Biomolecular Studies of Microbiological Processes Controlling Contaminant Fate and Transport from Hanford 300 Area Sediments

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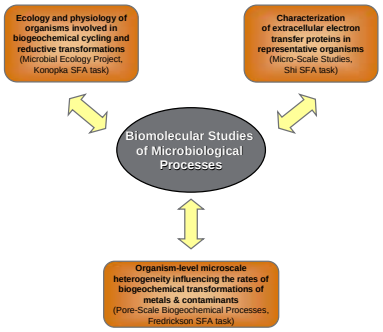
Project Goals

- Given the paucity of information regarding the microbiological processes controlling contaminant fate and transport at the Hanford site, we seek to isolate and identify relevant microorganisms involved in the biogeochemical cycling of metals and radionuclides.
- As part of the overall PNNL SFA objective, we will characterize the obtained strains of relevant microorganisms and elucidate the biological mechanisms catalyzing the pathways controlling the micro-scale distributions of metals and radionuclides in Hanford subsurface sediments.

Approach

- Use a combination of cultivation, genetic, biochemical, and microscopic techniques in conjunction with high-throughput proteomic and genomic technologies

Integration Between SFA Elements



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Isolation of the Culturable Hanford-Relevant Organisms from the July 2008 Hanford IFRC Deep Microbiology Characterization Borehole Sediments

- In collaboration with the Microbial Ecology project (Konopka), gradient enrichment cultures using a Hanford 300 Area synthetic ground water (SPP1 SGW) media, a cocktail of electron donors, and four terminal electron acceptors (iron, manganese, sulfate, or nitrate) were inoculated with soil from several depths of the Deep Microbiologic Characterization Borehole Sediments.
- We have selected three relevant depths where enrichments for both solid Fe(III) as HFO (hydrous ferric oxide) or Mn(IV) as MnO_2 were positive:
  - 1) Depth 1 reflects the organisms from the Hanford formation at ~31.5 feet below ground surface (bgs).
  - 2) Depth 2 reflects the organisms from the transition zone of the Ringold formation at ~57 feet bgs.
  - 3) Depth 3 reflects the organisms from the Ringold formation at ~126-127 feet bgs.

Gradient Enrichment Technique

SPP1 SGW	mMol/L
Potassium Bicarbonate	0.070
Magnesium Sulfate	0.210
Calcium Nitrate	0.180
Calcium Chloride	0.420
Sodium Sulfate	0.140
Sodium Bicarbonate	1.100

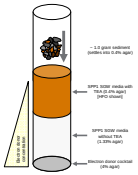
Buffer	mMol/L
HEPES (pH 7.8)	3

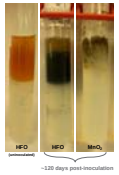
ML media components	mMol/L
Ammonium Chloride	1
Potassium Chloride	1.34
Sodium Phosphate	0.435

trace components
minerals stock
vitamins stock
Sodium Selenate
Fe(III)-NTA

electron donors (as cocktail)	mMol/L
Pyruvate	1
Caprylic Acid	1
DL-Malate	1
Sodium Formate	1
Sodium Acetate	1
Sodium Propionate	1
Succinate	1
DL-Lactate	1
n-Propanol	1
n-Butanol	1
Glycerol	1
Casamino Acids	1
Methanol	1
Ethanol	1

electron acceptors (individually)	mMol/L
HFO (1-2% Si-stabilized)	5
Manganese oxide	5
Sodium Sulfate	5
Sodium Nitrate	5

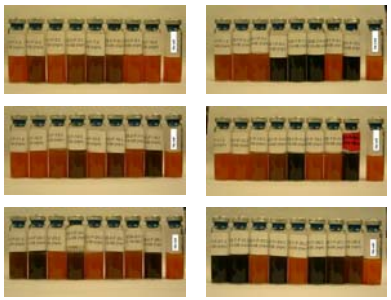




- For each of the three depths, an aseptic aliquot of the enrichment culture was collected and suspended in SPP1 SGW. Anaerobic liquid enrichment tubes containing each of the 14 individual electron donors with either Fe (HFO) or Mn (MnO_2) as the electron acceptor were inoculated with a 1:300 dilution. Tubes contained:
  - 5 mM of individual electron donor and 5 mM electron acceptor (HFO or MnO_2).
  - Casamino acids (CAS), added to a duplicate set of tubes as 0.5 mM supplement).
- The objectives of this task were to: a) eliminate electron donors that do not support metal reduction, b) ascertain if the addition of CAS is an added benefit to the enrichment media, and c) isolate pure cultures of Hanford-relevant organisms.

Donor Specific Liquid Enrichments

31.5 ft bgs | 57 ft bgs



- At different enrichment depths, differences existed in the electron donor profiles found to stimulate HFO reduction.
  - Many electron donors stimulated HFO reduction.
  - CAS supplement was an added benefit at ~50 µm concentration.
  - Malate and caprylic acid were not found to be good electron donors for HFO or MnO_2 reduction at any depth.
- After subsequent 1:200 transfers into fresh donor-specific liquid enrichment media, agar plates containing the SPP1 SGW media and soluble Fe(III) as the terminal electron acceptor were inoculated and monitored for anaerobic growth.
  - In some cases, colonies became visible in as few as 14 days, but growth was monitored until ~day 60.
  - Isolated colonies (137) were selected and transferred to fresh media (5 mM of individual electron donor and 5 mM HFO).
  - On some plates, black colonies, possibly indicative of FeS formation, were also observed.

Influence of SPP1 SGW?

- Does the presence of sulfate in the Hanford synthetic groundwater select for sulfate reducing bacteria (SRB) in SPP1 SGW enrichment tubes?
- Is indirect metal reduction via SRB occurring in enrichment tubes?
- Can metal reducing bacteria (MRB) also be isolated from the same enrichment tubes?
- Are SRB dominant over MRB in this groundwater system and 300 Area sediments?

- To ascertain the influence of the SPP1 SGW media, 13 putative isolates were inoculated into media with or without SGW.
  - After 43 days of growth, only one isolate was able to reduce HFO in media lacking the SPP1 SGW.
  - Several isolates reduce HFO in media containing SPP1 SGW.
  - Isolates currently being prepared for 16S sequence analysis.

Tubes lacking SPP1 SGW | Tubes containing SPP1 SGW

Preliminary Findings

- Several positive gradient enrichment cultures were obtained from the deep microbiology characterization borehole sediments using the electron donor cocktail.
- Enrichment cultures of iron, manganese, sulfate, and nitrate reducing organisms were obtained form the Hanford formation, Ringold formation at the oxic/anoxic interface, and the deep Ringold formation.
- Several individual electron donors stimulated the bioreduction of iron, manganese, sulfate, and nitrate. At each sample depth, differences in electron donor-specific bioreduction were found.
- Our findings suggest that SRBs may play an important role in the metal transformation reactions and overall microbiological process at the Hanford 300 Area.

Future Directions

- Isolate pure cultures of functionally relevant organisms and characterize for phylogenetic and physiologic properties.
- Characterize transformation reactions and pathways in laboratory and evaluate the extent to which these function *in situ* (via microenvironments and transition zones).
  - Done in collaboration with the Konopka and Fredrickson SFA projects and the Hanford IFRC.
- Integrate Hanford-relevant organisms and their pertinent biological processes into PNNL SFA pore- and molecular-scale mechanistic research projects.

For more information about the science you see here, please contact:

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