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Geochemical Explanation for the Prevalence of S^0 Reduction Among Many Fe(III)-Reducing Bacteria

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Abstract: Microbial reduction of ferric iron [Fe(III)] is an important biogeochemical process in anoxic aquifers. Depending upon groundwater pH, dissimilatory metal-reducing bacteria (DMRB) can also respire alternative electron acceptors to survive, including elemental sulfur (S^0). To understand the interplay of Fe/S cycling under alkaline conditions, we combined thermodynamic geochemical modeling with bioreactor experiments using Shewanella oneidensis MR-1. Under these conditions, S. oneidensis can enzymatically reduce S^0 but not goethite (α-FeOOH). The HS^- produced subsequently reduces goethite abiotically. Due to the prevalence of alkaline conditions in many aquifers, Fe(III) reduction may thus proceed via S^0–mediated electron-shuttling pathways whereby DMRB may require an active sulfate-reducing bacterial partner to respire.

One Sentence Summary: Under alkaline conditions, metal-reducing bacteria are shown to respire elemental sulfur rather than ferric iron.

Main Text:

Dissimilatory metal-reducing bacteria (DMRB) are diverse microorganisms that can use insoluble, extracellular substrates as electron acceptors for respiration (1, 2). Although DMRB can reduce a variety of chemical compounds, their ability to reduce ferric iron [Fe(III)] is their most studied trait. Fe(III) is common in the environment as insoluble (oxyhydr)oxide minerals such as ferrihydrite ($Fe(OH)_3$) or goethite (α-FeOOH) . The reductive dissolution of these minerals by DMRB produces highly reactive ferrous ions (Fe^{2+}), making Fe(III) reduction important to water quality (3), contaminant fate and transport (4), the biogeochemical cycling of carbon (5), and the geochemical evolution of the early Earth (6).

In addition to Fe(III), many DMRB strains can use elemental sulfur (S^0) as an electron acceptor. The ecological significance of S^0 reduction in aquifers, however, is poorly understood.

Although Fe(III) minerals are abundant in these environments, the steady-state concentration of S^0 is frequently below detection (7). Nevertheless, S^0 may still serve as a transient but important electron sink there (8). S^0 is also abundant in marine sediments where steep redox gradients allow the direct mixing of sulfidic waters with dissolved O_2, but it can be created in anoxic, freshwater systems by the reaction of dissolved sulfide with ferric minerals such as goethite (9). Many common DMRB in these environments (e.g. several Shewanella, Desulfuromonas, Geothrix, Pelobacter, and Geobacter spp.) can respire S^0 directly. Genetic evidence suggests that this ability is derived from an enzymatic mechanism distinct from the pathway used to reduce Fe(III) (10) and is therefore unlikely to be simply an incidental consequence of these microorganisms' ability to reduce transition metals. Rather, the common co-occurrence in metal reducers of the ability to reduce Fe(III) and S^0 suggests an evolutionary explanation linked to the ecology of the terrestrial subsurface, where metal-reducing microorganisms are frequently abundant (2).

Most microorganisms can respire using a variety of substrates, but their ability to use any one respiratory pathway depends on the amount of thermodynamic energy available from that reaction (11). The available energy can be calculated directly from the chemical activity of reactants and products in the metabolic reaction being catalyzed (12). For example, some geomicrobial reactions such as Fe(III) reduction are strongly proton-consuming and therefore much less energetically-favorable in alkaline environments (11).

Alkaline aquifers are common and serve as critical water resources, especially in arid regions where water-rock interactions drive the pH up to 8–10 (13). Furthermore, alkaline groundwater is often associated with high levels of arsenic, a toxic metal whose mobility in groundwater has been tied to the activity of Fe(III) and sulfate-reducing bacteria (SRB) (14).

To better understand the biogeochemistry of Fe and S in alkaline environments, we calculated the energy available to microorganisms from the reduction of Fe(III) and S^0 versus sulfate by creating a thermodynamic model of a pristine, anoxic, electron-donor-limited aquifer (Table S1). To test the model predictions regarding the effect of pH on the microbial reduction of Fe(III) and S^0, we inoculated pH-buffered suspensions of Fe(III)- and S^0-bearing minerals (goethite and rhombic S^0) with Shewanella oneidensis MR-1, a DMRB capable of reducing both. We chose strain MR-1 because a genetic mutant, PSRA1, contains an in-frame deletion of the gene psrA and is unable to respire S^0 (10). Additional information on methodology is available as Supplementary Online Materials.

