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Multiple Sulfur Isotope Fractionation by a Single Bacterial Strain During Sulfite Reduction, Oxidation, and Disproportionation

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The global sulfur cycle is paramount for investigating the Earth's surface environment, as it is tightly linked to surface redox states and the distribution of trace metals via the precipitation of sulfide minerals. Microorganisms are the primary drivers of this cycle, facilitating sulfur reduction, oxidation, and disproportionation metabolisms. While multiple sulfur isotope ratios serve as tracers for these processes, previous research has prioritized microbial sulfate reduction (MSR) due to its large $^{34}S/^{32}S$ fractionation. Despite conceptual models linking physiology to multiple isotope effects in MSR, even the measurements of multiple sulfur isotope fractionation factors during bacterial sulfur disproportionation (BSD) [1] and microbial sulfur oxidation [2] remain scarce, with only a single study available for each.

Sulfate reducing bacterial strain *Desulfovibrio desulfuricans* CSN is uniquely capable of growth via sulfite reduction, oxidation, and disproportionation and is hypothesized to utilize adenosine 5'-phosphosulfate reductase (Apr) and/or dissimilatory sulfite reductase (Dsr) as core enzymatic machinery across all three pathways [3,4], similar to MSR. In this study, the strain was cultivated under three distinct sulfite-utilizing regimes: reduction (with organic acid oxidation), oxidation (with nitrate reduction to ammonia), and disproportionation (with sulfite as the sole substrate). To achieve high-resolution isotopic data, we implemented a novel method to separate sulfite from sulfate with negligible isotopic fractionation and added $FeCO_3$ to the media to capture biogenic sulfide, preventing chemical reactions with sulfite.

Our measured isotopic data indicates that existing cellular-level models, parameterized using MSR-derived data, fail to fully predict the multiple sulfur isotopic fractionation factors of sulfite metabolisms. To address this discrepancy, we are constraining the fractionation factors associated with each enzymatic step to reproduce the observed BSD fractionation patterns. Furthermore, we are quantifying *apr* and *dsr* gene expression levels to test the hypothesis that these processes share the same core enzymes with MSR. Our findings provide a framework for updating existing cellular-level sulfur isotope fractionation models.

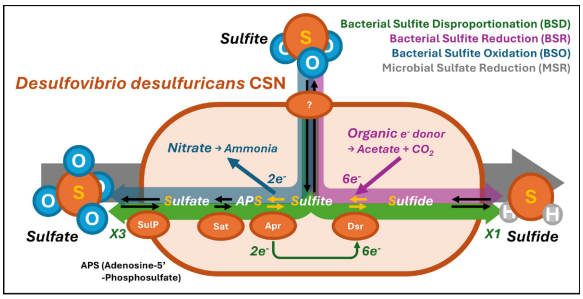

Figure 1. Hypothesized common enzymatic machinery facilitating sulfate reduction and sulfite metabolisms in *Desulfovibrio desulfuricans* CSN.

[1] Johnston *et al.* (2005), *American Journal of Science* **305**, 645-660.

[2] Zerkle *et al.* (2009), *Geochimica et Cosmochimica Acta* **73**, 291-306.

[3] Krämer & Cypionka (1989), *Archives of Microbiology* **151**, 232-237.

[4] Dannenberg *et al.* (1992), *Archives of Microbiology* **158**, 93-99.