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Supplemental Information: Incorporating Concentration-Dependent Sediment Microbial Activity into Methylmercury Production Kinetics Modeling

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Additional methods

Sediment Characterization

The wet/dry ratio for the sediments was determined by drying aliquots of the sediment overnight at 100°C. Sediments were removed from the drying oven, allowed to cool at room temperature, reweighed, and the wet/dry ratio was determined. To determine Loss on Ignition (%LOI), these same samples were then ashed in a muffle furnace at 550°C for 5 hours, cooled, removed, and reweighed. For sediment surface area determination and grain size characterization, aliquots of the bulk homogenized sample were dried in an oven at 80°C for over 24h. An aliquot of the dried bulk sediment was characterized for surface area via Brunauer-Emmet-Teller (BET) surface area analysis performed by QuantaChrome using a NOVA Touch 4LX.

Sediment grain size distribution

Dried bulk sediment was sieved through a sieve tower on a mechanical shaker for 30min. The sieve tower consisted of the following mesh size diameters: 4.76, 2, 0.841, 0.5, 0.149, 0.088, and 0.074 mm. The sediment retained on each sieve was weighed to determine the sediment grain size distribution.

Ferrozine determination of Fe(II) and Total Fe

Sediment samples were frozen at -20°C until preparation for analysis. The sediment was thawed inside the anaerobic chamber (98% N₂/2% H₂; Coy Labs). Sediment (0.5g) was weighed into a 15mL centrifuge tube, 5 mL of 0.5M HCl was added to the tube, and the tubes were shaken vigorously for 60min outside the glove box. The samples were taken off the shaker and allowed to settle. In the glovebag, the supernatant was decanted and filtered through a 0.2 \Box M PES filter. Aqueous samples were preserved at 1% HCl (v/v) and stored in at 4°C until preparation for analysis. Sediment extraction and aqueous samples were prepared for the ferrozine assay in the

same way. The calibration curve that was prepared by mixing the following solutions, 63 mL of HEPES buffer, 50 mL of 50 g/L Ferrozine, and varying amounts of 10ppm (Fe³⁺) standard solution prepared with a 1000 pm Ferric Nitrate solution (Fisher), and diluting to 2.5 mL with Milli-Q water. For Total Fe analysis, 125 uL of 2M HEPES buffer, 100 mL of 50 g/L Ferrozine solution, 250 mL of 10% (w/v) hydroxylamine solution, and <150 mL of sample solution were mixed together and diluted to 5mL with Milli-Q water. For Fe(II) samples, the preparation was the same except that the 10% (w/v) hydroxylamine solution was not added. The sample solutions were mixed well and kept in a dark area for 30 min before reading absorbance at 562 nm.

Gas Generation Incubations

Gas generation incubations were used to assess the general microbial activity of the sediments. Within 24h of collection 50 g of wet, bulk, homogenized sediment was weighed into plastic cups, which were placed into 1 qt mason jars that were equipped with septa in the lids. The jars were sealed in the anaerobic chamber and then were transferred outside the anaerobic chamber to incubate at room temperature over 68h. At each timepoint, 12mL of headspace gas was removed with a gas-tight syringe and transferred to vacutainers for storage. The headspace samples were analyzed for methane and carbon dioxide within 7 days of collection using an SRI 8610C Gas Chromatograph with a 1m molecular sieve packed column and a 2m silica gel packed column and FID detection.

Component	Units	Mean	SD
Са	mg L ⁻¹	46.8	6.55
Mg	$mg L^{-1}$	10.2	1.43
Na	$mg L^{-1}$	11	2.87
K	mg L^{-1}	3.12	0.67
Cl-	mg L ⁻¹	17.4	4.32
SO_4^{2-}	mg L ⁻¹	23.3	4.71
NO_3^-	mg L ⁻¹	14.7	5.87
Phosphate	$mg L^{-1}$	1.85	1.24
SRPa	$\mu g L^{-1}$	537	354
DIC^{b}	mg L ⁻¹	29.6	2.6
DOC^{c}	$mg L^{-1}$	2.2	0.563
pH	SU	7.81	0.393
Total Hg	ng L ⁻¹	87	50.4
Dissolved ^d Hg	$ng L^{-1}$	17.4	5.69
Total MMHg (summer)	ng L ⁻¹	0.519	0.177
Total MMHg (winter)	ng L ⁻¹	0.281	0.101
Dissolved ^d MMHg (summer)	$ng L^{-1}$	0.447	0.149
Dissolved ^d MMHg (winter)	ng L ⁻¹	0.202	0.0862