Our thermodynamic models show that under these hypothetical groundwater conditions, the reduction of Fe(III)-containing minerals is favored much more strongly at acidic pH than alkaline (Figure 1). With all three electron donors tested, goethite reduction yields as much energy as sulfate reduction at pH 8 but considerably less than S^0 reduction above pH 7. The reduction of ferrihydrite provides more energy per mole of substrate than reduction of goethite (Table S1), but this pathway also ceases to provide sufficient energy for respiration at roughly pH 9 for the conditions tested. Although the amount of energy available from these reactions also depends on the concentration of the electron donor being utilized, the strong correlation of pH with the amount of energy available from reducing ferric minerals shows that these means of respiration are likely to be much less favorable at the near-neutral to slightly basic pH of aquifers like the Columbia River Basalt Group (15) or the Continental Intercalaire aquifer (13). The reduction of S^0, in contrast, is energetically favorable at any pH and becomes more favorable with increasing pH.

Under the modeled conditions, the reduction of Fe(III) provides insufficient thermodynamic energy to support the respiration of DMRB at alkaline pH. Still, DMRB might

respire and grow under these conditions. Indeed, under laboratory conditions with abundant nutrients and large concentrations of electron donor and acceptor, microbial reduction of Fe(III) has been shown to occur at pH > 11 via microorganisms such as Geoalkalibacter and Anaerobranca (16). However, these idealized conditions differ markedly from those in most aquifers, where concentrations of organic acids such as acetate and formate are typically found in micromolar concentrations or less and the thermodynamic driving force is small (17).

In goethite-only bioreactors inoculated with wild-type S. oneidensis, considerably more Fe^{2+} was produced at pH 6.8 than pH 9.0 (Figure 2A). We attribute some reduction without added donor to the accumulation of residual reducing power in S. oneidensis cells during their initial growth in rich medium (see Supplementary Materials). At pH 6.8, however, more than twice as much Fe^{2+} was produced when formate was added versus the no-donor control; at pH 9.0, Fe^{2+} production was the same in control and donor-containing experiments. This result suggests that under the alkaline conditions tested, no respiratory reduction of goethite coupled to formate oxidation occurred, where our model predicts it to be thermodynamically unfavorable (Figure S1). As previously reported (10), the production of Fe^{2+} via goethite reduction did not differ between the PSRA1 mutant or the wild type (Figure 2A and 2B).

In bioreactors containing both goethite and S^0, the overall production of Fe^{2+} at pH 6.8 was nearly equivalent to that of goethite-only experiments at pH 6.8 for both the wild-type and PSRA1 (Figures 2C and 2D). At pH 9.0, however, the wild type produced nearly three times more Fe^{2+} when given formate compared to no-donor controls (Figure 2C). The rate at which Fe^{2+} accumulated was slower at pH 9.0, which is likely due to the slower reaction kinetics between sulfide and goethite at alkaline pH (18). In contrast, the amount of Fe^{2+} produced by PSRA1 at pH 9.0 differs little with or without S^0 (Figures 2B and 2D). Synchrotron-based measurement of sulfur

speciation by x-ray absorption spectroscopy confirmed that at pH 9.0, S^0 was reduced to sulfide by the wild type but not by PSRA1 (Figure 3), leading to the formation of mackinawite (FeS). Sulfide was detected in S^0-containing bioreactors of both wild-type and PSRA1 cells at pH 6.8, although for the mutant this likely resulted from the abiotic reaction of Fe^{2+} with S^0 to form mackinawite through a polysulfide intermediate (19). Our results indicate that, as predicted by the model (Figure 1), under alkaline conditions S. oneidensis can enzymatically reduce S^0 but not goethite. The production of Fe^{2+} at pH 9 is instead due to the abiotic reduction of goethite by sulfide produced through the enzymatic reduction of S^0, suggesting that Fe(III) reduction at alkaline pH proceeds via an indirect, sulfur-dependent electron shuttling pathway similar to those previously known to occur via flavins or humic substances (20).

