Biogeochemical and Molecular Mechanisms Controlling Mercury (Hg) Transformation in the Environment

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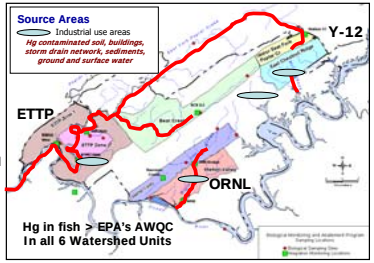
Oak Ridge National Laboratory – ERSP Science Focus Area

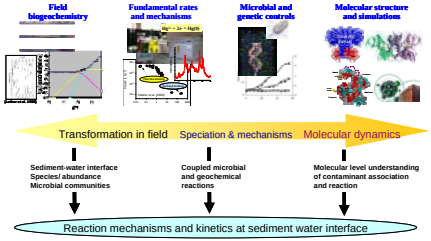
Background

- Mercury use at the Oak Ridge Y-12 National Security Complex in the early 1950s resulted in the loss of ~330,000 kg to the environment, 2/3 of which was released to soils and East Fork Poplar Creek (EFPC), originating inside the Y-12 facility
- Hg concentration in interstitial streambed water is >30 µg/L, and >400 mg/kg in sediments
- Subsurface mercury is a source of dissolved mercury in groundwater that contributes to surface water contamination
- **Knowledge gap:** Transformation mechanisms of inorganic mercury to methylmercury and net methylation rates are unknown in EFPC ecosystems, where methylmercury bioaccumulates and levels in fish exceed regulatory limits and pose health risks.
- **Research Needs:** As no clear relationship between methylmercury and total Hg concentrations in water is observed in EFPC, either methylmercury production is suppressed or demethylation is significantly higher than methylation in highly contaminated systems. Because inorganic mercury inputs cannot be eliminated, there is an urgent need to understand how inorganic Hg is transformed into methylmercury, and which processes control net methylmercury production.

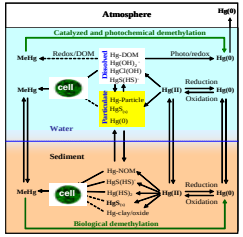
Mercury Concerns at Oak Ridge Reservation



ORNL SFA Program



Key questions to be examined
(1) How do abiotic reactions affect Hg speciation and complexation?
(2) Which products or forms of Hg are bioavailable for microbial methylation?
(3) What levels of Hg are required to initiate microbial demethylation and how can this process be tuned to favor demethylation?
(4) Which dominant microbes participate in methylation or demethylation and how do geochemical conditions affect these transformations?
(5) How do catalyzed chemical and photochemical reactions influence Hg speciation and transformations in water?

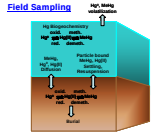


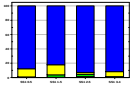
Goal: Close gaps in our knowledge regarding biogeochemical transformations of mercury, which will lead to solutions for mercury remediation challenges for DOE and the nation.

Approach: Multi-scale, multidisciplinary hypothesis-driven activities integrating geochemical, microbiological, molecular, and simulation sciences to achieve detailed objectives

Primary objectives: (i) Elucidate the rates, mechanisms and controls of abiotic and microbial processes affecting Hg speciation and transformation, and resolve how and which critical Hg precursors are produced and subsequently methylated at the sediment-water interface. (ii) Understand fundamental subcellular mechanisms by obtaining information on structure and dynamics of biological macromolecules involved in acquisition, transport and transformation of major Hg species and methylmercury.

Task I. Site Biogeochemistry

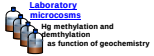




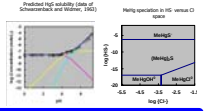
Functional gene arrays (with Task 3)

- Field Studies
  - Quantify geochemical gradients
  - Hg speciation and flux
  - Benthic flux chambers
- Microcosm Studies
  - Stable Hg isotopes to facilitate analysis
  - Transformation pathways
- Microbial Community Structure (with Task 3)
  - Functional gene arrays
  - Principal microbial communities
- Geochemical Modeling
- See poster by Brooks et al.