Table S1. Average composition of EFPC surface water at kilometer 5.4

^{*a*}soluble reactive phosphorous as phosphorous ^{*b*}dissolved inorganic carbon

^cdissolved organic carbon ^dpasses a 0.2 μ m pore size filter

Sediment	Sieve #	Sieve mesh (mm)	%mass passing	Classification
	4	4.76	89.5	Gravel
	10	2	68.8	Coarse Sand
	20	0.841	52.6	Medium Sand
Sadimant 1	35	0.5	45.4	Medium Sand
Sediment 1	100	0.149	23.6	Fine Sand
	170	0.088	11.5	Fine Sand
	200	0.074	0.4	Fine Sand
	Pan	N/A		Clay/Silt
Sediment	Sieve #	Sieve mesh	%mass	Classification
Scument	Sieve #	(mm)	passing	
	4	4.76	85.8	Gravel
	10	2	79.5	Coarse Sand
	20	0.841	55.2	Medium Sand
Sadimant 2	35	0.5	21.6	Medium Sand
Seament 2	100	0.149	3.1	Fine Sand
	170	0.088	0.4	Fine Sand
	200	0.074	0.2	Fine Sand
	Pan	N/A		Clay/Silt

Table S2. Sediment particle size distribution

Table S3. Sediment particle size categorization

Sediment	%Gravel	%Coarse Sand	%Medium Sand	%Fine Sand	%Clay/Silt
Sediment 1	10.47	20.76	23.36	44.96	0.67
Sediment 2	14.2	6.3	57.9	21.4	0

Rea	ction	log ₁₀ (K _{eq})
$FeS_{(s)} + 2H^+$	$Fe^{2+} + H_2S$	3.8
H_2S	$H^+ + HS^-$	-6.99
$FeS_{(s)} + H^+$	$Fe^{2+} + HS^{-}$	-3.19

Table S4. Mackinawite dissolution reaction added to the thermodynamic database for calculating saturation indices (Figure S11) (source: Lemire (2020)¹

Table S5. Free energy data used to calculate dissolution reaction for pyrite

Constituent	ΔG_{f}° (J mole ⁻¹) ^{<i>a</i>}	Source - Langmuir (1997) ²
FeS _{2,pyrite}	-160,247.2	Table A12.1
Fe^{2+}	-78,868.4	Table A12.1
HS ⁻	12,091.76	Table A12.3
<i>S</i> (0)	0	Reference state
SO_4^2	-74,4542.8	Table A12.3
H^+	0	Reference state
$O_{2(g)}$	0	Reference state
$H_2\bar{O}$	-237,140.752	Table A13.2

^{*a*}Values provided in Langmuir (1997) were converted from kcal mole⁻¹ to J mole⁻¹ with the conversion factor 4,184 J kcal⁻¹

Table S6.	Pyrite dissolution reaction added to	thermodynamic database	for calculating saturation indices
		(Figure S11)	

	Reaction		log ₁₀ (K _{eq}) ^a
$FeS_{2,(s)} + H^+$	\leftrightarrow	$Fe^{2+} + HS^- + S(0)$	-16.38
$S(0) + H_2 O$	\leftrightarrow	$0.5O_2 + H^+ + HS^-$	-43.67
$FeS_{2,(s)} + H_2O$	\leftrightarrow	$Fe^{2+} + 2HS^{-} + 0.5O_2$	-60.05
$0.5O_2 + 0.25HS^{-1}$	\leftrightarrow	$25SO_{4}^{2-} + 0.25H^{+}$	33.14
$FeS_{2,(s)} + H_2O \leftrightarrow Fe^{2+}$	\leftrightarrow	$Fe^{2+} + 1.75HS^{-} + 0.25SO^{2-}_{4} + 0.25H^{+}$	-26.91

