Research article

Microbial iron reduction as a method for immobilization of a low concentration of dissolved cadmium

Chaolei Yuan a, Tongxu Liu a, Fangbai Li a,*, Chengshuai Liu a, Huanyun Yu a, b, Weimin Sun a, Weilin Huang a, c

a Guangdong Key Laboratory of Integrated Agro-environmental Pollution Control and Management, Guangdong Institute of Eco-environmental Science & Technology, Guangzhou 510650, China
b Guangzhou Key Laboratory of Environmental Exposure and Health, School of Environment, Jinan University, Guangzhou 510632, China
c Department of Environmental Sciences, Rutgers University, New Brunswick, 08901, NJ, USA

ABSTRACT

Much attention has been paid to the relationship between microbial iron reduction and the behavior of cadmium (Cd) recently, but most previous research has employed unrealistically high Cd concentrations (e.g., 2–55 mg L$^{-1}$) and has failed to consider the effects of iron oxides and microbial cells together. We investigated the reduction of lepidocrocite by Shewanella oneidensis MR-1 in the presence of a low concentration of Cd using batch reactor systems. The results showed that with 422 mg L$^{-1}$ added dissolved Cd$^{2+}$, an initial 137 mg L$^{-1}$ decrease in aqueous Cd occurred due to adsorption onto lepidocrocite and that the further removal of remaining aqueous Cd occurred only in the system containing bacteria. This further decrease in aqueous Cd was unlikely to be caused by mineral transformation because the microbial reduction of lepidocrocite resulted in particle-size-increased (thus, specific-surface-area-decreased) lepidocrocite, and unlikely to be caused by the pH increase to 7.4 induced by iron reduction either because a pH-adsorption edge suggested that at pH 7.4, less than 60% of aqueous Cd can be adsorbed by lepidocrocite in the reactors. An adsorption isotherm showed a significant Cd adsorption capacity by S. oneidensis MR-1 cells, and we therefore attributed the further Cd removal to adsorption by S. oneidensis MR-1 cells. The results suggest that a realistically low concentration of Cd can be immobilized during microbial iron reduction by adsorption on iron oxides and microbial cells.

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1. Introduction

Cadmium (Cd) is a widespread metal pollutant in agricultural soils, and exposure to Cd in ways such as rice consumption can increase the risk of osteoporosis, renal diseases, and cancer (Maret and Moulis, 2013; Muehe et al., 2013a). Microbial iron reduction is an important process in saturated soils such as rice paddy soils (Yu et al., 2016). Iron oxides can adsorb Cd due to their large surface area, and therefore, the reductive dissolution of iron oxides may lead to the release of Cd (Muehe et al., 2013a; Yu et al., 2016). On the other hand, some studies have suggested that secondary mineralization during iron reduction could immobilize Cd. Li et al. (2016) investigated the reduction of Cd-loaded polyferric flocs by Shewanella oneidensis MR-1 and found that Cd$^{2+}$ was first released into the solution and was subsequently bound by secondary iron minerals that had formed. During the microbial reduction of ferrhydrite in the presence of Cd in solution, Cd was adsorbed to and/or co-precipitated within secondary carbonate minerals (Muehe et al., 2013a). Further, after incubation of a Cd-contaminated soil under reducing conditions with the addition of organic matter, Muehe et al. (2013b) found that Cd was probably immobilized in magnetite that formed during microbial Fe(III)-mineral reduction. Thus, iron reduction may not result in the release of Cd and the effects of iron reduction on Cd behavior need further investigation.

In addition to iron oxides, microbial cells also have a significant capacity to adsorb metals including Cd, owing to various functional groups (e.g., carboxyl, sulfhydryl, phosphoryl, and amide groups) on cell walls (Chang et al., 1997; Ha et al., 2010; Kaulbach et al., 2005; Mishra et al., 2010; Yu and Fein, 2015). However, the effects of microbial cells on Cd immobilization have hardly been considered together with those of iron oxides during microbial iron reduction. Moreover, the Cd concentrations in the experimental
systems of previous studies were usually too high (e.g., 2–55 mg L\(^{-1}\) \([\text{Li et al., 2016; Muehe et al., 2013a}]\)) compared to general Cd-contaminated soils. For example, total Cd concentration in European soils ranges between 0.06 and 0.6 mg kg\(^{-1}\) \([\text{Soco et al., 2016; Muehe et al., 2013a}]\). Cd-contaminated soils in China, most of the Cd-contaminated soils contains Cd concentration <0.78 mg kg\(^{-1}\) \([\text{Holmgren et al., 1993}]\), and in China, most of the Cd-contaminated soils contains <1 mg kg\(^{-1}\) Cd \([\text{Wang et al., 2015}]\). Thus, more attention should be paid to the fate of Cd that occurs in environmentally realistic concentrations (<1 mg kg\(^{-1}\) Cd).

