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Effects of Microbial Iron Reduction and Oxidation on the Immobilization and Mobilization of Copper in Synthesized Fe(III) Minerals and Fe-Rich Soils

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The effects of microbial iron reduction and oxidation on the immobilization and mobilization of copper were investigated in a high concentration of sulfate with synthesized Fe(III) minerals and red earth soils rich in amorphous Fe (hydr)oxides. Batch microcosm experiments showed that red earth soil inoculated with subsurface sediments had a faster Fe(III) bioreduction rate than pure amorphous Fe(III) minerals and resulted in quicker immobilization of Cu in the aqueous fraction. Coinciding with the decrease of aqueous Cu, SO_4^{2-} in the inoculated red earth soil decreased acutely after incubation. The shift in the microbial community composite in the inoculated soil was analyzed through denaturing gradient gel electrophoresis. Results revealed the potential cooperative effect of microbial Fe(III) reduction and sulfate reduction on copper immobilization. After exposure to air for 144 h, more than 50% of the immobilized Cu was remobilized from the anaerobic matrices; aqueous sulfate increased significantly. Sequential extraction analysis demonstrated that the organic matter/sulfide-bound Cu increased by 52% after anaerobic incubation relative to the abiotic treatment but decreased by 32% after oxidation, indicating the generation and oxidation of Cu-sulfide coprecipitates in the inoculated red earth soil. These findings suggest that the immobilization of copper could be enhanced by mediating microbial Fe(III) reduction with sulfate reduction under anaerobic conditions. The findings have an important implication for bioremediation in Cu-contaminated and Fe-rich soils, especially in acid-mine-drainage-affected sites.

Keywords: Bioremediation, microbial Fe(III) reduction, Cu immobilization, oxidation, red earth soils

Introduction

Mining activities of different scales in the regions of Southern China generate large quantities of waste tailings and acidic drainage and cause pollution by heavy metals (such as Cu, Pb, and As) in highly contaminated sites, spreading to local river sediments and agricultural soils [37, 40]. The release of such metals from polluted sites affected by acid-mine-drainage (AMD), which is usually generated by weathering a large volume of exposed sulfide-bearing reactive tailings, depends largely on sorption and desorption processes on the sediment mineral surfaces [16, 20]. Abundant iron oxides and hydroxides ((hydr)oxides) with a specific surface area and negative charge density have an important function in the sequestration of heavy metals into soils and sediments, particularly in red earth regions. However, the sequestrated metals in Fe(III) (hydr)oxide-rich soils and sediments are not always stable at soil–water interfaces, where the transition of anaerobic and aerobic conditions during water level fluctuation leads to significant biological activities, thereby altering the mineral surfaces as well as their adsorption properties [22].

Under anaerobic conditions, metal-reducing bacteria are able to utilize Fe(III) minerals by using a wide range of substrates (*e.g.*, acetate, ethanol, and glucose) as electron donors, thereby affecting the immobilization and/or release of heavy metals significantly [4]. Previous reports demonstrate that dissimilatory Fe(III)-reducing bacteria, such as *Geobacter sulfurreducens*, cause the reduction of Fe(III) to Fe(II) and lead to the direct release of metals adsorbed on the surface of iron oxides [18]. However, the rate and extent of biological Fe(III) reduction are influenced by various environmental factors (*e.g.*, sulfate) as well as the forms of Fe(III) oxide minerals [13, 14, 34]. Poorly crystalline iron oxides, such as amorphous iron oxides that are ubiquitously distributed in naturally occurring soils, exhibit a high ratio of reducible area to total surface area. The reductive dissolution and transformation of amorphous Fe(III) (hydr)oxides have a significant effect on the mobility of metallic contaminants under anaerobic conditions [18]. The solid-phase metals bound to the ferric minerals are partially transformed into dissolved-phase metals depending on the interactions of the metals with the iron oxide minerals, such as adsorption, surface precipitation, and coprecipitation (or substitution) [5]. However, the issue of whether the different interaction processes of heavy metals on the mineral surface affect the microbial reduction of amorphous Fe(III) minerals and the condition of heavy metals in Fe-rich soils, especially during the transition from anaerobic to aerobic conditions, remains unsolved to date.

It has been commonly thought that the microbial Fe(III) reduction concurs with a sulfate reduction process, because Fe(III) and sulfate are often relatively abundant in subsurface environments [8, 10]. Bethek *et al*. [1] reported that microbial iron reduction and sulfate reduction can simultaneously proceed without product limitation, due to the co-reaction of Fe(II) and sulfide products of both processes leading to formation of FeS precipitation. Although the reduction dissolution of Fe(III) (hydr)oxides may be accelerated in the presence of sulfate under pure culture condition, both Fe(III) reduction and sulfate reduction depend on the microbial community structure in natural environments [12, 23, 35]. So far, the stimulation influence of microbial iron reduction and sulfate reduction on the dissolution of Fe(III) minerals and mobilization of the heavy metals has not been well studied with mixed microbial populations in controlled laboratory conditions.

