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Microbial pathways for the mobilization of Mercury as Hg(0) in anoxic subsurface environments

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Three Chemical Forms of Mercury

methyl mercury	ionic mercury	elemental mercury
$CH_3Hg(I)$	$Hg(II)$	$Hg(0)$
↓	↓	↓
found in fish	water and rocks (cinnabar)	in thermometers
lipid soluble biomagnifies in food chain	water soluble, but sorbs to sediments and can precipitate	volatile, escapes to atmosphere
potent neurotoxin	renal toxin	least toxic

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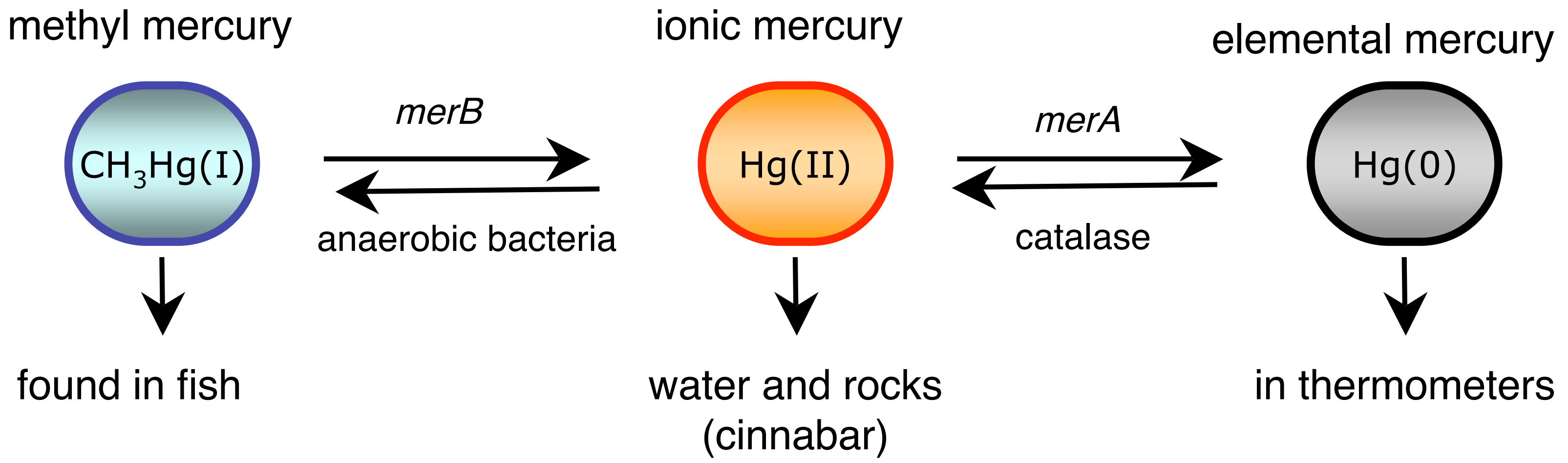
Microbially Mediated Mercury Transformations



methyl mercury

$CH_3Hg(I)$

found in fish

merB

anaerobic bacteria

ionic mercury

Hg(II)

water and rocks (cinnabar)

merA

catalase

elemental mercury

Hg(0)

in thermometers

lipid soluble biomagnifies in food chain	water soluble, but sorbs to sediments and can precipitate	volatile, escapes to atmosphere
potent neurotoxin	renal toxin	least toxic