During anaerobic respiration, iron-reducing bacteria use Fe(III) in iron oxides as an electron acceptor and produce Fe(II). The biogenic Fe(II) can induce the transformation of iron oxides \([\text{Hansel et al., 2003}]\). The co-existence of metal cations such as Cd(II) can influence microbial reduction and transformation of iron oxides. First, if the co-existing metal cations are toxic to cells, the activity of iron-reducing bacteria and thus Fe(II) generation will be constrained \([\text{Trevors et al., 1986}]\). Second, recent studies have shown that concomitant metal cations can inhibit Fe(II)-catalyzed mineral transformation, and the possible mechanisms include the competition between Fe(II) and concomitant metal cations for adsorption sites on the surface of iron oxides \([\text{Liu et al., 2016}]\). Lepidocrocite (γ-FeOOH) is an iron oxide-hydroxide that is found in many saturated soils \([\text{Schwertmann, 1988}]\). Laboratory incubation experiments show that the microbial reduction of lepidocrocite can produce various secondary minerals, such as magnetite, green rusts, vivianite, and goethite, depending on many factors such as the iron reduction rate, cell density, and medium composition \([\text{Bae and Lee, 2011; Li et al., 2012; O’Loughlin et al., 2010; Ona-Nguema et al., 2013; Lovley, 2013}]\). The total volume of the suspension was 40 ml per bottle for all the systems. The oxygen-repelled bottles were then sealed with rubber stoppers and aluminum lids and incubated in a shaker at 150 rpm and 30 °C in the dark.

2.2. Incubation setup

There were three batch reactor systems: γ-FeOOH incubated with Cd\(^{2+}\), γ-FeOOH incubated with MR-1, and γ-FeOOH incubated with MR-1 and Cd\(^{2+}\), which are referred to hereafter as systems Fe+ Cd, Fe+M, and Fe+M+Cd, respectively. The incubation was slightly modified after \([\text{Liu et al., 2011}]\). MR-1 cells were grown in lysogeny broth containing 10 g L\(^{-1}\) tryptone, 5 g L\(^{-1}\) yeast extract, and 10 g L\(^{-1}\) NaCl at 30 °C for 16 h under aerobic conditions to allow the bacteria to rapidly proliferate. Then, the cells were harvested and washed three times by centrifugation and re-suspension with a PIPES buffer medium (adjusted to pH 7.0 using KOH; autoclaved) containing 9.07 g homopiperazine-1,4-bis(2-ethanesulfonic acid) (PIPES), 10 ml vitamin solution, 10 ml mineral solution, and 4 ml 5 M sodium lactate solution in 1 L \([\text{Lovley, 2013}]\). A total of 0.0711 g lepidocrocite was weighed into 50-ml serum bottles. The bottles were sealed and autoclaved for 20 min at 121 °C, after which lepidocrocite remained unchanged as confirmed by XRD. Autoclaved PIPES buffer medium was then added to the bottles, and the suspension was bubbled with N\(_2\) (passed by a 0.22-μm membrane) for 40 min to repel oxygen. A 2-ml MR-1 cell suspension (OD\(_{600}\) = 2.0) was added for the systems with bacteria, and the systems with Cd\(^{2+}\) contained 422 μg L\(^{-1}\) Cd\(^{2+}\) (added as CdCl\(_2\) solution). This quantity of Cd was adopted because according to the environmental quality standard for soils in China, to ensure agricultural production and human health, the upper limit for the concentration of total soil Cd is 0.3 mg kg\(^{-1}\) for soils with pH < 7.5 or 0.6 mg kg\(^{-1}\) for soils with pH > 7.5 \([\text{Wang et al., 2015}]\). The total volume of the suspension was 40 ml per bottle for all the systems. The oxygen-repelled bottles were then sealed with rubber stoppers and aluminum lids and incubated in a shaker at 150 rpm and 30 °C in the dark.