The primary source of dissolved sulfide in the subsurface is microbial sulfate reduction (21), a process where the available energy is affected little by changes in pH (Figure 1). By reducing sulfate to HS^- in the presence of Fe(III) minerals in an alkaline aquifer, the respiration of SRB would create S^0 and allow DMRB like Shewanella spp. to respire (Figure 4). Many studies indicate that Fe(III) reduction and sulfate reduction co-occur frequently in the subsurface (22). Therefore, under alkaline conditions DMRB would depend on the activity of SRB to respire in a commensal or even mutualistic relationship (23). In addition to modern aquifers, such an interaction could have been important on the early Earth, where alkaline conditions are thought to have predominated in large areas of the ocean (24), and may have contributed to the formation of sedimentary pyrite during the Archean and early Proterozoic (25). The extreme alkalinity of the early oceans (pH >10) makes the direct, enzymatic reduction of Fe(III) even less likely to have been energetically favorable, and dissimilatory iron reduction alone probably would not be responsible for the production of Fe^{2+} there.

This ecological connection explains why many DMRB would maintain separate genetic pathways to respire Fe(III) and S^0. In the presence of active sulfate reduction and faced with an inability to respire Fe(III) due to energetic limitations, a microbe able to respire both S^0 and Fe(III) would have a competitive advantage. For example, the microbial reduction of the Fe(III) minerals ferrihydrite and goethite coupled to formate or acetate oxidation results in significant increases in pH due to H^+ consumption during the corresponding catabolic half reactions (Table S1). The ability to transition from enzymatic reduction of Fe(III) minerals at circumneutral pH to a S^0-reducing pathway at alkaline pH where Fe(III) minerals are thermodynamically unavailable for use as electron acceptors thus provides DMRB with a mechanism to sustain energy-generating electron transport processes over a much wider pH range (approaching nearly 4 orders of magnitude) than direct enzymatic Fe(III) reduction alone. Furthermore, at alkaline pH, Fe^{2+} ions are thought to sorb more strongly to the surfaces of iron oxides and thereby inhibit direct enzymatic reduction (26). Sulfide production through the reduction of sulfate and S^0 would strip these sorbed ions away and thereby circumvent the passivation of Fe(III) oxide surfaces, providing further evidence for the important of sulfate reduction to the reduction of Fe(III) oxides at alkaline pH.

Indirect Fe(III) reduction via a S^0 reduction pathway under alkaline conditions could be highly relevant to geologic carbon sequestration. In addition to their critical role as water resources, alkaline aquifers are primary targets for carbon capture and sequestration in the deep subsurface because they can mineralize injected supercritical CO_2 as carbonate minerals (27). This ability is derived from the superior pH buffering of alkali minerals in the aquifer, where groundwater becomes more acidic after injection of supercritical CO_2 (28). The reductive dissolution of Fe(III) minerals to aqueous Fe^{2+}—regardless of whether it is mediated biotically or abiotically—is critical to this process because these ions react with bicarbonate and precipitate as the mineral siderite,

thus trapping carbon in solid form (29). Assuming direct enzymatic reduction of ferric minerals is unlikely to occur in alkaline, oligotrophic environments (Figure 1), microbial sulfate reduction and the subsequent reaction of sulfide with ferric minerals to produce Fe^{2+} and S^0 (which itself could be re-reduced by DMRB to form additional sulfide) would be the primary mechanism responsible for producing Fe^{2+} and sequestering carbon.

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Fig. 1. Free energy change of microbial metabolisms in a hypothetical pristine aquifer. The amount of usable energy (ΔG_U) available to microorganisms from the reduction of S^0, Fe(III) minerals (ferrihydrite and goethite), and sulfate with either (A) formate, (B) acetate, or (C) hydrogen as an electron donor changes with pH. The dotted line at $\Delta G_U = 0$ kJ mol^{-1} represents the theoretical minimum energy required to support microbial respiration. Electron donating and accepting processes modeled are shown in D.

Fig. 2. Total Fe^{2+} production in bioreactor experiments. Experiments were conducted at pH 6.8 and 9.0 using S. oneidensis MR-1 wild type (A,C) and psrA-deficient mutant PSRA1 (B,D) as an inoculum. Bioreactors contained either 10 mM goethite alone (A,B) or 10 mM each of goethite and S^0 (C,D). Data points represent the average of triplicate bioreactors with error bars ± standard deviation.