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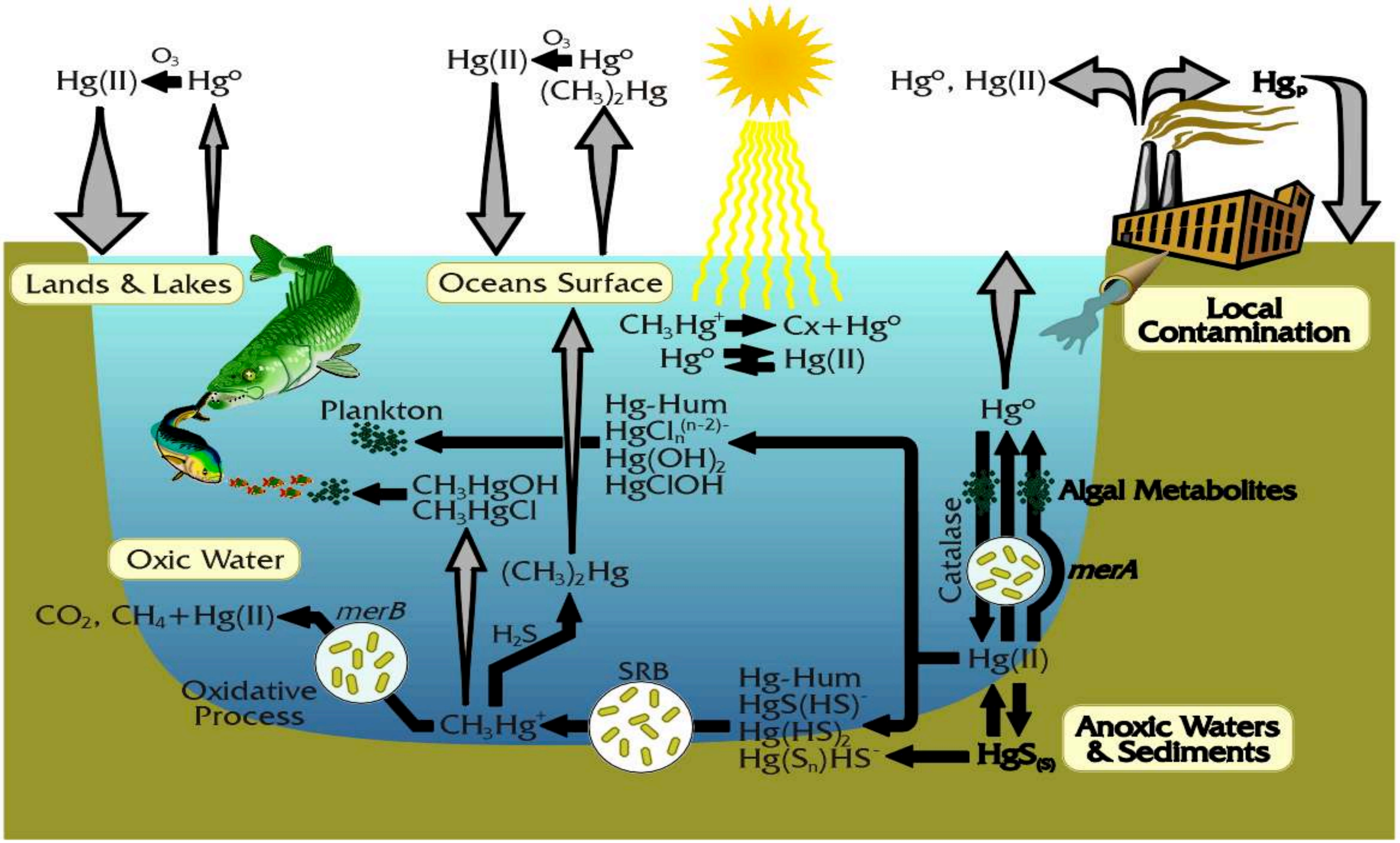
The Mercury Cycle in Surface Waters



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The Mercury Cycle in the Subsurface:

Precipitation of Hg(II):

- in the presence of sulfide, as HgS, cinnabar

Sorption:

- Hg(II) binds to organic matter, clays, metal oxides and oxyhydroxides

Mobilization:

- Southern Tuscany. Mobilization of Hg by seawater intrusion. Chloride releases geological Hg from Mt. Amiata Hg deposit

Methylation:

- Zone where streamwater mixes with groundwater important source of methylmercury in Lake Superior (Stoor *et al.* 2006)

Reduction:

- Kathmandu Valley - Presence of Hg(0) in deep fossilized groundwater with evidence of microbial activity