2.3. Analyses for Cd distribution and mineral transformation

Three reactors of each system were transferred into a glove box at prespecified time points. A 2-ml suspension was transferred to a 15-ml tube and mixed with 8 ml 0.5 M HCl end-over-end for 1 h. The mixture was then filtered with a 0.45-μm membrane, and the filtrate (HCl extract) was collected. The remaining 38-ml suspension was also passed through 0.45-μm membrane filters, and both the filtrate (aqueous fraction) and mineral were collected. The concentrations of Fe(II) and Cd(II) in the filtrates were measured via 1,10-phenanthroline colorimetric assay and inductively coupled plasma optical emission spectrometry (ICP-OES) \([\text{Optima 8000, PerkinElmer, USA}]\), respectively. The difference between the concentrations of Fe(II) or Cd(II) in the HCl extract and aqueous fraction was assigned as 0.4 M HCl-extractable Fe(II) or Cd(II). The collected mineral was air-dried in the glove box. The mineral in triplicate reactors was combined and then analyzed by XRD, infrared spectroscopy (IR), X-ray photoelectron spectroscopy (XPS), transmission electron microscopy (TEM), and Mössbauer spectroscopy. Details of the physical analyses are provided in the supplementary materials.

2.4. pH-adsorption edge and adsorption isotherms

The pH-adsorption edge for Cd adsorption on lepidocrocite was determined as follows. A 20-ml autoclaved PIPES medium with 422 μg L\(^{-1}\) Cd\(^{2+}\) and various pH between 6.1 and 7.5 (the useful pH range of PIPES) was added to 50-ml polyethylene tubes each containing 0.0356 g lepidocrocite. After ultrasonic dispersion, the tubes were shaken at 180 rpm and 30 °C in the dark for 24 h and were then centrifuged at 7200 g for 5 min. The supernatants were collected by passing through a 0.22-μm membrane for measurements of pH and Cd concentrations. An isotherm for Cd adsorption on lepidocrocite was obtained by incubating 0.0356, 0.0533, 0.0800, 0.1067, and 0.1778 g lepidocrocite in 20 ml PIPES medium with 422μg L\(^{-1}\) Cd\(^{2+}\) and pH 7.0 in a similar way. Cd adsorption by
Concentrations increased to 148 and 71 mg L\(^{-1}\) in Fe\(_{2}\) and 67 mg L\(^{-1}\) after 7 days, the Fe\(_{3}\) after 32 days, the pH of the suspension in the systems Fe\(_{2}\)-MR-1) and (b) attraction of mineral by a magnet. Fe\(_{2}\) and 0.4 M HCl-extractable Fe\(_{2}\) consistently low. In contrast, in the system Fe\(_{3}\) and in the systems Fe\(_{2}\)-MR-1, and Cd\(_{2}\) that did not contain bacteria, the concentrations of aqueous Cd after incubating 2, 4, 6, and 8 ml MR-1 cell suspension (OD\(_{600}\) = 2.0) in 40 ml N\(_{2}\)-bubbled PIPES buffer medium with 422 \(\mu\)g L\(^{-1}\) Cd\(_{2}\)- and pH 7.0 in 100-ml serum bottles for 48 h in a shaker at 150 rpm and 30 °C in the dark. After 48 h, the cell density in the system did not increase as revealed by OD\(_{600}\) because no electron acceptor was supplied for the bacteria (i.e., no iron oxide was added to the serum bottles).

3. Results and discussion

3.1. Lepidocrocite reduction and transformation

In the system Fe+Cd that did not contain bacteria, the concentrations of aqueous and 0.4 M HCl-extractable Fe\(_{2}\) remained consistently low. In contrast, in the system Fe+M, the concentrations of aqueous and 0.4 M HCl-extractable Fe\(_{2}\) increased to 167 and 67 mg L\(^{-1}\) within 7 days, and in the system Fe+M+Cd, the concentrations increased to 148 and 71 mg L\(^{-1}\) (Fig. 1). The increase in Fe\(_{2}\) demonstrated the microbial reduction of lepidocrocite. After 7 days, the Fe\(_{2}\) concentrations became relatively stable. After 32 days, the pH of the suspension in the system Fe+Cd was 7.01, and in the systems Fe+M and Fe+M+Cd, it was 7.40 and 7.44, respectively, because iron reduction is a proton-consuming process: CH\(_{2}\)O + 4FeOOH + 8H\(^+\) → CO\(_{2}\) + 4Fe\(_{2}\)+ + 7H\(_{2}\)O (Frommichen et al., 2004). The rate of microbial iron reduction in the first 7 days was slightly lower in the system with added Cd\(_{2}\) (1.25 mg Fe\(_{2}\)+ day\(^{-1}\)) than the system without added Cd\(_{2}\) (1.34 mg Fe\(_{2}\)+ day\(^{-1}\)) (Fig. 1), probably due to the toxicity of Cd to microbial cells.

