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Fate of Fe and Cd upon microbial reduction of Cd-loaded polyferric flocs by *Shewanella oneidensis* MR-1

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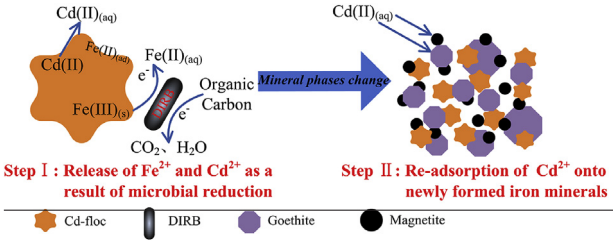
HIGHLIGHTS

- *Shewanella oneidensis* MR-1 has the ability to reduce Cd-loaded polyferric flocs.
- The microbial reduction of flocs induces the release of Fe^{2+} and Cd^{2+}.
- The newly formed iron minerals such as goethite and magnetite can re-immobilize Cd.

GRAPHICAL ABSTRACT



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ABSTRACT

Polyferric sulphate has been widely used for emergent control on incidental release of heavy metals such as Cd to surface water, causing precipitation of Cd-loaded polyferric flocs to the sediment. To date, little is known about whether the dissolution of the flocs in the presence of dissimilatory iron reducing bacteria (DIRB) can occur and how the dissolution influences the fate of Fe and Cd in the sediment. Here, we demonstrated that *Shewanella oneidensis* MR-1, as representative DIRB, has the ability to reduce the flocs, resulting in the release of Fe^{2+} and Cd^{2+} to the solution. Batch experiment results showed that the concentrations of Fe^{2+} and Cd^{2+} reached the maximum values at 48 h and then decreased over the remaining incubation time. The characterizations on the solid phase by the scanning electron microscopy coupled with energy dispersive spectrometer, X-ray diffraction, and X-ray photoelectron spectroscopy technologies revealed the formation of iron minerals such as goethite and magnetite as a consequence of microbial Fe(III) reduction. The newly formed iron minerals played a significant role in re-immobilizing Cd by sorption. These results imply that microbial reduction of polyferric flocs is an important contributor to the transport and transformation of metals in the sediment–water interface.

1. Introduction

Coagulation with chemicals such as polyferric sulphate (PFS) and poly aluminium chloride (PAC) is an important process that has been widely employed to treat surface water or wastewater contaminated with heavy metals (Song et al., 2006; Fu and Wang, 2011; Wu et al., 2011). The principle of the coagulation process lies in the charge neutralisation of negatively charged colloids derived from hydrolysis products of coagulants, followed by the adsorption of heavy metals onto amorphous hydroxide precipitates and the subsequent sedimentation (Duan and Gregory, 2003). Coagulation is frequently used for emergency responses to incidental release of hazardous substances such as Cd, Cr, and As to surface water, because it offers advantages of rapid process and ease of operation. For example, Cd spills to the Beijiang river (in 2005) and the Longjiang river (in 2012) resulted in Cd concentration ten times larger than the limiting permit, which has threatened water supply of many cities in South China. For the emergent control, the authorities urgently poured more than 300 tons of PFS and alkali

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into the water in an attempt to immobilize Cd by coagulation and precipitation.

It remains unknown whether Cd-loaded polyferric flocs present in the river sediments can be released as a consequence of the variation in geochemical conditions and what is the fate of Fe and Cd over a long period. One important factor contributing to the dissolution of the Fe(III) in Cd-loaded polyferric flocs should stem from the abundance of microbial iron metabolisms in sediment environments (Lovley et al., 2004). It is well recognized that dissimilatory iron reducing bacteria (DIRB) are able to reduce Fe(III)-bearing minerals coupled with oxidizing organic matter (Weber et al., 2006). Previous studies have shown that DIRB can respire with a variety of Fe(III)-containing (hydro)oxides such as ferrihydrite ($Fe^{III}_{5}HO_8{\cdot}4H_2O$) (Wolf et al., 2009), lepidocrocite (γ-$Fe^{III}OOH$) (Bae and Lee, 2013), goethite (α-$Fe^{III}OOH$) (Li et al., 2014), Magnetite ($Fe^{II}_{1}Fe^{III}_{2}O_4$) (Kostka and Nealson, 1995) and clay minerals containing Fe(III) (Kostka et al., 2002). This process results in the formation of Fe(II) and/or Fe(II)/Fe(III) secondary minerals depending on the geochemical environments (Fredrickson et al., 1998; Zachara et al., 1998). Since iron (hydro)oxides are important sorbents of heavy metals in the sediments (Peng et al., 2009; Muehe et al., 2013a), considerable efforts have been made to investigate how the microbial iron reduction affects the mobility of contaminated metals and the secondary mineral influences the re-sorption of them (Borch et al., 2010; Muehe et al., 2013a, 2013b).

To date, little is known about whether the dissolution of Cd-loaded polyferric flocs in the presence of DIRB can occur and what is the fate of Fe and Cd if microbial Fe(III) reduction takes place. To fill this gap, this study investigated *Shewanella oneidensis*-driven microbial reduction of Cd-loaded polyferric flocs by examining the changes in concentrations of aqueous Fe^{2+}, Fe^{3+} and Cd^{2+} as a function of incubation time, and further disclosed the newly formed iron minerals and their interactions with Cd^{2+} by performing the scanning electron microscopy coupled with energy dispersive spectrometer (SEM-EDS), X-ray diffraction (XRD), and X-ray photoelectron spectroscopy (XPS) analyses. The species *S. oneidensis* MR-1 was used because it is one of the most commonly known DIRB that have been found in the sediments (Myers and Nealson, 1988; Venkateswaran et al., 1999).

