Report for Contribution of Iron-Reducing Bacteria to Mercury Methylation in Marine Sediments.

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Project's activity and accomplishments:

Eighth International Mercury as a Global Pollutant, Madison, Wisconsin, 2006, Invited platform presentation. Fleming, Emily and Douglas Nelson. Is mercury methylation significant in molybdate-inhibited, iron-rich sediments?

Fleming, Emily and Douglas Nelson. Incomplete molybdate-inhibition of mercury methylation in marine sediments. Manuscript in preparation for submission to Applied and Environmental Microbiology.

Fleming Emily. Mercury methylation by sulfate-reducing and iron-reducing bacteria in culture and *in situ*. Ph. D. Dissertation. University of California at Davis In preparation, to be submitted in June 2007.

Summary of research project

Mercury is a global pollutant that when sufficiently bio-concentrated causes a dramatic drop in intellectual capacity humans (Clarkson, 2002) and a decrease in reproductive success in birds (Heath and Frederick 2005). The main sources of mercury contamination in the environment are via the weathering of rock exposed from mining activities and the industrial release of mercury through dumping of wastes or through atmospheric release and subsequent deposition. Deposition of mercury in California's aquatic ecosystems is a legacy of mining in the Coastal Ranges and in the Sierra Nevada foothills from the Gold Rush era. Through weathering of waste rocks and exposed mining areas, a suite of other metals (such as iron, manganese, aluminum) is transported, and these metals aid in the mobilization and cycling of mercury compounds. Mercury deposited in aquatic sediments is methylated mainly by anaerobic bacteria, and the resultant mono-methyl-mercury bio-accumulates to concentrations that may cause dramatic neurological effects in organisms higher in the food chain (Clarkson 2002). In the microbiological literature of the past 20 years, the conversion of inorganic mercury to methyl-mercury has been dogmatically been attributed to the activity of sulfate-reducing bacteria. This conclusion was deduced from the observation that sediments able to produce methyl-mercury when incubated anaero-

bicly failed to do so in the presence of molybdate, a specific inhibitor for sulfate reducing bacteria. Molybdate functions by disrupting sulfate-reducer energy metabolism (Compeau and Bartha 1985). The literature-based generalization reagarding the essential role of sulfate reducers in mercury methylation has been expanded to include a number of other marine, estuarine and freshwater sediments. Recently, running counter to this paradigm, we showed that incubation of iron-rich freshwater sediments from Clear Lake California with molybdate completely inhibited sulfate reduction yet failed to fully inhibit mercury methylation (only 32% - 42% of total methylation was inhibited). From similar sediment an iron-reducing bacterium was isolated and shown to methylate mercury at rates equivalent to those seen in sulfate-reducing bacteria (Fleming et al. 2006). In a follow-up to our findings by others, a number of strains of iron-reducing bacteria from culture collections were shown to methylate mercury (Kerin et al. 2003).

In some marine sediments iron-reducing bacteria have been shown to be responsible for up to 75% of the total carbon oxidation (Jensen et al. 2003). High concentrations of iron and mercury are transported into California's coastal sediments through weathering that may stimulate iron-reducing bacteria, and they, in turn, might contribute significantly to the overall mercury methylation activity in mercury-contaminated marine sediments. To determine the role iron-reducing bacteria play in mercury-methylation in California marine systems, we investigated sediments from of two mercury-impacted coastal estuaries, Walker Marsh in Tomales Bay and Guadalupe Slough in the South San Francisco Bay. Measurements of mercury concentration, sediment iron concentration, pore-water iron concentration and microbial activity, including rates of sulfate reduction and mercury methylation (both in the presence and absence of specific inhibitors) indicate that iron-reducing bacteria may be responsible for up to 50% of the total mercury methylation in these sediments. These findings are presented in more detail below.

Results and Discussion

Field surveys. Initially both field sites were surveyed and geochemical parameters were measured in both the solid and the aqueous phases. Pore-waters from both Guadalupe Slough and Walker Marsh ranged between 14-32‰ and 35-40‰ salinity, respectively, indicating significant marine influence. ICP-MS (Inductively Coupled Plasma Mass Spectroscopy) analysis for a variety of elements in pore-water from Walker Marsh showed signature elements typically present in acid rock drainage run-off plus those typical of sea-water (data not shown). Guadalupe Slough and Walker Marsh sediments are several kilometers down-stream from mines that formerly contained large deposits of cinnabar and

produced large quantities of mercury (Rytuba 2000). These marshes have sediment mercury concentrations that are average for those receiving mercury from point source contamination (**Table 1**). As a legacy of mining the tailings from the Gambonini Mine (upstream from Walker Marsh) contain mercury concentrations of 230ppm (Kim et al. 2000), and as water flows through these and similar materials in transit to San Francisco Bay or Tomales Bay mercury is deposted along the path in Guadalupe slough and Walker Marsh. Furthermore iron, which is characteristic of acid weathering of exposed mining rock, was observed in samples from in these two location at concentrations equal to (and in some instances higher than) those observed in other mercury impacted systems, (Long Island Sound and Lauretian trough; **Table 1**).