Fig. 3. Sulfur K-edge XANES spectra of S-containing bioreactors. Standards shown are (A) unreacted S. oneidensis MR-1 cells, (B) rhombic S^0, and (C) mackinawite (FeS). Samples are

shown from bioreactors containing both goethite and S^0 at pH 9.0 (D, E) or pH 6.8 (F, G) that were inoculated with cells of either the wild type (D, F) or PSRA1 mutant (E, G).

Fig. 4. Illustration of S^0-mediated Fe(III) reduction under alkaline conditions.

Supplementary Materials:

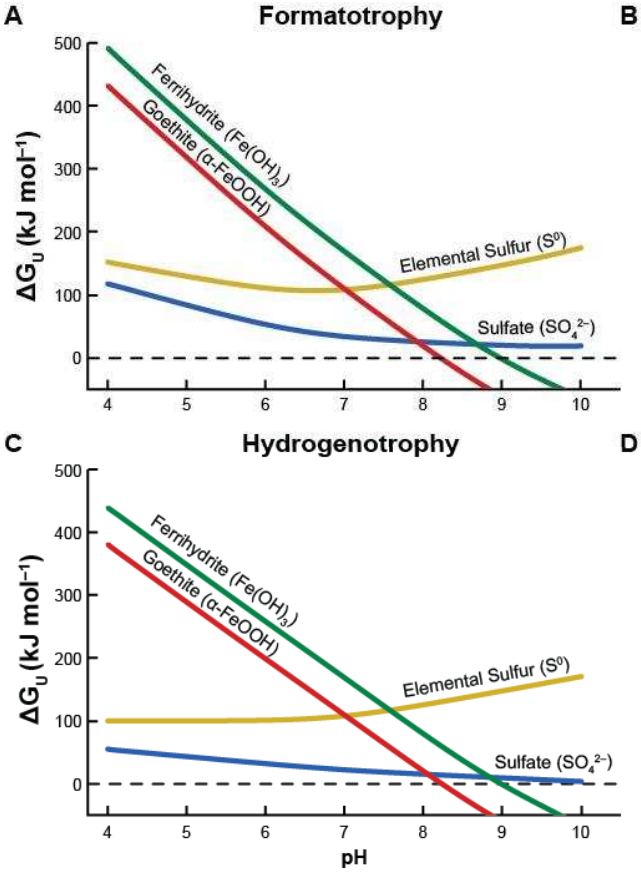
Materials and Methods

Table S1

Figure S1

References 31–44.

Figures



D

Electron donating half-reactions	ΔG° (kJ mol^{-1})
$CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$	144.18
$4CHOO^- + 4H_2O \rightarrow 4HCO_3^- + 8H^+ + 8e^-$	4.46
$4H_2(aq) \rightarrow 8H^+ + 8e^-$	–71.06
Electron accepting half-reactions	ΔG° (kJ mol^{-1})
$SO_4^{2-} + 9H^+ + 8e^- \rightarrow HS^- + 4H_2O$	–192.16
$4S^0 + 4H^+ + 8e^- \rightarrow 4HS^-$	48.17
$8Fe(OH)_3 + 24H^+ + 8e^- \rightarrow 24H_2O + 8Fe^{2+}$	–717.62
$8FeOOH + 24H^+ + 8e^- \rightarrow 16H_2O + 8Fe^{2+}$	–658.25