2. Materials and methods

2.1. Preparation of Cd-loaded polyferric flocs

The Cd-loaded polyferric flocs were synthesized by coagulation. Twenty milliliters of PFS solution (10% (m/v)) was added into 500 mL of 5 mg L^{-1} $Cd(NO_3)_2$ solution to coagulate Cd^{2+}. After a few seconds of rapid stirring, the pH value was adjusted to 6.8 using 5 M NaOH during slow stirring. The increasing time caused the gradual increase in the size of flocs due to flocculation. The Cd-loaded flocs were obtained after 4 h-gravity sedimentation.

2.2. Batch incubation experiments

S. oneidensis MR-1 purchased from ATCC (700550) was selected as the DIRB for the batch incubation experiments. Bacteria were stored in the agar slant medium at 4 °C, transferred to sterile Luria-Bertani (LB) medium (5 g L^{-1} beef extract, 10 g L^{-1} tryptone and 5 g L^{-1} NaCl) and grown aerobically for 18 h (30 °C, 150 rpm). Then 1 mL of the cultivated seed culture was used to inoculate another 50 mL of sterile LB medium and incubated aerobically (18 h, 30 °C, 150 rpm). Cells were harvested by centrifugation (3000 g, 10 min, 20 °C) and washed twice by 10 mM $NaNO_3$, once by sterile defined medium (DM) (Smeaton et al., 2009) containing: 1.34 mM KCl, 28 mM NH_4Cl, 0.68 mM $CaCl_2{\cdot}2H_2O$, 50 mM $NaClO_4{\cdot}2H_2O$, 24 mM Na-lactate (60% syrup) and 20 mM PIPES (1,4-piperazine diethanesulfonic acid) buffer. The supernatant of each washing step was decanted. Then the wet biomass was transferred to the sterile DM (pH = 7.3) to obtain cell suspensions with the concentration of 1 g L^{-1}.

For the batch experiments, 20 mL of cell suspensions and 2 mL of Cd-loaded flocs were added into a 100 mL-volume headspace vial. The control experiment containing 20 mL of sterile cell suspensions and 2 mL of Cd-loaded flocs was simultaneously performed. The total concentrations of Fe and Cd in each vial were 344.10 mg L^{-1} and 1.86 mg L^{-1}, respectively. All the vials were sealed with butyl rubber stoppers with aluminum seals, and then placed in a rotator for 288 h (30 °C, 120 rpm). Triplicate experiments were conducted to testify the reproducibility. Samples were taken at particular time intervals for chemical, electrochemical, and physical analyses. Inoculation and sampling were performed in anaerobic glove box (N_2, 99.99%) (YQX-II, Xinmiao Medical Instrument Manufacturing Company, Shanghai, China).

2.3. Analyses

The solution sample was filtered using 0.22 μm-sterile filters prior to aqueous analysis. A fraction of the filtrate (1 mL) was subject to immediate analysis for determining concentrations of Fe^{2+} and Fe^{3+} by the ultraviolet visible spectrophotometer (754 PC, Shanghai Spectral Instrument Company, China) at λ = 510 nm according to the o-phenanthroline photometry method (Harvey et al., 1955; Brand et al., 1998). Another fraction of the filtrate (2 mL) was kept at 4 °C after dilution using 5% nitric acid; then the concentration of Cd^{2+} was determined by the inductively-coupled plasma optical emission spectroscopy (ICP-OES) (5300DV, Perkin Elmer, USA). The remaining filtrate was transferred to a glass cup protected by nitrogen, followed by the electrochemical cyclic voltammetry (CV) test using the potentiostat (CHI660, Chenhua Instrument Company, Shanghai, China). All the CV curves were recorded within the potential window of −0.8 V to 0.8 V at a scan rate of 20 mV s^{-1}.

The sample slurries were collected, washed repeatedly with ultrapure water to remove organics and salts, and dehydrated subsequently with ethanol and vacuum freeze. Then the dried samples were subject to physical characterizations and Cd species analysis. The surface morphology and composition were examined using the scanning electron microscopy coupled with the energy dispersive spectrometer (SEM-EDS) (Melin, Zeiss, Germany). The crystallinity of the solid phase was evaluated using the X-ray diffraction (XRD) (D8 Advance, Bruker, Germany) equipment with Cu Kα radiation operating at 40 kV voltage and 40 mA current. The analysis of elemental composition and function groups available on the solid phase were performed using the X-ray photoelectron spectroscopy (XPS) technology. The XPS spectra were obtained by a multifunctional imaging electron spectrometer (Thermo ESCALAB 250XI, Thermo Fisher Scientific, Waltham, US) using a focused monochromatic Al Kα radiation (hν = 1486.6 eV) operating at 150 W.

The speciation analysis of Cd in the solid phase was performed by sequential extraction. The identification and quantification of Cd present in different forms in the solid phase were conducted according to the procedure proposed previously (Tessier et al., 1979; Muehe et al., 2013c; Liu et al., 2015). Specifically, the solid in the vial was collected after centrifugation and then vacuum freeze-dried. The dried powders were sequentially extracted in four fractions: (F1) extracted with 1 M $MgCl_2$ for 1 h (exchangeable phase); (F2) extracted with 0.1 M HCl for 0.5 h (iron oxide surface-