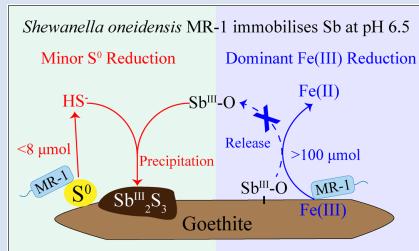


Iron(III) reducing bacteria immobilise antimonite by respiring elemental sulfur

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Abstract



Iron(III) reducing bacteria (IRB) are best known and most influential drivers for mobilising metal(lloid)s *via* reductive dissolution of iron(III)-containing minerals. Our study challenges this preconception and found that IRB immobilise metal(lloid)s by respiring elemental sulfur (S^0) even when Fe(III) reduction is prevailing under mildly acidic conditions. Antimony (Sb), a toxic contaminant, was chosen as an example of metal(lloid)s. Antimonite-adsorbed goethite was incubated with *Shewanella oneidensis* MR-1, a widely distributed IRB, in the presence of S^0 at pH 6.5. The results show that although the extent of Fe(III) reduction ($>100 \mu\text{mol}$) was over ten times greater than that of S^0 reduction ($<8 \mu\text{mol}$), it was S^0 reduction that immobilised Sb through Sb_2S_3 precipitation. Further, the thermodynamic calculation suggests that

such great impacts of marginal S^0 reduction can be extended to other metal(lloid)s *via* the formation of soluble thio-species or sulfide precipitates. This study redefines the role of IRB in the environmental fate of metal(lloid)s, highlighting the strong impacts from the marginal S^0 reduction over the central Fe(III) reduction.

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Introduction

Iron oxides are natural scavengers for toxic metal(lloid)s and iron reducing bacteria (IRB) mobilise these metal(lloid)s by facilitating the reductive dissolution of iron oxides (Kappler *et al.*, 2021). For example, elevated arsenic (As) (Kontny *et al.*, 2021), antimony (Sb) (Hockmann *et al.*, 2014), mercury (Hg) (Wang *et al.*, 2021) and cadmium (Cd) (Zhou *et al.*, 2020) in waters usually associate with increasing Fe(II) concentrations in the presence of IRB. In fact, many IRB, such as *Shewanella* and *Geobacter* spp., have an under-appreciated ability: elemental sulfur (S^0) respiration (Flynn *et al.*, 2014). Accordingly, the biogenic sulfide (HS^-) may either immobilise metal(lloid)s *via* sulfide precipitates or mobilise them by forming soluble thio-species (Planer-Friedrich *et al.*, 2020; Ye *et al.*, 2020; Helz, 2021). Previous studies suggested that S^0 is widely distributed in sediments and waters, and its concentration can be as high as 60 mM as summarised in Table S-1. S^0 usually co-exists with Fe(III) oxides in the environment where FeS oxidation occurs (Burton *et al.*, 2009) or where microbial sulfate reduction occurs in the presence of Fe(III) oxides (Burton *et al.*, 2011). Thus, rather than Fe(III) reduction alone, IRB influence the mobility of metal(lloid)s *via* coupled Fe(III) and S^0 reduction.

The extents of Fe(III) and S^0 reduction reveal their contribution in regulating the fate of metal(lloid)s. Our recent work found that IRB prefer to reduce S^0 under mildly alkaline conditions, and the biogenic sulfide greatly enhanced Sb release by the formation of thioantimonates (Ye *et al.*, 2022). In contrast, in the acidic environments, IRB prefer to respire Fe(III) rather than S^0 to conserve more energy (Flynn *et al.*, 2014). The effect of S^0

respiration by IRB is previously neglected and probably masked by appreciable Fe(III) reduction on the fate of metal(lloid)s. Thus, prevailing Fe(III) reduction (Cummings *et al.*, 2000) is commonly considered to drive the mobility of metal(lloid)s in mildly acidic sediments (Hockmann *et al.*, 2015), such as mining areas and acidic wetlands (Karimian *et al.*, 2018).

Contrary to the previous preconception, our study revealed that S^0 respiration by IRB even to a marginal extent can greatly shape the fate of metal(lloid)s under mildly acidic conditions. In detail, antimony (Sb), an emerging contaminant, is chosen as an example of metal(lloid)s. Antimonite-adsorbed goethite was incubated with *Shewanella oneidensis* MR-1, a typical Fe(III) reducing bacterium, in the presence of S^0 at pH 6.5. Our incubation and characterisation results suggest that although Fe(III) was the dominant electron acceptor and Fe(III) reduction was over ten times greater than S^0 reduction, Sb release was mainly inhibited by biogenic sulfide through the formation of Sb_2S_3 precipitates. The thermodynamic calculation further indicates that the strong effect of marginal S^0 reduction can be extended from Sb to other metal(lloid)s. This study breaks the stereotype that IRB influence the mobility of metal(lloid)s *via* Fe(III) reduction alone, and highlights the importance of S^0 respiration.

