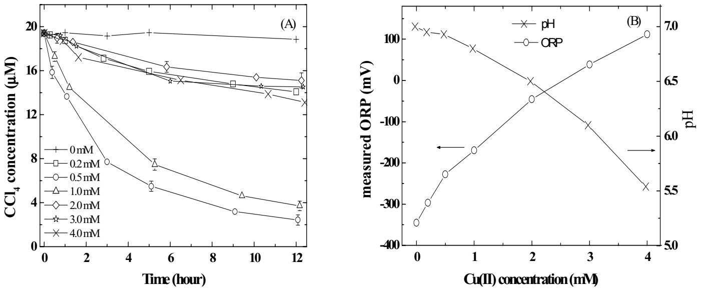

Figure 4-1. Effect of Cu(II) concentration on (**a**) the dechlorination of 20 µM CCl_4, and (**b**) the change in pH and ORP in anoxic buffered solution containing 3 mM Fe(II). HEPES (50 mM) was used to maintain the pH at 7.0 ± 0.1 in the range of 0-0.5 mM. The decrease in pH at Cu(II) concentrations higher than 0.5 mM is due to the production of high concentrations of protons

At lower concentrations, however, first order reaction kinetics was followed only within initial periods of the time. However, a same reaction kinetics assumptions for the CCl_4 dechlorination is considered for the simplicity at low Fe(II) concentrations. Figure 4-3 shows the rate constants for CCl_4 dechlorination as functions of the initial and the sorbed Fe(II) concentrations in the presence of 0.5 mM Cu(II) at pH 7. The k_{obs} increased slightly from 0.007 to 0.252 h^{-1} when the initial Fe(II) concentrations increased from 0.5 to 3 mM (Figure 4-3a). Further increase in Fe(II) concentration dramatically increased the dechlorination rate. The k_{obs} for CCl_4 dechlorination was 4 ~7 times higher than that with 3 mM Fe(II) when the initial Fe(II) concentrations were in the range of 4 ~ 5 mM. The increase in k_{obs} at high Fe(II) concentration is probably due to the formation of precipitates when anoxic solutions contained both Fe(II) and Cu(II).[9]

$$2\ Fe^{2+} + 2\ Cu^{2+} + 7\ H_2O \rightarrow Cu_2O + 2\ Fe(OH)_3 + 8\ H^+ \qquad (4\text{-}2)$$

According to equation (4-2), an amorphous ferrihydrite with cuprous oxide are formed when the molar ratio between Fe(II) and Cu(II) is 1. At high concentration of Fe(II), the excess ferrous ion would adsorb onto the surface of ferric oxide to form surface-bound iron species, and subsequently accelerates the dechlorination rate of CCl_4 at neutral pH.

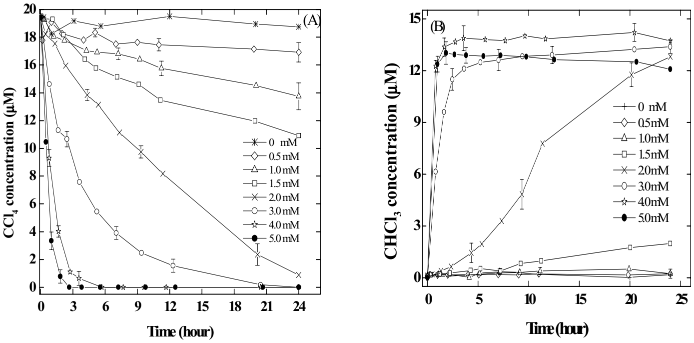

Figure 4-2. (**A**.) Effect of Fe(II) concentration on the dechlorination of 20 μM CCl_4 in the presence of 0.5 mM Cu(II) at pH 7.0 ± 0.1.(**B**.) The production of $CHCl_3$ as the major product in CCl_4 dechlorination by various concentrations of Fe(II) in the presence of 0.5 mM Cu(II) at pH 7.0 ± 0.1

Table 4-1. Concentrations of total, acid-extractable, dissolved, surface-bound and fixed Fe(II) in aqueous solutions containing 0.5 mM Cu(II) and various initial concentrations of Fe(II) after 24 h of mixing under anoxic condition. The pH of the system was controlled at 7.0 ± 0.1.

Concentrations of Fe(II) (mM)							
Initial Fe(II)	Fe(II)/Cu(II) [a]	Total Fe[b]	Acid extractable [c]	Dissolved	Surface-bound [d]	Fixed-Fe(II) [e]	Cu(I) (mM)
0.5	1	0.48±0.12	0.23±0.02	0	0.23	0.06	0.21
1.0	2	1.03±0.17	0.26±0.05	0.025±0.03	0.23	0.33	0.41
1.5	3	1.47±0.15	0.33±0.04	0.055±0.02	0.27	0.76	0.41
2.0	4	1.98±0.19	0.75±0.03	0.288±0.06	0.46	0.84	0.41
2.5	5	2.54±0.21	1.03±0.02	0.318±0.05	0.71	1.06	0.41
3.0	6	3.01±0.15	1.30±0.06	0.32 ±0.04	0.98	1.29	0.41
4.0	8	4.03±0.18	2.11±0.08	0.35 ±0.07	1.76	2.53	0.42
5.0	10	4.89±0.23	2.96±07	0.35 ±0.05	2.61	1.61	0.43

a: The molar ratio of initial Fe(II)/ Cu(II), b: Concentration of total iron species determined by ICP-OES, c: concentration of 1M HCl extractable Fe(II), d: surface-bound Fe(II) concentration = acid-extractable Fe(II) - dissolved Fe(II), e: fixed Fe(II) (un-disorbable Fe(II)) = initial Fe(II) – acid-extractable Fe(II) + oxidized Fe(II) that was assumed to be equal to determined Cu(I) concentration.

Table 4-1 shows the changes in the concentrations of dissolved and surface-bound Fe(II), after 24 h of the addition of various concentrations of Fe(II) into the buffered solution containing 0.5 mM Cu(II). The extractable Cu(I) concentrations at each systems are also shown in Table 4-1. The total extractable Cu(I) concentrations, with the exclusion of that from the 0.5 mM Fe(II)-amended solution, were in the range of 0.41 ~ 0.43 mM, implying that similar amounts of ferric oxides were produced. The lower recovery Cu(I) in the solution with 0.5 Fe(II) might be due to incomplete reduction of Cu(II). In contrast, the surface-bound Fe(II) concentrations increased with increasing initial Fe(II) concentration, which was in a good agreement with the k_{obs}. This means that Fe(II) species plays an important role in the dechlorination rate of CCl_4 in the presence of Cu(II) ions.

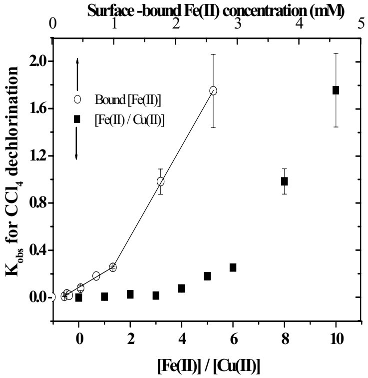

Figure 4-3. The pseudo first-order rate constant (k_{obs}) for CCl_4 dechlorination as functions of the ratio of [Fe(II)]/[Cu(II)] and the surface-bound Fe(II) concentration in aqueous solutions containing 0.5 mM Cu(II) at neutral pH.

Previous studies[6,7,9] showed that the reaction rate of CCl_4 in the heterogeneous Fe(II)-Fe(III) systems followed a linear relationship with respect to the surface-bound Fe(II) concentration. In this study, however, a two-linear phase relationship between k_{obs} and the surface-bound Fe(II) concentration was observed (Figure 4-3) which suggests that the dechlorination of CCl_4 in solutions containing Fe(II) and Cu(II) is controlled not only by the surface-bound Fe(II) concentration but also by the other factors with respect to the molar ratio of Fe/Cu. Several studies have reported that Fe(II) could be fixed to Fe(III)-containing mineral surfaces, resulting in the formation of different morphologies of Fe(III) oxides after a relative long contact time.[5,28,29] Jeon et al.[28] observed the interactions of dissolved Fe(II) with hematite surface which transformed to magnetite after a relative long time. Satapanajaru et al.[30] also reported that ferrihydrite could react with Fe(II) to form magnetite. In this study, the acid-extractable concentrations of Fe(II) in all batches were lower than the initial concentrations of Fe(II) after 24 h, and the difference between the total measured Fe(II) and the added amounts increased with increasing initial Fe(II) concentration. This shows the possibility of the fixation of Fe(II) and the change in mineral phase of amorphous ferric oxide to the crystalline ferric oxides at various Fe/Cu ratios.

4.3.3 Change in morphology of chemogenic solids at various Fe/Cu ratios.

In order to further understand the changes in the surface of chemogenic solids formed at various Fe(II)/Cu(II) ratios, SEM and XRPD were used to identify the surface morphology and the crystal phase of the precipitates, respectively. A high concentration of Cu(II) (3 mM) was used to react with Fe(II) in a range of stoichiometric ratios to generate sufficient amounts of chemogenic solids. Figure 4-4 shows the SEM images of chemogenic solids at various stoichiometries of Fe/Cu. The surface morphology of the precipitates varied at various Fe/Cu ratios, and the reactive species increased rapidly with increasing concentrations of Fe(II). Ferrihydrite was produced at Fe/Cu ratio of 1, while goethite crystals were clearly shown at Fe/Cu ratios of 4~6 and appeared to encrust a fraction of ferrihydrite with lath-like habit.

The XRPD patterns also showed the change in crystal phase of the chemogenic solid at various Fe(II)/Cu(II) ratios (Figure 4-5). The major peaks of cuprous oxide (Cu_2O) were observed at 36.42°, 42.22°, and 61.3° (2θ) and no distinct ferric oxide peak was identified at Fe/Cu ratio of 1.0. The SEM image of Fe(II) and Cu(II) mixture with a similar ratio shown in Figure 4-4a may represents those minerals. When the ratio of the Fe(II)/Cu(II) was increased to 4, at least three morphologies of mineral particles were observed. The peaks in the XRPD pattern (Figure 4-5) could be assigned to goethite, magnetite and cuprous oxides. Although the particles were difficult to be identified just by their shapes, the needle-like particles probably were goethite, which is in agreement with the peak pattern in the XRPD (Figure 4-5). Further increase in the concentration ratio of Fe(II)/Cu(II) to 6, the needle-like goethite particles were abundant, but with small particle sizes. Goethite is a well crystalline ferric oxihydroxide and has been demonstrated to effectively enhance the dechlorination efficiency and the rate of chlorinated compounds by Fe(II) when bound with the surfaces under anoxic conditions.[5,7,9] Therefore, the decrease in particle size my increase the surface area to sorb more Fe(II) that can increase the rate and efficiency of CCl_4 dechlorination. In addition, no XRPD peak related to goethite appeared at the Fe(II)/Cu(II) ratio of 10, revealing that an amorphous mineral phase with small particle sizes was formed. This means that the formation of amorphous mineral phase with very small particle size (Figure 4-4D and Figure 4-5) forms high concentration of surface bound Fe(II) that can increase the dechlorination of CCl_4. XRPD patterns were also observed at pH values ranging from 5.5 to 8.5 when the Fe/Cu ratio was 1 (Figure 4-6). This reflects the fact that the precipitates could be produced over a wide range of pH and that the Fe/Cu ratio is an important factor controlling the morphology of the precipitates. Several additional peaks, which can be assigned to magnetite and goethite, were observed when the molar ratios of Fe/Cu increased to 4 – 6 at neutral pH. Recently, Hansel et al.[31] reported the formation of goethite and magnetite when the biogenic Fe(II) was fixed over ferrihydrite in both column and batch systems. Tolchev et al.[32] also found that the chemical composition of precipitates was dependent on the concentration of Fe(II) or $Fe(OH)_2$ in the suspension.

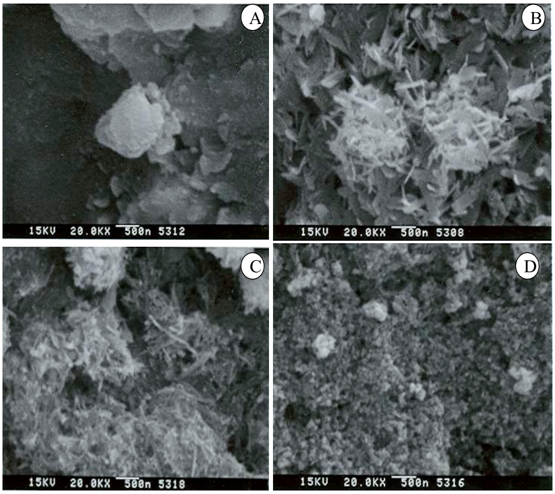

Figure 4-4. The SEM images of the precipitates in solutions containing various Fe(II) concentrations ranging from 0.5 to 5 mM with 0.5 mM Cu(II) at neutral pH. The stoichiometric relations between Fe(II) and Cu(II) were (**a**) 1:1, (**b**) 4:1, (**c**) 6:1, and (**d**) 10:1. All the precipitates were harvested after 48 h under anoxic conditions.

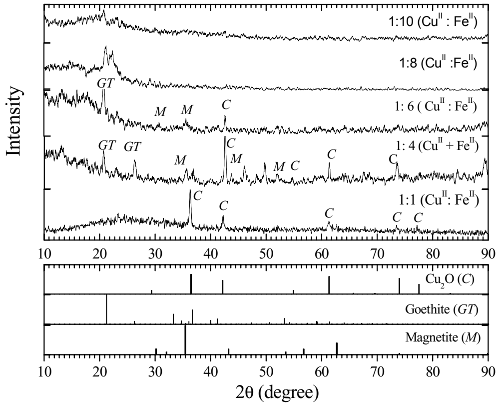

Figure 4-5. The XRPD patterns of the solid phases precipitated at various Fe(II)/Cu(II) ratios after 48 h of incubation

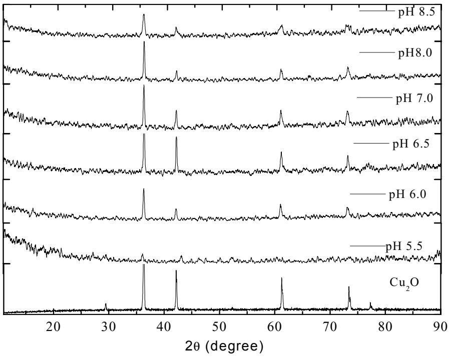

Figure 4-6. The XRPD patterns of the precipitates produced in the reaction of Fe(II) and Cu(II) at various pH values ranging from 5.5 to 8.5. The concentrations of Cu(II) and Fe(II) were 3 mM and the Fe(II)/Cu(II) ratio was 1., The peak pattern of Cu_2O is also shown for the comparison.

The results obtained from XRPD and SEM analysis clearly show that the addition of excess Fe(II) concentrations to solutions containing Cu(II) can form different mineral phases and morphologies of reactive surface-bound Fe(II) species, resulting in an acceleration of the CCl_4 dechlorination rate. However, no distinct peak was observed in XRD patterns when Fe/Cu ratios were increased to 8 –10, (Figure 4-5) presumably due to the presence of the amorphous oxides. Baltpurvins et al.[33] showed that the mineral phase conversion from ferrihydrite to crystalline ferric oxides was significantly hampered in the presence of chloride ions. In this study, the amendment of large amounts of Fe(II) as $FeCl_2$ increased the concentration of chloride ion in the system, and therefore, may inhibit the formation of crystal minerals. Ferrihydrite has a larger specific surface area than those of magnetite and goethite, therefore, the increase in surface-bound Fe(II) species associated with ferrihydrite due to sorption may increase the CCl_4 dechlorination rate at high Fe(II)/Cu(II) ratio. The mechanism for the formation of crystalline Fe(III) oxide in the presence of aqueous Fe(II) and Cu(II) ions is not well-understood. Previous studies reported that magnetite can be formed by the oxidation of $Fe(OH)_2$ under neutral or alkaline conditions[30] or from the oxidation of green rust.[30,34] The poorly crystalline iron oxides may also be transformed to goethite.[35] Mann et al.[36] reported that green rust, could be formed as an intermediate during the conversion of ferrihydrite to magnetite. In this study, a red- brown color was appeared at Fe/Cu ratio of 1, indicating the formation of Cu_2O and amorphous ferrihydrite as described by equation 4-2. The red-brown color then slowly changed to green-brown color during the first 12 h when excess molar concentration of Fe(II) was present. The green color is generally due to the formation of ferrous hydroxides or green rust. $Fe(OH)_2$ is generally formed by reaction of Fe(II) and OH^- ions under basic conditions. During the pre- equilibrium of 20h, the color changed to black-brown. This change is probably due to the oxidation of ferrous hydroxide to magnetite (equation 4-3), or the formation of green rust by reacting Fe(II) ions with Fe(III) species (equation 4-4). However, no greenish brown color was observed when the concentrations of Cu(II) and Fe(II) were similar. These results suggest that green rust could be formed as an intermediate and then oxidized to magnetite or goethite as shown in equation 4-5 and 4-6, respectively.

$$3\,Fe(OH)_2 \leftrightarrows Fe_3O_4 + 2H^+ + 2H_2O + 2e^- \qquad (4\text{-}3)$$

$$Fe(OH)_2^+ + 3FeOH^+ + Cl^- + 3OH^- + 2H_2O \leftrightarrows [Fe^{II}_3Fe^{III}(OH)_8][Cl\cdot 2H_2O] \qquad (4\text{-}4)$$

$$3[Fe^{II}_3Fe^{III}(OH)_8][Cl\cdot 2H_2O] \leftrightarrows$$
$$4\ Fe_3O_4 + 5e^- + 3Cl^- + 8H^+ + 14H_2O \quad (4\text{-}5)$$

$$[Fe^{II}_3Fe^{III}(OH)_8][Cl\cdot 2H_2O] \leftrightarrows 4\ \alpha\text{-}FeOOH + 3e^- + Cl^- + 4H^+ + 2H_2O \quad (4\text{-}6)$$

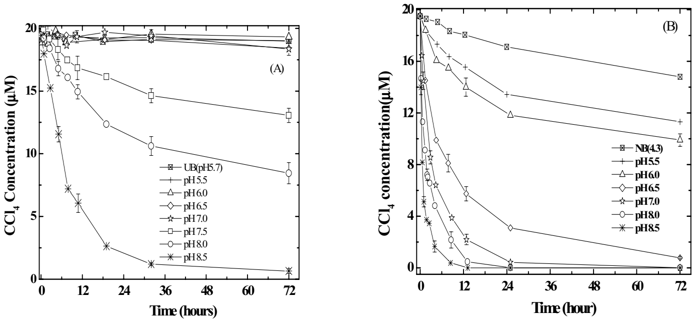

Figure 4-7. The effects of pH on the dechlorination of 20 μM CCl_4 by 3mM Fe(II) in (a) the absence and (b) the presence of 0.5 mM Cu(II). MES buffer was used to control pH at 5.0, 5.5 and 6.0. HEPES buffer was used to maintained pH at 7.0, 7.5 and 8.0. TRIS buffer was used for pH 8.5.

4.3.4 Effect of pH.

The pH may affect the formation of reactive chemogenic solids and the reactivity of surface sites on the Fe(III) mineral surfaces. Therefore, the effect of pH on CCl_4 dechlorination was examined, and the pH was maintained within the range of 5.5 ~ 8.5 with appropriate buffers. An un-buffered solution containing 3 mM Fe(II) and 0.5 mM Cu(II) at pH 4.5 was also used for comparison. Parallel experiments in the absence of Cu(II) were also conducted to elucidate the effect of possible precipitates on CCl_4 dechlorination.

Figure 4-7 illustrates the effect of pH on the dechlorination of CCl_4 by 3 mM Fe(II) in the absence and the presence of 0.5 mM Cu(II). In the absence of Cu(II), no apparent dechlorination of CCl_4 was observed over the pH range of 5.5 – 7.0 (Figure 4-7a), which is similar to reported results.[7] An obvious dechlorination of CCl_4 by Fe(II) occurred under alkaline condition and the k_{obs} for CCl_4 dechlorination increased from 0.0097 h^{-1} at pH 7.5 to 0.0946 h^{-1} at pH 8.5. The concentrations of $CHCl_3$

were within the range of 0.42 ~14.4 μM, which accounted for up to 71% of CCl_4 dechlorination (Table 4-2). In contrast to the system without Cu(II), CCl_4 dechlorinated to $CHCl_3$ over a wide range of pH when 0.5 mM of Cu(II) was amended into the solution. The efficiency and rate for CCl_4 dechlorination increased with increasing pH values, and the removal ratios of 26 ~ 96% were obtained in the pH range of 4.3 ~ 6.5. A nearly complete degradation of CCl_4 was observed after 24 h at neutral and basic pHs. The k_{obs} for CCl_4 dechlorination increased dramatically (150-fold) from 0.0057 h^{-1} at pH 4.3 to 0.856 h^{-1} at pH 8.5, showing that pH value has a great effect on CCl_4 dechlorination by Fe(II) in the presence of Cu(II).

Table 4-2. Comparison of the pseudo-first order reaction rate constant (k_{obs}) for CCl_4 dechlorination by 3 mM Fe(II) and production of $CHCl_3$ in the absence and the presence of 0.5 mM Cu(II) at various pH values.

pH	Without Cu(II)		With 0.5 mM Cu(II)	
	k_{obs} (h^{-1})[b]	Maximum detected $CHCl_3$ (μM)	k_{obs} (h^{-1})	Maximum detected $CHCl_3$ (μM)
UB[a]	0	0.42	0.0057	0.31
5.5	0	0.44	0.0170	0.42
6.0	0	0.41	0.0224	3.93
6.5	0	0.75	0.0827	12.28
7.0	0	0.77	0.2521	13.57
8.0	0.0209	8.45	0.3465	13.47
8.5	0.0946	14.44	0.8557	13.43

a: The measured pH values of the un-buffered solutions with and without the addition of 0.5 mM Cu(II) were 5.7 and 4.3, respectively. b: Zero values mean "not determined".