As reported in our previous study, Cu was one of the heavy metals that seriously contaminate the soil pore water near the drainage of a metal mining site in Datian County, Fujian Province, South China [6]. In the present study, the effects of microbial iron reduction and oxidation on the transformation of Cu in the presence of high level of sulfate were investigated with synthesized Fe(III) minerals and natural red earth soil, which is rich in amorphous Fe (hydr)oxides. A series of batch microcosm experiments were performed by inoculating subsurface sediments from a freshwater site in the red earth region of southern China. The dynamics of microbial Fe(III) reduction and sulfate reduction and their influences on the immobilization of Cu were assessed with Cu-adsorbed and Cu-coprecipitated amorphous Fe(III) minerals as well as Cu-saturated red earth soils. The change in the bacterial community composite was analyzed by comparing the denaturing gradient gel electrophoresis (DGGE) band patterns of 16S rRNA gene sequences to investigate the adaptation and potential role of the enriched microorganisms in the inoculated red earth soil. Oxidation incubation of the anoxic minerals and soils was then performed to evaluate the oxidation effect on the retention and/or release behavior of aqueous Cu and sulfate. The result of this study would provide new insight on the role of the biogeochemistry correlative to Fe(III) and sulfate-reducing conditions in the fate of heavy metals in soils and sediments, especially for AMD-affected and Cu-contaminated red earth regions.

Materials and Methods

Microcosm Study

Amorphous Fe(III) (hydr)oxide minerals were synthesized according to a previously reported method [15]. The mineralogical properties of the synthesized Fe(III) (hydr)oxides were characterized with a Panalytical X'Pert Pro X-ray diffraction system (XRD) equipped with monochromated CuKα monochromatic radiation at a tube voltage of 40 kV. The amorphous nature of Fe(III) oxide conformed to the acquired X-ray diffraction patterns.

A Cu-adsorbed mineral was prepared by equilibrium sorption with the synthesized amorphous Fe(III) oxides in a Cu-containing solution (Cu^{2+} = ~22 mg/l) that simulated the drainage of a metal mining site in the Datian County, Fujian Province, South China. For the synthesis of the Cu-coprecipitated amorphous Fe(III) mineral, 0.4 mol/l of $FeCl_3$ solution was premixed with the Cu^{2+}-containing solution and characterized as above.

Simultaneously, red earth soil was collected around the mining site. The soil properties were characterized, and the results are shown in Table 1. The mineralogical property of the collected red earth soil was characterized by XRD. The result demonstrated that Fe in the collected soil is presented primarily in the form of amorphous Fe (hydr)oxides. The collected soil was amended with 1% $CaCO_3$ (w/w), which has been used to limit acidification and enhance metal immobilization through the gradual diffusion and adsorption of sedimentary particulates as well as (co)precipitation,

Table 1. Properties of the investigated red earth soil from Datian County, Fujian Province, South China.

pH		5.3 ± 0.2	Mn		253.1 ± 2.0
Si		20.5 ± 9.5	Cu	mg/kg	55.0 ± 0.7
Al		9.2 ± 2.0	Zn		51.2 ± 6.5
Fe		4.9 ± 0.2	CEC	cmol/kg	3.58 ± 0.17
K	%	1.0 ± 0.1	Clay (<2 μm)		18.7 ± 1.5
Na		0.3 ± 0.0	Silt (2–20 μm)	%	47.4 ± 4.2
Mg		0.2 ± 0.0	Sand (0.02–2 mm)		33.9 ± 5.7
Ca		0.03 ± 0.0	TOC	g/kg	4.62 ± 0.2

such as the formation of metal-carbonate precipitations [27]. After saturation with the simulated Cu solution, the obtained red earth soil was freeze-dried and used as the incubation matrix.

The indigenous microbial community in the subsurface sediment was used as the inoculum source for microcosm batch experiments. A previously reported method of acquiring Fe(III)-reducing microorganisms was adopted [14]. Briefly, the surfacial sediment samples were collected in 250 ml serum bottles from a wetland region in Xiamen City, southern China. The bottles were capped immediately with butyl rubber stoppers after sample collection. The major soil minerals in the clay fraction of the surface sediments were found to be kaolinite and quartz, with Fe (hydr)oxides presented as coatings. Additional details regarding the elemental composition and particle distribution can be found in our previous study [36]. The obtained sediments were cultivated in 120 ml serum bottles with 80 ml of the medium under anaerobic conditions. The abiotic condition was achieved by sterilizing the subsurface sediments before the microcosm batch experiment. The basal anaerobic medium consisted of (g/l unless noted otherwise) KCl (0.1), Na_2HPO_4 (0.6), NH_4Cl (1.5), $NaHCO_3$ (2.5), and vitamin and trace element solutions (each 10 ml/l) [15]. A sterile anoxic solution of acetate was supplemented to a final concentration of 10 mmol/l. Then 0.6 g of the prepared Fe(III) minerals and 2.0 g of the freeze-dried soils were added into the serum bottle.

The culture was incubated anaerobically at room temperature after adjusting the solution pH to 6.8 to 7.0 with 0.5 mol/l of NaOH or HCl. To investigate the kinetic behavior of aqueous Fe(III) in the anoxic solution, 0.5 ml of the supernatant was obtained at a specific time. The variation of aqueous ferrous concentration was measured by the phenanthroline chlorometric method. A second sample of 0.5 ml was collected for the analysis of dissolved metals and sulfate concentrations determined with an Optima 7000 DV ICP-OES (PerkinElmer) and ICS-3000 ion chromatography system (Dionex Corp.), respectively.