Elevated Hg levels have appeared unexpectedly in groundwater

- **Taylor Road Landfill Superfund Site, Tampa Fl**,
  - January 2007 - Hg exceeds the MCL in a nearby observation well where groundwater flow was too slow to account for pattern.
- **Long Neck Water Company, Long Neck Peninsula, Delaware**
  - two production wells with Hg contamination, no known point source
- **Observation wells in Kentucky**
  - six wells with Hg higher than the MCL, with no known point source and no known geological Hg deposits

The Case in The Kirkwood Cohansey Aquifer, Southern New Jersey:

- More than 600 private domestic wells in nine counties have Hg concentration exceeding the USEPA MCL
- Current estimate - 1% of wells have Hg in excess of USEPA MCL. (400,000 private wells = 4,000 wells)
- Distribution of contaminated wells rules out point-source contamination
- ~ 10% Hg is present as Hg(0)

What do contaminated wells have in common?

- Contaminated well water shows impact from septic leachfields
- Elevated soluble iron correlates positively with elevated Hg

This work was performed by Julia Barringer, Zoltan Szabo, and others at the USGS in Trenton, NJ

Are there parallels between the Kirkwood Cohansey Aquifer and Current ERSP projects?

- In the Kirkwood Cohansey Aquifer, septic tanks provide a steady supply of electron donor to the aquifer, there is evidence for iron reduction, and Hg reduced and mobilized

- In ERSP projects, electron donors are added to the subsurface to stimulate microbial activity and immobilize metals and radionuclides

Will biostimulation at DOE sites result in the mobilization of Hg?

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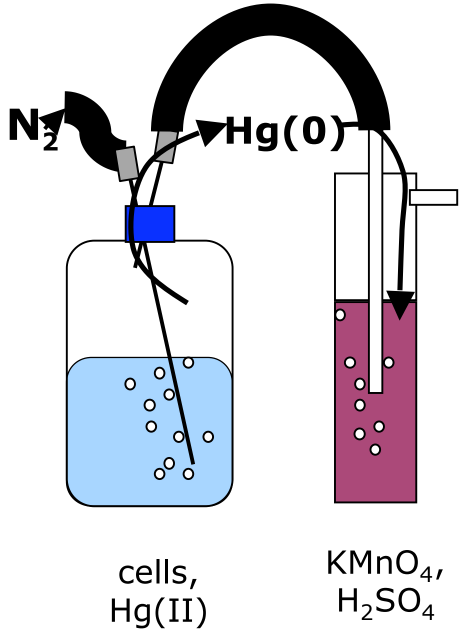
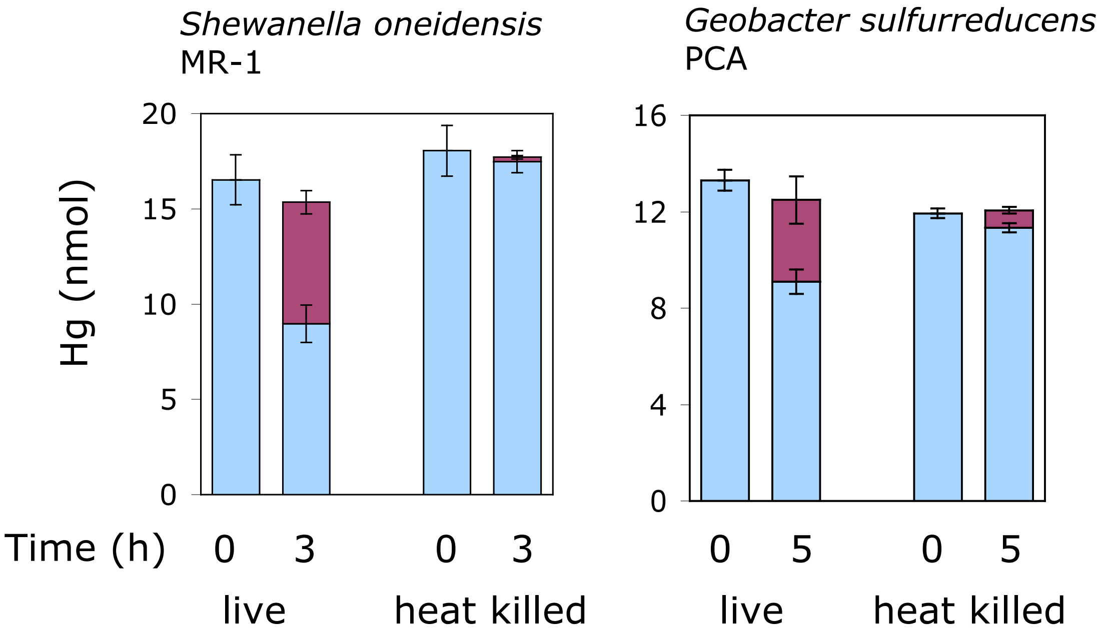
Reduction of Hg(II) to Hg(0) by model Dissimilatory Metal Reducing Bacteria