After 32 days, unlike in the system Fe+Cd, in the systems Fe+M and Fe+M+Cd where microbial iron reduction occurred, the color of the suspension became darker, and the iron oxides were attracted to a magnet (Fig. 2), suggesting the formation of magnetic material, probably magnetite, which has a black color (Bigham et al., 2002) and has been reported as a product of microbial reduction of lepidocrocite in many studies (Li et al., 2012; Yuan et al., 2017; Zegeye et al., 2007). However, XRD and IR analyses detected only lepidocrocite after 32 days in all the systems (Fig. 3). We then resorted to XPS and Mössbauer spectroscopy, but we still could not detect the presence of magnetite (see supplementary figures). The reason is probably that putative magnetite accounted for a very small fraction of the iron oxides and/or the magnetite was amorphous (Schwertmann and Cornell, 2000).

Various secondary minerals can be formed after the microbial reduction of lepidocrocite depending on reaction conditions as mentioned before. Particularly, the transformation of lepidocrocite in the presence of Fe\(_{2}\)+ can also result in recrystallized lepidocrocite (Pedersen et al., 2005, 2006). Using \(^{55}\)Fe labeling, Pedersen et al. (2005) observed rapid isotopic exchange between aqueous Fe\(_{2}\)+ and Fe in a 5-nm-sized lepidocrocite; after recrystallization, the mineral phase remained as lepidocrocite, but the crystal size increased to 15 or 20 nm. In this study, TEM images also showed that the particle size of lepidocrocite increased after microbial iron reduction (Fig. 4). This is corroborated by the narrowed XRD peaks of lepidocrocites in the systems Fe+M and Fe+M+Cd where iron reduction occurred compared to the system Fe+Cd where iron reduction did not occur (Fig. 3a), considering that narrowed XRD peaks indicate increased crystal sizes (Schwertmann and Cornell, 2000). Taken together, our data suggest that in this study, microbial iron reduction probably induced the recrystallization of

**Fig. 1.** Time-dependent concentrations of aqueous and 0.4 M HCl-extractable Fe(II) in batch reactor systems with (a) γ-FeOOH and Shewanella oneidensis MR-1 (hereafter MR-1) and (b) γ-FeOOH, MR-1, and Cd\(_{2}\)+. Error bars represent standard deviations.

**Fig. 2.** Appearance of reactors after 32 days which shows (a) the color of suspensions and (b) attraction of mineral by a magnet. Fe+Cd, Fe+M, and Fe+M+Cd indicate systems with (i) γ-FeOOH and Cd\(_{2}\)+, (ii) γ-FeOOH and MR-1, and (iii) γ-FeOOH, MR-1, and Cd\(_{2}\)+, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
lepidocrocite, with crystal-size-increased lepidocrocite as the major product and magnetite a very minor product. The presence of Cd did not have a significant effect on the iron oxide transformation during microbial reduction in this study (Fig. 3), which confirmed our third hypothesis, probably due to the very low Cd concentration (Cd/Fe = 0.019 mol.%).

3.2. Cd immobilization and mechanisms

In the system Fe+Cd, at the first sampling time point that was approximately 2 h after mixing lepidocrocite with 422 µg L⁻¹ dissolved Cd²⁺, the concentration of aqueous Cd²⁺ was 285 µg L⁻¹ (i.e., decreased by 137 µg L⁻¹) while the concentration of 0.4 M HCl-extractable Cd²⁺ was 140 µg L⁻¹ (Fig. 5a). This indicated rapid adsorption of 140 µg L⁻¹ dissolved Cd onto lepidocrocite in 2 h because 0.4 M HCl mainly extracts Cd adsorbed on the solid phase in the system (Jeon et al., 2003), and the great Cd adsorption capacity of lepidocrocite (113.6 µg Cd g⁻¹ lepidocrocite maximum) was demonstrated by an adsorption isotherm (Fig. 6a). After 2 h, the concentrations of aqueous and 0.4 M HCl-extractable Cd²⁺ became relatively stable in the system Fe+Cd.