Phylogenetic Analysis of Microbial Community in the Red Earth Soils

The genomic DNA in the inoculated red earth soils was extracted at the 50th day of incubation with a Mo-Bio UltraClean soil DNA Isolation Kit (Catalog No. 12800-50). To collect the inoculated red earth soil, the mixed suspension of cultures was sampled and centrifuged at 12,000 ×*g* for 10 min. The total DNA extracted from the original microbial community in the fresh subsurface sediments was set as a control. A segment of the bacterial 16S rDNA was amplified by PCR using the bacterial-specific forward primer 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) with a GC cap (CGC-CCG-GGG-CGC-GCC-CCC-GGG-CGG-GGC-GGG-GGC) and a universal reverse primer 519r (5′-CGTATTACCGCGGCTGCTGG-3′) [17]. PCR was performed in 25 μl PCR mixtures that included 12.5 μl of 2× Tag PCR MasterMix (Tiangen Biotech Co., Ltd., Beijing, China), 0.5 μl of each primer (20 pmol), 1 μl of genomic DNA (~50 ng), and 10.5 μl of sterile DNA-free water. The PCR temperature program was initiated with denaturing at 94°C for 5 min, followed by 10 cycles at 94°C for 30 sec, 65°C for 45 sec, and 72°C for 1 min; 20 cycles at 94°C for 30 sec, 55°C for 45 sec, and 72°C for 1 min; and a final extension of 72°C for 7 min, using a PCR Thermo Cycler (Model JC-96, China) [36].

The DGGE experiment was conducted with a universal mutation detection system. Each PCR production (40 μl) was loaded onto a 6% polyacrylamide gel containing a linear gradient from 30% to 40% (100% denaturant containing 7 mol/l urea and 40% formamide). DGGE was performed at 120 V for 12 h in 1× TAE buffer at 60°C. After electrophoresis, the DGGE gel was stained with a 1:10,000 dilution of SYBR Green I DNA gel stain for 30 min. The fluorescent image of the stained gel was acquired with an Ettan DIGE Imager. The DGGE bands were excised from the obtained gel and soaked overnight at 4°C in 50 μl of sterile DNA-free water. The extracted DNA from each selected band was amplified by PCR with the aforementioned primers but without the GC cap. The PCR products were sequenced at the DNA sequencing facility of Shanghai Majorbio Bio-Pharm Technology Co., Ltd. [36]. The phylogenetic associations of the obtained partial 16S rRNA sequences were analyzed with the Classifier and Sequence Match tools of the Ribosomal Database Project (http://rdp.cme.msu.edu/classifier/classifier.jsp) and with the Basic Local Alignment Search Tool (http://www.ncbi.nlm.nih.gov/BLAST/) [17].

Oxidation Experiment

After the microcosm experiment, aliquots (20 ml) of the

suspension of anoxic Fe(III) minerals and sediments were transferred into a 50 ml polyethylene tube. The suspended solutions were oxidized by vibrated incubation in a shaker at 150 rpm. All the anoxic minerals and sediment soils were oxidized for the same period of time. At particular intervals (0, 12, 24, 48, 72, 96, and 144 h), 1 ml of the supernatant was sampled and filtrated to analyze the concentrations of released Cu and sulfate. The same amount of fresh deionized water was supplemented into the incubated solution after each sampling for the oxidized solution to maintain a constant volume.

Speciation Analysis of Fe and Cu in the Red Earth Soil

The binding speciation of solid-fraction Fe and Cu in the investigated soil was determined by the sequential extraction method [31]. After the anaerobic and aerobic incubations, the obtained soils were respectively collected for the analysis of metal speciation. The exchangeable, carbonate-bound, organic matter/sulfide-bound, Fe/Mn oxide-bound, and residue-phase metals in the soil samples were extracted with 1 mol/l magnesium chloride, 1 mol/l sodium acetate/acetic acid, 0.04 mol/l hydroxylamine–hydrochloride in 25% (v/v) acetic acid, 3.2 mol/l ammonium acetate, and HNO_3-$HClO_4$, respectively. After each extraction, the concentrations of Fe and Cu were analyzed by ICP-OES (Optima 7000 DV; PerkinElmer).

Results and Discussion

Microbial Reduction and Cu Immobilization

The batch microcosm experiments were performed in pure-mineral systems that included Cu-adsorbed and Cu-coprecipitated Fe(III) minerals. The coprecipitated Fe(III) mineral had a greater microbial Fe(III) reduction rate than the Cu-adsorbed Fe(III) mineral after inoculation with subsurface sediments (Fig. 1A). During the initial 16 days of incubation, the aqueous Fe(III) concentration in the