Materials and Methods

The experiments were performed at pH 6.5 in the dark, and the experimental setup and analytical techniques were similar to our previous study (Ye *et al.*, 2022). Dissolved Fe(II), sulfide,

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antimonite, antimonate and thioantimonates were analysed periodically during the incubation. To extend the effect of marginal S⁰ reduction from Sb to other metal(loid)s, reaction path models were established to calculate the species of Sb, arsenic (As), tungsten (W), molybdenum (Mo), mercury (Hg), lead (Pb), zinc (Zn), cadmium (Cd) and copper (Cu) as a function of pH and the activity of sulfide in the presence of goethite. More details of the methods are shown in the *Supplementary Information (SI)*.

Results and Discussion

Prevalent Fe(III) reduction versus minor S⁰ reduction. During the incubation with *Shewanella oneidensis* MR-1 at pH 6.5 (Fig. S-2), the electron donor was formate and the electron acceptor was either goethite or S⁰. To determine the dominant electron acceptor in our experiments, dissolved Fe(II) and sulfide (HS⁻) were measured (Fig. 1) and the electrons accepted by goethite or S⁰ were calculated (Table S-2). In the abiotic control without MR-1 (Goe-Sb^{III}+S⁰), Fe(II) and sulfide were not detected (Fig. 1). In the presence of MR-1 (MR-1+Goe-Sb^{III}+S⁰), dissolved Fe(II) concentrations reached up to 527 ± 27 µM whereas sulfide was below 3 µM during the 192 hr incubation (Fig. 1a). Such a low sulfide concentration (<3 µM) would have a negligible effect on goethite reduction (Poulton *et al.*, 2004). At the end of incubation, 2.7 ± 0.5 µmol/g Fe(II) were adsorbed on solids and 26.6 ± 3.4 µmol/g sulfide were precipitated (Table S-2). Overall, goethite accepted 106.1 ± 5.3 µmol electrons from MR-1 while S⁰ accepted 14.4 ± 1.6 µmol. The order of magnitude difference in electrons suggested that goethite was the dominant electron acceptor at pH 6.5.

Consistent with the prevailing Fe(III) reduction, the thermodynamic calculations show that the free energy gain (ΔG_r) with goethite as the electron acceptor is higher than that with S⁰ (Fig. S-3), as detailed in SI. Previous studies also suggested that microbial Fe(III) reduction is the central pathway in acidic environments under anoxic conditions, and IRB are the dominant bacteria therein (Sun *et al.*, 2015). Inconsistent with the redox ladder concept (Peiffer *et al.*, 2021), minor S⁰ reduction still occurred although in a lower ΔG_r than goethite. However, no research has paid attention to the marginal S⁰ reduction by IRB in acidic environments.

Minor S⁰ reduction immobilises Sb. As a primary pathway, Fe(III) reduction has been reported to enhance Sb release due to a depletion of sorption sites (Hockmann *et al.*, 2014); however, the concentration of total dissolved Sb decreased from 9.07 ± 0.48 µM to 0.19 ± 0.07 µM in MR-1+Goe-Sb^{III}+S⁰

(Fig. 1b). In the abiotic control (Goe-Sb^{III}+S⁰; Fig. 1c), the Sb concentration slightly increased from 7.33 ± 0.27 µM to 8.95 ± 0.57 µM during the incubation. The dissolved Sb was mainly Sb^{III}-O, and no thiolated Sb species was detected by IC-ICP-MS (Fig. 1b-c). The decrease in dissolved Sb in MR-1+Goe-Sb^{III}+S⁰ was consistent with the loss of Sb^{III}-O (Fig. 1b).

The loss of dissolved Sb^{III}-O in MR-1+Goe-Sb^{III}+S⁰ was due to the formation of Sb₂S₃ as evidenced by Sb L₃-edge XANES analysis (Figs. 2a, S-4 and Table S-4). In detail, the first derivative peak at 4703 eV in Goe-Sb^{III}+S⁰ was attributed to Sb^{III}-O, in accordance with the initial Sb^{III}-O adsorbed on goethite (Fig. 2a). In the presence of MR-1, Sb^{III}-O was transformed to Sb₂S₃ at 4702 eV (Fig. 2a). Linear combination fitting (LCF) of XANES spectra further confirmed that 36 ± 4 % Sb₂S₃ were formed in MR-1+Goe-Sb^{III}+S⁰, whereas the proportion of Sb₂S₃ in Goe-Sb^{III}+S⁰ (5 ± 2 %) was negligible (Table S-4). In addition, approximately 51 ± 1 % and 39 ± 2 % Sb^{III}-O were oxidised to antimonate (Sb^V-O) in Goe-Sb^{III}+S⁰ and MR-1+Goe-Sb^{III}+S⁰, respectively (Fig. 2a and Table S-4). The abiotic oxidation was ascribed to the electron transfer between Sb^{III}-O and goethite (Yin *et al.*, 2021), as detailed in SI.

