

Biogeochemical and Molecular Mechanisms Controlling Mercury Transformation at a Contaminated Site in Oak Ridge, Tennessee, USA

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Mercury is a key metal contaminant on the Oak Ridge Reservation (ORR) due to its historic release to soil, sediment and stream environments. Biogeochemical factors controlling inorganic mercury transformation to the more toxic organomercuric species are not well understood at ORR and other contaminated sites globally. The ORNL Science Focus Area program centers around understanding coupled biogeochemical processes that determine net production of methylmercury (CH_3Hg^+). The main objectives are to (1) elucidate the rates, mechanisms, and controls of abiotic and microbial processes affecting Hg speciation and transformation, (2) resolve the critical Hg precursors that are produced and subsequently methylated, and (3) develop and validate subcellular models to understand the biochemical and biophysical mechanisms of transformation between Hg species and methylmercury.

We take a systems approach, examining processes occurring from the field scale down to the molecular scale. Field studies are focused on establishing the range of geochemical conditions in which critical transformations occur (see poster by Brooks et al.). Our work shows the importance of kinetics in systems that receive a constant source of inorganic mercury input. Both kinetics and speciation data will be critical in examining the cycling of mercury and the production of methylmercury in contaminated systems. These efforts are performed in parallel with, and contribute directly to, complementary studies of abiotic and microbial mercury transformation.

Studies of the mechanisms and geochemical controls on mercury speciation and transformation reveal that natural dissolved organic matter (DOM) plays an important role in the complexation, reactivity and redox transformation of mercury (see poster by Gu et al.). Even at low DOM concentrations (< 3 mg/L), DOM appears to dominate mercury speciation and reactivity in the UEFPC by forming strong Hg(II)-DOM complexes through the reactive thiol functional groups in DOM (Dong et al., 2010, *Environ. Chem.*, in press). The complexation is kinetically hindered. Reduced DOM is also found to be capable of reducing the mercuric ion Hg(II) to Hg(0) and forming strong Hg(0)-DOM complexes. Work is currently in progress to evaluate the potential impact of the formation of such complexes on biological production of toxic methylmercury (see poster by Schaefer et al.) and abiotic demethylation in the environment. Recent results show that Suwannee River NOM did not affect methylmercury production by the known methylator *Desulfovibrio desulfuricans* ND132 (see poster by Brooks et al.).

In our investigations of Hg methylating microbial communities, field samples have been collected from a known methylating area of the contaminated stream at ORR. Microbial community characterization using both a functional gene array (FGA) and 454 amplification and sequencing of 16S genes revealed pronounced phylogenetic and functional differences that appear to be related to seasonal trends (See poster by Elias et al.). With its draft genome sequence, the known methylating sulfate-reducing bacteria, *D. desulfuricans* ND132, is being used to elucidate the genes responsible for mercury methylation (See poster by Kucken et al.). The targeted approach has not yet identified a methylase, and we are in the process of creating a random Tn5 transposon mutant library with an attainable goal of >5000 mutants. We are also designing a second generation FGA that will allow detection of MerA and MerB genes, as demethylation is a key reaction in global mercury cycling.

Molecular, or subcellular, studies include OmcA, a dissimilatory metal reducing enzyme from *Shewanella oneidensis* MR-1, as well as various proteins and enzymes encoded in the *mer* operon, which confers bacterial mercury resistance. We have collected initial X-ray diffraction data to 2.6 Å resolution for OmcA. Small-angle X-ray scattering (SAXS) and molecular dynamics (MD) simulations have been used to characterize the structure and interdomain motions of the metalloregulator, MerR, and the mercuric reductase, MerA (See poster by Liang et al.). Quantum mechanical calculations have been used to show how the organomercurial lyase MerB catalyzes the demethylation of methylmercury (Parks et al., *J. Am. Chem. Soc.* 2009, 131, 13278).

Future work will involve functional genomics techniques to determine key microbial groups that influence methylmercury production under varying geochemical conditions. Investigation of structure and dynamics at the molecular level will reveal regulation mechanisms and the role of various subcellular components. Molecular simulation will be applied to determine key enzymatic mechanisms and extended to elucidate microbial methylation mechanisms identified by advanced genomic techniques. These studies will help in understanding the oxidation-reduction and methylation-demethylation transformations that determine the fate of Hg in sediment-water environments.