Laboratory microcosms



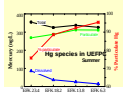
Geochemical modeling

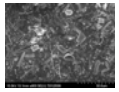


Deliverable: critical understanding of Hg flux, biogeochemical controls, and microbial determinants

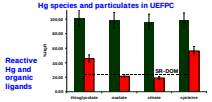
Task II. Fundamental Mechanisms and Transformations

- Hg and dissolved organic matter (DOM) complexation
  - Effect on Hg reactivity and kinetics
  - Hg DOM binding strengths and thermodynamics (with Task 4)
- Redox reactions and abiotic demethylation
  - Hg redox reactions and species transformation
  - Enriched stable isotope studies
  - Rates and controlling mechanisms (with Task 1)
- Geochemical controls on biological methylation (with Tasks 1 and 3)
  - Hg species and precursors for methylation
  - DOM and geochemical factors on methylation rates
- Photochemical and surface-catalyzed reactions
  - Effect of organic and inorganic ligands
  - Particulates and characterization
  - Free radicals
- See Poster by Gu et al.





Hg species and particulates in UEFPC

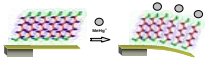


Deliverable: Critical understanding of dominant Hg species, bioavailability, and biogeochemical controls on rates and mechanisms of Hg methylation and demethylation

Compelling Research Needs - Program Directed Research

High-Throughput Screening Sensors for Microbial MeHg Production

- Functionalize piezoresistive microcantilevers with 1,6 hexanedithiol self-assembled monolayers.
- Interaction between the attached monolayer and methylmercury, confined to a single side of the microcantilever, causes a deflection of the cantilever and a change in the resistance of the piezoresistive material.
- Differential measurements were made by injecting methylmercury into a flow cell containing the functionalized microcantilever and a reference. A detection limit of 5 x 10^{-8} M was demonstrated in water.
- See Poster by Bange et al.



- **Need:** rapid, high-throughput detection of methylmercury to enhance other research tasks in the SFA

Task III. Microbial and Genetic Controls

- Elucidate the genetic determinants of methylmercury production and regulation (with Task 4)
  - Comparative gene expression, mutagenesis, and complementation
- Determine the effect of geochemical factors on gene regulatory networks for mercury methylation
  - Use whole genome microarrays to examine both biotic and abiotic effects on the methylating and non-methylating Desulfovibrio transcriptomic profiles
- Examine relationships among community structure, geochemical conditions, and methylmercury production in sediments collected from Hg contaminated sites (with Task 1)
  - Six sites sampled over 1 year period
  - Functional gene array
- Currently examining community analysis related to mercury contamination
  In the future will examine community structure compared to net methylation and demethylation
- See Poster by Palumbo et al.

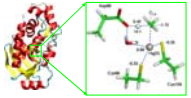
Desulfovibrio africanus (SEM by Dwayne Elias, U of Missouri)

Deliverable: Critical knowledge on genetic determinants of and geochemical factors influencing regulatory networks for microbial mercury methylation and demethylation

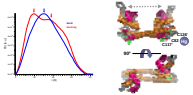
Task IV. Molecular Scale Studies and Computer Simulations

- Elucidate fundamental enzymatic and regulatory mechanisms to understand the processes of demethylation and reduction using quantum mechanical and classical simulations
  - Determine molecular mechanisms of MerB
  - Role of NmerA in the acquisition of Hg(II) by MerA
  - Transcriptional regulation of the *mer* operon by MerR
- Determine molecular structure and interactions of multiheme cytochromes in dissimilatory metal reducing bacteria
- Characterization of enzymes involved in the methylation of mercury (with Task 3)
- Computational investigation of ligand exchange reactions and the cleavage of Hg-C bonds in functional models (with Task 2)
- See Poster by Liang et al.

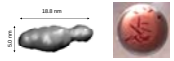
- Closed/open conformations of MerR



- MerB demethylation mechanism



- Mechanism of transcriptional activation by the microbial Hg(II) sensor MerR1



- Molecular structure of outer-membrane cytochromes from dissimilatory metal reducers

Mercury Resistance – Molecular Structure and Computational Simulations

Deliverable: Critical understanding of biomolecular mechanisms in Hg transformation (demethylation, reduction and methylation)

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