^aderived from free energy data listed in Table S5

Aqueous species					
Sediment 1	Day 0	Day 1	Day 2	Day 3	Day 4
HgS ₂ H ⁻	85.03	86.85	89.46	89.05	89.71
$Hg(SH)_{2(aq)}$	13.74	11.66	8.32	8.88	7.97
$\mathrm{HgS_2^{2-}}$	1.22	1.50	2.23	2.06	2.33
MeHgSH	98.45	99.31	99.06	99.54	99.43
MeHgRS ^b	1.43	0.60	0.78	0.32	0.41
MeHgS-	0.10	0.12	0.17	0.16	0.18
Sediment 2	Day 0	Day 3	Day 10	Day 14	
HgS ₂ H ⁻	50.90	53.76	70.10	66.17	
$Hg(SH)_{2(aq)}$	48.95	45.97	29.26	33.34	
$\mathrm{HgS}_{2^{2-}}$	0.21	0.25	0.67	0.52	
MeHgSH	99.80	99.85	99.90	99.80	
MeHgRS	0.00	0.00	0.00	0.00	
MeHgS-	0.02	0.02	0.04	0.03	

 Table S7. Calculated Hg and MMHg aqueous speciation^a during sediment incubations (presented as percent of total dissolved Hg or MMHg)

 a calculated using the Geochemists Spreadsheet within the Geochemists Workbench Community Edition and a database amended with Hg and MMHg speciation reactions.

^bRS represents the reduced sulfur groups in NOM. Their concentration was calculated as outlined in Dong et al., $(2010)^3$. Hg-RS species (HgRS⁺ and Hg(RS)₂) were considered in the calculations and their percent abundance ranged between ~10⁻¹² - 10⁻²⁰.



Figure S1. Sediment 1 and 2 particle size distribution.



Figure S2. Gas generation from wet sediment incubations. (a) CO₂, and (b) CH₄ generation. Each data point represents the average of triplicate incubation microcosms. Error bars represent one standard deviation.

16S: Abundant (>1%) Taxa by Phylum



Sample

Figure S3. 16S rRNA phylum in the initial sediment (day 0) and at the end of the methylation/demethylation assay. Blue bars represent the initial sediment. Red bars are the average of replicates (n=3 for Sediment 1, n = 2 for Sediment 2) at the end of the incubations. Error bars represent one standard deviation.



hgcA : All sequences by Phylum

Figure S4. hgcA sequences by phylum in the initial sediment (day 0) and end of the methylation/demethylation assay. Blue bars represent the initial sediment. Red bars are the average of replicates (n=3 for Sediment 1, n = 2 for Sediment 2) at the end of the incubations. Error bars represent one standard deviation.



Figure S5. Dissolved organic carbon (DOC) in sediment microcosms. Each data point represents the average of triplicate microcosms. Error bars represent one standard deviation.



Figure S6. Dissolved Fe(II) and Total Fe in (a) Sediment 1, and (b) Sediment 2 microcosms. Data points represent the average of triplicate microcosms. Error bars represent one standard deviation.



Figure S7. Dissolved (a) sulfate and (b) sulfide in sediment microcosms. Data points represent the average of triplicate microcosms. Error bars represent one standard deviation.



Figure S8. 16S rRNA Alpha Diversity in the initial sediments compared to the sediment at the end of the methylation/demethylation period.



Figure S9. (a) 16S rRNA Deltaproteobacteria by Order, NA = Unclassified, and (b) Deltaproteobacteria hgcA by order at the beginning (blue) and end (red) of the methylation/demethylation assay. Red bars represent the average ± 1 std. dev. of replicates (n=3 for Sediment 1, n = 2 for Sediment 2).

(a)

Euryarchaeota by Order (16S)



Figure S10. (a) 16S rRNA Euryarchaeota by order, NA = Unclassified, and (b) Euryarchaeota *hgcA* by order at the beginning (blue) and end (red) of the methylation/demethylation assay. Red bars represent the average ± 1 std. dev. of replicates (n=3 for Sediment 1, n = 2 for Sediment 2).



Figure S11. Saturation indices (SI) with respect to mackinawite (FeS), pyrite (FeS₂), and metacinnabar (β -HgS) solid phases over the course of the methylation-demethylation incubations for (a) Sediment 1, and (b) Sediment 2. Positive values indicate oversaturation with respect to the mineral phase. Minerals are considered to be at equilibrium when SI = 0 ± 0.05 if the thermodynamic data are well-known. Saturation indices were calculated using the Geochemists Spreadsheet within the Geochemists Workbench ⁴ Community Edition. The database was edited to include mackinawite following Lemire et al., (2020) ¹ and the equilibrium constant for pyrite was derived from free energy data given in Langmuir ² – see Table S4, Table S5, and Table S6.

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