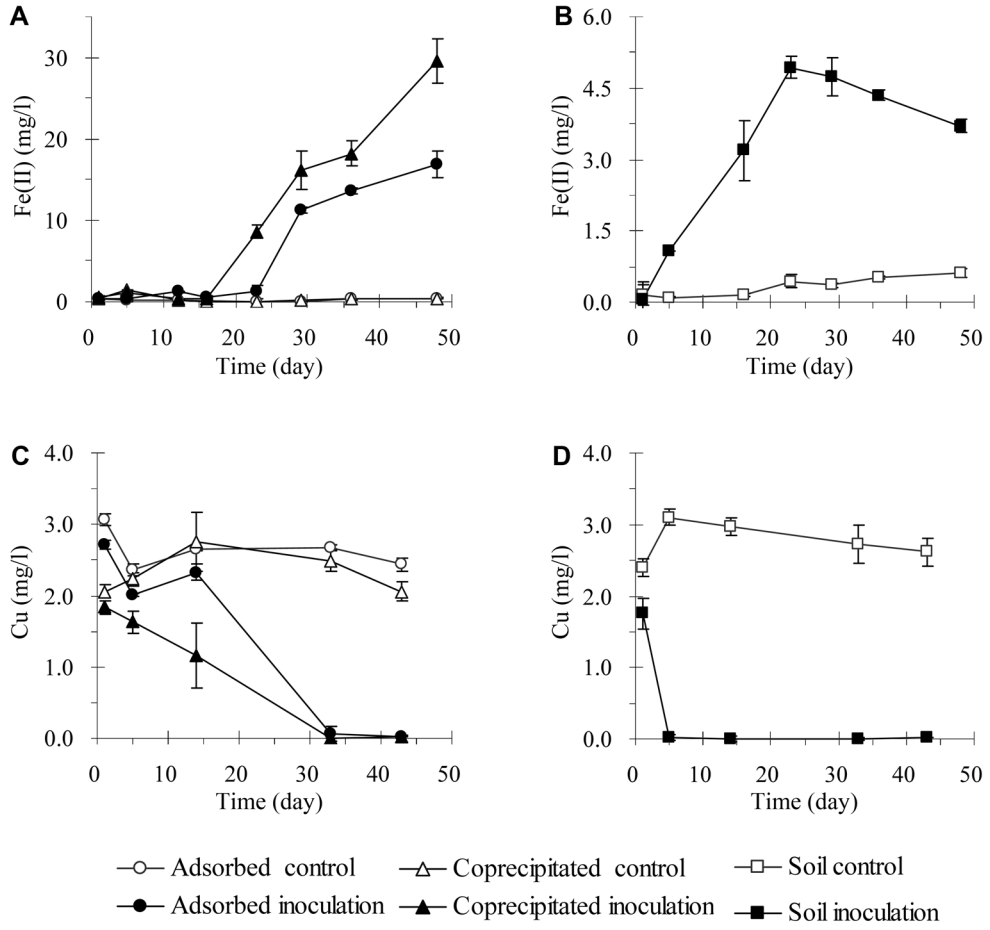

Fig. 1. Kinetics of aqueous-phase Fe(II) and Cu in the microcosm systems of the Cu-adsorbed and Cu-coprecipitated amorphous Fe(III) minerals (**A**, **C**), and the Cu-saturated red earth soil (**B**, **D**).
The control and inoculation indicate the treatments added with sterilized and fresh subsurface sediments, respectively.

microcosms exhibited no noticeable increase for both Cu-adsorbed and Cu-coprecipitated treatments. However, on the 23rd day of incubation, the aqueous Fe(II) in the Cu-adsorbed treatment increased to 1.2 mg/l and to 8.6 mg/l in the Cu-coprecipitated treatment. The difference in Fe(III) bioreduction between the Cu-adsorbed and Cu-coprecipitated inoculations was probably due to the alteration in the available ferric sites on the surface of Fe(III) minerals for microbial reduction. The adsorbed Cu was mostly distributed on the outer surface of the amorphous Fe(III) mineral and covered part of the bioreducible ferric sites, leading to a decrease in their availability. On the other hand, the coprecipitated Cu was mainly distributed on the inner surfaces of the amorphous Fe(III) minerals and had a minimal effect on the bioreducible sites. A similar phenomenon was reported by Masue–Slowey *et al*. [21], in which Al coprecipitation in ferrihydrite had a minimal effect on microbial reduction relative to pure ferrihydrite in pure cultured *Shewanella* sp.

Compared with the synthesized Fe(III) minerals, Fe(III) bioreduction with red earth soil required a relatively shorter starting time. On the fifth day of incubation, the aqueous Fe(II) of the inoculated soil increased to 1.1 mg/l and reached the highest level of 4.9 mg/l on day 23 (Fig. 1B). The relatively short starting time for Fe(III) bioreduction can be attributed to the easy adaption of the inoculated sediment microbes to the familiar soil environment. In addition, the organic matter content in the red earth soil can be used by microbes as an electron donor and/or electron shuttling to facilitate biological Fe(III) reduction [32, 33].

The Fe(II) concentration increased continuously in the treatments with synthesized minerals and reached 30.5 mg/l after incubation. However, in the soil treatment, the Fe(II) concentration began to decrease on the 29th day of incubation and eventually decreased to 3.5 mg/l after incubation. This result suggests that the bioavailability of Fe(III) minerals in the red earth soil cannot sustain the microbial reduction. In addition, the aqueous Fe(II) generated by bioreduction formed mixed Fe(II)-Fe(III) compounds or secondary Fe minerals, such as $Fe_4(OH)_{10}$ or $Fe_3(OH)_8$, siderite ($FeCO_3$), pyrite (FeS), and magnetite (Fe_3O_4) [11, 14, 24, 25].

