Co-reduction of Fe(III) and S0 drives Fe-S biomineral formation and phosphate mobilisation

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Abstract

Microbially mediated iron and sulfur cycling have impacted redox transitions and the bioavailability of nutrients throughout Earth’s history. Here, we incubated Geobacter sulfurreducens in the co-presence of ferricyanide and S0 at pH 6.5, 7.2 or 8.0. Microbial reduction of Fe(III) and S0 resulted in a shift from ferruginous (Fe2+–rich) to sulfidic (sulfide-rich) conditions and the precipitation of mackinawite, greigite and vivianite. The initial pH controlled the timing of the ferruginous-sulfidic transition and the relative abundance and crystallinity of the formed minerals. Vivianite formation was attributed to phosphate initially added to the medium. Phosphate showed a dynamic cycle, with low dissolved concentrations initially due to sorption to ferrhydrite, followed by vivianite precipitation under ferruginous conditions, and a significant release under sulfidic conditions. Co-reduction of Fe(III) and S0 therefore regulates Fe-S biomineral formation and P bioavailability, which could be particularly important to consider in microbial mats and the sulfate-poor Archean ocean.

Introduction

Microbially mediated Fe and S cycling are vital parts of Earth’s history that affected the oceanic transition between ferruginous and sulfidic conditions, as well as playing an integral role in modern biogeochemical cycles of greenhouse gases, nutrients and contaminants (Lepot, 2020; Kappler et al., 2021). Many species, including model organisms Geobacter and Shewanella, are capable of linking the Fe and S cycles through the reduction of Fe(III) minerals and elemental sulfur (S0). This metabolic flexibility enables them to survive under alkaline conditions and even to carry out the anaerobic oxidation of methane (Alcott et al., 2014), resulting in Fe sulfide biomineral formation (Wang et al., 2018; Nie et al., 2020; Ye and Jing, 2022; Liu et al., 2023). Investigations of coupled reduction of Fe(III) and S0 are important for mineral biosignatures and their impact on nutrient bioavailability, especially in environments where S0 could be an important electron acceptor such as in microbial mats and sulfate-poor Archean oceans (Troelsen and Jørgensen, 1982; van Gemerden et al., 1989; Philippot et al., 2007; Galić et al., 2017). The mobilisation and bioavailability of phosphate in particular is affected by Fe and S biogeochemistry with direct consequences to primary productivity, climate and Earth’s redox evolution (Aldcott et al., 2022).

Microbial Metabolism Drives Geochemical Shifts

To investigate biomineral formation during co-reduction of Fe(III) and S0, G. sulfurreducens was incubated in the co-presence of ferricyanide (30 mM) and S0 (60 mM) with acetate as excess electron donor. Different initial pH values (6.5, 7.2 or 8.0) were employed. Over 42 days of incubation, all cultures exhibited a change from dark brown to black, indicating transformation of ferricyanide to reduced Fe minerals (Fig. S-1). From initial pH values of 6.5 and 7.2, pH increased to 7.1 and 7.5, respectively. At initial pH of 8.0, the pH slightly decreased to 7.9. From here onward, the cultures will be referred to by their initial pH values for simplicity.

At the beginning, solid phase extractable Fe (6 M HCl) amounted to 29.1 ± 2.2 mM (n = 12) with no Fe(II) components detected. Within 15 days, the Fe(II)/Fe(III) percentages of the HCl extractable Fe approached 100 %, indicating Fe(III) reduction (Fig. S-2). Note that the total HCl extractable Fe decreased over time, and we attributed this primarily to sampling inhomogeneity due to mineral aggregation. Fe(III) reduction was accompanied by the release of Fe2+ into solution, reaching maxima of 1000–2000 μM depending on initial pH (Fig. 1). Dissolved Fe2+ showed a decline to <500 μM within 12 days after the maximum. Once Fe2+ reached low levels, dissolved sulfide started to...
increase, reaching maxima of 320–420 µM. Solid phase Fe sulfides were not quantified but were detectable by scanning electron microscopy (SEM), X-ray diffraction (XRD) and Mössbauer spectroscopy (Figs. 2, 3).

Overall, cultures at different pH values exhibited similar geochemical trends with differences in timing and the amount of Fe(II) and sulfide released to the solution. Parallel abiotic controls showed neither Fe(II) nor sulfide production (Fig. S-2). The geochemical evolution can be divided into two stages. In the first stage, ferruginous conditions dominated as ferrihydrite was reduced and Fe(II) accumulated in solution. In the second stage, sulfidic conditions were observed once reactive Fe(III) surfaces were exhausted and sulfide accumulated in solution. The time at which the shift from ferruginous to sulfidic conditions occurred differed depending on the pH values, following the order: pH 7.2 (30 days) > pH 6.5 (22 days) > pH 8.0 (19 days).

Mirroring this trend, the highest maximum Fe(II) followed the order: pH 7.2 (2000 µM) > pH 6.5 (1500 µM) > pH 8.0 (1100 µM). Maximum dissolved sulfide followed the opposite trend: pH 7.2 ≈ pH 6.5 (320 µM) < pH 8.0 (420 µM).