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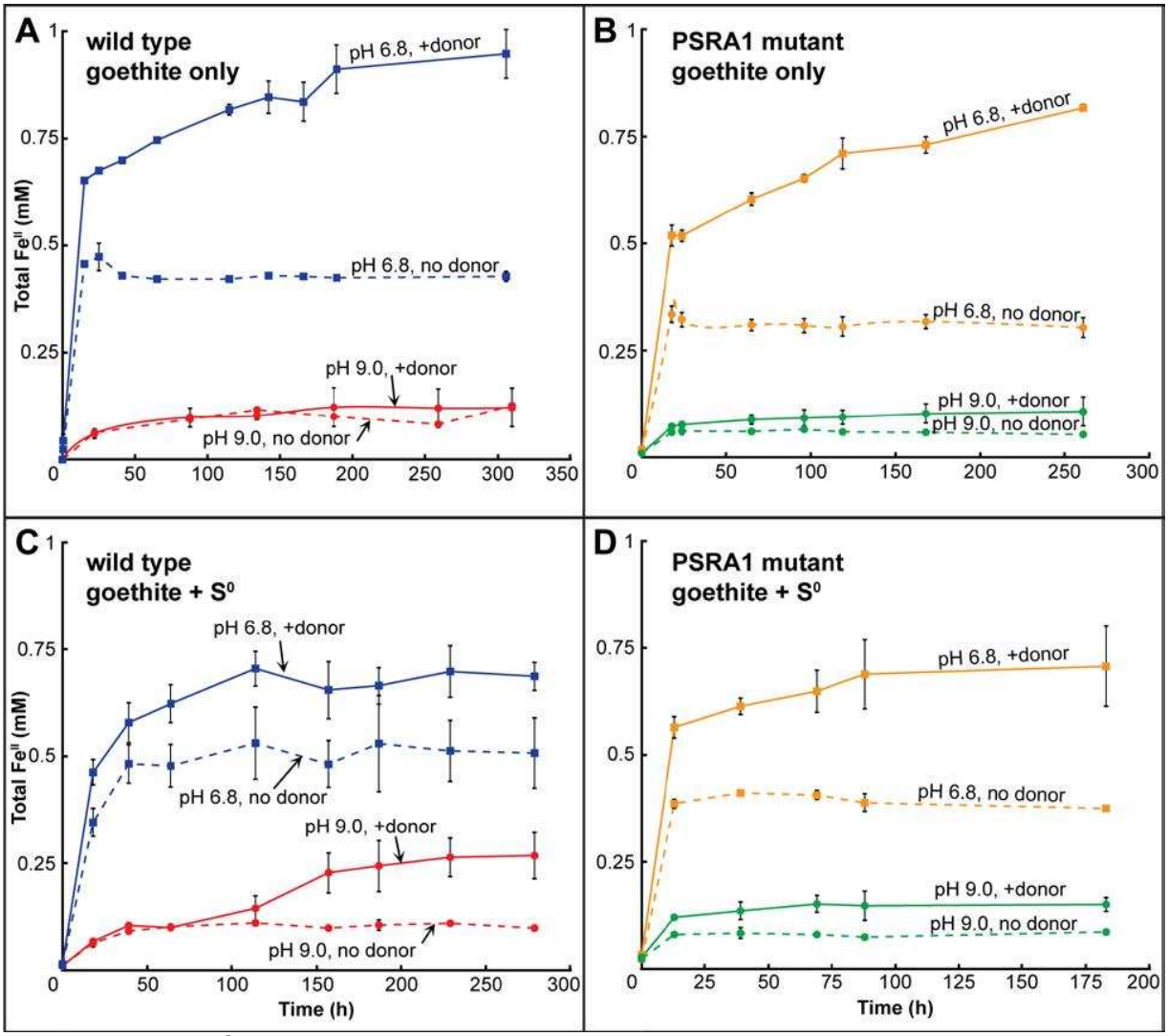
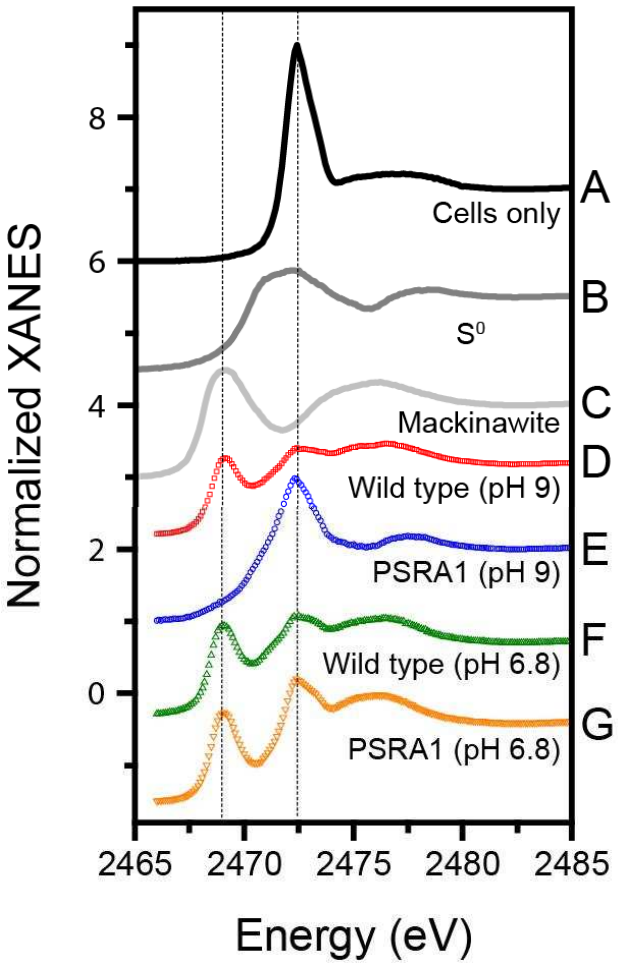