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Do these DMRB have *merA?*

- *Shewanella oneidensis* MR-1 does not have a *merA* gene in its genome

- *Geobacter sulfurreducens* PCA has two genes annotated as *merA*, *merA-1* and *merA-2*
  - we have several reasons to believe that these genes do not encode an active MerA mercuric reductase
  - currently, we are knocking out these genes to confirm that reduction of Hg(II) to Hg(0) proceeds by a different mechanism

Differences between Hg(II) reduction by MR-1 and the *mer* system

A *mer* operon was introduced to MR-1 on a plasmid to facilitate comparison.

A *mer* operon increases Hg(II) resistance 50 fold in MR-1. (25 vs 0.5 μM)

strain	Initial specific reduction rates (nmol min^{-1} mg protein^{-1}) in medium containing Hg(II) at:	
	25 μmol L^{-1}	0.3 μmol L^{-1}
MR-1 with mer operon	16.3 ± 1.3	1.60 ± 0.32
MR-1	2.0 ± 0.6	2.56 ±0.17
MR-1, autoclaved	0.7 ± 0.4	0.28 ± 0.04
uninoculated media	0.4 ± 0.5	0.10 ± 0.04

Reduction of Hg(II) by MR-1 is not an inducible process:

- **Exposed to Hg(II): 3.14 ± 0.25 nmol min^{-1} mg protein^{-1}**
- **Unexposed:: 3.07 ± 0.35 nmol min^{-1} mg protein^{-1}**

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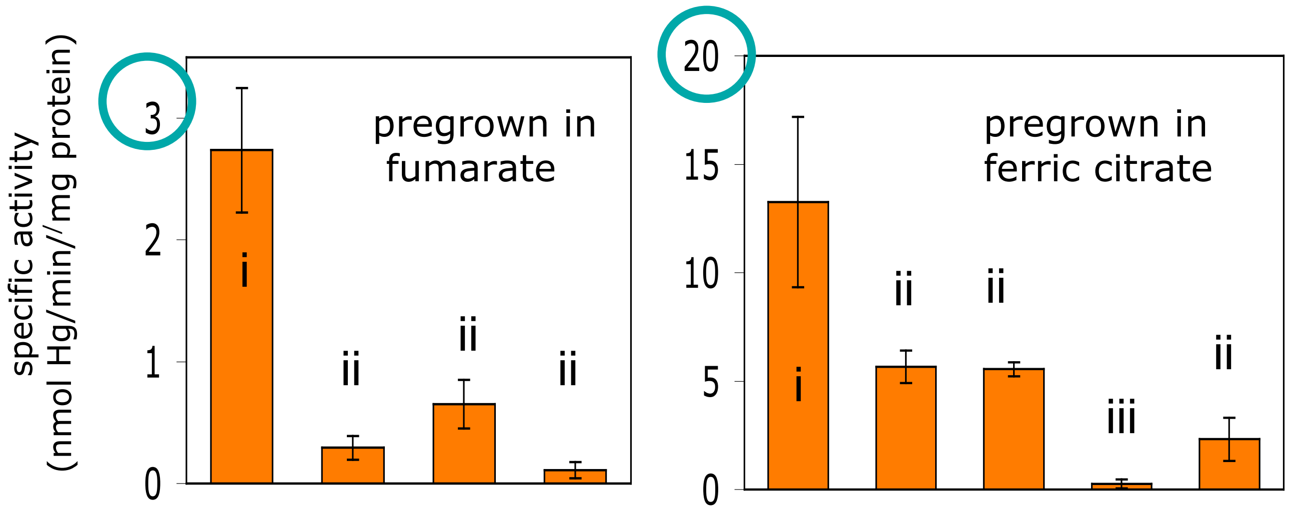
Reduction of Hg(II) by MR-1 is enhanced in iron reducing conditions