The mass balance calculations show that 1.52 ± 0.62 µmol Sb^{III}-O were precipitated in the presence of MR-1 at the end of incubation, but 4.30 ± 0.48 µmol Sb as Sb₂S₃ were detected by XANES analysis. The much higher amount of Sb as Sb₂S₃ than the loss of dissolved Sb^{III}-O suggests that not only dissolved, but also adsorbed Sb^{III}-O, reacted with biogenic sulfide to form Sb₂S₃ precipitates. Consistent with this observation, the precipitation of Sb₂S₃ may readily occur in mildly acidic environments, such as paddy soils, wetland, groundwater and lake/river systems where sulfide was commonly less than 10 µM (Figs. 3a, S-6a). Actually, if sulfide or pH slightly increased, instead of Sb₂S₃, soluble thioantimony (Sb-S) would be prevalent (Figs. 3a, S-6a), resulting in enhanced Sb release (Ye *et al.*, 2022).

Unlike our results, a previous study ascribed the immobilisation of Sb to incorporation of Sb^V-O into the newly formed secondary iron phases (*i.e.* feroxyhyte and goethite) (Burton *et al.*, 2019). However, that pathway is not suitable in our system because the phase transformation of goethite was not significant. In detail, Fe XANES LCF analysis suggest that 99 ± 0 % of Fe phases remained as goethite and negligible iron sulfides (1 ± 1 % of total Fe) were formed at the end of incubation (Fig. S-5 and Table S-5). S K-edge XANES also confirmed the negligible formation of iron sulfides (4 ± 1 % of total S; Fig. S-5 and Table S-6). The newly formed iron sulfide may co-precipitate with or re-adsorb Sb, but its low content (1 ± 1 % of total Fe) limited its contribution to Sb mobility (detailed in SI).

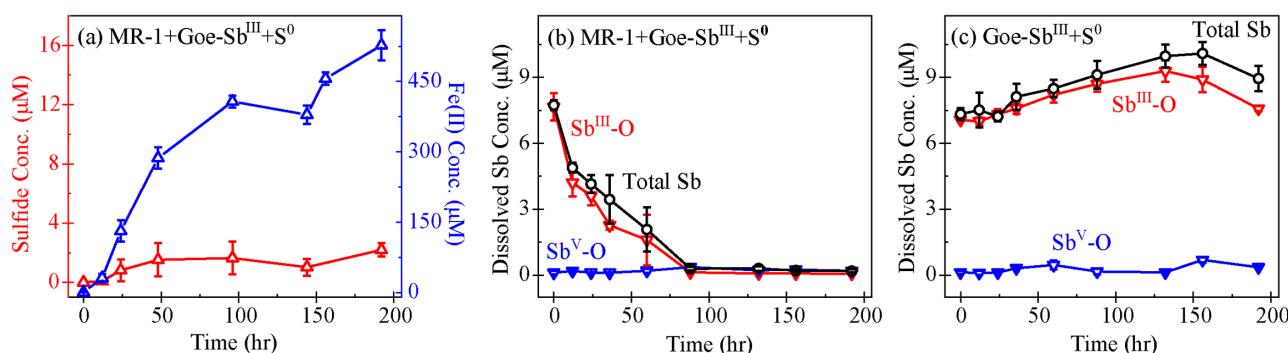


Figure 1 (a) Changes in dissolved sulfide (red) and Fe(II) (blue). (b) Total dissolved Sb (black), Sb^{III}-O (red), and Sb^V-O (blue) during incubation in MR-1+Goe-Sb^{III}+S⁰ at pH 6.5. (c) Changes in dissolved Sb species in abiotic control Goe-Sb^{III}+S⁰. Fe(II) and sulfide were not detected in Goe-Sb^{III}+S⁰.