Coinciding with the biological Fe(III) reduction, the aqueous Cu concentration in the inoculated treatments decreased successively in the order of red earth soil > Cu-coprecipitated mineral > Cu-adsorbed mineral (Figs. 1C and 1D). Compared with the sterilized controls, 95% of aqueous Cu was removed from the aqueous phase on the 33rd day of incubation for the treatments with inoculation. For the inoculated red earth soil, aqueous Cu dropped by more than 95% in the first 5 days of incubation compared with the relatively constant Cu concentration in the abiotic control. The immobilization of Cu can be attributed to the adsorption interaction of aqueous Cu with the mixed Fe(II)-Fe(III) compounds, as reported previously [24, 25]. In addition, Cu can be removed by the formation of metal-sulfide coprecipitates as sulfate reduction in the incubation (Fig. 2); Cu-sulfide precipitates could be generated during the reduction processes [29].

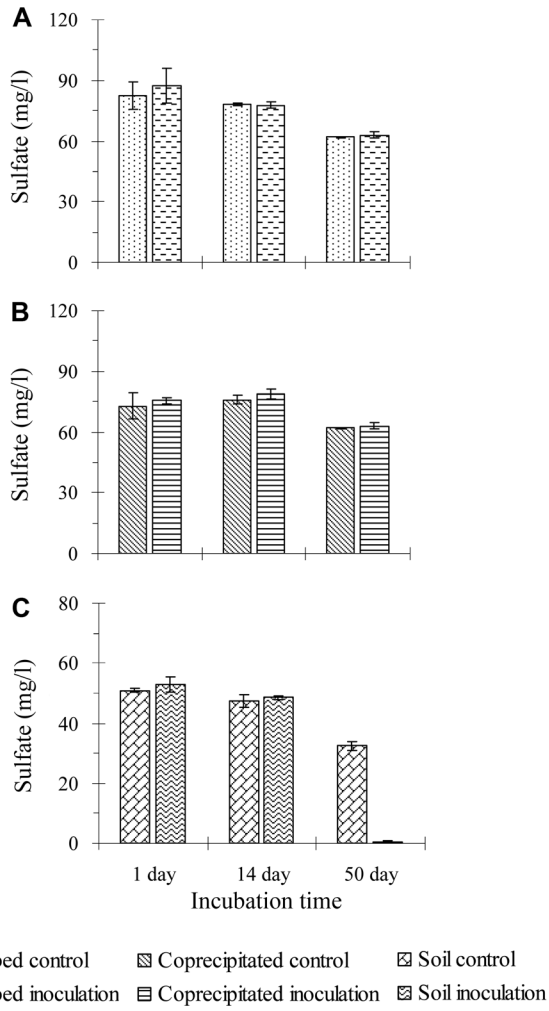

Fig. 2. Concentration change of aqueous-phase sulfate in the treatments of the Cu-adsorbed (**A**) and Cu-coprecipitated (**B**) amorphous Fe(III) minerals, and the Cu-saturated red earth soil (**C**) during anaerobic incubation.
The control and inoculation indicate the treatments added with sterilized and fresh subsurface sediments, respectively.

Change in the Microbial Community Composite in the Red Earth Soil

A shift in the population of enriched bacteria was observed with a distinguished pattern in the DGGE bands between the control and inoculated red earth soils (Fig. 3). As determined by the Classifier and Sequence Match tools of the Ribosomal Database Project (RDP-II; release 10), members of Betaproteobacteria were the predominant bacterial communities in the control soil. However, the bacterial populations in the inoculated soil mainly included Sphingobacteriales, Burkholderiales, Rhizobiales, Sphingomonadales, and Caulobacterales. Phylogenetic analysis of these excised band sequences revealed that the predominant bacteria related to *Bacteroides* sp., *Massilia* sp., *Methylobacterium* sp., *Sphingomonas* sp., and *Caulobacter* sp. were enriched in the inoculated red earth soil (Table 2). These newly enriched bacterial populations from the inoculated surfacial sediments may help accelerate the bioreduction processes of Fe(III) and sulfate in the red earth soil. Some of the enriched bacteria, including *Bacteroides*, *Massilia*, and *Sphingomonas*, have been reported to aid in mediating the biological reduction and cycling of environmentally relevant elements, such as As(V), Fe(III), SO_4^{2-}, and NO_3^- [3, 19, 26, 30]. For example, Shirokova and Ferris [26] reported that the enriched bacteria, including *Bacteroides*, could be reponsible for the reduction and biogeochemical cycling of iron and sulfur within groundwater systems on the Canadian Shield. Therefore, the enriched microorganisms in the inoculated red earth soil were considered to have contributed to the promotion of the reduction processes of

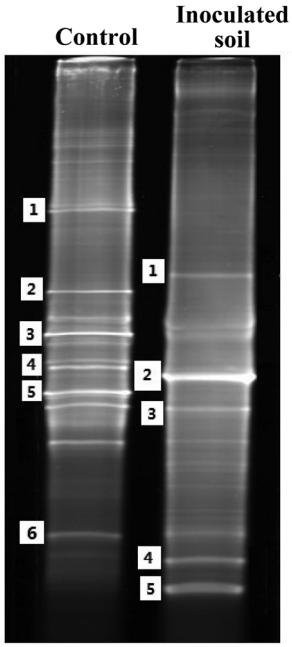

Fig. 3. Denaturing gradient gel electrophoresis analysis of partial 16S rRNA sequences of microorganisms from the inoculated red earth soil, sampled at 50th day of incubation.
The inoculated red earth soil was collected by centrifuging the mixed suspension of cultures at 12,000 ×*g* for 10 min. The control represents the original microbial community in the fresh subsurface sediments.