TEA	fumarate	FeOOH	none	fumarate	FeOOH	FeOOH	none	FeOOH	FeOOH
preincubation	-	-	-	-	+	-	-	+	-
autoclaved	-	-	-	+	-	-	-	+	+

- biosynthesis of macromolecules
- reduction of Hg(II) by Fe(II)/Fe(III) complexes

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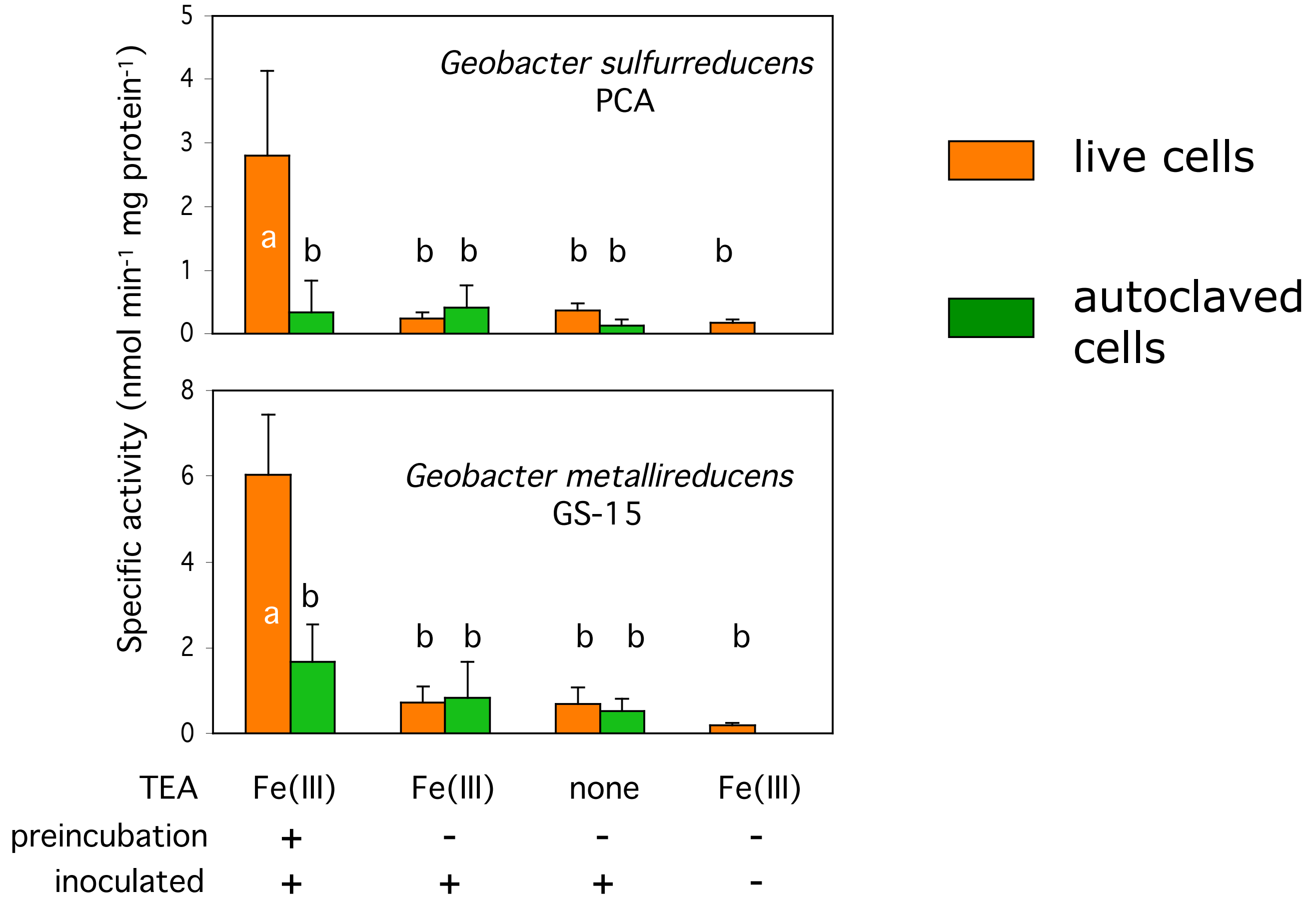
Geobacter spp. also require preincubation in Fe(III) for reduction of Hg(II)