The increase in k_{obs} at high pH values may be attributed to the formation of precipitates. Figure 4-8 illustrates the sorbed and dissolved concentrations of Fe(II) in the absence and in the presence of Cu(II) after 72 h. In the solution containing 3 mM Fe(II) without Cu(II), a total Fe(II) concentration of 3 mM was detected as the dissolved fraction at pH 5.5 ~7.0, showing that no sorbed Fe(II) was observed in the solutions. A slight green color was observed when pH values were higher than 7.5. The sorbed Fe(II) accounted for 25 ~ 40 % of the initial Fe(II) in the solutions at pH 7.5 ~ 8.5, which is in a good agreement with the increase in k_{obs}. Previous studies also showed that the increase in dechlorination efficiency of chlorinated compounds at high pH was due to the increasing sorption density of Fe(II) onto Fe(III) oxides.[5,7] However, the total Fe(II) concentrations at various pH levels were determined to be 3.0 ± 0.014 mM (n = 8), which was equal to the initial amounts of Fe(II). Previous studies discussed the formation of different Fe(II) species using pH–Eh diagrams.[30,37] The most favorable Fe(II) species in the pH range of 4 ~ 6 under anoxic condition is dissolved Fe(II). Green rust is formed within the pH range of 6.5 ~ 8, while $Fe(OH)_2$ is a most abundant species at high pH values. In this study, the measured ORP was +180 mV in un-buffered solution, and then gradually decreased to –20 mV at pH 5.5 and to – 500 mV at pH 8. These results suggest that no significant amount of ferric oxide was formed in the absence of Cu(II), and the sorbed amounts of Fe(II) could be green rust or $Fe(OH)_2$.

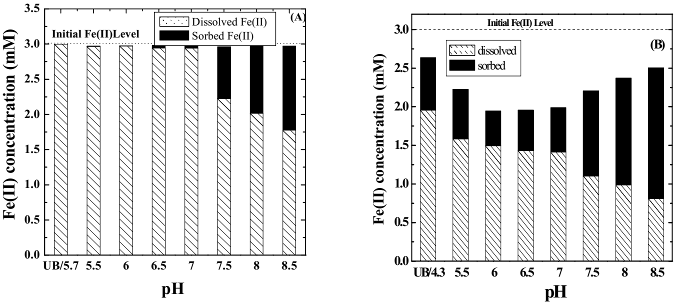

Figure 4-8. Fractions of the dissolved and sorbed concentrations of Fe(II) (a) in the absence and (b) in the presence of 0.5 mM Cu(II) at various pH values after 72 h.

Unlike the systems without Cu(II), the concentrations of the acid extractable Fe(II) in the presence of 0.5 mM Cu(II) were lower than 2.5 mM after the termination of dechlorination processes. The Cu(I) concentrations in serum bottles were in the range of 0.44 ~ 0.5 mM, and the total Fe concentration determined by ICP-OES were 3.0 ± 0.15 mM (n = 8), clearly showing that the decrease in Fe(II) is mainly due to the formation of ferric oxides. In the un-buffered system where the pH was 4.36 (Figure 4-8b), the sorbed Fe(II) was found but the dechlorination efficiency of the dechlorination of CCl_4 was quite low (Figure 4-7b). This may be due to the protonation of the surface-bound Fe(II) within the acidic pH range. The protonated surface-bound Fe(II) species are less reactive compared to deprotonated species.[3] It is noted that the formation of ferric oxide is most prevalent at pH 6.0 ~ 7.0 and gradually decreases with the increasing pH. The SEM images showed different morphologies of ferric oxides at various pH values (Figure 4-9), due to the fixation of Fe(II). In addition to ferric oxids, Fe-Cu oxides may also be formed. However, such mineral phases were not identified in this study.

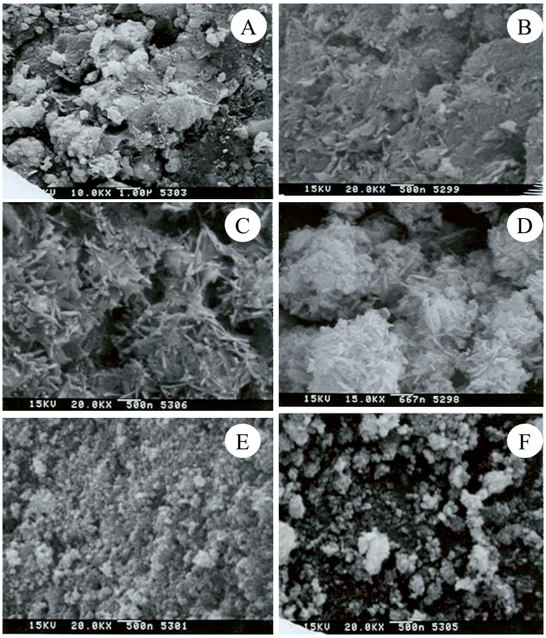

Figure 4-9. Changes in morphology of ferric oxides produced from the reaction of 3mM Fe(II) and 0.5 mM Cu(II) at various pH ranging from 5.5 to 8.5.

4.4 ENVIRONMENTAL SIGNIFICANCE

In natural anoxic environments, several biogeochemical redox processes may take place in parallel, giving rise to several potential reductants for the dechlorination of subsurface contaminants. The Fe(II)-Fe(III) cycle is one of the most important redox processes and could be the dominating removal processes for the chlorinated contaminants under anoxic conditions. Recently, the impact of transition metals on the reductive dechlorination of chlorinated compounds by structural Fe(II) species has been addressed. Jeong and Hayes[24] showed that the addition of transition metal ions including Cu(II), Ni(II), Zn(II), Cd(II) and Hg(II) increased the dechlorination rates of hexachloroethane (HCA) in the presence of mackinawite (FeS). The catalytic activities of Cu(II), Au(III) and Ag(I) in the reduction of chlorinated alkanes by green rust were also demonstrated.[38,39] A possible mechanism for the enhanced CCl_4 reduction is the formation of a galvanic couple involving the zerovalent metal and green rust. Although the concentrations of Cu(II) used in this study are higher than those naturally available Cu(II), the results of this study clearly indicate the potential use of low concentration of Cu(II) to enhance the dechlorination efficiency and rate of CCl_4 by aqueous Fe(II) in the contaminated subsurface environments. Dissolved Fe(II) can react with Cu(II) to chemically produce different compositions of Fe(III) oxides to accelerate the dechlorination of CCl_4.

Several studies[40,41] have demonstrated the possibility of natural attenuation of CCl_4 in the contaminated aquifers. The observed half-lives for dechlorination rage between 5 and 15 days, which are much slower than the results obtained in this study. In contaminated groundwater where Cu(II) and chlorinated hydrocarbons coexist, ferric oxides are present as alternative electron acceptors by DIRB to reductively dissolve Fe(II). The dissolved Fe(II) can be adsorbed onto the surface of ferric oxides to form the surface-bound Fe(II) species or react with Cu(II) to generate new reactive surface sites for the dechlorination of chlorinated hydrocarbons.

Permeable reactive barriers (PRB) are receiving a great attention as a possible innovative technology for *in situ* cleanup of ground water contamination. Zerovalent iron is the most frequently utilized material, accounting for approximately 45% of PRB applications.[42] The Fe(II) species generated by oxidation of zerovalent iron are also thought to play a key role in the long-term reactivity of metal iron reactive walls for the remediation of aquifers contaminated with chlorinated solvents.[42-45] Several ferric oxides, including magnetite, goethite and green rust have been observed as products during zero valent-treatment.[30,46,47] Recently, laboratory experimental results showed the effect of transition metal ions including Cu(II) on the dechlorination of chlorinated compounds by zerovalent iron[48]. The synergistic effect of dissolved Cu(II) and Fe(II) on the reduction of CCl_4 shown in this study may enhance our understanding of the role of Fe(II) and the long-term reactivity of Fe^0 systems in the dechlorination processes for chlorinated organic contaminants. In conclusion, our results clearly show the high reactive nature of dissolved Fe(II) in the dechlorination of CCl_4 in the presence of Cu(II) ion under anoxic conditions. In the contaminated subsurface, the Fe(II) concentrations can be higher to 1.4 – 3 mM.[17] This gives impetus to facilitate the development of processes that could be used for the coupled detoxification of chlorinated hydrocarbons and metal ions.

4.5 SUMMARY

The reductive dechlorination of CCl_4 by dissolved Fe(II) in the presence of Cu(II) ions was investigated to understand the synergistic effect of Fe(II) and Cu(II) on the dechlorination processes. No CCl_4 was dechlorinated in acidic or neutral solutions of 3 mM Fe(II). However, the dechlorination efficiency of CCl_4 by Fe(II) increased with increasing Cu(II) concentrations over the range 0.2 to 0.5 mM. A further increase in Cu(II) concentration lowered the CCl_4 dechlorination efficiency and only 35 % of the initial CCl_4 was removed within 12 h when the Cu(II) concentration was increased to 4 mM. Analysis of total Fe(II), ORP and Cu(I) suggested that oxidation of Fe(II) by reducing Cu(II) to C(I) occurs. At high concentrations of Cu(II), the rate and efficiency of CCl_4 dechlorination decreased due to oxidation large amount of Fe(II) and becoming the medium more acidic. CCl_4 dechlorination increased with increasing dissolved Fe(II) concentration in the presence of 0.5 mM Cu(II) at neutral pH. When the Fe(II)/Cu(II) ratio was increased from 1 and 10, the k_{obs} increased 250-fold from 0.007 h^{-1} at 0.5 mM Fe(II) to 1.754 h^{-1} at 5 mM Fe(II). XRPD and SEM analyses showed that Cu(II) can react with Fe(II) to produce different morphologies of ferric oxides, and subsequently accelerate the dechlorination rate of CCl_4 if the [Fe(II)]/[Cu(II)] was greater than 1. Amorphous ferrihydrite was observed when the stoichiometric ratio of Fe(II) to Cu(II) was 1, while green rust, goethite and magnetite were formed when the Fe(II)/Cu(II) molar ratio reached 4–6. Also, the dechlorination of CCl_4 by dissolved Fe(II) is pH-dependent. In the absence of Cu(II), CCl_4 was dechlorinated only in the basic solutions and the k_{obs} for CCl_4 dechlorination ranged between 0.0097 and 0.0946 h^{-1} at pH 7.5 - 8.5. However, CCl_4 could be dechlorinated by Fe(II) in a wide range of pH values in the Cu(II)-amended solutions, and the k_{obs} increased from 0.0057 h^{-1} at pH 4.3 to 0.856 h^{-1} at pH 8.5, which was16 times greater than that in the Fe(II) solutions without Cu(II). Results obtained in this study enhance the understanding of the role of Fe(II) with dissolved Cu(II) and the long-term reactivity of PRB systems in the dechlorination processes for chlorinated organic contaminants .

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Chapter 5

ENHANCED DECHLORINATION OF CHLORINATED METHANES AND ETHENES BY GREEN RUST WITH COPPER IONS

Enhanced dechlorination of carbon tetrachloride (CCl_4), tetrachloroethene (C_2Cl_4) and trichloroethene (C_2HCl_3) by Cu(II) amended green rust chloride (GR(Cl)) was investigated. The crystallization and chemical speciation of the secondary mineral phase produced from the GR(Cl)-Cu(II) system were characterized by XRD and XPS, respectively. All the chlorinated aliphatics followed first-order kinetics in the dechlorination. An 80% of initial concentration (20 μM) of CCl_4 was dechlorinated by GR(Cl) (0.0015g mL^{-1}) at pH 7.2 within 20 h with the observed rate constant (k_{obs}) of 0.0808 h^{-1}. Addition of 0.5 mM Cu(II) completely dechlorinated CCl_4 during 35 min. The k_{obs} was 84 times greater than that in the absence of Cu(II). Chloroform ($CHCl_3$), the major chlorinated product in CCl_4 dechlorination, was accumulated up to 13 μM in the GR(Cl) suspension. However, the addition of Cu(II) into GR(Cl) suspension could completely dechlorinate $CHCl_3$ within 9 h. The solid phase analysis by XRD suggested that addition of Cu(II) increase the ability of green rust conversion to magnetite providing more electrons for reducing reactions and also the reduction of Cu(II) to elemental Cu. It also found that Cu(I) is formed and released into the liquid phase. The efficiency for the C_2Cl_4 dechlorination by GR(Cl) was relatively slower and only 33% of initial C_2Cl_4 (17.8 μM) was dechlorinated during 40 days. Addition of 0.5mM Cu(II) into GR(Cl) suspension enhanced the reactivity of GR(Cl) and a 93% of the initial C_2Cl_4 was dechlorinated within 40 d with k_{obs} of 0.0852 h^{-1}, which was 4.7 times higher than that without the addition of Cu(II). The major chlorinated product of C_2Cl_4 dechlorination was C_2HCl_3 and addition of 1.0 mM Cu(II) into GR(Cl) could increase the k_{obs} for C_2HCl_3 dechlorination up to 7 times. The increase in k_{obs} was proportional to Cu(II) concentration ranged between 0.1 and 1.0 mM. When the Cu(II) concentration exceeded 1.0 mM, the k_{obs} for both C_2Cl_4 and C_2HCl_3 dechlorination decreased coupling with the decrease in pH. The pH of the GR(Cl) system was an important factor for the dechlorination and the rate constants increased with increasing pH within the studied range of 5.5 ~ 9.0

pH. However, the highest efficiency and rate of dechlorination was obtained at near neutral pH whenCu(II) was added in to GR suspension.. The results obtain in this study clearly shows that green rust can reduce chlorinated hydrocarbons including CCl_4, C_2Cl_4 and C_2HCl_3 and inorganic cation Cu(II). The couple reduction of chlorinated compound and Cu(II) shows a synergistic effect on the dechlorination.

5.1 INTRODUCTION

The reduction of environmental hazardous chemicals by ferrous ions has recently received a great attention as a potential pathway for the natural attenuation. In addition to the dissolved and surface-bound Fe(II) species, mixed-valence iron minerals mainly magnetite and green rust (GR) also contain a large portion of Fe(II) in structures[1,2] and may contribute to the attenuation and treatment of reducible contaminants in soil or ground water. Green rust (GR) is a double layered hydroxides built upon $Fe(OH)_2$-like sheets with anionic interlayer that form platy crystals. The characteristics of the green rust depend on the type of anion presented in the interlayer.[3,4] Based on the interlayer anion, green rust can be divided into two types: type I (GR1) contains halogen ions (e.g. Cl^-) and planar molecules (e.g., CO_3^{-2}), whereas Type II contains three-dimensional molecules (e.g SO_4^{-2}). The divalent anions containing green rust have a general formula of

$$[Fe^{II}_{(6-x)}Fe^{III}_{x}(OH)_{12}]^{x+}[(A)_{x/n};yH_2O]^{x-} \tag{5-1}$$

where x = 0.9 – 4.2, A is an anion with *n* valence state and *y* is varying numbers of interlayer water molecules with typical values of 2 – 4[5,6,7,8,] GR(Cl) is rhombic and GR(SO_4) and GR(CO_3) are hexagonal. Abdelmoula et al.[8] reported that the chemical formula of GR(Cl) is $[Fe_3^{II}Fe^{III}(OH)_8][Cl^{\cdot}2H_2O]$, where the Fe(II)/Fe(III) stoichiometric ratio is 3 deviating from that for divalent anion containing green rust.

All the green rusts have reactive Fe(II) groups that can act as reductant. Green rusts are naturally produced by both abiotic and biotic reactions under alkaline and neural suboxic conditions. Abiotically, GRs are formed as intermediate product in the formation of iron oxides during the oxidation of iron(II) at neutral and weakly alkaline pHs[9], the abiotic reductive dissolution of Fe(III) oxyhydroxides by Fe(II)[10], and the transformation of poorly crystalline hydrous ferric oxides by Fe(II) sorption.[9,11] Biological formation of GRs has been observed in the process of microbial oxidation of Fe(II)[12,13] and in the reductive dissolution of

Fe(III) minerals during Fe(III) mineral reduction by dissimilatory iron reducing bacteria (DIRB).[14,15-18] The finding of frequent availability of green rusts in anoxic hydromorphic soils where DIRB are among the most metabolically active bacterial species is a direct evidence of GR formation by biological Fe(III) reduction.[19-21] Green rust is also formed in the process of both microbial and abiotic induced corrosion of steels.[7,22] Moreover, green rust is thought to play a vital role in the redox reactions in the systems contain surface-bound Fe(II) associated with Fe(III) minerals.[23-25] Depending on the environmental conditions such as pH, available type and concentrations of anions or cations, and oxidation condition, GRs can be further oxidized to other minerals. Magnetite is usually found as the major oxidation product of green rusts under suboxic condition at near neutral or alkaline pH.[3,18,26-31]

Recently, green rusts have been used as a possible natural reductant to reduce various classes of chemical compounds in the contaminated groundwaters and soils because of their abundance in hydromorphic soils and strong reduction potentials. The target compounds those have been reported to be reduced by green rust include inorganic such as U(VI)[26], Se(VI)[27,28], Cr(VI)[29-31], nitrate[9,32] and chlorinated hydrocarbons such as chlorinated ethanes[4,5,29,33], chlorophenyl hydroxylamine[33], and chlorinated ethenes.[34,35] In contrasts to the surface-bound Fe(II) species, GR has the capability to dechlorinate both chlorinated methanes and ethenes and produces low concentrations of chlorinated intermediates in the dechlorination process.[35] Recently, O'Loughlin et al. found that the chlorinated methanes and ethanes can be more effectively dechlorinated by GR(SO_4) in the presence of transition metal ions including Ag(I), Au(II), Cu(II) and Hg(II).[3,4,36] The dechlorination by GR(SO_4) was much faster and produced less chlorinated products in the presence of metal ions compared to GR(SO_4) alone, presumably due to the formation of nano-sized zerovalent metal particles.[3,36] This finding gives impetus that GRs is a possible mineral for the natural attenuation of chlorinated organic compounds together with toxic inorganic ions. However, the effect of environmental conditions on dechlorination of chlorinated hydrocarbon by GR has received less attention. Moreover, green rust has been identified on the surface of zerovalent iron in the process of corrosion under oxygen-limited conditions and it could play a vital role in long-term performance of permeable reactive barriers (PRBs). Rafait et al.[7] found the formation of GR(Cl) in aqueous corrosion of metal iron in the presence of chloride ions. However, most of reported works have only focused on the reactivity of GR(SO_4) for the dechlorination. Although GR(Cl) has been used for the reduction of other inorganics such as Cr(VI)[30], nitrate[32], the reactivity of

GR(Cl) has not been studied on the dechlorination of chlorinated hydrocarbons.

The objective of this study was to investigate the reactivity of green rust chloride GR(Cl) on the reduction of chlorinated methanes and ethenes including carbon tetrachloride (CCl_4), trichloroethylene (C_2HCl_3) and tetrachloroethylene(C_2Cl_4) in the absence and presence of Cu(II) ion. The environmental parameters including pH, concentrations of GR(Cl) and chlorinated compounds, and the concentration of Cu(II) on the dechlorination rate and efficiency were also studied. X-ray powder diffractometer (XRPD) and X-ray photoelectron spectrometer (XPS) were used to identify the crystalline properties and changes in chemical species of the solid phase in the GR(Cl) suspensions. Also, the changes in morphologies of solids were studied using transmission electron microscope (TEM).

5.2 MATERIALS AND METHODS

5.2.1 Chemicals.

The following chemicals used in this study were used as received without further treatment. Carbon tetrachloride (CCl_4 > 99.8%, GC grade), chloroform ($CHCl_3$ > 99.8%, GC grade), $CuCl_2{\cdot}2H_2O$ and tris (hydroxymethyl) aminomethane (TRIS buffer) were purchased from Merck Co.(Darmstadt, Germany). Trichloroethylene (C_2HCl_3) (> 99.8% GC grade), tetrachloro-ethene (C_2Cl_4) (99.8%, GC grade), $FeCl_2{\cdot}4H_2O$ (99%), N-(2-hydroxyethyl) piperazine-N-(2-ethanosulfonic acid (HEPES) (99.5%), 2-(N-morpholine)-ethanosulfonic acid (MES) (> 99.5%), hydrochloric acid (HCl) (37%), and sodium hydroxide (NaOH) (> 98%) were obtained from Sigma-Aldrich Co. (Milwaukee, WI). Bathocuproinedisulfonic acid disodium salt ($C_{26}H_{18}N_2Na_2O_6S_2$) (90%) was purchased from Fluka (Buchs, Switzerland). All the chemical solutions were prepared using high purity deoxygenated deionized water (Millipore, 18.3 Ωcm) with vacuum and N_2 (>99.9995%) purging system.[37-39]

5.2.2 Synthesis and characterization of GR(Cl)

Green rust chloride was synthesized by partial oxidation of ferrous chloride solution at neutral pH[1]. Briefly, 49.7 g of vacuum-desiccated $FeCl_2{\cdot}4H_2O$ was dissolved in 1-L of deionized water in a 2-L beaker placed on a magnetic stirrer at an ambient condition. After the ferrous salt was completely dissolved, a solution of 1M NaOH was added drop wise using stopcock funnel until the pH was maintained at 7.0 ~ 7.2 with vigorous stirring the suspension. The suspension of the dark blue-green precipitate was then transferred into a 1-L bottle capped with screw cap. The bottle was repeatedly vacuumed (5 x 10 $^{-3}$ mm Hg) followed by N_2 purging for several times with continuous stirring of the suspension. The suspension was kept anaerobically for one week and the pH was re-adjusted to7.0 ~ 7.2 using NaOH solution. After the pH was stable, 50 mL aliquots of the suspensions were transferred from the sealed bottle into a centrifuge tube using a N_2-purged 100-mL plastic syringe. The supernatant was removed by centrifugation at 10,000 rpm for 10 min under N_2 atmosphere. The GR(Cl) pellets in the centrifuge tubes were washed using deoxygenated deionized water for 5 times to remove the residual Fe(II) in solution. After the washing, the GR(Cl) suspension was transferred into a 500-mL serum bottle under N_2 purge. The bottle was capped followed by vacuuming and N_2 purging repeatedly for several times and then was stored under N_2 atmosphere.