Table 2. Phylogenetic association of partial 16S rRNA sequences excised from denaturing gradient gel electrophoresis bands for the control and inoculated red earth soil after anaerobic incubation.

	Class	Family	Genus	CRAN	Identities (%)	Sequence length
	Control					
No-1	Betaproteobacteria	Oxalobacteraceae	Unclassified	EU446139	97	533
No-2	Unclassified	Unclassified	Unclassified	JX574811	99	531
No-3	Betaproteobacteria	Oxalobacteraceae	*Janthinobacterium*	AB252072	98	533
No-4	Betaproteobacteria	Oxalobacteraceae	*Naxibacter*	JF970594	94	533
No-5	Unclassified	Unclassified	Unclassified	AB623231	100	527
No-6	Bacilli	Streptosporangiaceae	*Microbispora*	JX978443	91	508
	Inoculated soil					
No-1	Sphingobacteria	Bacteroidaceae	*Bacteroides*	AB623231	99	527
No-2	Betaproteobacteria	Oxalobacteraceae	*Massilia*	FN386769	98	533
No-3	Alphaproteobacteria	Methylobacteriaceae	*Methylobacterium*	AY358007	99	510
No-4	Alphaproteobacteria	Sphingomonadaceae	*Sphingomonas*	HQ436497	99	482
No-5	Alphaproteobacteria	Caulobacteraceae	*Caulobacter*	NR074208	88	485

CRAN = closest relative accession numbers.

ferric iron and sulfate during anaerobic incubation, which then induced Cu immobilization.

Oxidation and Remobilization of Cu

The anoxic matrices were exposed to air for a short period to evaluate the stability of immobilized Cu under aerobic conditions. Within 12 h of air exposure, the aqueous Fe(II) concentration decreased to an undetectable level for all the treatments (Figs. 4A and 4B), indicating Fe(II) oxidation and precipitation. The aqueous Cu concentrations in the inoculated treatments increased for both Cu-adsorbed and Cu-coprecipitated minerals (Fig. 4C). Within 24 h of oxidation, the aqueous Cu increased to over 1.2 mg/l for the Cu-adsorbed treatment and over 1.5 mg/l for the Cu-coprecipitated treatment. The remobilization of Cu could be due to the loss of adsorption sites in the mixed Fe(II)-Fe(III) compounds (such as $Fe_4(OH)_{10}$ or $Fe_3(OH)_8$).

Compared with the synthesized minerals, aqueous Cu in the red earth soil exhibited a more gradual increase during oxidation (Fig. 4D). The Cu concentration increased to 0.5 mg/l within 24 h of oxidation, suggesting that the immobilized Cu in the red earth soil was partly released into the aqueous fraction under oxidation conditions probably because of the oxidation and dissolution of Cu and sulfide coprecipitates. This explanation is supported by the fact that the release of SO_4^{2-} was observed in the red earth soil after oxidation (Fig. 4). Aqueous sulfate in the inoculated soil increased from 0.5 to 13 mg/l after anaerobic incubation (within 144 h of oxidation). In previous studies, the oxidation of anoxic sediments led to the release of heavy metals from iron-rich sediments [2]. Simpson *et al.* [28] reported that the oxidation of metal sulfide phases (*e.g.*, CuS and FeS) is the major source of metal release to oxic waters. Lin *et al.* [13] also suggested that the transformation of sulfur and organic compounds contributes to the increase in dissoluble and exchangeable Cu in rice rhizosphere.