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Conclusions: *S. oneidensis* MR-1 and *G. sulfurreducens* PCA

- MR-1 and PCA reduce Hg(II) to Hg(0) by a mechanism unrelated to the *mer* operon
  - *Thus, profiling merA will give an incomplete picture of Hg(II) reduction potential in the environment*

- Reduction of Hg(II) in iron reducing conditions requires a preincubation step in insoluble iron
  - *This may be due to a coupled biotic/abiotic pathway involving reactive iron species*

Hg(II) reduction in enrichment cultures

Goal: To assess the potential for Hg(II) reduction by nitrate and iron reducing microbial communities

Enrichments constructed with sediments from the background area of the FRC under nitrate and iron reducing conditions

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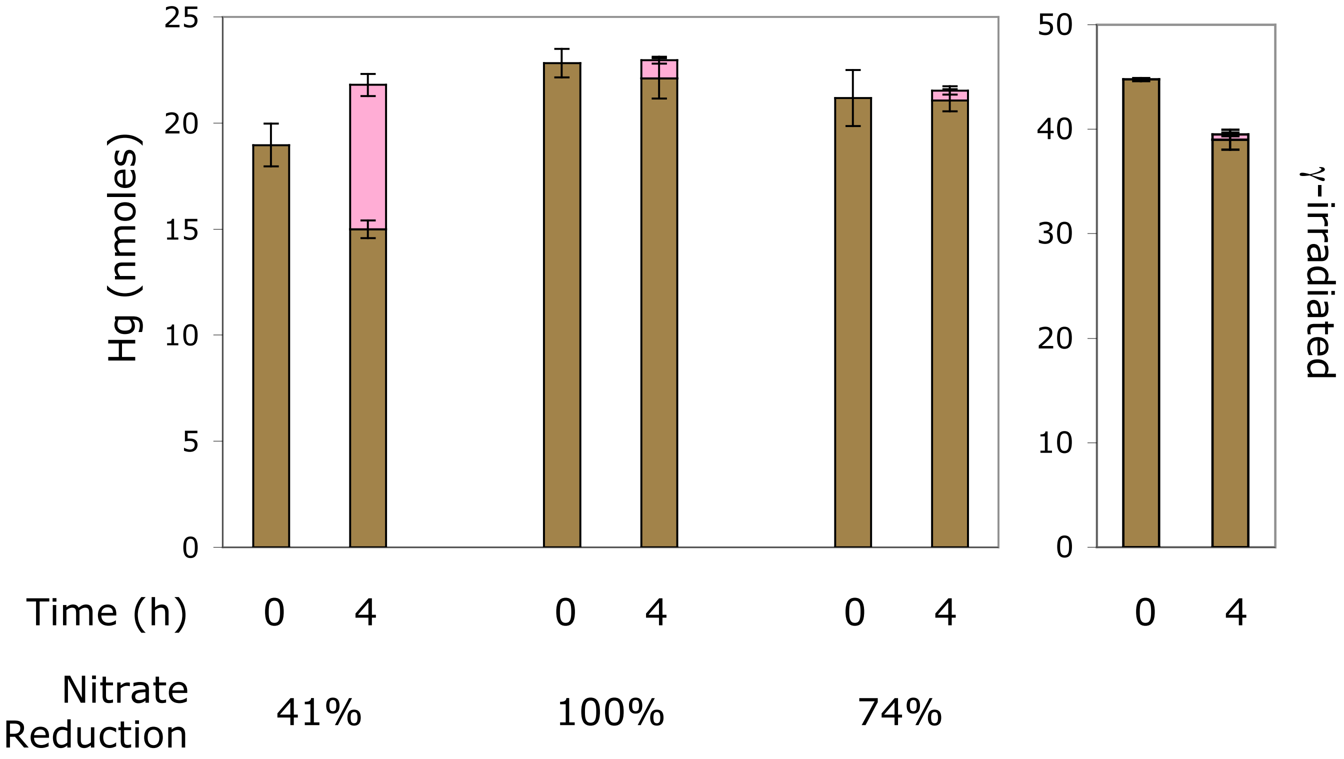
Hg(II) reducing potential in nitrate reducing enrichments