5.2.3 Quantification of GR(Cl) concentration

The Fe(II)/Fe(III) ratio of synthesized GR(Cl) was determined to quantify the GR(Cl) concentration. The stock suspension of GR(Cl) was withdrawn using a 1-mL N_2-purged syringe and was delivered into a 70-mL serum bottle containing 49 mL of deoxygenated deionized water. One milliliter of the well-mixed suspension was then withdrawn by a N_2-purged 1-mL syringe and the dissolved and total concentrations of the Fe(II) in GR(Cl) suspension was determined by the ferrozine method.[37-39] The total iron (both Fe(II) and Fe(III)) concentration of the GR(Cl) suspension was also determined after adding 1mL of 10% hydroxylammonium chloride to reduce Fe(III) to Fe(II).[34] The concentration of Fe(III) was calculated from the difference in the total Fe(II) concentration of the suspension before and after the reduction. The determined dissolved and total Fe(II) concentrations were 3.75 mM and 16.65 mM, respectively. This depicts that the Fe(II) concentration in GR(Cl) was 12.9 mM. After reducing the Fe(III), the total Fe(II) concentration was found to be 20.71 mM. Since the solubility of Fe(III) is quite low at neutral pH, it can be assumed that the 4.05 mM of Fe(III) was from the GR(Cl). Therefore, the molar ratio of Fe(II)/Fe(III) in GR(Cl) is 3.17, which is in a good agreement with the reported value of 3.[5] This also means that the concentration of GR(Cl) (w/v) in the serum bottle that was treated with 1mL of stock GR(Cl) suspension and contained a total liquid phase volume of 50mL was 0.0015 g mL^{-1}. The high concentration of the dissolved Fe(II) in the suspension is probably due to dissolution of GR(Cl) or due to slow desorption of Fe(II) that had been sorbed on to solid GR(Cl) surfaces from the Fe(II) ions in the solution during the synthesis process. 5.2.4 Dechlorination Experiments Dechlorination of chlorinated methanes and ethenes by GR(Cl) was studied using 70-mL serum bottles those were purged continually with a gentle flow of N_2.[37] Buffer solution that was degassed by repeated vacuuming and N_2 purging in sealed 1-L bottles were delivered in to serum bottles using N_2 purged 50-mL air tight plastic syringe. Then, 1.0 mL aliquot of GR(Cl) from the stock suspension was delivered in to the serum bottle using N_2-purged 1-mL plastic syringe. To study the effect of GR(Cl) concentration on the dechlorination, different volumes of GR(Cl) stock suspension (ranging 0.25 mL ~ 4.00 mL) were delivered and followed by a 0.5 mL of $CuCl_2$ from the deoxygenated 150 mM stock solution was introduced using N_2- purged 1-mL syringe. In the studies of the effect of Cu(II) concentration on the dechlorination by GR(Cl), various volumes of $CuCl_2$ were injected. The total liquid volume in the serum bottle was maintained at 50mL. The serum bottles were sealed with Teflon lined rubber septa and aluminum crimp caps. Then the bottles were incubated for equilibrium in an orbital shaker with shaking speed of 150rpm at 25 ºC in dark. Upon 20 h of equilibrium, target organic compound (CCl_4, C_2HCl_3 or C_2Cl_4) was injected from the stock solutions in degassed methanol, using 50-µL gas-tight glass syringes. For the blank controls target organic compounds were also injected in to serum bottles contained only 50 mL of buffer solutions. After the chlorinated compound was injected, serum bottles were placed on the incubator and were subjected for the analysis of target chlorinated compounds as well as Fe(II) concentrations and copper ion concentrations at different time intervals. For the periodical analysis of Fe(II) and copper ions, separate bottle sets were used. After the termination of the dechlorination studies pH and the Oxidation-reduction potentials (ORP) in the bottles were measured upon opening bottles under gentle purge of N_2 at 25 °C

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aliquot of GR(Cl) from the stock suspension was delivered in to the serum bottle using N_2-purged 1-mL plastic syringe. To study the effect of GR(Cl) concentration on the dechlorination, different volumes of GR(Cl) stock suspension (ranging 0.25 mL ~ 4.00 mL) were delivered and followed by a 0.5 mL of $CuCl_2$ from the deoxygenated 150 mM stock solution was introduced using N_2- purged 1-mL syringe. In the studies of the effect of Cu(II) concentration on the dechlorination by GR(Cl), various volumes of $CuCl_2$ were injected. The total liquid volume in the serum bottle was maintained at 50mL. The serum bottles were sealed with Teflon lined rubber septa and aluminum crimp caps. Then the bottles were incubated for equilibrium in an orbital shaker with shaking speed of 150rpm at 25 ºC in dark. Upon 20 h of equilibrium, target organic compound (CCl_4, C_2HCl_3 or C_2Cl_4) was injected from the stock solutions in degassed methanol, using 50-µL gas-tight glass syringes. For the blank controls target organic compounds were also injected in to serum bottles contained only 50 mL of buffer solutions. After the chlorinated compound was injected, serum bottles were placed on the incubator and were subjected for the analysis of target chlorinated compounds as well as Fe(II) concentrations and copper ion concentrations at different time intervals. For the periodical analysis of Fe(II) and copper ions, separate bottle sets were used. After the termination of the dechlorination studies pH and the Oxidation-reduction potentials (ORP) in the bottles were measured upon opening bottles under gentle purge of N_2 at 25 °C.

5.2.5 Analytical techniques

Headspace analytical technique was performed for the analysis of target organics and their chlorinated byproducts. For the analysis of CCl_4 and its major byproduct, 50 µL of gas from the headspace of reactor bottle was withdrawn using a 100-µL gas-tight glass syringe. In the case of C_2Cl_4 and C_2HCl_3 analysis, 60 µL volume of headspace was withdrawn. The headspace samples were immediately injected into the gas chromatograph (GC) equipped with electron capture detector (ECD) and flame ionization detector (FID) (Perkin-Elmer, Autosysem, Norwark, CT). A 60-m VOCOL fused-silica megabore capillary column (0.545 mm × 3.0 µm, Supelco Co.) was used for the separation of organic compounds and the column was simultaneously connected with both ECD and FID using Y-splitter. For the study of concentration effect on the dechlorination, high C_2Cl_4 concentrations (up to 81 µM) were used and only FID was used for C_2Cl_4 analysis. In such case the column was disconnected from ECD and both the column end and ECD connector were capped. The column temperature was maintained isothermally at 90 °C with nitrogen (N_2) as the carrier gas. The injector temperatures of ECD and FID were maintained at 350°C and 250 °C, respectively. The concentrations of chlorinated compounds in the buffer solutions were calculated using external standard method by preparing known concentrations of chlorinated hydrocarbons under the identical condition using procedures those used for the analysis of samples from the reactor bottles. The relative standard deviations (RSD) of ECD analysis were within 10% and that for FID analysis were within 5%. Control blanks were used to test the possible leakage of target compounds during the incubation.

XRPD for the characterization of mineral phases was performed using an X-ray diffractometer (Regaku D/max-II B) and a Cu Kα-radiation source (λ = 1.54056 Å) that was operated with 30 kV voltage and 20 mA current to identify the crystal phases of the precipitate. The precipitates were allowed to settle down after the termination of dechlorination experiment and the supernatant was removed carefully from the sealed bottles using the N_2–purged syringe. After drying the precipitate by a gentle stream of N_2, samples were mounted on a glass sample holder using small amounts of grease. A drop of glycerol was immediately added on the mounted powder layer to minimize the reaction with oxygen. The scan range for all samples was between 5 and 90° (2θ) at a scanning speed of $4°min^{-1}$. For the morphology studies of the solid phase in GR(Cl)-Cu(II) suspension using transmission electron microscopy (TEM), samples were treated in the same way that for XRD analysis.

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. The acidified samples were centrifuged at 10,000 × g for 10 min to remove particles and Fe(II) contents were determined by colorimetric method using ferrozine as chelating agent. The complex has the maximum absorption at 562 nm.[37-39] Absorptions were measured using UV-visible spectrophotometer (Hitachi, U3010). The dissolved fraction of Fe(II) was determined in the filtrates (0.2-μm cellulose acetate filter) acidified with 0.5 mL of 1 M HCl, and the sorbed Fe(II) was calculated from the difference between total and dissolved concentrations. Also, the total concentrations of iron species were determined using inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer, Optima 3000XL).

The concentration of extractable Cu(I) was determined using the bathocuproinedisulfonic acid method with minor modifications[40]. A 1 mL of aliquot was withdrawn by a N_2-purged plastic syringe and immediately added into the mixture containing 1 ml of 10 % tatrate solution and 0.5 mL of 1 % bathocuproinedisulfonic acid solution. After 30 min of reaction, the mixture was centrifuged at 10,000 × g for 5 min. The Cu(I) concentration in the supernatant was then determined colorimetric method at 483 nm. Standard solutions of Cu(I) were prepared using 10 % hydroxylammonium chloride as a reductant to reduce $CuCl_2$ solutions.

5.3 RESULTS AND DISCUSSION

5.3.1 Dechlorination of CCl_4 by GR(Cl)

Green rust has a high capability for CCl_4 dechlorination. Figure 5-1 shows the dechlorination of CCl_4 by GR(Cl) and the production of chloroform ($CHCl_3$) in the presence and absence of 0.5 mM Cu(II) at pH 7.2. A 80% of the initial CCl_4 (20 μM) was dechlorinated by GR(Cl) within 20 h in the absence of Cu(II). Chloroform was identified as the major product for CCl_4 dechlorination. The maximum detected $CHCl_3$ concentration was 13.5 μM, which accounted for 85% of the CCl_4 dechlorination. O'Loughlin et al.[3] also reported a similar percentage of mass balance for CCl_4 dechlorination by GR(SO_4) suspension (0.0015 g ml^{-1}) after 70 h of incubation. However, the mass balance of CCl_4 dechlorination by GR(Cl) is higher than that by structural surface-bound iron system.[37] The disappearance of CCl_4 followed first order reaction kinetics and the pseudo-first-order rate constant (k_{obs}) for CCl_4 dechlorination was 0.0808 h^{-1}, which is close to the upper margin of range of the reported k_{obs} values ($0.47 \times 10^{-5} \sim 2.18 \times 10^{-5}\ s^{-1}$) by GR($SO_4$)[3,41] The addition of Cu(II) into the GR(Cl) suspension greatly enhanced the dechlorination efficiency and rate of CCl_4. A nearly complete dechlorination of CCl_4 by GR(Cl) suspensions was observed within 35 min in the presence of 0.5 mM Cu(II). The k_{obs} for CCl_4 dechlorination was 6.779 h^{-1}, which was 84 times higher than that in GR(Cl) system without Cu(II) amendment. The concentration of $CHCl_3$ increased rapidly with a concomitant disappearance of CCl_4. The maximum $CHCl_3$ concentration of about 13 μM was observed at 30 min and decreased with time. A complete degradation of $CHCl_3$ was also observed after 10 h of incubation. The dechlorination also followed first-order kinetics and the k_{obs} for $CHCl_3$ dechlorination was 0.3513 h^{-1}. However, no other chlorinated product was detected by GC-ECD, presumably due to the production of non-chlorinated products. Several studies investigated the degradation pathways of chlorinated hydrocarbon including chlorinated methanes including carbon tetrachloride in the presence of iron compounds. [3,42,43] CCl_4 can be converted in to non-chlorinated products methane by repeated hydrogeneolysis of chloroform or form formate (CHCOO-) through hydrolysis of dichlorocarbines as described in Chapter 1.

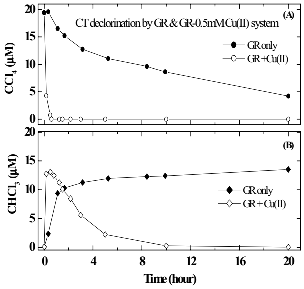

Figure 5-1. Dechlorination of 20 μM carbon tetrachloride (CCl_4) by green rust chloride (GR(Cl)) (0.0015 g mL^{-1}) at pH 7.2 in the absence and presence of 0.5 mM Cu(II). (a) The concentration of CCl_4 and, (b) The concentration of chloroform (CF) as the major by product.

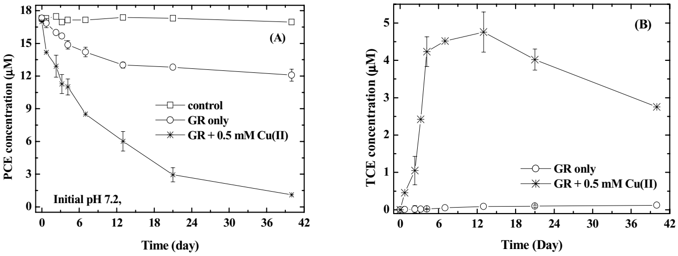

Figure 5-2. Dechlorination of C_2Cl_4 (17.8 μM) by GR(Cl)(0.0015 g mL^{-1}) in the absence and presence of 0.5 mM Cu(II), at pH 7.2. (a) C_2Cl_4 concentration profile and (b) C_2HCl_3 formation as the major chlorinated product.

5.3.2 Dechlorination of chlorinated ethenes by GR(Cl).

The reductive dechlorination of chlorinated ethenes by $GR(SO_4)$ has been previously studied.[34] However, the reactivity of GR(Cl) towards the dechlorination and the effect of Cu(II) on the reductive dechlorination of chlorinated ethenes remain unclear. Figure 5-2 shows the dechlorination of 18 μM C_2Cl_4 by GR(Cl) suspension (0.0015g mL^{-1}) in the absence and presence of Cu(II) ion at pH 7.2. C_2Cl_4 was dechlorinated rapidly during the first 12 days and then a slow dechlorination was observed. A degradation efficiency of 33% was observed after 40 days of incubation, which is similar to the previous studies.[34] The k_{obs} for C_2Cl_4 dechlorination was calculated to be 0.0182 d^{-1}. Only a trace amount of C_2HCl_3 was detected by GC-ECD, as the major chlorinated intermediate for C_2Cl_4 dechlorination, which accounted for a 2% of C_2Cl_4 dechlorination. Lee and Batchelor[34] recently reported that no C_2HCl_3 was detected during the dechlorination of 190 μM C_2Cl_4 by GR(SO_4). However, acetylene and ethylene with the total carbon mass balance of 70% had been observed as the major products. This suggests that β-elimination could be the major dechlorination pathway for C_2Cl_4 by green rust.

Similar to the degradation of CCl_4 by GR(Cl), the dechlorination efficiency and the rate of C_2Cl_4 was significantly enhanced in the presence of 0.5 mM Cu(II). A 93% of the initial C_2Cl_4 concentration (18 μM) was dechlorinated by GR(Cl) at 0.5 mM Cu(II) within 40 days od incubation. The dechlorination reaction followed the pseudo first-order kinetics and the rate constant (k_{obs}) for C_2Cl_4 dechlorination was 0.0852 d^{-1}. C_2HCl_3 was also found to be the major chlorinated byproduct identified by GC-ECD headspace analysis during C_2Cl_4 dechlorination by GR(Cl)-Cu(II) system. A maximum C_2HCl_3 concentration of 4.75 μM was observed at the13th day of the reaction and gradually decreased to 2.75 μM after 40 d of incubation, showing that the amendment of 0.5 mM Cu(II) ions in to the suspension can significantly enhance the dechlorination capability of green rust.

5.3.3 Concentration effect of Cu(II)

The dechlorination efficiency of C_2HCl_3 by GR(Cl) increased from 50% at 0.5 mM Cu(II) to 62 % at 1 mM Cu(II) after 35 d of incubation. However, further increase in Cu(II) concentration resulted in the decrease in the efficiency of C_2HCl_3 dechlorination, presumably due to the consumption of the reactive Fe(II) sites on GR(Cl) by Cu(II). Moreover, the dechlorination of C_2HCl_3 followed first-order reaction kinetics. Figure 5-4 shows the k_{obs} for C_2HCl_3 dechlorination as a function of Cu(II) concentrations.

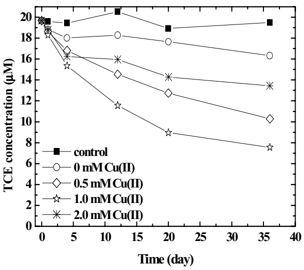

Figure 5-3. Dechlorination of 20 µM C_2HCl_3 by GR(Cl) (0.0015 g mL^{-1}) in the absence and presence of Cu(II) (0.5 mM ~ 2.0 mM). The pH of the system was maintain at 7.2 using HEPES (50 mM) buffer at 25 °C

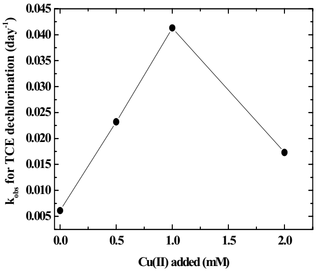

Figure 5-4. The effect of the added Cu(II) concentration (0 mM ~ 2.0 mM) on the observed rate constant (k_{obs}) for dechlorination of 20 µM C_2HCl_3 by GR(Cl) (0.0015 g mL^1). The pH of the system was maintained at 7.2 using HEPES (50 mM) buffer at 25 °C in the dark

A linear relationship between k_{obs} and the added Cu(II) concentration ranging between 0 and 1.0 mM was observed. Further increase in Cu(II) concentration up to 2 mM decreased the k_{obs}, clearly showing that the amendment of high concentration of Cu(II) inhibits the dechlorination of C_2HCl_3 by GR(Cl). The relationship between Cu(II) concentration and dechlorination capability of GR(Cl) was further investigated within a wide range of Cu(II) concentrations from 0.1 to 5 mM using C_2Cl_4 as the target compound. Also, a high concentration of GR(Cl) suspension (0.003 g mL^{-1}) was added and the pH in GR suspension was maintained at pH 7.2 using 50 mM HEPES buffer. Figure 5-5 shows the dechlorination of C_2Cl_4 by GR(Cl) at various concentrations of Cu(II). Similar to the dechlorination of C_2HCl_3, the dechlorination efficiency of C_2Cl_4 increased from 41% at 0.1 mM to 91.6% at 1 mM within 40 day. Further increase in the concentration of Cu(II) decreased the rate and efficiency of C_2Cl_4 dechlorination. When the concentration of amended Cu(II) was 5.0 mM, the dechlorination capability of GR(Cl) was lower than that in the un-amended system. The k_{obs} for C_2Cl_4 dechlorination as a function of Cu(II) concentration is shown in Figure 5-6. The k_{obs} values were proportional to the Cu(II) concentration and increased linearly from 0.0084 d^{-1} in the absence of Cu(II) to 0.138 d^{-1} at 1 mM Cu(II). Addition of high concentration of Cu(II) lowered the k_{obs} for C_2Cl_4 dechlorination, presumably due to the oxidation of Fe(II) in GR(Cl) and the decrease in pH of the system. Figure 5-6 also shows the decreased concentration of Fe(II) due to the addition of various concentrations of Cu(II) into the GR(Cl) suspension.

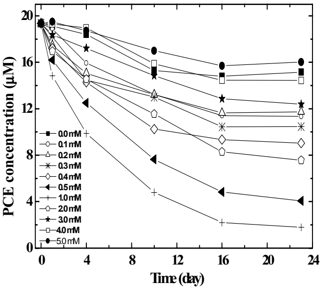

Figure 5-5. The Effect of the concentration of Cu(II)(0~5.0 mM) amended with GR(Cl) (0.003 g mL^{-1}) on the dechlorination of C_2Cl_4 (19 μM) in pH 7.2 50 HEPES (50mM) buffer solution.

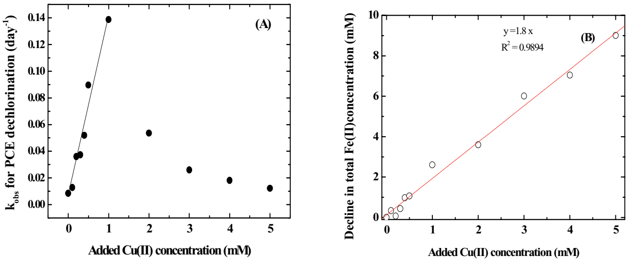

Figure 5-6. Effect of Cu(II)(0 ~ 5.0 mM) amended with GR(Cl) (0.003 g mL^{-1}) in 50 mM HEPES (initial pH=7.2) buffer solution on (a) rate constant (k_{obs}) for the dechlorination of C_2Cl_4 (19 μM) and (b) the decrease in total Fe(II) concentration.