Table 1. Total mercury from sediments with marine or from Point Source Contamination

Sediment type	**Total sediment Hg^{2+} (μg g sediment dw^{-1})**	**Percent methylation rate of added Hg^{2+} (% d^{-1})**	**Total extracted Fe in sediments (μmol g sediment dw^{-1})**	**Reference**[1]
Laurentian trough , Canada, marine,	0.001-0.014	n d	109-240[4]	(1)
Clear Lake, CA, USA, freshwater	3-183	n d	n d	(2)
San Carlos Creek, CA, USA, freshwater	0.5-1.0	n r	n d	(3)
Long Island Sound, NJ, USA marine	0.5-3	0.1-7[3]	121-191[5]	(4)
Almadén Spain, run-off creek, freshwater	3-2300	0.88-9.6[3]	n r	(5)
Guadalupe slough,CA, USA, brackish	0.087-0.76	n d	183-1709[5]	This study
Walker Marsh, CA, USA, marine	0.134-2.28	1.00-1.6	22-586[5]	This study

References[1], (1) Gobeil and Cossa 1993, (2) Suchanek et al. 1998, (3) Marvin- Di Pasquale et al. 2000, (4) Hammerschmidt et al. 2004, (5) Gray et al. 2004
[2]Atmospheric contamination
[3]Determined with mercury isotopes but not at tracer levels
[4]extracted with hydroxyl amine
[5]extracted with HCl for one hour
nd not determined
nr not reported

Biogeochemistry of sediments. Mercury present in muscle tissue of *Hemigrapsus* sp. crabs from Walker Marsh showed evidence of bio-accumulation (Nelson, unpublished data), indicating production in these sediments of methyl-mercury (the form of mercury hypothesized to be bio-accumulated). As mentioned previously mercury-methylation has been almost universally attributed to the activity of sul-

fate-reducing bacteria. Initially, to determine the activity of sulfate-reducing bacteria in these sediments we measured sulfate-reduction rates using a radiotracer technique (Fossing and Jørgensen 1989). We measured rates of sulfate-reduction in Walker Marsh sediments, and determined the minimum concentration of molybdate that fully inhibited sulfate-reduction. To produce homogeneous distribution of inhibitor, sediments were homogenized (± amendments) and incubated in gas-tight plastic bags incubated under an argon atmosphere (Hansen et al. 2000). Sulfate Reduction rates in similarly incubated unamended sediments were comparable to those observed by others in coastal sediments (**Table 2**.). In Walker sediments additions of 3mM and 30mM of sodium molybdate knocked out sulfate reduction completely and 0.3mM inhibited rates by 35 to 83%.

Table 2. Sulfate reduction rates determined via bag incubation from various environments.

Site description	Observed Sulfate Reduction rate (nmol cm^{-3} day^{-1})	Carbon oxidation attributable to iron reduction (%)	Total extracted iron from sediments (μmol g sediment dw^{-1})	Reference[1]
Continental margin sediments	15	32	0-90[2,4]	(1)
190-380m deep	41	51	0-105[2,4]	
Continental margin sediments	54	5	100-170[3,5]	(2)
25-56m deep	50	75	80-250[3,5]	
Estuararine sediments surface	240-1104	n r	n r	(3)
Walker marsh estuarine sediments	540	n d	22-586[3]	This study

Reference[1], (1)Thamdrup and Canfield 1996, (2) Jensen et al. 2003, (3) Pallud and Van Cappellen 2006
[2]Oxalate extractable iron
[3] HCl extractable iron,.
[4] porosity of 0.75 and granular density of 2.67 was assumed
[5] granular density of 2.67 was assumed

Molybdate acts to inhibit sulfate reducing-bacteria by competing with sulfate in the first step in the dissimilatory sulfate reduction pathway , which is an ATP-consuming reaction. The resultant molybdenum compound is unstable and decomposes with a resulting "futile" consumption of ATP. Continuous exposure of sulfate-reducing bacteria to molybdate causes depletion of cellular ATP pool resulting in cell death (Peck 1959;1962). Commonly in the literature, molybdate is added at concentrations equimolar to the *in situ* sulfate concentrations (Oremland and Capone 1988). In contrast, in Walker Marsh sediments we observed that when molybdate reached 10% of *in situ* sulfate levels, sulfate reduction was completely inhibited. To avoid adversely impacting the numerous sediment microbes that employ sulfate reduction pathways for assimilatory purposes, we employed molybdate at a concentration that was just high enough to fully inhibit sulfate-reduction.