… addition of Hg halted denitrification in all three microcosms

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Microbial community analysis in sediment and nitrate reducing enrichments

Clone library	**RFLP Pattern**	**No. of clones (% of library)**	**Blastn search results (identity)**
Background sediments	I	30 (66.7%)	*Zoogloea* spp. (97%)
	II	11 (24.4%)	*Herbaspirillum* spp. (95%)
	III	4 (8.9%)	Uncultured *Escherichia* spp. (99%)
Denitrifying enrichment	I	125 (84.5%)	*Zoogloea* spp. (97%)
	II	12 (8.1%)	*Herbaspirillum* spp. (95%)
	III	11 (7.4%)	Uncultured *Comamonadaceae* spp. (99%)

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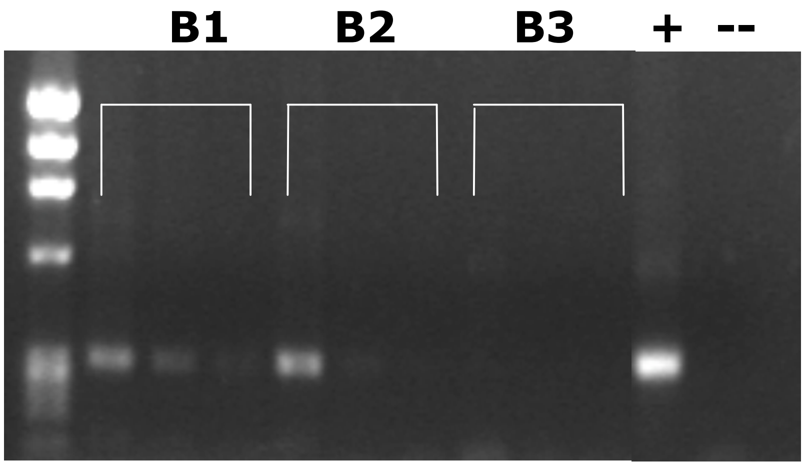
Presence of *merA* genes in nitrate reducing enrichments

- Six new primer sets were designed that cover the entire known diversity of *merA* genes.