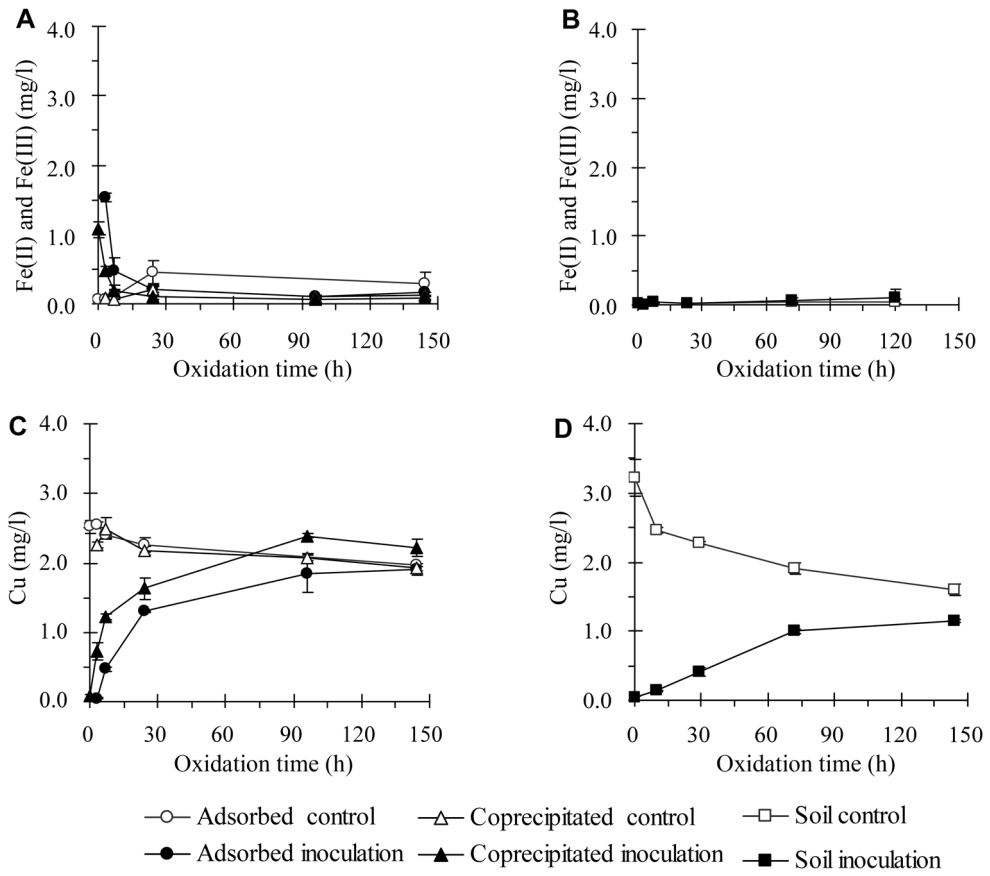

Fig. 4. Kinetics of aqueous Fe(II), Fe (III), and Cu from the Cu-adsorbed and Cu-coprecipitated amorphous Fe(III) minerals (**A**, **C**), and the Cu-saturated red earth soil after short-term oxidation (**B**, **D**).

The inoculation treatments represent the different anoxic substrates with fresh subsurface sediments. Controls represent treatments of the anoxic substrates containing sterilized subsurface sediments.

Therefore, the release of aqueous-phase SO_4^{2-} and Cu in the present study indicated the occurrence of oxidation and dissolution of Cu and sulfate coprecipitates in the red earth soil treatment.

However, the decrease in aqueous Cu and sulfate in the sterile soil exhibited a different trend from that in the inoculated soil during oxidation (Figs. 4D and 5C). This result could be due to the increased adsorption sites for anion and cation in the variable-charged red soils. A similar phenomenon has been reported previously, in which the opposite-charged sites on the surfaces of variable-charged red soil particles exhibited a strong adsorption interaction in various anions and cations [7, 39].

Speciation of Cu in the Red Earth Soil

Although the speciation of Fe in the red earth soil exhibited minimal change in the non-inoculated and inoculated treatments after anaerobic incubation and oxidation, the changes in solid-fraction Cu were significant in the inoculated red earth soil (Fig. 6). The inoculated soil exhibited an increase of 52.9% in organic matter/sulfide-bound Cu and a decrease of 48.7% in Fe/Mn oxide-bound speciation compared with the abiotic control (Fig. 6C). No significant change (less than ±1%) was found for exchangeable and carbonate-bound Cu. Comparison of the speciation of solid Cu from anaerobic with aerobic conditions revealed that Fe/Mn oxide-bound Cu increased by 18.8% in the inoculated soil, whereas organic matter/sulfide-bound and carbonate-bound Cu decreased by 53.6% and 4.9%, respectively (Fig. 6D). The significant change in organic/sulfide-bound Cu in the inoculated red earth soil supports the finding that the immobilization and release of Cu are related to the generation and oxidation dissolution of Cu-sulfide coprecipitates. Similarly, Koski *et al*. [9] found that the release of heavy metals, including Cu, is enhanced in porewater because of the oxidation of sulfide-rich debris in the sediment. Therefore, the immobilization and/or remobilization of Cu in the red earth soil in the present study could have been regulated by microbial iron reduction coupled with sulfide reduction and short-term oxidation.

In conclusion, a distinguished pattern in microbial iron reduction and Cu immobilization was observed in the synthesized amorphous Fe(III) minerals and red earth soils. The Cu-coprecipitated mineral with inoculated surface sediments exhibited a greater extent of microbial Fe(III) reduction compared with the Cu-adsorbed inoculation treatment. The Cu-saturated red earth soil required a relatively short starting time for microbial Fe(III) reduction. A decrease in the aqueous Cu concentration in the inoculated