Addition of 0.5ppm divalent mercury, essential to measuring methyl-mercury production, inhibited sulfate-reduction by 54% (in the absence of molybdate). For mercury-amended sediments from Walker

Marsh (and in parallel with rates observed with sediments incubated in the absence of mercury), 3mM molybdate was completely inhibitory and 0.3mM molybdate decreased sulfate reduction rates by 74-91% vs no-molybdate controls. Mercury methylation in uninhibited sediments occurred at rates seen in other systems (**Table 3.**) At 0.3mM and 3mM added molybdate, mercury methylation rates decreased by 22 to 47%, respectively. If sulfate-reducing bacteria were the only microbes responsible for methylation one would expect full inhibition at 3mM molybdate. This incomplete inhibition of mercury methylation observation has not been reported for other marine systems (**Table 3**) but has been observed in at least one mine-impacted sediment (**Table 3**) and a few freshwater systems (Winfrey and Rudd 1990; Kerry et al.1991; Fleming et al. 2006).

Table 3. Inhibition of sulfate-reduction and mercury methylation processes by MoO_4^{2-} in anaerobic incubations of saline environments

Source of mercury contamination	SO_4^{2-} (mM)	MoO_4^{2-} (mM)	Inhibition of SO_4^{2-} reduction[2] (%)	Inhibition of Hg-methylation (%)	Reference[1]
Mine impacted point source[3] (NV)	0.5	0.7	n r	40[4] 60[5] 35[6]	(1)
Atomspheric deposition (NJ)	~4	20	100	95	(2)
Atomspheric deposition (NJ)	0.1-19	20	n d	95 or more	(3)
Industrial contamination, point source (GA)	14	100	92	~90	(4)
Mine impacted, point source, Walker Marsh (CA)	17-20	0.3 3.0	35-83 100	n d	This study
Mine impacted, point source, Walker Marsh (CA)	20-23	0.3 3.0	74-91 100	22 47	This study

[1]References, (1)Bonzongo et al., 1996 (2)Compeau & Bartha, 1985 (3) Compeau & Bartha, 1987 (4) King et al. 1999 .
[2]Percent inhibitions calculated relative to the rate of the same process in the absence of molydate
[3]These sediments are naturally exposed to a variety of oxyanions, including MoO42- and selection for resistance to uncoupling by this compound is expected in sulfate reducers
[4]pH 5.5 [5]pH 6.0, [6]pH 8.5
nd not determined
nr not reported

Extractable iron may be indicative of reducible-iron available, for both biotic and abiotic iron-reduction. Concentrations we observed are equivalent to those seen in systems were iron-reduction was determined to be the dominant pathway for anaerobic carbon oxidation (**Table 2**). In sediments from Walker Marsh, enrichments with media rich in reducible-iron yielded several genera of bacteria that were present in high numbers and were identified through molecular sequencing to be members of the genera *Desulfuromans* and *Pelobacter* (Fleming unpublished data). Members of these genera have been demonstrated to reduce iron and play a significant role in carbon cycling in marine sediments (Jensen et. al. 2003; Lovely et al. 1997) Recently, closely related species of iron-reducing bacteria from culture collections were shown to methylate divalent mercury (Kerin et al. 2006). Based on the

possibly widespread ability of these organisms to methylate mercury and their presence in these sediments, mercury methylation that is not attributable to sulfate-reducing bacteria may well be due to the activity of these iron-reducing bacteria.

Wider implications. Transport of mercury in aquatic sediments is aided by manganese, iron or aluminum colloids, and these metals have been found to co-occur in mercury-contaminated sediments (Gobeil and Cossa 1993; Mason et al. 1993). Iron and mercury were observed in both the pore water and the solid phase of Walker Marsh and Guadalupe Slough sediments. Bioturbation of the upper layers of marine sediments can result in both the cycling of iron and manganese and the transport of mercury to deeper layers of the sediment. Additionally, via bioturbation, iron can be reduced and re-oxidized multiple times supplying ample substrates for iron-reducing bacteria (Thandrup 2000). The recently described ability of iron-reducing bacteria to methylate mercury in freshwater sediments (Fleming et al. 2006), in marine sediments (this study) and in pure cultures from culture collections (Kerin et. al. 2006) expands the kinds of anaerobic bacteria and terminal electron acceptors known to be important in the production of methyl-mercury. Models of methyl-mercury production and remedial strategies need to be modified to account for our new findings.

Unfortunately there is no known specific inhibitor of microbial iron-reduction, which would aid in unequivocally assigning a portion or all of the molybdate-insensitive methyl-mercury production to iron-reducing bacteria. Based on our observations, one or more groups other than sulfate-reducing bacteria are responsible for significant mercury methylation in these sediments. In the context of the recent literature we suggest that in iron-rich, mercury-contaminated sediments iron-reducing bacteria play a significant role in marine mercury methylation in these sediments. Other mercury contaminated marine systems (**Table 1**), known to produce methyl-mercury, reportedly contain high concentrations of potentially reducible iron, thereby providing a potential substrate for active iron-reducing bacteria. Thus, a portion of the total methylation potential in these sediments may be also attributable to these microorganisms. However, extension of our findings to anaerobic marine sediments in general requires much additional field work.

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