

# Sulfur and oxygen stable isotope geochemistry and sulfide oxidation kinetics of *A. ferrooxidans* under controlled atmospheres

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The environmental geochemistry of sulfide oxidation carries important water quality implications for the release of heavy metals through the direct dissolution of pyrite (FeS<sub>2</sub>) and the acidification of natural waters. Under acidic conditions, the process is largely microbially mediated through the coupling of S and Fe oxidation to the reduction of molecular O<sub>2</sub>. *Acidithiobacillus ferrooxidans* is a well-known acidophilic S-oxidizer that has long been studied for its ability to drive environmental sulfide oxidation and formation of acid rock-drainage. The sulfur and oxygen isotope geochemistry of sulfate (SO<sub>4</sub><sup>2-</sup>), the primary end product of sulfide oxidation, provides a fingerprint for tracking the environmental activity of *A. ferrooxidans*. The δ<sup>34</sup>S values of sulfate, in particular, can be used to understand the sources of sulfide minerals in surface environments. Furthermore, sulfate S and O isotope geochemistry reflect the pathway of mineral oxidation, including the direct incorporation of molecular O<sub>2</sub>, through the oxidation of sulfur intermediates (e.g., SO<sub>3</sub><sup>2-</sup>, S<sub>2</sub>O<sub>3</sub><sup>2-</sup>). Novel stable isotope tools, including the minor isotope of oxygen (<sup>17</sup>O) provide an additional means of tracking sulfide oxidation pathways through the unique D<sup>17</sup>O signatures of O<sub>2</sub> and H<sub>2</sub>O that are incorporated into sulfate.

Here, we present the results of growth experiments with *A. ferrooxidans* DSM 583 at ambient (~21%) and sub-ambient (<5%) O<sub>2</sub> concentrations to calibrate the kinetics of sulfide oxidation, sulfate stable isotope geochemistry (δ<sup>34</sup>S, δ<sup>18</sup>O, D<sup>17</sup>O), and product aqueous geochemistry. *A. ferrooxidans* DSM 583 was provided with pure mineral FeS<sub>2</sub> and a pyrite bearing shale (Tyler Formation, Wisconsin) as electron donor substrates for S-oxidation. The results of the oxidation of these pure minerals and natural host rocks provide much needed perspective on sulfate and heavy metal release in ecologically sensitive environments that are currently being explored for critical mineral extraction, can be used to estimate the fraction of O<sub>2</sub> directly incorporated into sulfate, and add further geochemical context in the use of sulfate stable isotopes for studying surface sulfur cycling in anthropogenically influenced watersheds.