Moreover, Table 5-1 demonstrates the concentrations of total Fe(II) (1M HCl extractable Fe(II)) and Cu(I) in the GR(Cl) systems with various concentrations of Cu(II). The total Fe(II) concentrations decreased with increasing Cu(II), showing that Fe(II) was oxidized by Cu(II). It is noted that a slight decrease in Fe(II) was observed in the GR(Cl) suspension without Cu(II). This oxidation may be due to the formation of secondary mineral phase on the surface of green rust. Elsner et al.[33] has recently observed a partial conversion of $GR(SO_4)$ into a black-coloured secondary mineral phase (probably magnetite) in neutral MOPS-buffered medium. In this study, however, no visible color change was observed in the GR(Cl) suspension in HEPES buffer at pH 7.2. Instead, the amendment of Cu(II) in the GR(Cl) suspension changed the colour from green to black with increasing Cu(II) concentration, presumably attributed to the formation of magnetite.[36] Particularly, the colour was completely changed to black when the Cu(II) concentration was above 2 mM. Moreover, a good linear relationship between Cu(II) concentration and the decreased Fe(II) concentration was observed (Figure 5-6b). The slope of 1.8 depicts that more than one Fe(II) is oxidized by one Cu(II) suggesting that Fe(II) in the GR(Cl) suspension could reduce Cu(II) into both Cu(I) and elemental Cu:

$$2\ \mathrm{Fe(II)}_{\mathrm{GR(Cl)}} + \mathrm{Cu(II)} \rightarrow 2\ \mathrm{Fe(III)}_{\mathrm{Mag}} + \mathrm{Cu(0)} \qquad (5\text{-}2)$$

$$\mathrm{Fe(II)}_{\mathrm{GR(Cl)}} + \mathrm{Cu(II)} \rightarrow \mathrm{Fe(III)}_{\mathrm{Mag}} + \mathrm{Cu(I)} \qquad (5\text{-}3)$$

$$\mathrm{Fe(II)}_{\mathrm{dissolved}} + \mathrm{Cu(II)} \rightarrow \mathrm{Fe(III)} + \mathrm{Cu(I)} \qquad (5\text{-}4)$$

Table 5-1. The total Fe(II) concentrations (initial and after 30 h equilibrium) and extractable Cu(I) concentration in green rust suspension (0.003g mL^{-1}) at various concentrations of Cu(II).

Added Cu(II) concentration (mM)	Extractable Cu(I) concentration (mM)	Total Fe(II) concentration (mM)	
		Initial	After 30h
0		1.70	9.65
0.1	.069	1.70	9.32
0.2	.105	1.70	9.59
0.3	.091	1.70	9.22
0.4	.127	1.70	8.69
0.5	.126	1.70	8.19
1.0	.184	1.70	7.05
2.0	.186	1.70	6.05
3.0	.236	1.70	3.65
4.0	.244	1.70	2.61
5.0	.249	1.70	0.65

Previous studies showed that Cu(II) could be reduced to metallic Cu by the oxidation of Fe(II) in GR(SO_4).[3,4,36] However, a possible mechanism for such reaction remains unclear. Moreover, the generation of Cu(I) from the reduction of Cu(II) in the surface-bound Fe(II) system has been observed.[37] These implies that Cu(II) could be reduced to both Cu(I) and Cu(0) by structural Fe(II) in GR(Cl). To elucidate this possibility, the Cu(I) concentration in the solution was first measured after the equilibrium. The Cu(I) concentration increased rapidly from 0.05 to 0.2 mM when Cu(II) concentration increased from 0.1 to 1 mM (Table 1). With the increase in Cu(II) concentration to 3 mM, the generated Cu(I) maintained at a relative stable concentration of 0.26 mM. This behaviour is different from the surface-bound Fe(II) and the previous studies using GR(SO_4).[3,4] In this study, the decrease in Fe(II) concentration is much higher than production of Cu(I), implying that the system may produce metallic Cu. Therefore, the solid phase was analyzed for possible Fe(II) and Cu species.

Beside the oxidation of Fe(II), another plausible reason for the decreased rate constant at high Cu(II) concentration is the changes in pH and redox potentials in GR(Cl) system. Figure 5-10 shows that change in pH and redox potential of the aqueous solution in GR(Cl) suspensions with various concentrations of Cu(II) after the termination of experiments. The pH values decreased with increasing Cu(II) concentrations. At low concentration of Cu(II), the pH values decreased from 7.2 in the absence of Cu(II) to 6.98 pH at 1 mM Cu(II). Also, the measured redox potential increased gradually from –555 to –500 mV when the added Cu(II) concentration increased from 0 to 1 mM. Further increase in Cu(II)concentration decreased the pH to lower than 7 and increased the redox potential up to –380 mV at 5 mM Cu(II). Therefore, the decrease in the C_2Cl_4 dechlorination by GR(Cl) at high Cu(II) concentration may be due to the decrease in pH and the increase in redox potential.

5.3.4 Solid-phase analysis of GR(Cl)–Cu(II) suspension.

XRPD and XPS were used to identify the crystal phases and chemical species of the solid in the GR(Cl) systems. As shown in Figure 5-7, two peaks centered at 962.9 and 953 eV were clearly shown in the XPS spectra, which could be assigned as Cu $2p_{3/2}$ and Cu $2p_{1/2}$,repectively. This means that the major copper species in Cu(II)-amended GR(Cl) system should be metallic Cu (Cu°). In order to characterize the crystalline properties of Cu and Fe in the heterogeneous system, the solid phases were further characterized by XRPD. Two green rust peaks at 12.08° and 23.596° 2θ were clearly appeared in the XRPD patterns in the absence of Cu(II). Hansen et al.[32] also observed similar XRPD pattern for GR(Cl). Addition of Cu(II) changed the crystalline property of green rust. Peaks at 35.46°, 43.31°, 56.96° and 62.79° 2θ, which represented magnetite (Fe_3O_4) were observed when various concentrations of Cu(II) were amended into the suspensions (Figure 5-8). Also, the major peaks for elemental Cu at 43.47°, 50.37° and 73.99° 2θ were observed. Perhaps the third major peak for magnetite at 43.34° 2θ and the first dominant peak for elemental Cu at 43.47°2θ might be difficult to be identified separately. It is noted that no

Cu(I) species (Cu_2O) was identified in XRRD pattern or XPS spectra, presumably due to the low concentration of Cu(I) (0 ~ 0.24 mM). The TEM images of GR(Cl) in the absence and presence of 0.5 mM Cu(II) are shown in Figure 5-9. In the absence of Cu(II), GR(Cl) can be seen in rhomboidal shape and in addition some cubic shape magnetite particles also can be seen due to possible oxidation of GR(Cl). In contrast to GR(Cl) in the absence of Cu(II), some additional particles with 10~20 nm sizes were appeared when 0.5 mM Cu(II) was added (Figure 5-9b). Those particle might be Cu^0 or Cu(I) containing mineral species.

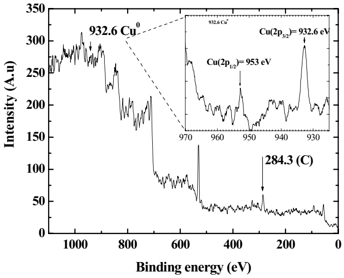

Figure 5-7. XPS spectra for the identification of Cu species in the solid-phase of the suspension of GR(Cl) with 0.5mM Cu(II)

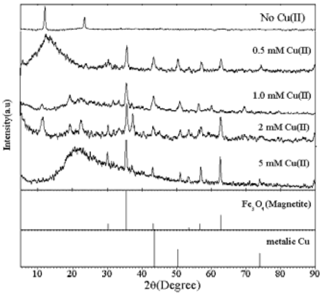

Figure 5-8. XRD pattern of the solid phase of GR(Cl) suspension in the presence of various Cu(II) concentrations

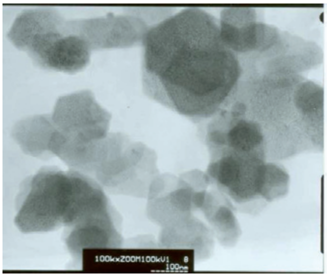

Figure 5-9a. The TEM image of the GR(Cl)

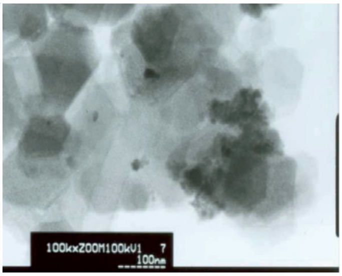

Figure 5-9b.TEM image of the GR(Cl) amended with 0.5mM Cu(II)

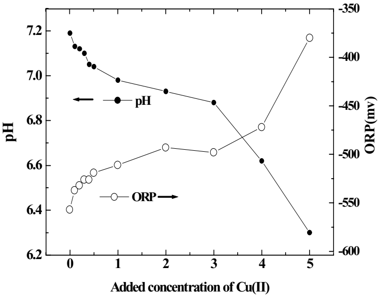

Figure 5-10. The effect of Cu(II) on the pH and oxidation reduction potential (ORP) of the GR(Cl) (0.003g mL^{-1}) suspension. The initial pH was maintained at 7.2 using 50 mM HEPES buffer.

5.3.5 Effect of pH on C_2Cl_4 dechlorination

Green rust (GR(Cl)) is a metastable iron mineral and the hydrated green rust is composed of hydroxide functional groups those could be protonated or deprotonated during the changes in pH values.[34] Also, the structural Fe(II) concentration in green rust is dependent on the pH of the suspension. Figure 5-11 shows the effect of pH on the dechlorination of C_2Cl_4 by GR(Cl) in the absence and presence of 0.5 mM Cu(II). In the absence of Cu(II), the dechlorination efficiency of C_2Cl_4 by GR(Cl) (0.0015 g mL^{-1}) slightly increased from 18.3% at pH 5.5 to 28.7% at pH 9 during 31 days. Trace amounts of C_2HCl_3 within the concentration range of 0.04 ~ 0.12 μM were observed after the termination of experiments (Table 5-2). Moreover, a relatively low concentration of C_2HCl_3 was observed at high pH value, suggesting that further dechlorination of C_2HCl_3 at high pH could be occurred. A previous study has showed that rate constant for C_2HCl_3 dechlorination could increase 3 times when pH was increased from 6.8 to 9.2.[34] Generally, the yield of the dechlorination products was high at the elevated pH conditions during the dechlorination of chlorinated hydrocarbons by surface-bound Fe(II) systems.[44] In contrast, the results obtained in this study using GR(Cl) shows a low concentration of C_2HCl_3 as a byproduct of C_2Cl_4 dechlorination and it may be due to the fast dechlorination of C_2HCl_3 at higher pH conditions. The effect of pH for the dechlorination of C_2Cl_4 by GR(Cl) in the presence of Cu(II) was also studied. The dechlorination efficiency and rate of C_2Cl_4 increased rapidly from 19.8% to 73.6% when the pH values increased from 5.5 to 7.2. Further increasing the pH from 7.2 to 9.0, however, decreased the efficiency of C_2Cl_4 dechlorination to 42.9% (Figure 5-11b). Higher concentrations of C_2HCl_3 ranging between 2 and 5.2 μM were observed in the presence of Cu(II) after 31 days of reaction when compared to that in the absence of Cu(II). The produced C_2HCl_3 concentration also increased in the pH range of 5.5 ~ 7.0 and then decreased at high pH values. It is noted that the C_2HCl_3 concentration in GR(Cl) suspension at pH 7.2 reached the maximum concentration of 4.87 μM in the 5th day of incubation and then decreased to 2.70 μM during experimental course.

Table 5-2. Effect of pH on the dechlorination of C_2Cl_4 by GR(Cl), and the maximum concentration of detected C_2HCl_3 as the major chlorinated product.

	GR(Cl) in the absence of Cu(II)			GR(Cl) with 0.5 mM Cu(II)		
pH	degraded C_2Cl_4 (μM)	C_2HCl_3 (μM)	(C_2HCl_3/ decayed C_2Cl_4)%	degraded C_2Cl_4 (μM)	C_2HCl_3 (μM)	[C_2HCl_3] / [degraded C_2Cl_4] %
5.5	1.68	0.04	2.38	3.3	0.11	3.33
6.0	3.04	0.12	3.86	6.6	2.95	4.69
7.2	3.98	0.089	2.24	12.26	4.87	39.72
8.0	4.36	0.061	1.40	10.06	5.24	52.08
9.0	4.78	0.049	1.025	7.15	2.08	29.09

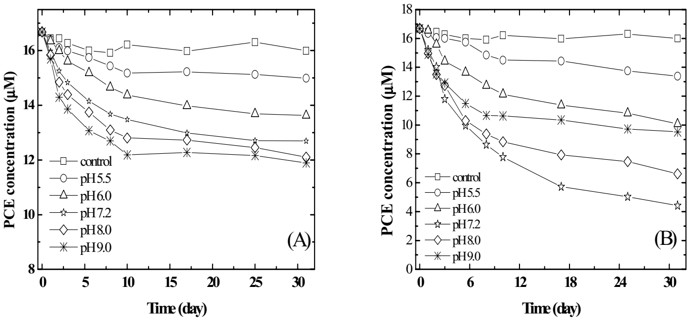

Figure 5-11. The effect of pH on the dechlorination of C_2Cl_4 by GR(Cl)(0.0015 gmL^{-1}) (a) in the absence of Cu(II) and (b) in the presence of 0.5 mM Cu(II).

The dechlorination of C_2Cl_4 by GR(Cl) at various pH values followed pseudo first-order kinetics. The increase in the k_{obs} for C_2Cl_4 dechlorination by GR(Cl) was proportional to the increase in pH values (Figure 5-12). In the absence of Cu(II), the k_{obs} for C_2Cl_4 dechlorination increased slightly from 0.01 d^{-1} at pH 5.5 to 0.037 d^{-1} at pH 9.0. In contrast to the suspensions without Cu(II), the k_{obs} increased from 0.008 d^{-1} to 0.0825 d^{-1} when pH increased from 5.5 to 7.2 and then slightly decreased to 0.063 d^{-1} at pH 9.0 when 0.5 mM Cu(II) was added into the suspensions(Figure 5-12). Although no obvious difference in k_{obs} between the GR(Cl) suspensions in the presence and absence of Cu(II) was observed at pH 5.5, a significant increase in rate constants was observable when Cu(II) was amended into the suspensions in the pH range of 6 ~7. Maximum reactivity on C_2Cl_4 dechlorination was found at near neutral pH in the presence of Cu(II).

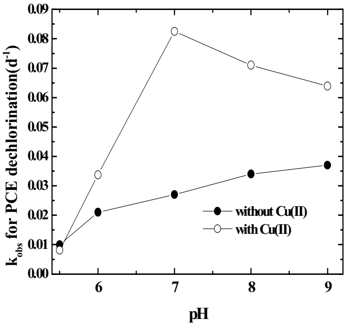

Figure 5-12. The comparison of the observed rate constant (k_{obs}) for C_2Cl_4 dechlorination by GR(Cl) (0.0015 g mL^{-1}) suspension in the absence and presence of 0.5 mM Cu(II) at various pH conditions.

The dechlorination of C_2Cl_4 by GR(Cl) at various pH values followed pseudo first-order kinetics. The increase in the k_{obs} for C_2Cl_4 dechlorination by GR(Cl) was proportional to the increase in pH values (Figure 5-12). In the absence of Cu(II), the k_{obs} for C_2Cl_4 dechlorination increased slightly from 0.01 d^{-1} at pH 5.5 to 0.037 d^{-1} at pH 9.0. In contrast to the suspensions without Cu(II), the k_{obs} increased from 0.008 d^{-1} to 0.0825 d^{-1} when pH increased from 5.5 to 7.2 and then slightly decreased to 0.063 d^{-1} at pH 9.0 when 0.5 mM Cu(II) was added into the suspensions(Figure 5-12). Although no obvious difference in k_{obs} between the GR(Cl) suspensions in the presence and absence of Cu(II) was observed at pH 5.5, a significant increase in rate constants was observable when Cu(II) was amended into the suspensions in the pH range of 6 ~7. A maximum reactivity on C_2Cl_4 dechlorination was found at near neutral pH in the presence of Cu(II). Figure 5-13 shows the changes in total Fe(II) in GR(Cl) suspension at various pH values ranging between 5 and 9. The decrease in total Fe(II) between the 0.5 mM Cu-amended and un-amended systems ranged from 1 to 1.8 mM, presumably due to the two-electron transfer between Cu(II) and Fe(II) (equation 5-2). It is noted that the decreased amount of Fe(II) at neutral pH is highest. The decrease in total Fe(II) could be due to the fixation of Fe(II) onto the ferric oxide. Several studies have reported that Fe(II) could be fixed to Fe(III)-containing mineral surfaces, resulting in the formation of different morphologies of Fe(III) oxides after a relative long contact time.[5, 28, 29] Jeon et al.[28] observed the interactions of dissolved Fe(II) with hematite surface which transformed to magnetite after a relative long time. Satapanajaru et al.[30] also reported that ferrihydrite could react with Fe(II) to form magnetite. These means that the decrease in Fe(II) at neutral pH could produce new Fe(II)-Fe(III) reactive surfaces in the GR suspensions resulting from the fixation of Fe(II) on the GR(Cl) oxidation products (magnetite), and subsequently increases the dechlorination efficiency and rate of chlorinated hydrocarbons. This is similar to the trend of C_2Cl_4 dechlorination in Cu(II)-GR(Cl) suspensions at various pHs. When Cu(II) was added into the GR(Cl) suspensions, the color changed from blue-green to black-blue-green at neutral pH, while no color was changed at high pH values.

5.3.6 The concentration effect of GR(Cl) on C_2Cl_4 dechlorination

Figure 5-14 shows the concentration effect of GR(Cl) on the C_2Cl_4 dechlorination in the absence and presence of Cu(II) at neutral pH. The concentrations of GR(Cl) used in this study were in the range of 0.00075 ~ 0.009 g mL^{-1}. In the absence of Cu(II), the dechlorination efficiency of C_2Cl_4 slightly increased with the increase in GR(Cl) concentration and only 18 ~ 21% of the initial C_2Cl_4 were dechlorinated after the incubation of 66 days. In contrast to the dechlorination in the GR(Cl) suspension without Cu(II), the dechlorination efficiency of C_2Cl_4 increased from 17% at 0.00037 g mL^{-1} of GR(Cl) to 84% at 0.009 g mL^{-1}. The k_{obs} for C_2Cl_4 dechlorination as a function of GR(Cl) concentration is shown in Figure 5-15. In both suspensions (with and without Cu(II) amendment), the k_{obs} increased rapidly in the GR(Cl) concentration range of 0.00037 ~ 0.0015 g mL^{-1} and then levelled off when the GR(Cl) concentration was further increased to 0.009 g mL^{-1}. This behaviour may be due to the high C_2Cl_4 concentration used in this study. At high C_2Cl_4 concentration, all the reactive Fe(II) sites in GR(Cl) may be occupied by target organic compounds (C_2Cl_4) molecules. If the reason is that, the use of low concentration of target compounds would increase the k_{obs}. Therefore, the concentration effect of the target organic (C_2Cl_4) on the dechlorination by GR(Cl) was studied.

The decrease in total Fe(II) concentration in GR(Cl) (0.0015g mL^{-1}) suspension in the absence and presence of 0.5 mM Cu(II) during the pre-equilibrium time

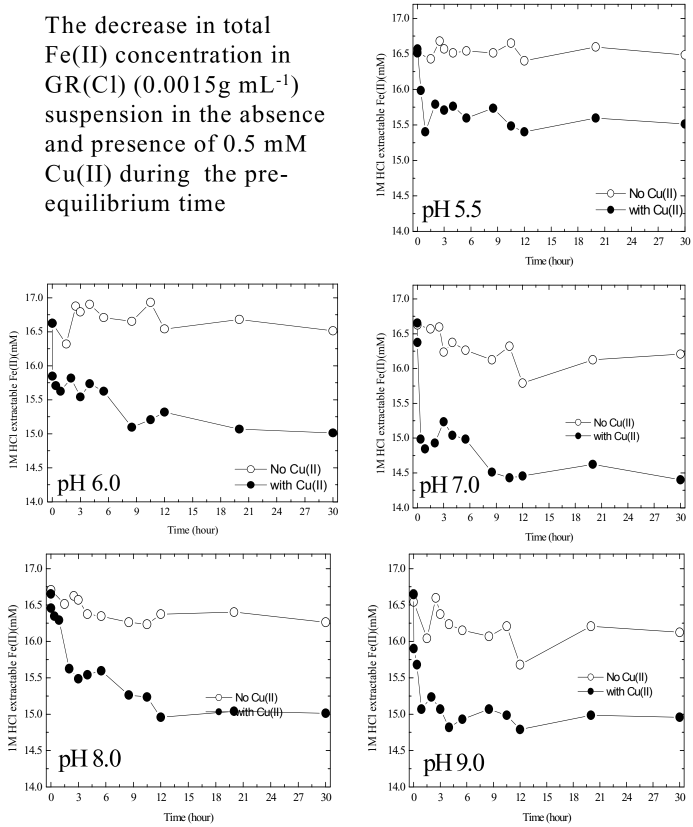

Figure 5-13. The concentration profile of total Fe(II) in GR(Cl) suspensions at various pH (5.5~9.0) in the absence and presence of 0.5 mM Cu(II).

