Genomic and synchrotron based investigation of metal immobilization during fermentation-supported sulfate reduction

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J.O. Sharp (PI), R. Almstrand, D. Drennan - *Colorado School of Mines;*

S.M. Webb and J.R. Bargar – *Stanford Synchrotron Radiation Lightsource*

Subsurface and bioreactor systems containing an abundance of solid organic substrates offer a rich environment in which to study the influence of microbial ecology on sulfide-driven metal immobilization. These syntrophic systems rely on cellulolytic and saccharolytic fermenters to metabolize lignocellulosic materials into small organic acids and alcohols. Resulting soluble products are in turn utilized as electron donors by sulfate-reducing bacteria (SRB) who produce sulfide that can react with soluble contaminants such as zinc, copper and nickel to form insoluble metal-sulfides. In this research, we employed seven ex situ pilot-scale and six lab-scale sulfate-reducing bioreactors amended with different solid substrate permutations that received circumneutral, Zn-laden mining-impacted water. Next generation sequencing was used in conjunction with multivariate statistics to identify temporal and spatial phylogenetic trends with the ability to correlate to metabolic drivers of community composition. Despite conservation in the overall organoheterotrophic community across substrate permutations (varying percentages of woodchips and alfalfa) results indicated more recalcitrant lignocellulosic substrates selected for higher ratios of bacteroidetes to firmicutes as well as less pronounced sulfate reduction and Zn immobilization. *Ruminococcus sp.* and *Dysgonomonas sp.*, frequently linked to cellulose and cellobiose fermentation, were present in all columns regardless of substrate, although *Ruminococcus sp*. was preferentially correlated with woodchip content. In contrast, *Treponema sp.* was predominantly encountered in alfalfa-containing columns. Synchrotron-based analysis revealed geochemical patterns where Zn and Ni correlated spatially with S, indicative of sulfide-driven metal immobilization. S-speciation analysis was also used to differentiate between metal-bound sulfide, sulfate associated with metals, and organically bound sulfate. In addition, metallo-labeling of specific microbial genera using biotinylated 16S rRNA targeted DNA-probes, followed by incubation with a streptavidin-nanogold conjugate and subsequent gold-enhancement (Gold-FISH) was improved by targeting multiple ribosomal sites, thus significantly increasing the signal to noise ratio without severely affecting the redox chemistry of the microbe-mineral interface.