In the system Fe+M+Cd, the concentration of aqueous Cd²⁺ at the first sampling time point was even lower (207 µg L⁻¹) compared to the system Fe+Cd (Fig. 5), probably due to additional Cd²⁺ adsorption by MR-1 cells. The aqueous Cd²⁺ then decreased dramatically from 207 to 12 µg L⁻¹ within 2 days and further to under the detection limit on day 7. Correspondingly, the concentration of 0.4 M HCl-extractable Cd²⁺ increased to 385 µg L⁻¹ after 7 days. Namely, after an initial decrease by approximately 200 µg L⁻¹, the remaining 207 µg L⁻¹ aqueous Cd transferred into adsorbed Cd during microbial iron reduction. This adsorption was unlikely to be caused by mineral transformation because after 32 days, the particle size of lepidocrocite increased, which leads to decreased specific surface area. The increase in the pH of the suspension to 7.4 after 32 days could not explain the further decrease in aqueous Cd either, because a pH-adsorption edge suggested that at pH 7.4, less than 60% of aqueous Cd could be adsorbed by lepidocrocite in the reactors (Fig. 7). Thus, we speculated that the remaining 207 µg L⁻¹ aqueous Cd was adsorbed by MR-1 cells. This was supported by the Cd adsorption capacity of MR-1 cells (9.3 µg Cd ml⁻¹ MR-1 cells (OD₆₀₀ = 2.0) maximum) as shown by an adsorption isotherm (Fig. 6b). And Cd adsorption by MR-1 cells has also been reported by other researchers (Ha et al., 2010; Mishra et al., 2010; Yu and Fein, 2015). Considering microbial regeneration during iron reduction, for instance, Muehe et al. (2013a) observed that the cell number of iron-reducing bacteria increased by approximately two orders of magnitude after 7 days during ferrihydrite reduction, the

![Fig. 3. XRD and IR patterns of minerals on day 32. Lepidocrocite was the only identifiable mineral in all the systems.](image1)

![Fig. 4. TEM images of lepidocrocites after 32 days in batch reactor systems with (a) γ-FeOOH and Cd²⁺, (b) γ-FeOOH and MR-1, and (c) γ-FeOOH, MR-1, and Cd²⁺.](image2)
adsorption of Cd by MR-1 could be significant. The probable increase in the MR-1 cell number after 7 days in this study could be suggested by the generation of Fe^{2+} in reactor systems. The results, therefore, supported our first hypothesis that microbial lepidocrocite reduction can immobilize a low concentration of aqueous Cd and our second hypothesis that adsorption by microbial cells contributes significantly to Cd immobilization. The change in the spatial distribution of Cd during the microbial reduction of lepidocrocite in this study is shown in a conceptual model (Fig. 8).

During microbial iron reduction in this study, there was no initial increase and subsequent decrease in aqueous Cd, as shown for the cases reported by Li et al. (2016) and Muehe et al. (2013a). In the experiment conducted by Li et al. (2016), the substrate for Shewanella oneidensis MR-1 was Cd-loaded polyferric flocs (i.e., Cd was not added in dissolved form), and thus, Cd was released to the solution after reductive dissolution of the solid phase. In the study by Muehe et al. (2013a), within 9 days, approximately 40% of Fe in ferrihydrite was transformed to Fe(II), so a significant amount of Cd that was adsorbed on ferrihydrite shortly after the addition of aqueous Cd could be released into the solution again, which was amplified by the large concentrations of added Cd (11 or 55 mg L^{-1}). The greater extent of iron reduction observed by Muehe et al. (2013a) compared to the one in this study (<15% after 32 days) might be attributed to the different types and concentrations of iron oxides and iron-reducing bacteria employed.

3.3. Environmental implications

This study demonstrated that during microbial iron reduction, adsorption by 20 mM lepidocrocite can immobilize approximately...
one-third of 400 μg L⁻¹ dissolved Cd²⁺, and the remaining Cd²⁺ could be adsorbed by bacterial cells (Fig. 8). This could provide insight for the management of realistic Cd-polluted paddy soils. First, an extended flooding period is recommended for rice cropping in Cd-polluted areas, and the effectiveness has been attributed to increased soil pH and the formation of Cd₅ (Fulda et al., 2013; Li et al., 2017; Sun et al., 2007). This study highlighted the potential contributions from iron oxides and especially their microbial reducers, considering that iron oxides are abundant (Loeppert and Inskeep, 1996) and iron-reducing bacteria are widespread in paddy soils (Yu et al., 2016). Second, the stimulation of microbial iron reduction by, for example, adding iron oxides or electron donors (e.g., organic matter) to the soil may be useful to reduce Cd bioavailability in paddy soils that contain a relatively low concentration of Cd. To answer this question, further soil and field experiments are required.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jenvman.2018.04.023.

References