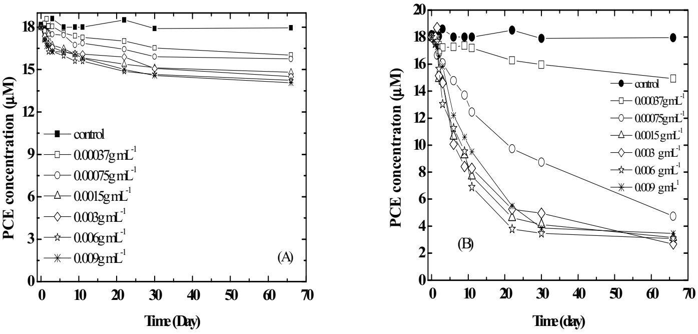

Figure 5-14. Dechlorination of C_2Cl_4 by various concentrations (0.0037~0.009 g mL^{-1}) at pH 7.2 of GR(Cl) (a) in the absence and (b) in the presence of 0.5 mM Cu(II).

5.3.7 The effect of target organic (C_2Cl_4) concentration

The effect of C_2Cl_4 concentration on the dechlorination rate and efficiency of C_2Cl_4 was further investigated using various initial concentrations within the range of 6.6 ~ 81 μM. Figure 5-16 shows the concentration profiles of various concentrations of C_2Cl_4 by GR(Cl) (0.0015 g mL^{-1}) suspensions in the presence and absence of 0.5 mM Cu(II). Only a 10 ~23 % of the initial C_2Cl_4 were dechlorinated during 16 days of incubation in the absence of Cu(II), showing that the dechlorination efficiency of C_2Cl_4 is independent on the initial concentration of C_2Cl_4. Similar dechlorination patterns were also observed in the presence of 0.5 mM Cu(II). Although 91% of the initial C_2Cl_4 was removed after 16 days, the dechlorination efficiency of C_2Cl_4 was observed to be 63 ~67 % in the concentration range of 19.2 ~ 81 μM. However, the initial rate of C_2Cl_4 dechlorination increased linearly with increasing initial C_2Cl_4 concentration. In the heterogeneous surface-mediated dechlorination reactions, the reactive sites on the surface are the source of reductive capacity. Therefore, the degradation rate of the target compound would be proportional to the concentration of the target compound interacted with the surface reactive sites. When considering the reaction rate constant, the highest rate constant (k_{obs}) at low concentration of target organics means that the surface reactive sites are completely occupied with target organic at high concentrations and become inactive after the reaction was completed. As it was previously anticipated, when the low concentration of C_2Cl_4 was used as the initial concentration both initial dechlorination rate and k_{obs} increased. As shown in Figure 5-17, the initial C_2Cl_4 dechlorination rate increased proportional to the initial concentration of C_2Cl_4, but the k_{obs} decreased first and then maintained at a nearly constant level, which agreed with the hypothesis (Figure 5-18). This behavior also could be the reason for the slow dechlorination in the latter period of the incubation, compared to the first period of 10~15 days. However, considering the natural reductants under sub–surface conditions, continuous formation of reactive Fe(II) sites by surface uptake of dissolved Fe(II) on Fe(III) containing minerals or reductive dissolution of Fe(III) minerals by dissimilatory iron reducing bacteria (DIRB) may form new reactive mixed-valence iron minerals such as green rust.[11,16,45] Therefore, in contrast to that in the laboratory batch experiments, the dechlorination reaction rate constant could be maintains at a certain level without decreasing for a long time under natural sub surface conditions.

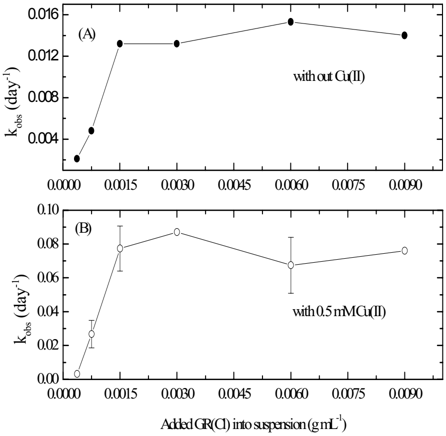

Figure 5-15. Effect of GR(Cl) concentration (0.0037 ~ 0.009 g mL^{-1}) on the observed rate constant (k_{obs}) for C_2Cl_4 dechlorination in 0.5 mM Cu(II) in the absence and presence of 0.5 of Cu(II).

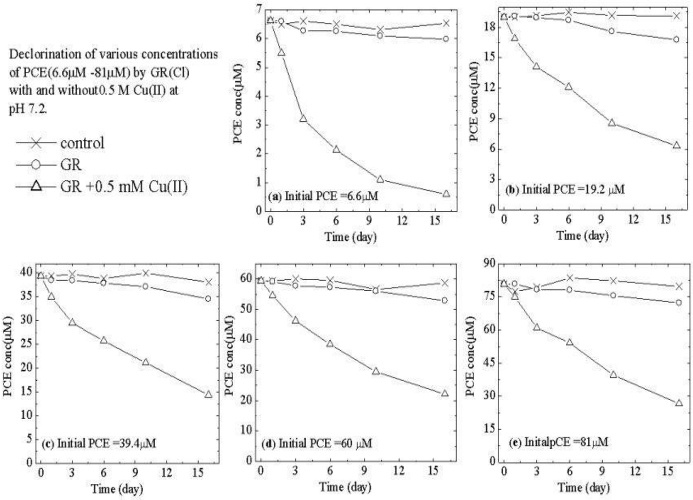

Figure 5-16 Declorination of various concentrations of C_2Cl_4 (PCE) (from 6.6 to 81.0 μM) by GR(Cl) (0.0015 g mL-1) in the absence and presence of 0.5 mM Cu(II) at pH 7.2

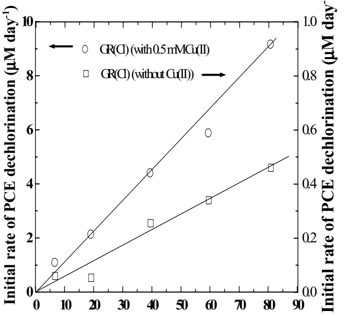

Figure 5-17. The initial rate of the dechlorination at various initial concentrations of C_2Cl_4 (6.6 ~ 81.0 μM) by GR(Cl) (0.0015g mL^{-1}) in the presence and absence of 0.5 mM Cu(II) at pH 7.2

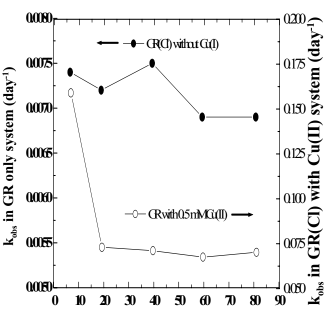

Figure 5-18. The rate constant (k_{obs}) for the dechlorination of various initial concentrations of C_2Cl_4 (6.6~81.0 μM) by GR(Cl) (0.0015g mL^{-1}) in the presence and absence of 0.5 mM Cu(II) at pH 7.2

5.4 ENVIRONMNTAL SIGNIFICANCE

Green rust is a mixed valence metastable iron oxide which contains up to 75% of Fe(II) iron in the subsurface[1,2] and can be formed abiotically by partial oxidation of Fe(II), interactions of Fe(II) ions with Fe(III) minerals and reduction of Fe(III) or oxidation of Fe(II) by bacterial activity.[9,11,12,14] Green rust plays an important role in biogeochemistry of the subsurface environments mainly related to iron cycling as well as the fate and transport of environmental contaminants under subsurface environments due to its sorption capacity and reducing power for reductively transforming various kinds of priority pollutants including chlorinated hydrocarbons[3,34,41] and metal ions.[2,26-28,30,32] However, dechlorination of chlorinated methanes and ethenes by green rust has not been addressed systematically by evaluating the effect of environmental parameters on the dechlorination reaction. A recent study reported that the rate and efficiency of the dechlorination of chlorinated alkanes could be enhanced in the presence of Ag(I), Cu(II) and Ag(II). The present study addresses the dechlorination of both chlorinated methanes and ethenes by green rust chloride and their enhanced reductive transformation into less chlorinated products in the presence of Cu(II) ions. Also the results of present study show that green rust can reduce Cu(II) into both Cu(I) and Cu(0), thus enhancing the dechlorination efficiency and rate of CCl_4, $CHCl_3$, C_2Cl_4 and C_2HCl_3. It is noted that green rust could be formed in both biotic and abiotic corrosions of metallic iron[7,22], which is the dominant material that has been used in innovative permeable reactive barrier (PRB) technique for the *in-situ* remediation of contaminated ground water. Recently the effect of transition metal ions including Cu(II) on the reactivity of PRB has reported [46]. Since both metal ions, and chlorinated compounds coexist in the contaminated sites, the reactive surfaces on the iron particles may form metal-green-rust complexes and catalyze the dechlorination reactions. Therefore, the results obtained in this study may be important to evaluate the enhanced reactivity in both natural attenuation of contaminants by green rust available in the subsurface conditions, and the enhanced reactivity and the long-term performance of metal iron permeable reactive barriers.

5.5 SUMMERY

Dechlorination of CCl_4, and C_2HCl_3 by green rust chloride (GR(Cl)) was observed. Addition of Cu(II) in to the GR(Cl) significantly increased the dechlorination of these all of chlorinated compounds. All the chlorinated aliphatics followed first-order kinetics in the dechlorination. In the CCl_4 dechlorination, 80% of initial concentration (20 μM) was dechlorinated by GR(Cl) (0.0015g mL^{-1}) at pH 7.2 within 20 h with the observed rate constant (k_{obs}) of 0.0808 h^{-1}. Addition of 0.5 mM Cu(II) completely dechlorinated CCl_4 during 35 min. The k_{obs} was 84 times greater than that in the absence of Cu(II). Chloroform ($CHCl_3$), the major chlorinated product in CCl_4 dechlorination, was accumulated up to 13 μM in the GR(Cl) suspension. However, the complete dechlorination of $CHCl_3$ occurred within 9 h by GR(Cl) in the presence of Cu(II). The efficiency for the C_2Cl_4 dechlorination by GR(Cl) was relatively slower and only 33% of initial C_2Cl_4 (17.8 μM) was dechlorinated during 40 days. The addition of 0.5mM Cu(II) into GR(Cl) suspension dechlorinated a 93% of the initial C_2Cl_4 within 40 d with a k_{obs} of 0.0852 h^{-1}, which was 4.7 times higher than that in GR(Cl) suspension without the addition of Cu(II). The major chlorinated product of C_2Cl_4 dechlorination was C_2HCl_3 and addition of 1.0 mM Cu(II) into GR(Cl) could increase the k_{obs} for C_2HCl_3 dechlorination up to 7 times. The increase in k_{obs} was proportional to Cu(II) concentration ranged between 0.1 and 1.0 mM. When the Cu(II) concentration exceeded 1.0 mM, the k_{obs} for both C_2Cl_4 and C_2HCl_3 dechlorination decreased coupling with the decrease in pH. The pH of the GR(Cl) system was an important factor for the dechlorination and the rate constants increased with increasing pH within the range of 5.5 ~ 9.0. However, the highest efficiency and rate for dechlorination was obtained at near neutral pH when Cu(II) was added. The solid phase analysis by XRD suggested that addition of Cu(II) increase the ability of green rust conversion to magnetite providing more electrons for reducing reactions. XRD analysis of solid phase showed that Cu(II) in GR(Cl) suspensions was reduced to elemental Cu. It also found that Cu(I) is formed in the liquid phase. This means that green rust can reduce Cu(II) into both Cu(I) and Cu(0), thus enhancing the dechlorination efficiency and rate of CCl_4, $CHCl_3$, C_2Cl_4 and C_2HCl_3.

5.6 REFERENCES

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Chapter 6

REDUCTIVE DECHLORINATION OF CARBON TETRACHLORIDE BY BIOGENIC FERROUS SPECIES UNDER MICROBIAL IRON REDUCING CONDITIONS BY ***Geobacter sulfurreducens***

Enhanced dechlorination of carbon tetrachloride (CCl_4) by biogenic Fe(II) species in the presence of Cu(II) ions under microbial Fe(III)-reducing condition was investigated using *Geobacter sulfurreducens* as dissimilatory iron reducing bacteria (DIRB). The biological reduction of solid Fe(III) oxides was facilitated by electron shuttling compounds. Both 0.5 mM cysteine and 10 μM AQDS facilitated the microbial Fe(III) reduction. Due to the side reaction of cysteine with Cu(II) for the dechlorination, AQDS was used as electron shuttling compound for biological Fe(III) reduction. *G. sulfurreducens* reduced Fe(III) oxides in the presence of Cu(II) (up to 0.6 mM). However, the efficiency of F(III)-reduction significantly decreased in the presence of Cu(II). The observed decrease in Fe(II) formation may be due to low recovery of generated Fe(II), because of the oxidation of biogenic Fe(II) to Fe(III) coupling with the reduction of Cu(II) to Cu(I) was evidenced by the presence of Cu(I) in Cu(II) amended bacterial Fe(III) reducing system. *G.sulfurreducens* was tested for the possible use of CCl_4 as the electron acceptor. However, only 30% of the initial CCl_4 (3.5μM) was dechlorinated within 16 days by G.*sulfurreducens* in the presence of acetate as electron donor. Whereas 92% of the initial CCl_4 was dechlorinated within 5 days when Fe(III) oxide was provided as electron acceptor. The dechlorination kinetics of CCl_4 was not observable to follow first-order kinetic under the biological Fe(III)-reducing condition due to the simultaneous occurrence of the formation of reactive Fe(II) species and the dechlorination reaction. However, addition of CCl_4 in to the bacterial system after biogenic Fe(II) was formed (within the range of 2.5~2.8 mM), dechlorination reaction followed first-order reaction kinetics. Also addition of Cu(II) into thesystem increased the dechlorination. Results obtained in this study provide valuable evidences that the biogenic Fe(II) species can simultaneously reduce Cu(II) and CCl_4

significantly and the presence of Cu species can significantly increase the efficiency of CCl_4 dechlorination during microbial Fe(III)-reduction.

6.1 INTRODUCTION

Microbial reductive dehalogenation plays a pivotal role in the detoxification of chlorinated hydrocarbons in contaminated ground water and soil. In this process, chlorinated hydrocarbons are served as terminal electron acceptors by bacteria (halorespiration) or are undergone co-metabolic transformation.[1] Laboratory and field studies have proven that reduction of organic pollutants may occur by non-biological (abiotic) chemical reactions in subsurface environments under anoxic condition.[1,2] Recently, the biological processes of Fe(III) reduction coupling with the oxidation of organic matter by dissimilatory iron reducing bacteria (DIRB) have been shown to produce substantial amounts of Fe(II) and mixed-valence iron minerals such as magnetite ($Fe^{II}Fe^{III}{}_2O_4$) in the subsurface environments.[3-5] Heijman et al.[6] found that the biogenic magnetite produced by Fe(III) reducing process of *Geobacter metallireducens* could reduce niroaromatic compounds. Also, the reduction of nitro aromatic compounds due to the surface-mediated abiotic reaction of Fe(II) species generated by DIRB in aquifer soil column has been reported.[7] Microbially dissimilatory Fe(III) reducing process regenerates Fe(II) species on the surface of iron minerals those are consumed by the reduction of contaminants.[7,8] The biological regeneration of Fe(II) species is necessary for the continuous maintain of long-term reactivity of the surface-bound and structural Fe(II) species. However, organic compounds are important as carbon source for DIRB during biological Fe(III) reduction. Elevated concentrations of Fe(II) and depletion of Fe(III) have been observed in contaminated sediments and aquifers containing aromatic hydrocarbons in petroleum contaminated sites indicating that the Fe(III) reducing bacteria may easily grow in the aquifers contaminated with organic compounds..[9-12] Generally, it is believed that polycyclic aromatic hydrocarbons (PAHs) are resistant to biological degradation under anoxic conditions, but investigations showed that DIRB can oxidize naphthalene to CO_2 coupling to Fe(III) reduction.[12] In addition to those organics, many Fe(III)-reducing bacteria also can use hydrogen as electron donor. Therefore, Fe(III)-reducing bacterial species can survive in different environments according to the availability of electron donors.

Also few species of DIRB belonging to *Geobacteriacea* have been reported to reductively transform tetrachloroethene (C_2Cl_4) and trichloroethene (C_2HCl_3) by using these compounds as the sole electron acceptors coupling with the oxidation of organic compounds.[13] However it is not known whether *Goebacter sulfurreducens* can use chlorinated compounds as electron acceptor.

Not only organic compounds but also transition metal ions complexed with dissolved organic matter (DOM) often exist in the contaminated aquifers.[14,15] Few studies have focused attention on the possible reduction of other metal species by DIRB as electron acceptors besides Fe(III) minerals. Up to now the metal ions including U(VI), Mn(IV), Co(III), Cr(VI), and Tc(VII) have been reported to be reduced by Fe(III)-reducing bacteria coupling with organic compound or hydrogen oxidation.[16,17] Recently, DIRB was found to reduce Au(III) to metallic gold (Au°).[18] However, the effect of other metal ions available in the aqueous phases, on the Fe(III) reduction is still unclear. This issue is important because certain transition metal ions such as Cu (II), are known to be toxic for microbial activity.[19] Also, the effect of such metal ions on the abiotic dechlorination of chlorinated compounds by biogenic Fe(II) should be elucidated, because in the previous chapters, it was found that transition metal ions can increase the reactivity of surface-bound Fe(II) systems towards reduction of contaminants.

Members of the genus *Geobacter* are the dominant metal-reducing microorganisms in a variety of anaerobic subsurface environments and have been shown to involve in the bioremediation of both organic and metal contaminants.[20] It was previously considered that Fe(III)-reducing microorganisms must come into direct contact with Fe(III) oxides in order to reduce Fe(II) oxides. However, recent studies have suggested that electron-shuttling compounds may facilitate the need for the Fe(III) reducing bacteria to establish direct contact with Fe(III) oxides.[21] *Geobacter metallireducens*, was found to have an alternative strategy to contact with Fe(III) by forming flagella and pili only when they grow on insoluble Fe(III)oxide.[22] However, more recently it was found that *Geobactor* species such as *Geobacter metallireducens* does not produce electron-shuttles or Fe(III) chelators.[21] Therefore, addition of some electron shuttling compound are necessary to facilitate the reduction of solid Fe(III) minerals by DIRB.

Humic substances (humics) and the humics analogue, anthraquinone-2,6-disulfonate (AQDS) were found to stimulate microbial reduction of synthetic poorly crystalline Fe(III) oxide under laboratory conditions.[23,24] Moreover, a recent study found that cysteine an essential amino acid in living cells acts as electron carrier to stimulate the reduction of iron (III) oxides by *G. sulfurreducens.*[25] Generally, cysteine is also amended into strictly anaerobic microbial cultures to maintain O_2-free conditions in many anaerobic microbial experiments.

Geobacter sulfurreducens was first isolated form surface sediment of a hydrocarbon –contaminated ditch in Norman, Okla, USA.[26] *G. sulfurreducens* was also first introduced as strictly anaerobic species and non-fermentative, nonmotile, gram-negative rod shaped. This species also grew in a defined medium containing acetate as an electron donor and Fe(III)-porphyrine, Fe(III) oxyhydroxide, Fe(III)citrate, elemental sulfur, Co(III)-EDTA, fumarate, or malate as the sole electron acceptors.[26] *G. sulfurreducens* also coupled the oxidation of hydrogen to the reduction of Fe(III). However, the complete genome sequence of *G. sulfurreducens* recently revealed unsuspected capabilities, including evidence of aerobic metabolism, one-carbon and complex carbon metabolism, motility and chemotactic behavior.[27]

The major objective of this study was to understand the abiotically reductive dechlorination of carbon tetrachloride (CCl_4) by the biogenic Fe(II) species under microbial Fe(III) reducing condition using *Geobacter sulfurreducens* and to evaluate the effect of Cu(II) on the dechlorination process. Cysteine or AQDS was added into the system as electron shuttling compounds to increase the efficiency of biological Fe(III) reduction. The possible microbial dechlorination of CCl_4 was also tested. Amorphous ferric oxide is well -known to be reduced by *G. sulfurreducens*. However, the reduction of other crystalline iron minerals by this bacterium is not clear. Therefore, goethite and hematite, the most available and abundant crystalline Fe(III) oxides were also used to understand the capability of Fe(III) reduction and dechlorination of CCl_4 by *G. sulfurreducens.*

6.2 MATERIALS AND METHODS

6.2.1 Chemicals

Carbon tetrachloride (CCl_4, > 99.8%, GC grade), $CuCl_2{\cdot}2H_2O$ (99%), $FeSO_4.7H_2O$ (99%), Na_2HPO_4 (>99%), NH_4Cl (99.8%), $CaCl_2.\ 2H_2O$ (99.5%), KCl (> 99.5%), sodium acetate (CH_3COONa)(> 99%) were purchased from Merck Co. (Darmstadt, Germany). $FeCl_2{\cdot}4H_2O$ (99 %), $FeCl_3{\cdot}6H_2O$ (99%), L-cysteine hydrochloride ($C_3H_7NO_2S.\ HCl$) (> 98%), $MgCl_2.6H_2O$ (> 99%), fumarate disodium salt ($C_4H_2O_4Na_2$) (99%), N-(2-

hydroxyethyl)-piperazine-N'-(2-ethanosulfonic acid (HEPES) (99.5%), $Fe(NO_3).9H_2O$ (> 98%), HCl (37%), HNO_3 (65 %), ferrozine monosodium salt ($C_{20}H_{13}N_4O_6S_2Na$), ammonium acetate(CH_3COONH_4) and 9,10-anthaquinone-2,6-disulfonic acid disodium salt ($C_{14}H_6O_8S_2Na_2$) (AQDS)(> 98%) were purchased form Sigma-Aldrich Co. (Milwaukee, WI). Bathocuproinedisulfonic acid disodium salt ($C_{26}H_{18}N_2Na_2O_6S_2$, 90 %) was purchased from Fluka (Buchs, Switzerland). NaCl (> 99.8%), $NaHCO_3$ (> 99.7%) were received from Riedel-de-Haën (Germany). All the chemicals obtained were used without further purification.

