

## Bacterial H<sub>2</sub>S Generation in Oil Sands Process Wastes: Where Does it Begin?

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Reclamation of tailings, wastewater, and afflicted land represents one of the largest challenges facing the Alberta Oil Sands industry. Syncrude Canada Ltd., one of the largest producers in the Alberta Oil Sands, is assessing a novel reclamation strategy involving the establishment of a freshwater fen overlying a sand-cap topped, gypsum (CaSO<sub>4</sub>·2H<sub>2</sub>O)-amended, composite tailings (CT) waste material. However, a recent phenomenon resulting in the unpredicted generation of H<sub>2</sub>S gas in CT dewatering wells has indicated the importance of constraining microbial S dynamics in the tailings system. On-going work is establishing the occurrence of microbially-linked S cycling in CT undergoing reclamation. However, this research will investigate Fe and S biogeochemistry and the hypothesized connections with porewater H<sub>2</sub>S generation from fluid fine tailings (FFT), the gypsum-free precursor to CT, via a series of microcosm experiments. FFT is microbially rich and contains iron-rich clays; both constituents could be involved in S biogeochemical cycling within CT. Thus, in order to establish the relative roles of microbial and geochemical factors in FFT that may contribute to observed CT H<sub>2</sub>S generation in situ, experimental microcosms were established to assess: i) microbial links to tailings porewater H<sub>2</sub>S generation, and ii) the implications of gypsum amendment on porewater H<sub>2</sub>S generation in variably microbially amended FFT treatments. Results of these experiments establishing the linkages between FFT and microbial S cycling prior to the amendment of gypsum and reclamation activities will be discussed.

## Unraveling the Genetic Basis of an Ancient Geochemical Biomarker

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### Abstract

Based on phylogenetic and geochemical evidence, the cycling of inorganic sulfur compounds was likely among the first metabolisms used by life. Sulfur isotope fractionation is a geochemical biomarker which has been observed in marine sediments dating back more than 2.5 billion years, but the cellular-level processes responsible for the fractionation effect are not well understood. Utilizing bioinformatic approaches in combination with measurements of sulfur isotope fractionation in pure cultures we seek to make quantitative links between SRB and their geochemical biomarkers.

The intrinsic sulfur isotope fractionation effect,  $\epsilon$ , is essentially a measure of the preferential use of <sup>32</sup>S-substituted sulfate over <sup>34</sup>S-substituted sulfate expressed in parts per thousand, usually measured on pure cultures grown under optimal conditions. Much effort has gone into expanding the range of measured values of  $\epsilon$  by subjecting SRB to a wide range of physiological stress, but this approach has failed to elucidate the underlying genetic basis of sulfur isotope fractionation. The mechanism is thought to involve kinetic limitation at the reaction sites of critical enzymes or the uptake of sulfur species, but there also appears to be an overarching energetic association such that SRB that completely oxidize their electron donors to CO<sub>2</sub> tend to have larger  $\epsilon$ . However, this relationship is confounded by a phylogenetic split between two major Orders of SRB: within the Desulfobacterales, complete oxidation is the norm; within the Desulfovibrionales, complete oxidation is the exception. The interaction between the evolutionary history of SRB, their mode of metabolism, and their intrinsic fraction effect points towards the need for a genomic approach to unravel the basis of sulfur isotope fractionation.

Analyses of a set of genomes from 24 sulfur-metabolizing and 37 non-sulfur-metabolizing Bacteria and Archaea have identified gene families which may play a role in setting the magnitude of sulfur isotope fractionation effects. Though the members are phylogenetically divergent, genomes of SRB with moderate  $\epsilon$  (10–20 per mille) and small  $\epsilon$  (<10 per mille) each contain conserved gene clusters not found in the other genomes, including regulatory proteins and conserved hypothetical proteins. Unfortunately, a dearth of genome sequences from high- $\epsilon$  species (>20 per mille) limits our ability to further investigate the genetic basis of these large fractionation effects. The Desulfobacteraceae is a major lineage of SRB that is under-represented in genome sequences but which includes 90% of known SRB with  $\epsilon$  >20. Presently we are expanding the representation of complete genomes by sequencing *Desulfobacula phenolica*, *Desulfospira joergensenii*, and *Desulfotignum balticum*, which have large  $\epsilon$  values of 37, 26, and 21, respectively. Our ongoing biogeochemical measurements continue to expand the range of known fractionation effects, and sequencing of these SRB will increase the phylogenetic coverage of fractionation effects observed in vitro.