Figure 2. Total Fe^{2+} production in bioreactor experiments. Experiments were conducted at pH 6.8 and 9.0 using Shewanella oneidensis MR-1 wild type (A,C) and psrA-deficient mutant PSRA1 (B,D) as an inoculum. Bioreactors contained either 10 mM goethite alone (A,B) or 10 mM each of goethite and S^0 (C,D). Data points represent the average of triplicate bioreactors with error bars ± standard deviation.



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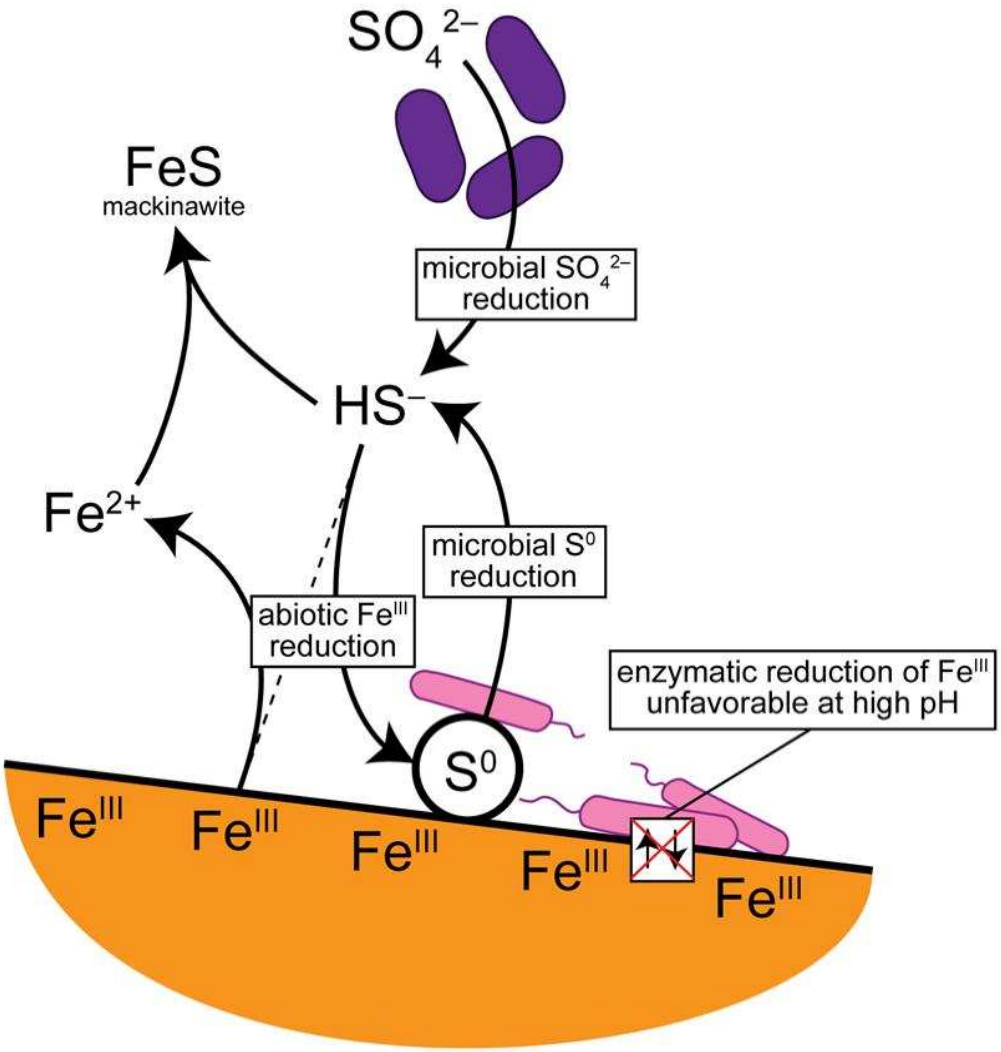

Figure 4. Illustration of S^0-mediated Fe(III) reduction under alkaline conditions.

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