6.2.2 Microorganism and Cultivation

The dissimilatory Fe(III)-reducing bacterium *G. sulfurreducens* was a courtesy from prof. Schink (Universität Konstanz, Germany). Bacteria were cultivated in bicarbonate-buffered mineral medium (pH 7.1 ± 0.1). The medium was prepared by adding Na_2HPO_4 (0.2g), NH_4Cl (0.25g), NaCl (1.0g), $MgCl_2.6H_2O$ (0.4g), $CaCl_2.2H_2O$ (0.15g), KCl (0.5g) for a 1 L of anoxic bicarbonate (30mM) buffer solution. Sodium acetate (CH_3COONa) (30mM) and sodium fumarate ($C_4H_2O_4Na_2$)(20mM) were added as electron donor and electron acceptor, respectively, in the medium. The bottle was tightly sealed using rubber septum and screw cap. The head-space was filled with N_2/CO_2 (80/20) and autoclaved. After the solution was cooling to room temperature under the anoxic condition, 1 mL of the anoxic solution of trace elements in the following concentrations (g L^{-1}) $Fe(NH_4)_2(SO_4)\cdot6H_2O$ (0.8), $CoCl_2\cdot6H_2O$ (0.2), $ZnSO_4\cdot7H_2O$ (0.2), $CuCl_2\cdot2H_2O$ (0.02), $NiCl_2\cdot6H_2O$ (0.02), $Na_2MoO_4\cdot2H_2O$ (0.02) and a 1 mL of selenite-tungstate solution (Na_2SeO_4,0.02 gL^{-1} and Na_2WO_4,0.02gL^{-1}) were injected through 0.2 μM sterile membrane filter (PTFE). Then, the medium was transferred in to 50 mL serum bottles those were vacuumed and sealed with rubber septum and aluminum crimp cap using 50 mL sterile airtight plastic syringe and needles in the sterile lamina-flow hood. The total volume of the mineral medium transferred into serum bottle was about 45 mL remaining 5 mL of headspace filled with N_2/ CO_2 (80/20, v/v). Then *G. sulfurreducens* was inoculated into culture medium and the serum bottles were incubated at 25 ± 1 °C. The bacterial growth was tested by optical density at 662 nm using UV-visible spectrophotometer (Hitachi UV 3003).

6.2.3 Fe(III) reduction experiments

Biological Fe(III) reduction by *G. sulfurreducens* was carried out using poorly crystalline ferric oxide (amorphous ferrihydrite) to study the biogenic Fe(II) mediated dechlorination of CCl_4. Moreover, goethite and hematite were also used to study the possible microbial Fe(III) reduction by *G. sulfurreducens*. Serum bottles (70 mL) capped with Teflon lined rubber septa and aluminum crimp caps were used in Fe(III) reducing experiments. Bacterial medium was prepared by following the similar procedure used for the preparation for microbial culture. No other trace metals or mineral solutions were added to avoid the complex effects during the Fe(III) reducing process. Instead of bicarbonate buffer, organic buffer (50mM HEPES) was used to maintain the solution pH at 7.0 ± 1. No fumarate was added into the solution since Fe(III) oxides was to be added as terminal electron acceptor. Fe(III) oxides were introduced into serum bottles from their sterile stock suspensions under anoxic conditions using N_2-purged sterile plastic syringes. The final concentrations of the added Fe(III)oxides were 10 mM. To facilitate the Fe(III) reduction by *G. sulfurreducens,* L-cysteine (0.5 mM) or AQDS (10μM) was added from anoxic stock solution in water. *G. sulfurreducens* was inoculated by injecting 1 mL of bacterial culture in the stationary phase. Because cysteine can abiotically reduce Fe(III) to Fe(II)[28], blank controls (without bacterial cells) were also used to quantify the Fe(III) mineral reduction by cysteine. In control experiments, medium of the bacterial culture was introduced into serum bottles through 0.2 μM sterile membrane filter (PTFE) to normalize all the possible effect from the components in the culture except the bacteria. To understand the toxic effect of Cu(II) on the biological Fe(III) reduction, Cu(II) was added in to the serum bottles within the concentration range of 0.1 ~ 0.6 mM. Carbon tetrachloride (CCl_4) was added into the serum bottles prepared in anoxic methanol solution in order to study the bacterial Fe(III) reduction. The maximum volume of added methanol was 10 ~ 20 μL. The total liquid phase in the serum bottles were maintained at 50 mL remaining 20 mL of headspace. The serum bottles were incubated horizontally in an orbital shaker at 150 rpm and at 25°C in the dark.

6.2.4 Dechlorination experiments

Abiotic dechlorination of CCl_4 by biogenic Fe(II) species was studied by analysis of the concentration of CCl_4 in the Fe oxides amended serum bottles. CCl_4 from the stock solution in methanol was injected as the target organic to be the final concentration of 3 μM. The CCl_4 concentrations were determined periodically. Because cysteine and reduced-form of quinone compounds also can reduce chlorinated compounds[29] [30], control experiments were performed in the absence of bacteria. In the similar way some serum bottles were treated with bacteria without the amendment of Fe(II) oxides to understand the possibility of using CCl_4 as terminal electron acceptor.

6.2.5 Analytical methods

Analytical methods for chlorinated compounds, Fe(II) concentration, and Cu(I) concentrations were similar to the methods described for those analyses in previous chapters (from chapter 2 to chapter 5). Concentration of cysteine in Cu(II) solutions was measured using 5, 5'-dithiobis(2-nitrobenzoic acid) (DTNB) method.[31,25] Briefly, anoxic solution in the serum bottle was withdrawn by 1-mL N_2-purged syringe and 0.1 mL of aliquots was mixed with 1 mL anoxic solution of 1mM DTNB in 50 mM phosphate buffer at pH 8.5. Then the mixture was allowed to develop the color complex for 10 min and the absorption at 412nm was measured by UV-Visible spectrophotometer. This method can be used to detect the lowest concentration of 6 μM.

After bacterial Fe(III) reduction in the ferrihydrite suspensions, the contents in the bottles were withdrawn by 50 mL syringe and injected into 50 mL centrifuge tubes under nitrogen atmosphere. Then it was separated by centrifugation at 10,000 rpm for 10 mins. The solid-phase was dried with gentle stream of N_2 and the crystal property of dried powder was analyzed by XRD (Regaku D/max-IIB) using Cu Kα-radiation source with 30 kv voltage and 20 mA current. The powder samples treated with the similar procedure were also subjected to examine the morphology using scanning electron micros copy (SEM) (Topcon ABC-150S).

6.3 RESULTS AND DISCUSSION

6.3.1 Reduction of various Fe(III) oxides by *Geobacter sulfurreducens*

Figure 6-1 shows the reduction of various Fe(III)-oxides by *G. sulfurreducens* at neutral pH. HEPES buffer (50mM) was used to maintain the pH at 7.0 ± 0.1. According to the measured Fe(II) concentrations, the biological Fe(III) reduction seems more favored on poorly crystalline ferrihydrite in comparison with crystalline Fe(III) oxides including goethite and hematite. The maximum concentration of Fe(II) in the ferrihydrite suspension was 2.25 mM after 20 d of incubation, which is rather consistent with the reported results using 30 mM bicarbonate buffered medium with cysteine as an electron shuttling compound in biological Fe(III) reduction.[25] It is noted that ferrihydrite also could be abiotically reduced by cysteine.[28,32,33] Therefore, the blank system without addition of bacterial cells was used for comparison. In the blank control system, observed low concentration of Fe(II) due to the reduction of ferrihydrite was up to 0.5 mM during 20 days of incubation indicating that a large fraction of Fe(II) formation in bacterial system is mainly from the bacterial Fe(III) reduction. Although the reduction of Fe(III) oxides in both goethite and hematite-amended bacterial systems was observed, the detected Fe(II) concentrations were lower than 0.5 mM, depicting that crystalline Fe(III)-oxide is more resistant to be reduced by *G. sulfurreducens*. Reduction of crystalline Fe(III) minerals including magnetite and hematite by facultative DIRB species such as *Pseudomonas spp*.[34], or *Shewanella putrefaciens* has been reproted[35-38] . Moreover, the Fe(II) concentrations detected in the blank controls due to abiotic Fe(III) reduction by cysteine were also quite lower in crystalline Fe(III) systems compared to that in ferrihydrite system, indicating that the reduction of crystalline Fe(III) by cysteine also slower than that of poorly crystalline Fe(III) oxides. Previous study also reported that the rate of ferrihydrite reduction was higher than that for goethite.[28] Therefore, poorly crystalline Fe(III) oxide was selected to study the effect of other factors in the further experiments.

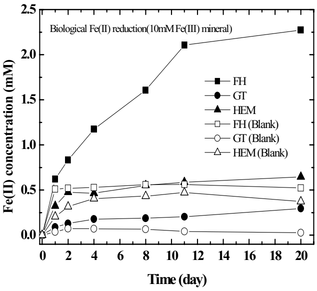

Figure 6-1. Reduction of 10mM Fe(III) minerals including ferrihydrite (FH), goethite(GT) and hematite (HEM) by *G. sulfurreducens* at pH 7 (50 mM HEPES) in the presence of 0.5 mM cysteine.

6.3.2 Abiotically reductive dechlorination of CCl_4 under microbial Fe(III) reducing condition

Because the reductive dechlorination is mediated by Fe(II)/Fe(III) systems and the regeneration Fe(II) is required to form new reactive Fe(II), the simultaneous occurrence of biological Fe(III) reduction and abiotic dechlorination catalyzed by Fe(II)/Fe(III) system are needed to evaluated. Recent studies have used biogenic Fe(II) species for the remediation of chlorinated hydrocarbons.[39,40] Figure 6-2 shows the reduction of ferrihydrite by *G. sulfurreducens* in the presence of 3.5 μM CCl_4. In a comparison of the ferrihydrite reduction in the absence and presence of CCl_4 (Figure 6-1 and Figure 6-2,) it is clear that amendment of CCl_4 lowered the production of Fe(II). A 38% of decrease in the maximum Fe(II) concentration due to the addition of CCl_4. Moreover, the reduction of Fe(III) in the blank controls did not change by addition of CCl_4 showing that the decrease in Fe(II) reduction in the presence of CCl_4 is probably due to the inhibition of bacterial process of Fe(III) reduction. However, the added concentration of CCl_4 did not completely inhibit the Fe(II) reduction, implying that CCl_4 is not extremely toxic at low concentrations to *G. sulfurreducens*.

A parallel series of serum bottles was set up to understand CCl_4 reduction coupling to biogenic Fe(II) resulted by the reduction of various Fe(III) oxides by *G. sulfurreducens* . As shown in Figure 6-3, the concentrations of CCl_4 decreased with time in every Fe(III)-oxide system both in the absence and presence of *G. sulfurreducens*. Particularly, the dechlorination of CCl_4 was higher in ferrihydrite (FH)-amended suspensions. The concentrations of CCl_4 in FH systems with and without the inoculation of bacterial cells were 0.25 μM and 1.5 μM after 20 d, respectively, which corresponded to 92 % and 58.84 % of CCl_4 dechlorination. Interestingly, the CCl_4 concentration profiles in the goethite and hematite systems were similar in the presence and absence of bacteria, depicting that microbial activity is not significant in these two Fe(III) oxide systems for the dechlorination.

The high efficiency of CCl_4 reduction in the systems containing poorly crystalline Fe(III) in the presence of *G. sulfurreducens* gives impetus to the reactivity of biogenic Fe(II). In the previous chapters it has been found that the dechlorination of chlorinated compounds by various Fe(II) systems can be increased significantly, when Cu(II) ion is amended into the suspension. Therefore, the effect of Cu(II) on the dechlorination of CCl_4 under microbial Fe(III) reduction was also evaluated.

6.3.3 Influence of copper ions on the growth of *G. sulfurreducens* and the microbial Fe(III) reduction

Copper is an essential trace element for living cells and poses little threat at background levels with trace amounts. However, it is detrimental to the enzymatic metabolic activities at elevated concentrations. The effect of Cu(II) on the growth of *G. sulfurreducens* was tested in the anoxic culture medium which contained 30 mM acetate as carbon source (electron donor) and 20 mM fumarate as terminal electron acceptor. The optical density of the bacterial culture at 662 nm was used to evaluate the bacterial population density. Table 6-1 shows the optical density of *G. sulfurreducens* in the absence and presence of 0.5mM Cu(II). It is obvious that the growth of *G. sulfurreducens* strongly decreased with the addition of Cu(II). However, bacteria still could metabolize acetate using fumarate as the terminal electron acceptor in the presence of Cu(II) and the optical density after 10 days were 0.583, implying that *G. sulfurreducens* has capability to survive using acetate and fumarate as electron donors and acceptors ,respectively in the presence of 0.5 mM Cu(II) implying possible Fe(III) reduction in the presence of Cu(II).

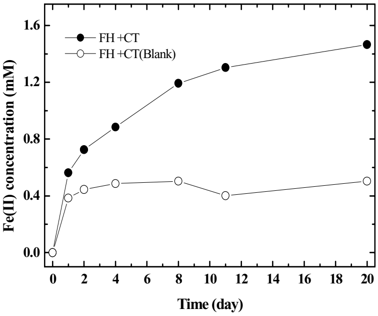

Figure 6-2. Reduction of 10 mM ferrihydrite by *G. Sulphur-reducens* in the presence of 3.5 µM carbon tetrachloride (CCl_4) and 0.5 mM cysteine at pH 7.

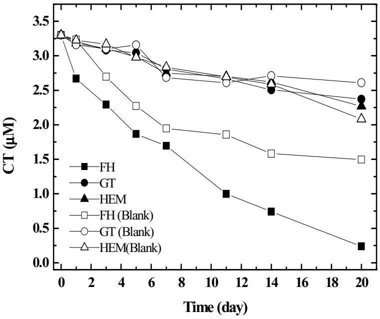

Figure 6-3. Dechlorination of CCl_4 under bacterial Fe(III) reducing condition of *G. sulfurreducens*. Fe(III) minerals (10mM) including ferrihydrite (FH) , goethite (GT) and hematite(HEM) were used as electron acceptor and 30 mM acetate was used as

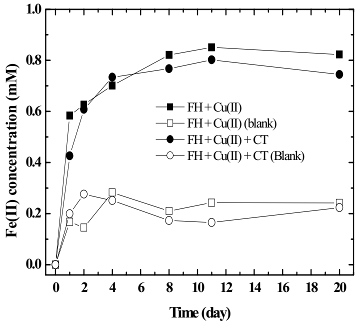

Figure 6-4. Effect of Cu(II) (0.5 mM) on the reduction of ferrihydrite (FH) (10mM) by *G.sulfurreducens* at pH 7.0 in the absence and presence of CCl_4. Cysteine (0.5 mM) was added as electron shuttling compound.

Table 6-1. Changes in the optical density of the culture medium of *G. sulfurreducens* in the presence and absence of 0.5 mM Cu(II).

Time (day)	Absorption (at $\lambda = 662$mm)	
	without Cu(II)	with 0.5 mMCu(II)
0	0	0
2	0.024	0.011
4	0.046	0.033
6	0.306	0.112
8	0.657	0.347
10	0.696	0.583

In order to understand the influence of Cu(II) on the biological Fe(III) reduction, only ferrihydrite was used because of the efficiency of ferrihydrite reduction was much higher than that of other Fe(III) oxides. Figure 6-4 demonstrates the Fe(II) reduction by *G. sulfurreducens* in the presence of 0.5 mM Cu(II). Parallel experiments were run in the absence and presence of 3.3 mM CCl_4 to understand the effect of Cu(II) on the Fe(III) reduction. A significant increase in Fe(II) concentration was obtained in ferrihydrite system when 0.5 mM Cu(II) was added. The maximum concentration of Fe(II) was around 0.8 mM, which was higher than the blank control of 0.2 mM Fe(II). An apparent decrease in Fe(II) concentration was observed in Cu(II)-amended systems when compared Figures 6-1 and 6-2. Figure 6-4 also illustrates that addition of CCl_4 has a little effect on the production of Fe(II) concentration. However, biological Fe(III) reduction still seems possible in the presence of both CCl_4 and Cu(II). Therefore, CCl_4 dechlorination by biogenic Fe(II) also may be possible.

6.3.4 Dechlorination of CCl_4 in the presence of Cu(II) under microbial Fe(III) reducing condition.

Figure 6-5 shows the concentration profile of CCl_4 in Cu(II)-amended ferrihydrite suspensions in the presence and absence of *G. sulfurreducens*. A complete disappearance of CCl_4 was observed within 20 d in microbial system, while only 76% of initial CCl_4 was dechlorinated in the absence of *G. sulfurreducens*. It is apparent that the addition of Cu(II) enhanced the dechlorination efficiency and rate of CCl_4 in the microbial system and the pseudo first-order rate constant (k_{obs}) was 0.2362d^{-1}(Table 6-2). Similar to the microbial system, both the rate and efficiency also increase in blank control system and k_{obs} was 0.1175 d^{-1}. Also, the enhanced ratio of k_{obs} in blank controls is higher than that in microbial batches (Table 6-2). This decrease in the enhanced ratio of k_{obs} may be due to the inhibition of microbial activity by Cu(II).The reduction of Cu(II) to Cu(I) by other reducing agent (probably cysteine) in the solution may be another possibility. It could be an abiotic reaction catalyzed by the copper species complexed with the medium, the third assumption is microbial metabolism of CCl_4 in the presence of Cu(II).

In order to confirm the possible reasons, dechlorination of CCl_4 was studied in the absence of Fe(III) oxides with and without *G. sulfurreducens* in a medium containing 30 mM acetate and 0.5 mM cysteine at pH 7. Figure 6-6 shows the effect of 0.5 mM Cu(II) on the dechlorination of CCl_4 by *G. sulfurreducens* without the addition of Fe(III) oxides. In the absence of Cu(II) ion, the CCl_4 concentration decreased from 3.5 µM to 2.5 µM and 2.8 µM after 20 d , which accounted for 23% and 15% of the initial CCl_4 in bacterial and blank control systems, respectively. This small difference in dechlorination efficiency of between DIRB-amended and un-amended systems indicates the low dechlorination capacity of *G.sulfurreducens using* CCl_4 as the sole electron acceptor. Interestingly, in the presence of 0.5 mM Cu(II), the dechlorination efficiency and rate of CCl_4 significantly enhanced both in microbial system and blank control. Also, the contribution of bacterial activity on the CCl_4 dechlorination is negligible compared to the blank control, suggesting that copper complexed with the cysteine should be responsible for the abiotic CCl_4 dechlorination.[41]

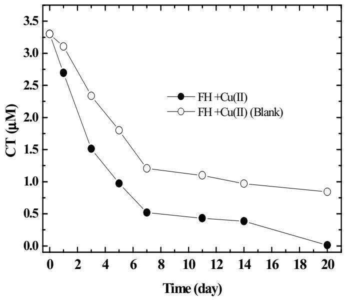

Figure 6-5. Dechlorination of CCl_4 during the reduction of ferrihydrite (FH)(10mM) by *G. sulfurreducens* in the presence of 0.5 mM Cu(II) at pH 7.with 30mM acetate as electron donor and 0.5 mM cysteine as electron mediator.

Table 6-2. The pseudo-first order rate constant (k_{obs}) for the CCl_4 dechlorination under microbial Fe(III) reducing condition. 10 mM ferrihydrite was reduced in the absence and presence of 0.5 mM Cu(II).

system	Without Cu(II)	With 0.5mM Cu(II)	Enhanced ratio
Microbial	0.1181	0.2362	2
Blank(control)	0.0553	0.1175	2.12

The dechlorination of chlorinated methanes by cysteine as bulk reductant in the presence of various electron mediators such as iron porphyrine and quinone compounds has been reported.[29,42] However, the cysteine complexed with Cu(II) ions on the dechlorination of chlorinated compounds is not well-known. To confirm the dechlorination of CCl_4 due to the cysteine-copper complexes, a parallel experiments were performed by injecting 20 μM CCl_4 into the anoxic solutions contained 0.25 mM cysteine with 0.5 mM Cu(II) and 0.5 mM Cu(II) alone at neutral pH. Figure 6-7 shows the dechlorination of CCl_4 in the cysteine-Cu(II) system.

A complete dechlorination of CCl_4 was observed after 23 d in the solution containing cysteine and Cu(II), whereas no significant dechlorination of CCl_4 was observed in the solution with 0.5 mM Cu(II) alone during the incubation time. When 0.25 mM cysteine was mixed with 0. 5 mM Cu(II) before CCl_4 injection, no detectable cysteine concentration was found in the solution. The limit of detection (LOD) of DTNB method used to detect cysteine was 6 μM, showing that most cysteine was oxidized into cystine, by Cu(II). The concentration of Cu(I) was 0.24 mM, which is nearly similar to the concentration of added cysteine. These results conclude that the dechlorination of CCl_4 should be due to the formation of possible Cu(I)–cystine complex or Cu(I) species. The reduction of Cu(II) to Cu(I) coupling to cysteine oxidation can be describe in equation (6-1).

$$2\,\mathrm{Cu(II)} + 2\,\mathrm{cysteine} \rightarrow 2\mathrm{Cu(I)} + \mathrm{cystine} + \mathrm{H}^{+} \qquad (6\text{-}1)$$

In this experiment, low concentration of cysteine was used to understand the effect of the product of equation 6-1. The blank controls which containing 0.5 mM cysteine did not dechlorinate CCl_4, implying that he product of reaction 6-1 is responsible for the dechlorination of CCl_4. However, equation (6-1) is a side reaction for CCl_4 dechlorination. The objective of this work is to study the dechlorination of CCl_4 by biogenic Fe(II) and possible synergistic effect of Cu(II) in Fe(II)/Fe(III) systems. Therefore, cysteine was not suitable to serve as an electron shuttling compound to increase the biological Fe(III) oxide reduction in this study.

