# Upstream Enzymatic Reactions in Microbial Sulfate Reduction

DEREK A. SMITH[1]*, DAVID A. FIKE[1], DAVID T. JOHNSTON[2], ALEXANDER S. BRADLEY[1,3]

[1]Department of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, MO 63130 USA (*correspondence: derek.smith@wustl.edu)

[2]Department of Earth and Planetary Sciences, Harvard University, Cambridge, MA 02138 USA

[3]Division of Biology and Biomedical Sciences of Earth and Planetary Sciences, Washington University in St. Louis, St. Louis, MO 63130 USA

The sulfur cycle is one of the oldest biologically mediated processes on earth and is critical for the oxidation of organic matter in modern and ancient oceans. Through the transformation and burial of oxidized and reduced sulfur, the sulfur cycle is essential in regulating the overall redox state of the Earth's surface. The sedimentary stable sulfur isotope record is one of the best-preserved continuous records of Earth's history; however, deciphering this record has been challenging due to non-unique isotopic signatures of sulfur cycling processes. One of the most important metabolic processes often invoked is microbial sulfate reduction (MSR). MSR is a multi-enzymatic reaction network that partitions the isotopes of sulfur and oxygen as a consequence of both the flux of material through its enzymes and the fractionation imposed by each individual enzyme.

We undertook a multi-faceted approach to assess the impacts that the first two enzymes in the MSR pathway impart on the mass flux and isotopic discrimination of sulfur and oxygen. Two different sets of genetically engineered mutants were employed for growth studies, those with: i) perturbed ability to transport sulfate into the cells via the deletion of suspected sulfate permease proteins, and ii) enhanced expression of the second enzyme of the MSR network, sulfate adenylyl transferase, that is dependent on intracellular redox state. Changes in the isotopic fractionations produced by the mutants that did not correlate with growth rate and could not be fully explained by changes in sulfate reduction rate may have resulted from differences in the material flux through the MSR network. The work further illustrates the utility of genetic engineering and highlights the complexities that the regulation of enzymes can have on phenotypic expressions that may be preserved in the environment and geologic record.