The different timings and concentrations of Fe(II) and sulfide observed depend on a number of interrelated pH dependent processes including microbial preference of Fe(II) over S, Fe(II) adsorption to ferrihydrite, sulfide mediated ferrihydrite reduction and Fe mineral precipitation. These processes can lead to secondary phenomena such as Fe(II) catalysed recrystallisation, decreased reactivity from the FeS surface coating, and formation of polysulfides (Peiffer et al., 2015). Notably, the observed geochemical trends do not follow straightforward predictions based on initial pH (Supplementary Information). Further extensive investigation at the nanometre (e.g., transmission electron microscopy) and molecular (e.g., speciation via synchrotron) scales, coupled to a biogeochemical model will be needed to elucidate the specific mechanisms.

**Formation of Biogenic Mackinawite and Greigite, but not Pyrite**

Mineral products were identified using a combination of magnetic testing, XRD, SEM and Mössbauer spectroscopy. XRD patterns of samples at day 21 and 42 indicated the presence of S, which decreased over time at all pH values, signifying continuous microbial S reduction (Fig. 3a). Additionally, XRD and Mössbauer identified mackinawite (FeS) as a major product (20–70 % of solid phase Fe). Black colourations typical of Fe sulfides were commonly observed to form coatings on S(S). Dashed vertical lines denote the shift from ferruginous to sulfidic conditions.

The mechanism and kinetics of biogenic Fe sulfide mineral transformation are important to understand with respect to their implications for biosignatures and elemental cycling (Picard et al., 2016; Nie et al., 2020). Poorly-crystalline mackinawite typically forms first from the reaction between Fe(II) and sulfide at low temperatures, followed by its transformation to greigite and pyrite over time (Son et al., 2022). In our cultures, the XRD patterns for mackinawite were most pronounced at pH 7.2. XRD data showed clear patterns for greigite only at pH 6.5 at day 21. Positive identification in other cultures was complicated due to the overlap of the main greigite signal with mackinawite at ~35° 2θ and the generally broad patterns resulting from low crystallinity. Consistent with this interpretation, crystalline greigite particles were not observed by SEM. Other minerals such as magnetite (Fe₃O₄) and pyrite were not detectable within 21–42 days of incubation (Supplementary Information).

We report the first instance of greigite (Fe₃S₄) formation by *Geobacter*. The presence of a magnetic mineral was first suggested via attraction of the minerals to a hand magnet held next to the bottles. Strong magnetism was observed at all pH values starting from day 11 (Fig. S-1) but decreased noticeably after 27 days (Supplementary Information). Greigite was identified by Mössbauer spectroscopy, making up 17 ± 5 % of solid phase Fe. Black colourations typical of Fe sulfides were commonly observed to form coatings on S(S). Dashed vertical lines denote the shift from ferruginous to sulfidic conditions.

![Figure 1](image-url) Geochemical evolution of dissolved Fe(II), total sulfide and phosphate at (a) pH 6.5, (b) pH 7.2, and (c) pH 8.0. Dashed vertical lines denote the shift from ferruginous to sulfidic conditions.

Geochem. Persp. Let. (2023) 24, 27–32 | [https://doi.org/10.7185/geochemlet.2301](https://doi.org/10.7185/geochemlet.2301)
crystalline mackinawite is expected. With longer incubation, the cultures shifted to sulfidic conditions, promoting additional mackinawite formation. However, this mackinawite exhibited lower crystallinity and contributed to broader XRD patterns reflective of the bulk mixture.

Greigite is increasingly recognised as a common and stable phase in nature and as an important intermediate for pyrite formation (Subramani et al., 2020). We found the highest percentage of greigite at the intermediate pH of 7.2 (53 % of solid phase Fe). This is in contrast with the expectation that lower pH, including around the microenvironments of sulfate reducing bacteria, could promote mackinawite’s transformation to greigite (Bourdoiseau et al., 2011; Mansor et al., 2019). Instead of pH, we suggest that the crystallinity of the precursor mackinawite is the main controlling factor of transformation kinetics (Csákberényi-Malasics et al., 2012; Miller et al., 2020). As discussed, we observed mackinawite with the highest crystallinity at pH 7.2. Our data showed that precipitation under ferruginous conditions enhances mackinawite’s crystallinity and its transformation to greigite, confirming recent predictions from density functional theory (Son et al., 2022).