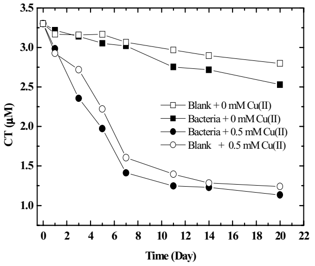

Figure 6-6. Dechlorination of CCl_4 by *G. sulfurreducens* in the absence of Fe(III) oxide at pH 7 in the absence and presence of 0.5 mM Cu(II). Cysteine (0.5 mM) and acetate (30mM) were used as electron mediator and electron donor, respectively.

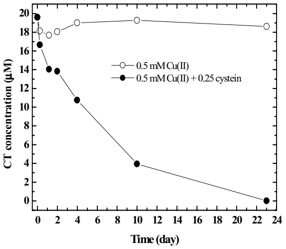

Figure 6-7. Dechlorination of 20 µM CCl_4 by cysteine(0.25 mM) with Cu(II) (0.5 mM) at pH 7.

A complete dechlorination of CCl_4 was observed after 23 d in the solution containing cysteine and Cu(II), whereas no significant dechlorination of CCl_4 was observed in the solution with 0.5 mM Cu(II) alone during the incubation time. When 0.25 mM cysteine was mixed with 0. 5 mM Cu(II) before CCl_4 injection, no detectable cysteine concentration was found in the solution. The limit of detection (LOD) of DTNB method used to detect cysteine was 6 μM, showing that most cysteine was oxidized into cystine, by Cu(II). The concentration of Cu(I) was 0.24 mM, which is nearly similar to the concentration of added cysteine. These results conclude that the dechlorination of CCl_4 should be due to the formation of possible Cu(I)–cystine complex or Cu(I) species. The reduction of Cu(II) to Cu(I) coupling to cysteine oxidation can be describe in equation (6-1).

$$2\ Cu(II) + 2\ \text{cysteine} \rightarrow 2Cu(I) + \text{cystine} + H^+ \qquad (6\text{-}1)$$

In this experiment, low concentration of cysteine was used to understand the effect of the product of equation 6-1. The blank controls which containing 0.5 mM cysteine did not dechlorinate CCl_4, implying that he product of reaction 6-1 is responsible for the dechlorination of CCl_4. However, equation (6-1) is a side reaction for CCl_4 dechlorination. The objective of this work is to study the dechlorination of CCl_4 by biogenic Fe(II) and possible synergistic effect of Cu(II) in Fe(II)/Fe(III) systems. Therefore, cysteine was not suitable to serve as an electron shuttling compound to increase the biological Fe(III) oxide reduction in this study.

6.3.5 Microbial reduction of Fe(III)oxides using AQDS as electron shuttling compound

Besides cysteine, several quinone and related compounds[23,43] have been proven to act as electron shuttling compounds. AQDS (2,6-anthraquinone disulphonate) has been found to be the most efficient electron shuttling compound that facilitated the reduction of insoluble Fe(III) oxides by *G. metallireducens* which the first found dissimilatory iron reducing bacteria (DIRB).[23] Studies also found that quinone-like compounds are excreted by DIRB in the process of Fe(III) reduction.[44] In the present study 10 μM of AQDS was added in to the solution containing 10mM Fe(III)oxide suspensions and 30mM acetate as the carbon source for bacteria.

As can be anticipated, the microbial reduction of Fe(III) using AQDS as electron shuttling compound was faster than that with cysteine. The concentration profile of Fe(II) shown in Figure 6-8 clearly shows that ferrihydrite was the most effectively reduced Fe(III) oxide mineral which generated around 4.5 mM Fe(II) during 9 days of incubation and then, maintained at constant. The Fe(II) ions were possible to deposit or sorb on the surface of Fe(III) mineral to form a barrier which could further decrease the rate and efficiency of microbial Fe(III) reduction.[45,46] The influence on the Fe(III) reduction may depends on the composition of Fe(II) coverage.[47] In the absence of bacteria (blank control), the formation of Fe(II) was quite low indicating that the increase in the Fe(II) concentration in microbial system is due to the bacterial Fe(III) reduction. Additionally it proves that, not like cysteine-added systems, AQDS has less effect on abiotic Fe(III) reduction. Several studies reported that quinone compounds in the reduced form could be oxidized coupling with the reduction of Fe(III).[23,48,49] In this study, however, such a reaction is unlikely because the reduced form of AQDS (AHQDS) was not added into the system. It is noted that not only amorphous ferrihydrite but crystalline Fe(III) minerals (goethite and hematite) were also reduced to a certain extent by *G. sulfurreducens* in the presence of AQDS. The reduction of hematite increased rapidly followed by slow reduction after 15 d and the Fe(II) concentration was 3.2 mM after 24 d of incubation.

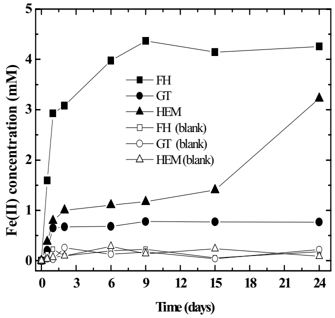

Figure 6-8. Reduction of 10 mM Fe(III) oxides by *G. sulfurreducens* at pH 7. The ferric oxides (10 mM) used were ferrihydrite (FH), goethite,(GT), and hematite (HEM). 30 mM acetate and 10 µM AQDS were added to serve as electron donor and electron shuttling compound, respectively.

In the goethite suspension, the microbial reduction was low but it was observable when compared to that in the cysteine- amended system (Figure 6-1). The blank controls showed similar low concentrations (lower than 0.2mM) of Fe(II) in every Fe(III) oxide systems depicting that *G .sulfurreducens* can reduce hematite and ferrihydrite significantly in the presence of AQDS. Ferrihydrite (FH) is the easily reducible Fe(III) mineral.

During FH reduction, the original brown color gradually transferred into black color, which indicates that the product mineral could be magnetite. To confirm the mineral phase and to study the morphology of the product, the separated solid phase was dried under N_2 -atmosphere and characterized by x-ray diffractometer (XRD) and scanning electron microscopy (SEM). Figures 6-9 and 6-10 show the XRD pattern and the SEM image of biogenic iron mineral-phase resulted from the microbial reduction of ferrihydrite using AQDS as electron shuttling compound to facilitate the Fe(III) reduction. Figure 6-9 shows that the XRD pattern of the product was similar to that obtained from pure magnetite synthesized by standard method. The SEM image indicates that in addition to magnetite some small needle-like minerals phase also presence, presumably due to formation of goethite.

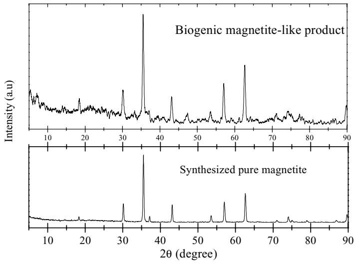

Figure 6-9. The comparison of the XRD patterns between biogenic and chemically synthesized magnetite. Biogenic magnetite was produced by reduction of ferrihydrite by *G. sulfurreducens* in the presence of 10 μM AQDS.

6.3.6 Dechlorination of carbon tetrachloride under microbial Fe(III) reducing condition using AQDS as electron shuttling compound.

Since all the Fe(III) oxides were reduced by microbial activity in the presence of 10μM AQDS, it is necessary to evaluate the microbial ability of Fe(II) reduction in the presence of CCl_4. A similar experimental system of the microbial Fe(III) reduction that was described previously was used. CCl_4 (3.5 μM) was injected into the serum bottles. The Fe(II) concentrations shows in Figure 6-11 depicts that nearly 4.5 mM, 3.2 mM and 1.1 mM of Fe(II) were formed in ferrihydrite, hematite, and goethite systems respectively, during 24 d in the presence of CCl_4 and AQDS. These concentrations were similar to those in the absence of CCl_4 (Figue6-8) revealing that the addition of CCl_4 has little effect on the microbial activity of *G. sulfurreducens* when AQDS was used as electron shuttling compound.

Figure 6-12 illustrates the dechlorination of 3.5 μM CCl_4 under the microbial Fe(III)- reducing condition. No significant decrease in CCl_4 concentration was observed after 16 d of incubation in the serum bottles without the addition microbial cells. CCl_4 was effectively dechlorinated in hematite where nearly complete CCl_4 was removed during 16 days. However, the CCl_4 concentrations in goethite and hematite systems were around 0.5 and 1.5 µM after 16 d of incubation, which accounted for 86 % and 57 % of initial CCl_4 removal, respectively. According to the magnitude of the reduction efficiency of CCl_4 , microbial iron oxide systems were in the descending order of Hem > Goe > FH.

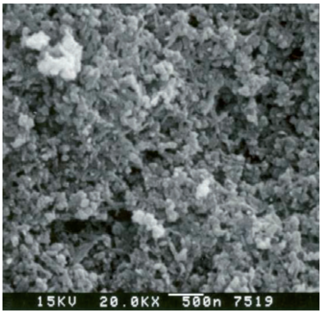

Figure 6- 10. The SEM image of the mineral produced from the reduction of ferrihydrite by *G. sulfurreducens* in the presence of 10 µM AQDS

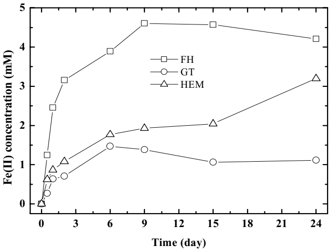

Figure 6-11. Reduction of Fe(III) minerals by *G. sulfurreducens* in the presence of 3.5 µM CCl_4 at pH 7. The ferric oxides (10mM) used were ferrihydrite(FH), goethite(GT), and hematite (HEM). The system contained 30mM acetate and 10 µM AQDS as electron donor and electron shuttling compound, respectively.

This sequence is different from that for microbial Fe(III) reduction in which ferrihydrite could produce higher amount of Fe(II) than hematite and goethite. This difference is probably because, although the surface-bound Fe(II) species are supposed to be responsible for the dechlorination, there is difference in reactivity in the different iron mineral systems. This phenomena has been discussed in chapter 2 using surface-bound Fe(II) associated with different Fe(III) oxides where crystalline iron minerals have better dechlorination capabilities than that of poorly crystalline ferrihydrite. Moreover, *G. sulfurreducens* reduced hematite more efficiently than goethite, depicting that intrinsic reactivity of surface-bound Fe(II) species is dependent on type of iron oxide. However, it is complicate to predict which species of Fe(II) is reactive for CCl_4 dechlorination under microbial Fe(III) reducing condition because the biogenic minerals may not be similar to the pure minerals. Also, the composition of biogenic mineral phase is dependent on several factors including the types of buffer and available ions in the aqueous phase. For example, siderite ($FeCO_3$) would be produced if bicarbonate buffer was used, while the mineral phase would change to vivianite ($FePO_4$) or magnetite (Fe_3O_4) if phosphate or organic buffer was used.[25,50] In this study, the biogenic Fe(II) containing mineral resulted by microbial reduction of ferrihydrite was identified to be magnetite. The dechlorination of CCl_4 by Fe(II) species may also be facilitated by electron shuttling compound (AQDS) where Fe(II) species can be oxidized to Fe(III) coupling with the reduction of AQDS to AHQDS.[51]

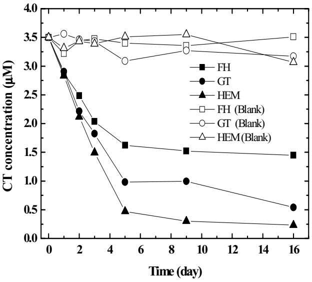

Figure 6-12. Dechlorination of 3.5 μM CCl_4 under the biological Fe(III) reducing condition. 10 mM Fe(III) oxides, ferrihydrite (FH), goethite (GT), hematite (HEM) were reduced by *G. sulfurreducens* at pH 7(50 mM HEPES). The system contained 30mM acetate and 10mM AQDS as electron donor and electron shuttling compound, respectively.

According to Figure 6-12, the decrease in CCl_4 dechlorination followed two linear phases. This means that observable dechlorination does not obey first-order kinetics. This may be due to the simultaneous occurrence of the increase in the reactive Fe(II) concentration by continuously formation of Fe(II) from biological Fe(III) reduction wile the dechlorination by biogenic Fe(II) is undergoing. Chloroform was found as the major chlorinated product. However, the maximum concentration of $CHCl_3$ was below or around 1 μM in all the systems. No additional peaks were observable in the GC-ECD chromatograph in the headspace analysis suggesting that non-chlorinated product may be formed. A recent study reported the formation of $CHCl_3$ as the sole chlorinated product, which accounted for around 30% of dechlorinated CCl_4, by isolated biogenic magnetite.[39]

The low recovery of less chlorinated compound might be due to the partially sorption of CCl_4 on the Fe(III) mineral particles or on the bacterial surface. The blank controls in Figure 6-12 showed that the concentration of CCl_4 did not decrease obviously in the presence of Fe(III) oxides, depicting that ferric oxides only sorb trace amounts of CCl_4 which was not significantly observable. To study the possible contribution of *G.*

sulfurreducens to the disappearance of CCl_4 by sorption or by enzymatic metabolism, 3.5 µM of CCl_4 was injected into two series of serum bottles containing all the components identical that contained in Fe(III)-systems, except the Fe(III) oxide minerals. Blank controls were also performed without the addition of bacterial cells. As depicted in Figure 6-13, an obvious dechlorination of CCl_4 was observed in the bacterial systems without the addition of Fe(III) mineral. Trace amount of $CHCl_3$ was found in the GC-ECD chromatograph suggesting that the decrease in CCl_4 concentration may be due to bacterial metabolism of CCl_4. It is also the first time to report that *G. sulfurreducens* can reduce dechlorinated compound. However, the comparison of Figures 6-12 and 6-13 , shows that the abiotically reductive dechlorination of CCl_4 by biogenic Fe(II) plays an important role in the microbial Fe(III)-reducing environment.

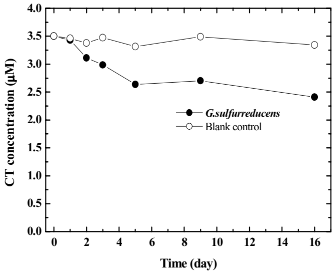

Figure 6-13. Biological dechlorination of CCl_4 by *G. Sulphurreducens* in the absence of other electron acceptor at pH 7. Acetate (30 mM) was added as electron donor in anoxic HEPES buffer (50 mM).

6.3.7 Effect of Cu(II) ion on the dechlorination of carbon tetra-chloride under microbial Fe(III) reducing condition using AQDS as electron shuttling compound.

The effect of Cu(II) ions on the microbial Fe(III) reduction and the dechlorination of CCl_4 was further investigated. Ferrihydrite was selected as the Fe(III) source to study the effect of Cu(II) on the microbial Fe(III) reduction because it is efficiently reducible by *G. sulfurreducens*. Figure 6-14 shows the reductive dissolution of ferrihydrite by *G. sulfurreducens* in the presence of Cu(II). It is clear that, in the presence of Cu(II), the Fe(III) reduction is mainly contributed by biological reaction because blank controls do not produce significant concentration of Fe(II). A previous studies found that iron reducing bacteria could reduce goethite even in the presence of toxic metal ions such as Cd, Pb, Cr and As.[52] However, the detrimental effect of toxic metal ions on the *G. sulfurreducens* may be dependent on the type of heavy metal. No significant different in Fe(II) concentrations between batches in the absence and presence of CCl_4 was observed. However, Cu(II) strongly influenced the biological Fe(III) reduction by *G. sulfurreducens*. Comparison of Fe(II) concentrations between solutions in the presence and absence of Cu(II) (Figures 6-11 and 6-14) shows that the maximum Fe(II) concentration from ferrihydrite reduction decreased from 4.2 mM in the absence of Cu(II) to 1.2 mM at 0.5 mM Cu(II) indicating 70% decrease in microbial Fe(III) reduction. A decrease in Fe(III) reduction due to the addition of Cu(II) was also observed in cysteine amended suspension (40% decrease). The inhibitive effect of microbial Fe(III) reduction by *G. sulfurreducens* in the presence of Cu(II) may be due to several possibilities. Firstly, Cu(II) toxicity may be detrimental to the metabolism and impeded the enzymatic activities of *G. sulfurreducens*. However, as it was shown in Table 6-1, bacteria could grow in the culture medium in presence of 0.5 mM Cu(II) indicating that Cu(II) is not detrimental to metabolism. Secondly, the Fe(III) reduction can be inhibited by precipitation. At neutral pH, Cu(II) is easy to precipitate (as discussed in Chapter 2) and the precipitate may cover the surface of Fe(III) oxide surfaces. Also when Fe(III) was reduced to Fe(II) in the presence of Cu(II), this Fe(II) again can be oxidized into Fe(III) by reducing Cu(II) to Cu(I), giving low estimates for Fe(II) concentration. The high toxicity of the resultant Cu(I) by the oxidation of Fe(II) may be the another possibility. It is known that Cu(I) is more toxic for living cells than Cu(II).[53] This may affect the Fe(III) reduction and the final Fe(II) concentration.

To understand the concentration effect of added Cu(II) on the microbial Fe(III) reduction by *G. sulfurreducens*, a series of experiments were carried out by adding various concentrations of Cu(II) (0.1 ~ 0.6 mM) into the anoxic suspensions containing 10 mM ferrihydrite (FH), 30 mM acetate and 10μM AQDS in the presence and absence of *G. sulfurreducens.* The concentrations of Fe(II) and Cu(I) were also detected periodically. Figures 6-15 and 6-16 demonstrate the Fe(II) concentrations detected in the bacterial inoculated and blank systems respectively. The 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 2.2 mM at 0.6 mM of Cu(II) clearly showing that with the increase in the added Cu(II) concentration, the efficiency of F(III) reduction decreased gradually. The Fe(II) reduction efficiencies were almost similar after 25d of incubation when the Cu(II) concentration ranged between 0.3 and 0.6 mM, suggesting that the microbial activity was hampered.

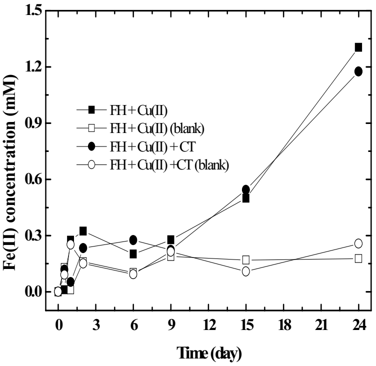

Figure 6-14. Reduction f 10 mM ferrihydrite (FH), by *G. Sulphurreducens* in the presence of 0.5 mM Cu(II) using 10mM AQDS as electron shuttling compound with and without amendment of 3.5 µM CCl_4 in the buffered (50 mM HEPES) solution at pH 7.

The extractable Cu(I) concentrations in the serum bottles in the presence of various concentrations Cu(II) (0.1 ~ 0.6 mM) were also determined (Figures 6-17 and 6-18) The concentrations of extractable Cu(I) in the bacterial system were in the range of 0.04 ~0.15 mM. Whereas only 0.01~ 0.02 mM Cu(I) were obtained in the blank control systems reflecting that a considerable concentration of Cu(I) was formed due to the microbial Fe(III) reduction in the Cu(II) amended systems. The low Cu(I) (< 0.02mM) in the batches without the addition of Cu(II) may be due to the possible interferences. This result is consistent with the result of Fe(II) concentration produced from Fe(III) reduction, depicting that Cu(I) was formed mainly from the reduction of Cu(II) by the oxidation of Fe(II) that has been discussed in Chapters 3 and 4.

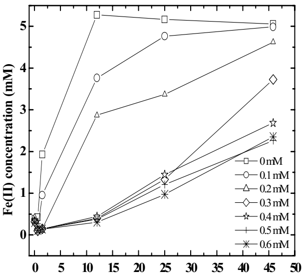

Figure 6-15. Reduction of 10mM ferrihydrite (FH) by *G. Sulphur-reducens* in the presence of various concentrations (0.1~ 0.6 mM) of Cu(II) at pH 7. 30 mM acetate and 10μM AQDS were used as electron donor and electron shuttling compound, respectively.

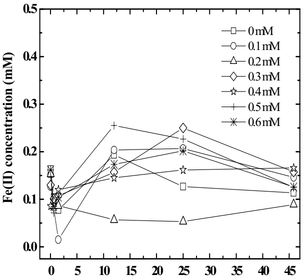

Figure 6-16. Reduction of 10mM ferrihydrite (FH), without *G. sulfurreducens* in the presence of various concentrations (0.1~ 0.6 mM) of Cu(II) at pH 7. 30 mM acetate and10 μM AQDS were also contained in the suspensions.

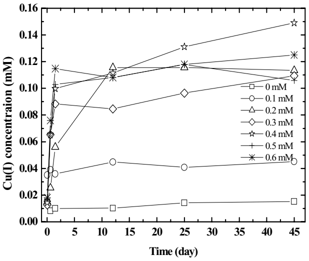

Figure 6-17. Formation of Cu(I) during the biological reduction of 10 mM ferrihydrite by *Geobacter sulphur-reducens* at neutral pH in the presence of various concentrations of Cu(II) (0.1~0.6mM). 30 mM acetate and 10μM AQDS were used as electron donor and electro shuttling compound.