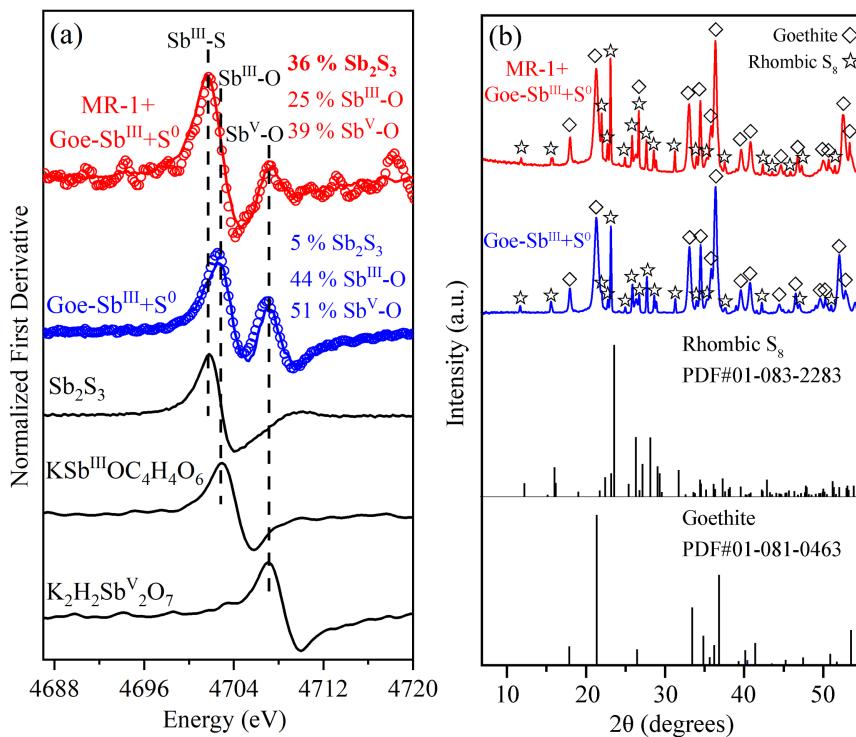


Figure 2 (a) Observed (circles) and linear combination fitting (lines) for the first derivative of normalised Sb L₃-edge XANES for samples at the end of incubation. Spectra for standard references are also shown for comparison. The results of linear combination fitting analysis are shown in Table S-4. (b) Synchrotron X-ray powder diffraction pattern recorded from the solid in MR-1+Goe-Sb^{III}+S⁰ and Goe-Sb^{III}+S⁰ at the end of incubation.