Pyrite, the most common Fe sulfide mineral in the environment, was not formed in our cultures within 42 days, similar to in pure cultures of sulfate reducing bacteria (Picard et al., 2016). Nonetheless, studies with sulfur cycling bacteria have demonstrated pyrite formation from sulfidation of Fe(III) phosphates within one month (Berg et al., 2020; Duverger et al., 2020). Pyrite precipitation was attributed to microbial production of

Figure 2  SEM micrographs of microbe-mineral associations. (a) $S^0$ aggregates consisting of ~20 μm globules and Fe mineral aggregates consisting of nanometre-sized structures. (b) G. sulfurreducens atop a $S^0$ globule. Note the close spatial association with aggregates of Fe-O minerals and FeS-rich minerals with pseudo-honeycomb structure. (c) A naturally fractured sample highlighting the FeS-rich coating on a $S^0$ globule. (d) The FeS-rich coating appeared to peel off, revealing the smoother surface texture of $S^0$ compared to FeS. (e) Typical morphology of Fe-P-O-rich minerals (vivianite). (f) A higher magnification of the radial blade-like morphology of vivianite (from the boxed region in (e)).
extracellular polymeric substances (EPS) that concentrated key ingredients for pyrite formation locally and the continuous formation of Fe$^{2+}$, S$^{0}$ and polysulfides from slow sulfide mediated Fe(III) dissolution. *G. sulfurreducens* is also known to produce EPS that can bind cations, especially in the presence of Fe(III) minerals (Stöckl et al., 2019; Tomaszewski et al., 2020). Furthermore, the co-presence of Fe(III) and S$^{0}$ should have led to high polysulfide concentrations that enhance pyrite formation. However, it is possible that rapid reduction of Fe(III) and S$^{0}$ (and potentially polysulfides) by *G. sulfurreducens* may have prevented the accumulation of intermediates necessary for fast pyrite formation. Further kinetic based studies and comparison with a pyrite forming culture will be necessary to elucidate factors controlling pyrite formation (Supplementary Information).

**Microbial Fe-S Metabolism leads to Phosphate (Im)Mobilisation**

Besides Fe sulfides, XRD and Mössbauer spectroscopy revealed the presence of vivianite [Fe$_3$(PO$_4$)$_2$·8H$_2$O] at all pH values, making up 7–28% of solid phase Fe (Fig. 3). The formed vivianite had radial blade-like structures up to 50 μm in length (Fig. 2e, f). Vivianite formation is explained by the addition of phosphate to the medium, which resulted in P/Fe ratio of 0.15 in our experiments, comparable to the ∼0.10 ratio typical in nature (Kraal et al., 2022). Despite the addition of 4.4 mM phosphate, initial dissolved phosphate was <200 μM (Fig. 1). We attributed this to strong phosphate adsorption to ferrhydrite (Wang et al., 2013; Kraal et al., 2022). The concentrations dropped within a few days to near detection limit, coincident with the rise in Fe$^{2+}$, attributed to vivianite formation. Dissolved phosphate then showed a marked increase coincident with sulfide release to solution.

The sequence of biogeochemical processes in the experiments can be summarised as follows (Fig. 4). First, phosphate from the growth medium was rapidly adsorbed to ferrhydrite. *G. sulfurreducens* reduced ferrhydrite and S$^{0}$ in the presence of excess electron donor, with more S$^{0}$ reduction at pH 8.0 compared to lower pH values. In the early ferruginous stage, sulfide concentration was kept low as it reacted rapidly with ferrhydrite, contributing to Fe(III) reduction, Fe$^{2+}$ release to solution, and the precipitation of mackinawite, greigite and vivianite. The cultures progressed into the late sulfidic stage as reactive Fe(III) became fully exhausted. In addition to further Fe sulfide formation, this biogeochemical switch resulted in vivianite dissolution, releasing phosphate into solution. This supports previous observations in which the switch from ferruginous to sulfidic conditions has been recognised to increase the bioavailability of phosphate (Duverger et al., 2020; Alcott et al., 2022).

Overall, our study demonstrated that co-reduction of Fe(III) and S$^{0}$ leads to formation of mackinawite, greigite and vivianite, but not pyrite within 42 days. Initial pH affected the length and timing of the ferruginous-sulfide transition, resulting in differences in the crystallinity and relative abundance of the mineral products. The transition from ferruginous to sulfidic

**Figure 3** (a) XRD patterns of precipitates at Days (D) 21 and 42. Vertical lines denote 2θ positions specific to certain minerals, with colours corresponding to vivianite (‘V’, light blue), mackinawite (‘M’, black), S$^{0}$ (‘S’, yellow) and greigite (‘G’, grey). (b) Solid phase Fe distribution based on Mössbauer spectroscopy at 77 K.
conditions was associated with phosphate release. Similar processes, driven by the activity of whole microbial communities instead of a single species, are likely important to consider for micro-niches in modern sediments and microbial mats. These processes are further applicable to SO4-poor Archean oceans and ultimately the investigation of Fe-S biominal signatures and the bioavailability of important nutrients that affected primary productivity and Earth’s biogeochemical evolution.

**Acknowledgements**

This study was supported by the DFG (SPP 1833, Emmy Noether Programme, 1450/3-1, DU 1450/3-2, DU 1450/7-1, JPD; INST 37/1027-1 FUGG, AK) as well as the Excellence Strategy of the German Federal and State Governments (EXC2124, 390983184; Tuebingen Structural Microscopy Core Facility; AK, MM, PI, SF, JS). We thank Prof. DJ. Lunter and Yali Liu for assistance with Raman analysis.

**Editor: Juan Liu**

**Additional Information**

Supplementary Information accompanies this letter at https://www.geochemicalperspectivesletters.org/article2301.

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