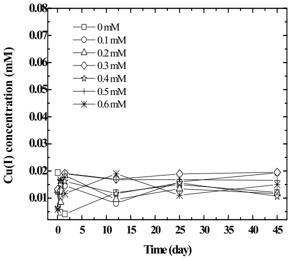

Figure 6-18. Formation of Cu(I) in blank controls (without bacterial cells) contained 10 mM ferrihydrite at neutral pH in the presence of various concentrations of Cu(II) (0.1~ 0.6mM). 30 mM acetate and 10μM AQDS were also presence in the suspension.

According to the result obtained in this study, one can suggest that biological Cu(II) reduction also could be occurred when coupled with the biological Fe(III) reducing process. Previous studies have demonstrated the possibility of several metals such as Mn (IV), U(VI), Tc(VII) Cr(VI) and Co(III) ions by DIRB.[16,17,54] The reduction of Mn(IV), U(VI), Tc(VII) by *G. sulfurreducens* was also reported.[26,55,56] However, no study has been reported on the microbial reduction of Cu(II). The results obtained in this study supports the hypothesis of microbial Fe(III) reduction in the presence of Cu(II) and the resultant Fe(II) could reduce Cu(II) to Cu(I). In the previous chapters the effect of Cu species on the dechlorination of CCl_4 by different Fe(II) systems were discussed. Therefore, the dechlorination of CCl_4 under the microbial iron- reducing conditions and the impact of Cu(II) on the dechlorination efficiency was also studied using ferrihydrite as the model Fe(III) mineral. Figure 6-19 shows the dechlorination of 3.5μM CCl_4 under the biological Fe(III)-reducing condition in the presence of 0.5 mM Cu(II) at neutral pH. A rapid dechlorination of CCl_4 was observed in bacterial inoculated serum bottles while the decrease in concentration of CCl_4 concentrations in the blank was insignificant. Nearly 83% of the initial CCl_4 was dechlorinated during 2 days and then slowly decreased to lower than the detection limit after 16 d. Under the similar conditions in biological Fe(III) reducing environment however, only 28% of CCl_4 was dechlorinated in the absence of Cu(II) (Figure 6-13). It is noted that although the addition of Cu(II) lowered the formation of Fe(II), the dechlorination of CCl_4 was fast in the presence of Cu(II). Trace amounts of $CHCl_3$ were found to be the major chlorinated product identified by GC-ECD.The first stage for CCl_4 dechlorination seems like linear which is as the same as that in the absence of Cu(II) because of the increase reactive Fe(II) with CCl_4 reduction simultaneously.

In order to study the kinetics of CCl_4 dechlorination by biogenic Fe(II) in the presence of Cu(II) other experiment with high concentration (40 μM) of CCl_4 was conducted. At first, 10 mM ferrihydrite was allowed to reduce by G. sulfurreducens in the HEPES buffered medium at pH7 ± 0.1 in the presence of 30 mM acetate and 10 μM AQDS. After incubation for two days, the Fe(II) concentrations were determined and were in the range of 2.58 ~ 2.79 mM. Subsequently Cu(II), and CCl_4 were added to the batches to get the concentrations of 0.5 mM and 40μM respectively Figure 6-20 shows the dechlorination of 40 μM CCl_4 by biogenic Fe(II) oxides in the absence and presence of 0.5 mM Cu(II). In the absence of Cu(II), CCl_4 concentrations decreased from 40 μM to 33.9 μM and 30.7 μM when, the Fe(II) concentrations were 2.58 and 2.65 μM respectively, depicting that only 15% ~ 23% of the initial CCl_4 was dechlorinated within 72 h by biogenic minerals without Cu(II). Addition of 0.5 mM Cu(II) significantly enhanced the efficiency and rate of CCl_4 dechlorination and 89% ~ 98.5% of the initial CCl_4 were dechlorinated during 72 hours. This CCl_4 dichlorination followed first – order kinetics and the k_{obs} for CCl_4 dechlorination were within the range of 0.04 ~ 0.05 h^{-1}.

In this study the effect of Cu(II) on the dechlorination under Fe(III)-reducing condition was evaluated using only ferrihydrite because *G. sulfurreducens* can effectively reduce ferrihydrite more than crystalline Fe(III) oxides during the limited time in the experimental course. If crystalline Fe(III)oxides were used in the presence of Cu(II) the efficiency of dechlorination may be much higher. In conclusion, this study shows the possibility of simultaneous reduction of Cu(II) and carbon tetrachloride due to biogenic Fe(II) under bacterial Fe(III) reducing conditions simultaneously with the synergistic effect of of copper ions on the CCl_4 dechlorination under microbial Fe(III) reducing condition.

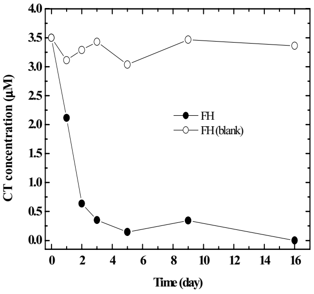

Figure 6-19. Dechlorination of 3.5 μM CCl_4 under the biological Fe(III) reducing condition in the presence of 0. 5 mM Cu(II) at pH 7.0. Acetate (30 mM) and 10 μM AQDS were amended as electron donor and electron shuttling compound, respectively.

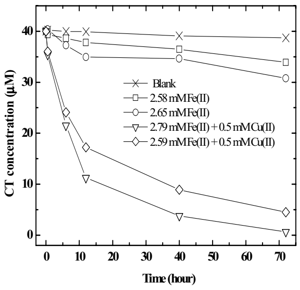

Figure 6-20. Dechlorination of CCl_4 (40μM) by biogenic Fe(II) in the absence and presence of CT and 0.5 mM Cu(II). Biogenic ferrous were the product mineral of the reduction of 10mM ferrihydrite by *G. Sulphur-reducens.*

6.4 ENVIRONMENTAL SIGNIFICANCE

Dissimilatory iron reducing bacteria (DIRB) plays a significant role in the environment in different ways, such as oxidation of various types of contaminants.[12,10] Coupling with reduction of natural Fe(III) oxide mineral, hydrogen and other metal ion species.[16,17] Due to the reduction of Fe(III) oxides, various types of surface-bound Fe(II) species and mixed-valence iron minerals are generated, which have reducing capacity to reduce various types of priority pollutants including chlorinated hydrocarbons. During the reductive transformation of those chlorinated compounds, reactive Fe(II) species are consumed, but DIRB can regenerate the reactive Fe(II) sites on Fe(III) oxides.. However, the effect of metal ions on the microbial reductive dissolution of Fe(III) minerals received less attention. This issue is environmentally important due to several reasons. Firstly, transition metal ions often co-exist with chlorinated hydrocarbons in the contaminated sites Secondly; iron oxides have strong sorption capacity towards the sorption of transition metal ions. Thirdly, transition metals have been investigated to assess their impact on the reductive dechlorination rates by structural Fe(II) containing minerals [57-59] and surface-bound Fe(II).[60] The understanding of the effects of transition metal ions on the microbial dissolution and simultaneous dechlorination of chlorinated hydrocarbons by biogenic Fe(II) provides more strong impetus on the possibilities for the enhanced remediation of chlorinated hydrocarbons under microbial iron-reducing conditions. This study provide evidences that, in the presence of Cu(II) lower than 0.6mM, *G.sulfurreducens* could reduce Fe(III) oxides to some extent and convert ferrihydrite to magnetite to form biogenic Fe(II)species. A rapid increase in reductive dechlorination of carbon tetrachloride in the biogenic Fe(III)species can occur under reducing condition in the presence of Cu(II) ions. The abiotic dechlorination by the biogenic Fe(II) species was found to be the dominant dechlorination reaction

In summary, the microbial Fe(III)-reduction can catalyze the abiotic dechlorination of chlorinated compounds significantly. Addition of Cu(II) ions decreased the Fe(III) reducing efficiency by *G. sulfurreducens*. However, the added Cu(II) can be reduced to Cu(I) by Fe(II) oxidation, and subsequently enhances the dechlorination rate significantly under the microbial Fe(III) reducing conditions

6.5 SUMMARY

G. sulfurreducens could reduce Fe(III) in all the iron oxides used in this study and the efficiency of Fe(III) reduction was in the descending order of ferrihydrite > hematite>goethite. Both 0.5 mM cysteine and 10 μM AQDS facilitated the microbial Fe(III) reduction. Hower, maximum Fe(II) concentration due to Fe(III)reduction by *G. sulfurreducens* increased 1.7 and 4 times in ferrihydrite and hematite systems when AQDS was used instead of cysteine. Also the abiotic Fe(III) reduction decreased. Due to the side reaction of cysteine with Cu(II) for the dechlorination, AQDS was used as electron shuttling compound for biological Fe(III) reduction. *G. sulfurreducens* reduced Fe(III) oxides in the presence of Cu(II) (up to 0.6 mM). However, the efficiency of F(III)-reduction significantly decreased in the presence of Cu(II). The observed decrease in Fe(II) formation could be due to low recovery of generated Fe(II), because of the oxidation of biogenic Fe(II) to Fe(III) coupling with the reduction of Cu(II) to Cu(I) was evidenced by the presence of Cu(I) in Cu(II) amended bacterial Fe(III) reducing system. *G. sulfurreducens* degrade 30% of the initial CCl_4 (3.5μM) was dechlorinated within 16 days by G.*sulfurreducens* in the presence of acetate as electron donor. Whereas 92% of the initial CCl_4 was dechlorinated within 5 days when Fe(III) oxide was provided as electron acceptor indicating that CCl_4 dechlorination under microbial Fe(III) reducing condition is mainly due to the abiotic reaction by biogenic Fe(II). The dechlorination kinetics under the biological Fe(III)-reducing condition did not follow first-order kinetic due to the simultaneous occurrence of the formation of reactive Fe(II) species and the dechlorination reaction. However, addition of CCl_4 in to the bacterial system after biogenic Fe(II) was formed (within the range of 2.5~2.8 mM), dechlorination reaction followed first-order reaction kinetics. Although the addition of 0.5 mM Cu(II) decreased the efficiency of microbial Fe(III) reduction by 70%, the CCl_4 dechlorination rate increased from 0.5 $\mu M\ d^{-1}$ to 1.45 $\mu M\ d^{-1}$ in ferrihydrite reducing system. Results obtained in this study provide valuable evidences that the biogenic Fe(II) species can simultaneously reduce Cu(II) and CCl_4 significantly and the presence of Cu species can significantly increase the efficiency of CCl_4 dechlorination during microbial Fe(III)-reduction.

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Chapter 7

CONCLUSIONS

Results obtained in this study clearly demonstrate the influence of Cu(II) ions on the dechlorination of chlorinated hydrocarbons by surface-bound iron species under anoxic conditions. Fe(II) in the dissolved form did not dechlorinate carbon tetrachloride (CCl_4) significantly at near neutral pH conditions. However, surface-bound Fe(II) species dechlorinated CCl_4 with different reactivities, depending on the mineral types. In this study, Fe(II) bound with crystalline iron oxides is found to be more reactive than that with amorphous ferrihydrite. The product formation ratio with regards to CCl_4 conversion to chloroform $CHCl_3$ () also depends on the type of Fe(III) oxide to which Fe(II) was bound. Goethite has the highest reactivity towards dechlorination of CCl_4, while the dechlorination capability of ferrihydrite is the least. Addition of 0.5 mM Cu(II) to 3 mM Fe(II) associated with 10 mM iron oxide systems significantly enhanced the dechlorination efficiency and rate of chlorinated hydrocarbons. The increase in the k_{obs} due to Cu(II) addition were 100 times in goethite, 30 times in hematite, 3.2 times in ferrihydrite, and 119 times in magnetite systems in the presence of 3 mM Fe(II) at pH7.2. No dechlorination of CCl_4 by Cu(II) alone confirms the synergistic effect on the dechlorination by Cu(II) and surface-bound iron species.

The reactivity of Fe(II)/Fe(III) systems on the dechlorination of chlorinated methanes and ethenes used in this study are compared with results obtained in the literature is compared in Table 7-1 with some experimental conditions. As shown in Table7-1, the reactivity (k_{obs}) of the Fe(II) associated with goethite suspensions towards the dechlorination of CCl_4 was within the range of the values available in the literature. GR(Cl) seems slightly high reactivity for the dechlorination of CCl_4 compared with the k_{obs} in GR(SO_4) in the literature. In addition to the type of green rust, the type of buffer also may affect the reactivity of green rust system. As shown in the table 7-1, in the case of controlling pH with out the use of buffers (just by adjusting the initial pH using acids and bases) the final pH of the green rust suspension may change. Specially when the metastable iron hydroxides can be partially dissolved in the water and hence changes in the pH may occur gradually. Therefore, in comparison of the reactivities of pH buffered GR(Cl) system with those from the literature results obtained in un-b uffered green rust systems, some deviations in the reactivity are reasonable.

Table7-1. Comparison of the reactivities of various Fe(II)/Fe(III) systems for the dechlorination of chlorinated methanes and ethenes and experimental conditions used in this study with those data reported in the literature.

Fe(II)/Fe(III) system	Reference	Target organic	Experimental conditions and reactivity	Present study
Dissolved Fe(II)	a	CCl_4	$[CCl_4]_0$= 0.7µM, $[Fe(II)]_0$= 1.85 $T_{½}$ for CCl_4 ≈ 4 d, pH=7.0	$[CCl_4]_0$= 20mM, $[Fe(II)]_0$= 3mM pH ≤ 7.0 no reduction
Goethite/Fe(II)	b	CCl_4	$[Goe]_0$= 11.2mM (55 m^2L^{-1}) , pH=7.0 (10 mM PIPES), $[Fe(II)]_0$ =3mM, k_{obs} = 0.016 h^{-1}	$[Goe]_0$= 10mM (25.6 m^2L^{-1}) , pH=7.0 (50 mM HEPES), $[Fe(II)]_0$ =3mM, k_{obs} = 0.036 h^{-1} k_{sa} = 0.028 h^{-1} m^{-2}
Goethite/Fe(II)	c	CCl_4	$[Goe]_0$= 86mM (110 m^2L^{-1}) pH=6.8 (30 mM MES), $[Fe(II)]_0$ =1mM, k_{sa} = 0.138 h^{-1} m^{-2}	
Goethite/Fe(II)	d	CCl_4	$[Goe]_0$= 16mM (25 m^2L^{-1}) pH=7.2 (25 mM MOPS), $[Fe(II)]_0$ =1mM, k_{sa} = 0.016 h^{-1}	
Green rust	e	CCl_4	pH~8.0, [GR (SO4)]= not available k_{obs} = 0.016 h^{-1} ~ 0.78 h^{-1}	pH~7.2, [GR (Cl)]= 0.0015 g mL^{-1} k_{obs} = 0.080 h^{-1}
Green rust	f	CCl_4	PH=7.6 $[GR(SO_4]$ = 0.005 g mL^{-1} k_{obs} =0.0612 h^{-1}	
Green rust +Cu(II)	f	CCl_4	PH=7.6 $[GR(SO_4]$ = 0.005 g mL^{-1} [Cu(II)] = 0.1 mM k_{obs} =9.72 h^{-1}	pH~7.2, [GR (Cl)]= 0.0015 g mL^{-1} [Cu(II)] = 0.5 mM k_{obs} = 6.99 h^{-1}
Green rust+Cu(II)	f	$CHCl_3$	PH=7.6 $[GR(SO_4]$ = 0.005 g mL^{-1} [Cu(II)] = 0.1 mM k_{obs} =0.162 h^{-1}	pH~7.2, [GR (Cl)]= 0.0015 g mL^{-1} [Cu(II)] = 0.5 mM k_{obs} = 0.3513h^{-1}
Green rust	g	C_2Cl_4	PH=7 (No buffer) $[GR(SO_4]$ = 0.007g mL^{-1} k =0.0195 d^{-1}	pH~7.2(50mM HEPES), [GR (Cl)]= 0.0015 g mL^{-1} k_{obs} = 0.0182 d^{-1}
Green rust	g	C_2HCl_3	PH=7 (No buffer) $[GR(SO_4]$ = 0.007g mL^{-1} k =0.0132 d^{-1}	pH~7.2(50mM HEPES), [GR (Cl)]= 0.0015 g mL^{-1} k_{obs} = 0.006 d^{-1}

* *Note: the literature mentioned are available in the reference list in chapter-1 as follows a: (118), b: (109), c:(136), d: (111), e:(124), f: (125), g: (121)*

The environmental parameters including pH and Fe(II) concentration controlled the reactivity in Fe(II)/Fe(III) systems both in the presence and absence of Cu(II). The relationship between initial Fe(II) and k_{obs} followed

Langmuir-Hinshelwood kinetics, showing that Fe(II) is responsible for the dechlorination reaction either in the absence or presence of Cu(II). Chloroform ($CHCl_3$), the major chlorinated product in the CCl_4 dechlorination, also could be dechlorinated into less chlorinated products by the amendment of Cu(II) into surface -bound Fe(II) systems, depicting that Cu(II) plays an important role in detoxification of chlorinated compounds.

The rate constant (k_{obs}) for the CCl_4 dechlorination in Fe(II)-goethite system has linear relationship with the concentration of amended Cu(II) up to 0.5 mM and further increase in Cu(II) concentration decreased the rate constant and the pH of the system. Analysis of total Fe(II) in the suspension showed that Fe(II) was oxidized to Fe(III). The presence of Cu(I) in the suspension proved that Cu(II) was reduced to Cu(I) coupling with Fe(II) oxidation. This means that new Fe(III) minerals and Cu(I) species are formed in the surface–bound Fe(II) system. The solid species were found to be mainly responsible for the enhanced dechlorination when Cu(II) was amended into goethite system. It also found that the surface-bound Fe(II) concentration in goethite suspension slightly increased even the total Fe(II) concentration decreased. The analysis of solid-phase by XRD and XPS suggests that amorphous ferrihydrite and cuprous oxides (Cu_2O) are produced when Fe(II) oxidation coupled with Cu(II) reduction. According to these results, it can be concluded that the enhanced dechlorination in Cu(II)-amended Fe(II)-iron oxide suspension is due to the formation of new solid phases. The produced Fe(III) minerals increases the surface-bound Fe(II) concentration and catalytic Cu(I) with surface-bound Fe(II) subsequently enhances the dechlorination efficiency and rate of CCl_4. From the environmental point of view, this gives positive impetus for the coupled remediation of chlorinated products and inorganic metal ions using synergistic effect of Cu(II) and surface-bound iron species.

The synergistic effect of Cu(II) in the Fe(II) solution was also investigated without amendment of Fe(III) oxides. Either Fe(II) or Cu(II) alone did not dechlorinate CCl_4 at neutral pH. However, the addition of Cu(II) into Fe(II) solution at various Fe(II)/Cu(II) ratios significantly enhanced the dechlorination efficiency and rate of CCl_4. The solid phase analyses by XRPD and XPS suggests the mixture contained Cu_2O and several Fe(III) oxides. Amorphous ferrihydrite was produced at Fe(II)/Cu(II) ratio of 1, while goethite and magnetite were formed when the Fe(II)/Cu(II) molar ratios reached 4 – 6. Further increase the stoichiometric relation of Fe(II)/Cu(II) to 10 again transformed the iron oxide to ferrihydrite.

Amendment of Cu(II) ions into green rust (GR(Cl)) suspensions also enhanced the dechlorination of carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), tetrachloroethene (C_2Cl_4) and trichloroethene (C_2HCl_3). Although those compound could be reduced by GR(Cl) alone with slow dechlorination rates, the addition of Cu(II) into GR(Cl) system increased the k_{obs} for dechlorination up to 84 times for CCl_4, 4.7 times for C_2Cl_4 and 7 times for C_2HCl_3. However, the chemical species of Cu(II) after the dechlorination in the GR(Cl) system is different from that in the surface-bound Fe(II) and dissolved Fe(II) systems. Cu(II) was reduced to both Cu(I) and Cu(0) coupling to oxidation of green rust to magnetite, thus enhancing the dechlorination efficiency and rate of chlorinated hydrocarbons.

Since Cu(II) ions have the synergistic effect on various types of Fe(II)/Fe(III) systems including surface-bound Fe(II), dissolved Fe(II) and mixed–valence green rust, the effect of Cu(II) on the dechlorination of CCl_4 by biogenic Fe(II) species under microbial Fe(III) reducing condition was also evaluated. Although addition of Cu(II) into bacterial Fe(III) reducing environment inhibited the Fe(III) reducing process to a certain extent, the dechlorination efficiency and rate of CCl_4 was enhanced in the presence of AQDS. This enhanced effect is mainly due to the formation of biogenic magnetite and aqueous Fe(II) complexes during the microbial reductive dissolution of ferrihydrite. The biogenic Fe(II) species can simultaneously reduce Cu(II) and CCl_4 and the presence of Cu(I) species can increase the dechlorination efficiency of CCl_4 during the biological Fe(III) reducing reaction.

In summary, the results obtained in this study clearly show the feasibility of coupled degradation of chlorinated hydrocarbons and transition metals under the iron-reducing conditions. The synergistic effect of dissolved Cu(II) and Fe(II) species on the reduction of chlorinated hydrocarbons may enhance our understanding of the role of Fe(II) and the long-term reactivity of zerovalent iron system in the dechlorination processes. Fe(II) ions as well as iron oxides are abundant in the natural environments. The association of Fe(II) with iron oxide can form the reactive surface-bound iron species to serve as an reductant in natural attenuation processes. In the contaminated subsurface environments, the Fe(II) concentrations can be higher up to 1.4 ~3 mM. This gives impetus to facilitate the development of processes that could be used for the coupled detoxification of chlorinated hydrocarbons and metal ions.

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