- We were able to amplify *merA* from microcosms B1 and B2 using a set of primers specific for gram negative bacteria and *Firmicutes*.
- *merA* was not detected in unamended background soil.
- Thus, enrichment for nitrate reducers also enriches for *merA.*

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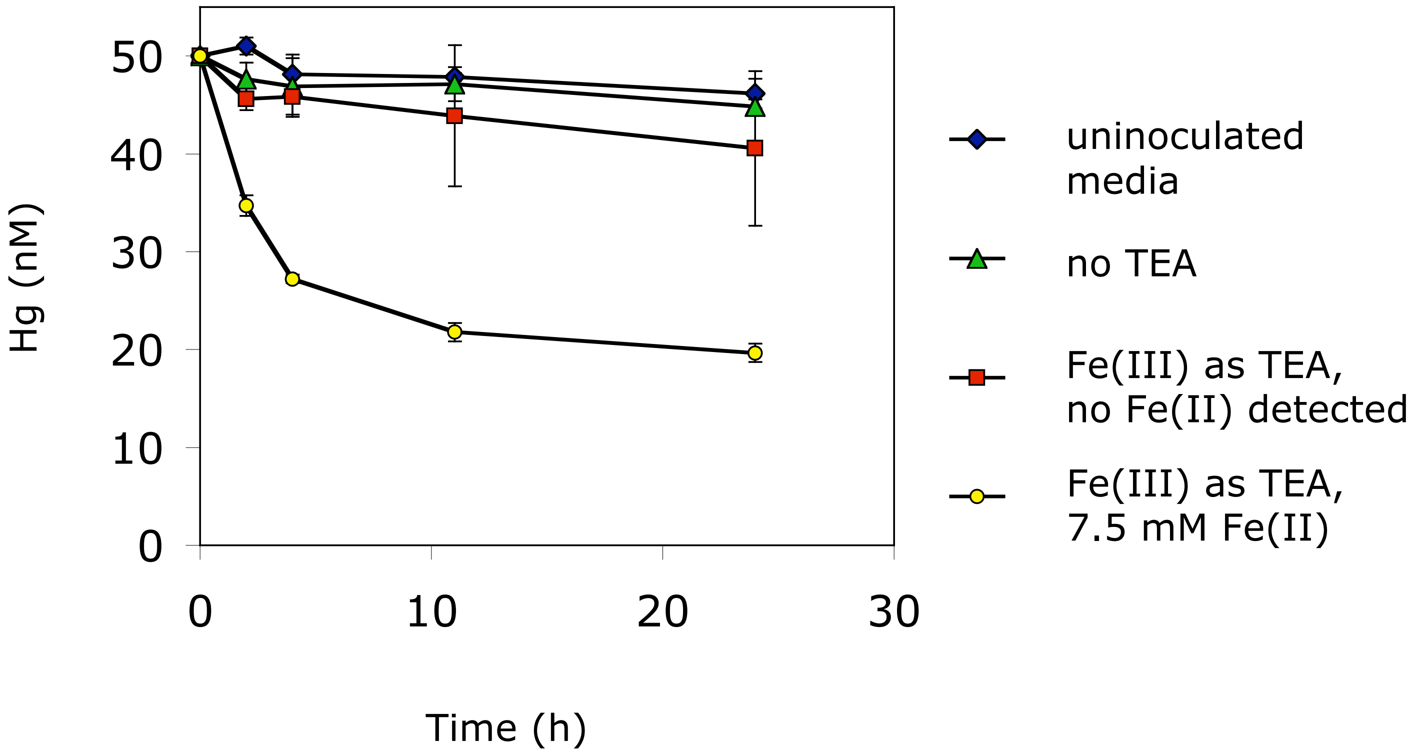
Hg(II) reducing potential in iron reducing enrichments



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Preliminary Conclusions and Questions

- Enriching for denitrifiers enriches for *mer* genes
  - these communities may have the capacity to reduce Hg(II) to Hg(0)
  - This could potentially mobilize mercury into groundwater

- Under iron reducing conditions, there is a potential to reduce Hg(II) to Hg(0)
  - microbial community analysis is pending

Major questions to be answered in the environment

- How toxic is Hg(II) to subsurface microbial communities?
  - what levels of Hg will harm metal and radionuclide reducing communities?
  - how do microbial communities adapt to the presence of Hg in the subsurface?
- Is presence of *merA* genes and transcripts a good predictor of Hg reducing potential?
- Does reduction and mobilization of Hg occur in iron reducing conditions by a coupled biotic / abiotic pathway?

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