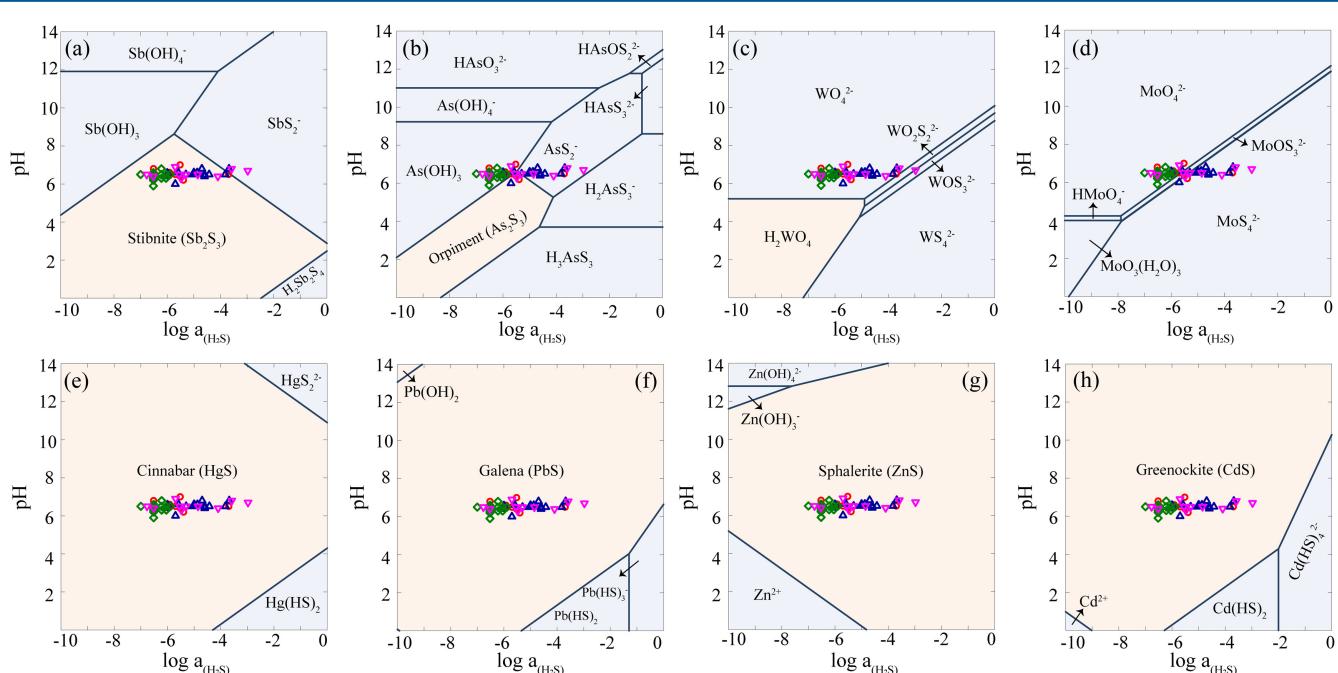


Figure 3 Species of (a) Sb, (b) As, (c) W, (d) Mo, (e) Hg, (f) Pb, (g) Zn and (h) Cd in the reaction with different log activity (H_2S) and pH values in the presence of goethite at 25 °C. The activities of these metal(loid)s were set as 10 μM . The red, blue, green and magenta symbols represent the sulfide concentrations in mildly acidic paddy, wetland, groundwater and lake/river systems, respectively. The data are from references in Table S-7.

In addition, synchrotron X-ray diffraction (SXRD) did not resolve new Fe minerals other than goethite and rhombic S₈ in both Goe-Sb^{III}+S⁰ and MR-1+Goe-Sb^{III}+S⁰ samples at the end of incubation (Fig. 2b).

A recent study demonstrated that Sb^V-O can readily incorporate into the goethite structure during Fe(II) catalysed recrystallisation without phase transformation (Burton *et al.*, 2020). This phenomenon, which involves relatively rapid

interactions between goethite and Fe(II), may have contributed to the immobilisation of Sb under our experimental conditions. However, an assessment of the quantitative contribution of Fe(II)-induced goethite recrystallisation to Sb immobilisation in our experiment is beyond the scope of the present study. Despite this possible uncertainty, it is clear from our results that a substantial amount of Sb was certainly immobilised *via* Sb₂S₃ precipitation due to the IRB-driven S⁰ reduction.

Strong impacts from weak S⁰ reduction on metal(lloid)s mobility. Since environmental acidification is inevitable with increasing CO₂ emission (Terhaar *et al.*, 2020), the effect of marginal S⁰ reduction under mildly acidic conditions should be paid more attention. This effect can be extended to other metal(lloid)s including arsenic, tungsten, molybdenum, mercury, lead, zinc and cadmium. To justify the proposition, reaction path modelling was performed to predict the speciation change of these metal(lloid)s at 10 µM (Fig. 3) and 1 µM (Fig. S-6) as a function of pH and the activity of sulfide. As shown in our thermodynamic calculations (Figs. 3b-d, S-6b-d), soluble thiolated species such as thioarsenic (As-S), thiotungsten (W-S), and thiomolybdenum (Mo-S) would occur in multiple low sulfide (<0.1 mM) environments. Consistent with our results, As-S has been detected in paddy soil porewaters when sulfide <10 µM at pH 6.5, and the As-S formation was promoted by S⁰ (Wang *et al.*, 2020). In general, the formation of As-S and W-S may enhance their mobility (Mohajerin *et al.*, 2014; Ye *et al.*, 2020), whereas Mo-S is less mobile than Mo-O due to its higher affinity for sulfide minerals and natural organic matter (Smedley and Kinniburgh, 2017).

On the other hand, S⁰ reduction leads to precipitation of sulfide minerals, such as cinnabar (HgS), galena (PbS), sphalerite (ZnS), and greenockite (CdS) (Figs. 3e-h, S-6e-h). These minerals immobilise metal(lloid)s due to minor S⁰ reduction (~10 µM), resulting in decoupling of Fe(II) and metal(lloid)s release to the aqueous phase during the microbial Fe(III) reduction. However, most previous studies attributed the decoupling of Fe(II) and metal(lloid)s release to re-adsorption on, or incorporation into, the newly formed iron minerals (Hockmann *et al.*, 2020). This study provides a new perspective that IRB shape the mobility and transformation of metal(lloid)s *via* S⁰ respiration. Even under mildly acidic conditions where Fe(III) reduction is prevailing, the underappreciated and marginal S⁰ reduction plays a vital role in the mobility of metal(lloid)s.

Author Contributions

LY conceived the study, performed the experiments, interpreted the data and wrote the first draft; CJ contributed to the work design, data interpretation and manuscript revision.

Competing interests:

The authors declare no competing financial interest.

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Additional Information

Supplementary Information accompanies this letter at <https://www.geochemicalperspectivesletters.org/article2215>.



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