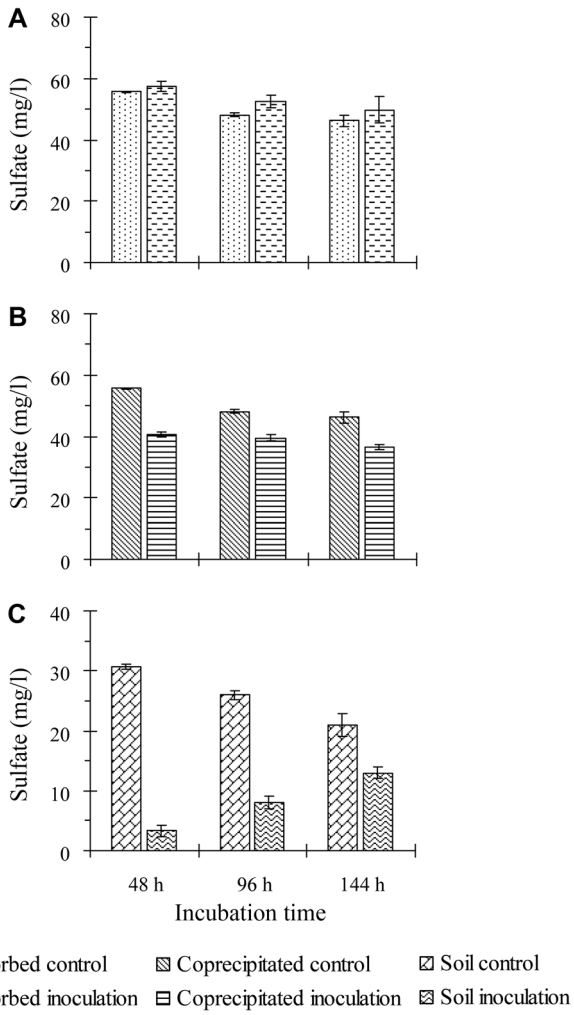

Fig. 5. Aqueous sulfate in the treatments of the Cu-adsorbed (**A**) and Cu-coprecipitated (**B**) amorphous Fe(III) minerals, and the Cu-saturated red earth soil (**C**) during short-term oxidation.

The inoculation treatments represent the different anoxic substrates with fresh subsurface sediments. Controls represent treatments of the anoxic substrates containing sterilized subsurface sediments.

treatments was observed in the order of red earth soil > Cu-coprecipitated mineral > Cu-adsorbed mineral. The enriched microorganisms in the inoculated red earth soil revealed the potential cooperative effect of microbial iron and sulfide reduction on Cu immobilization. When exposed to air for a short period, the immobilized Cu was unstable and could remobilize into the aqueous fraction with the recovered aqueous sulfate. The significant conversion of organic/sulfide-bound Cu under anaerobic and aerobic conditions demonstrated that the immobilization and remobilization of Cu are closely related to the generation

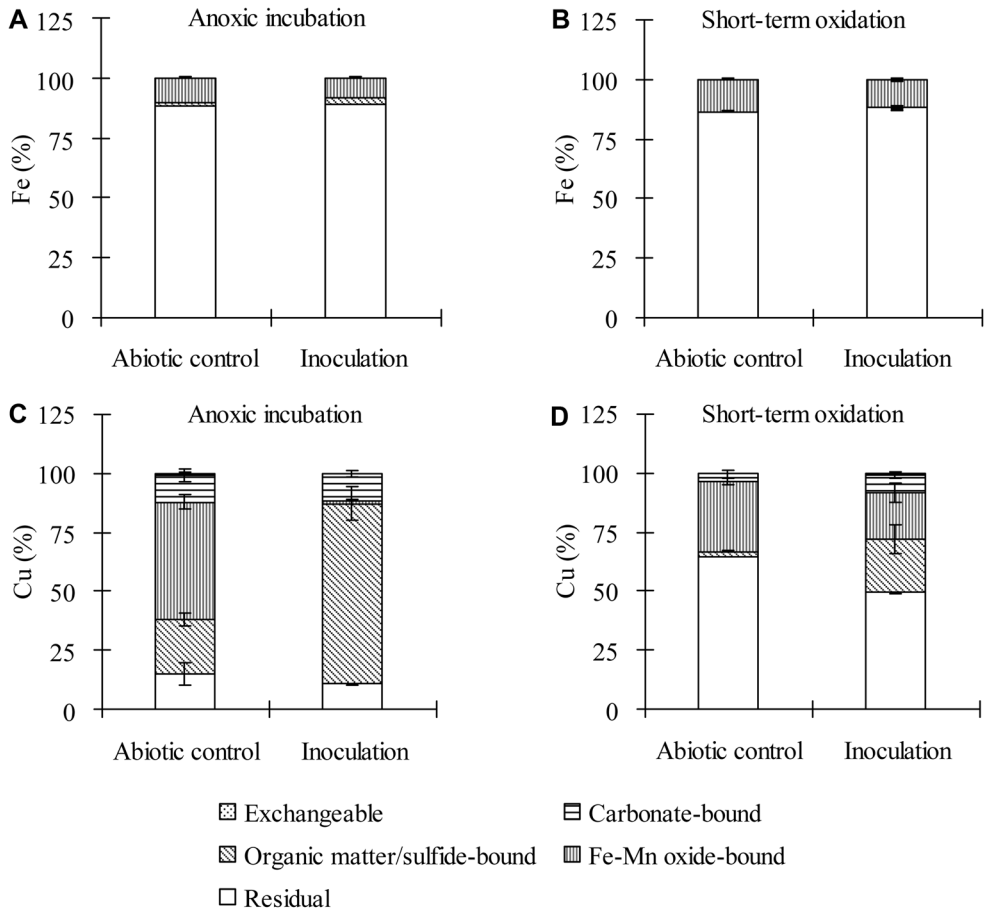

Fig. 6. Speciation of solid-fraction Fe and Cu in the red earth soil at the end of anoxic microcosm incubation and short-term oxidation.

The abiotic control (**A**, **C**) represents the red earth soil treated with sterilized subsurface sediments after 50 days of anoxic incubation. The anoxic soil containing the sterilized subsurface sediments was used as an abiotic control (**B**, **D**) after 6 days of oxidation.

and oxidation dissolution of Cu-sulfide coprecipitates in the inoculated red earth soil. These findings could have an important implication for bioremediation in Cu-contaminated and iron (hydr)oxide-enriched soils, especially in AMD-affected regions.

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