Supporting Information

to

Sulfurization of Dissolved Organic Matter Increases Hg-S-DOM Bioavailability to a Hg-Methylating Bacterium

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Table SI-1. Recovery and UV-VIS spectral characteristics of sulfurized DOM by solid phase extraction (SPE). Slope ratio (S_R) is the ratio of the slope of the natural log transformed spectra in the wavelength range 275-295 nm divided by the slope in the range 350-400 nm. S_R is strongly correlated with the size and aromaticity of DOM as described in Helms et al. (2008).[1] The reported error on S_R was determined based on the relative standard errors of the linear fits to the natural log transformed spectra in each wavelength range.

Sample	Measured S/C ratio	SPE Recovery (%)	S_R (slope ratio)
SRHA (unsulfurized)	3.42	33.3	0.67±0.02
SRHA	4.12	34.6	0.67±0.02
SRHA	4.73	34.8	0.68±0.02
SRHA	6.12	32.8	0.66±0.02
SRHA	5.83	31.6	0.70±0.02
IHSS SRHA (no SPE)	not measured	not applicable	0.65±0.02
SRFA (unsulfurized)	1.88	67.3	0.69±0.02
SRFA	3.80	54.2	0.72±0.02
SRFA	4.22	53.5	0.69±0.02
SRFA	4.08	55.8	0.63±0.02
SRFA	5.69	52.5	0.70±0.02
IHSS SRFA (no SPE)	not measured	not applicable	0.82±0.05
NLFA (unsulfurized)	3.12	46.2	0.78±0.06
NLFA	3.98	46.6	0.76±0.06
NLFA	8.83	29.4	0.74±0.09
NLFA	12.8	44.9	0.84±0.05
IHSS NLFA (no SPE)	not measured	not applicable	0.65±0.04
PLFA (unsulfurized)	11.0	78.6	0.99±0.08
PLFA	10.4	75.6	0.79±0.06
PLFA	14.5	75.0	0.76±0.05
PLFA	12.5	79.9	0.85±0.06
PLFA	12.2	81.3	0.96±0.09
IHSS PLFA (no SPE)	not measured	not applicable	0.91±0.06

Table SI-2. Quality control data for total Hg (THg) and methylmercury (MeHg) analyses. Instrument detection limit determined as three times standard deviation of blank.

Parameter	Result
Me^{201}Hg instrument detection limit	0.11±0.18 pg (0.02 ng/L for 5 mL sample)
Distillation blanks for Me^{201}Hg	0.02±0.02 ng/L
Relative percent difference for duplicate MeHg analyses	7.4±6.2% (n = 5 pairs)
MeHg recovery for NIST 1566b (oyster tissue)	139±8% (n = 6 determinations)
^{201}THg instrument detection limit	0.37±0.38 ng/L
Digestion blanks for ^{201}Hg	0.02±0.04 ng/L
Relative percent difference for duplicate THg analyses	5.4±2.7% (n = 4 pairs)
THg recovery for NIST 2709a (San Joaquin soil)	91.6±22.0% (n = 8 determinations)

Instrument detection limit calculated as three times the standard deviation of reagent blanks.

Description of Equilibrium Speciation Modeling

Equilibrium speciation modeling was performed in MINEQL+ v. 4.6 (Environmental Research Software). Equilibrium constants were critically selected using the most up to date information on Hg(II)$_i$ complexation in natural waters. The solubility product for metacinnabar (HgS(s)) was recently reevaluated by Drott *et al.*[2] and reported as log K = 36.8, 1.2 log units lower than that reported in the NIST Critical Database.[3] Following Skyllberg,[4] we have assumed that Hg(II)$_i$ forms linear two-coordinate complexes with DOM thiols with a log K = 42.0. In this approach, we ignore the contribution of weaker O- and N- donor ligands in the DOM pool. This approach is justified for two reasons: 1) DOM/Hg ratios are sufficiently high in these experiments, such that binding will be dominated by stronger S-donor ligands[5]; 2) All solutions contain μM concentrations of sulfide further diminishing the contributions of weak Hg(II)$_i$-binding ligands. [RSH]$_T$ was estimated based upon [DOC], the measured S/C ratio, and the assumption that strong Hg(II)-binding thiols could be estimated based on the concentration of exocyclic sulfur in each DOM sample. Manceau and Nagy[6] determined S speciation using X-ray absorption near edge spectroscopy (XANES) for 3 out of the 4 isolates used in this study (and S speciation for the humic acid fraction of the Nordic Lake sample). The percentage of total S as reduced exocyclic S ranged from 23.6 to 46.9% (mean = 32.2±10.5%). We further assume that DOM S speciation is independent of total S content – recent data from Hoffmann *et al.*[7] and Poulin et al.[8] suggests, however, that the fraction of total S in reduced forms increases with increasing sulfurization. In that case, our application of a single conversion factor for total S to reduced S may underestimate the true contribution of DOM thiols to Hg(II)$_i$ binding. Other input parameters for modeling can be found in Table SI-3 below; for sulfide concentration, the mean of initial and final (t=3 h) concentrations were input into the speciation model. In modeling Hg-cysteine complexation, some reports suggest the possibility of a tris Hg(cys) complex (likely Hg(Hcys)$_3^-$).[9] Unfortunately, no thermodynamic data are available for this purported complex. Kõszegi-Szalai and Paál[10] reported equilibrium constants for Hg-penicillamine complexes, including a Hg(Hpen)$_3^-$ complex with a log K of 75.3 at *I* = 0 M. Given their similar structures (differing only in the two CH$_3$—substituents at the 3-position for penicillamine), we can evaluate the potential contributions of a Hg(Hcys)$_3^-$ complex to Hg(II)$_i$ speciation using the log K for the Hg(Hgpen)$_3^-$ complex. Using this approach, we find that Hg(Hcys)$_3^-$ is not likely to be a significant species under our experimental conditions ([^{201}THg], [H$_2$S]$_T$, [cys]$_T$, and pH), and we do not include this species in our modeling. A summary of important thermodynamic data for speciation modeling can be found in Table SI-3 below.

Table SI-3. Thermodynamic data for equilibrium speciation modeling. Equilibrium constants for Hg-Cl and Hg-OH complexes were taken directly from the MINEQL+ database.

Reaction	log K	Reference
Hg-sulfide Aqueous Speciation		
$Hg^{2+} + 2HS^- = Hg(SH)_2^0$	39.1	Drott *et al.*[2]
$Hg^{2+} + 2HS^- = HgS_2H^- + H^+$	32.5	Drott *et al.*[2]
$Hg^{2+} + 2HS^- = HgS_2^{2-} + 2H^+$	23.2	Drott *et al.*[2]
Metacinnabar Precipitation		
$Hg^{2+} + HS^- = HgS(s) + H^+$	36.8	Drott *et al.*[2]
Hg-DOM Complexation		
$Hg^{2+} + 2RS^- = Hg(SR)_2$	42.0	Skyllberg[4]
$RS^- + H^+ = RSH$	10.0	Skyllberg[4]
Hg-CYS Complexation		
$Hg^{2+} + 2H^+ + 2CYS^{2-} = Hg(HCYS)_2^0$	64.1	Starý and Kratzer.[11]
$Hg^{2+} + 2CYS^{2-} = Hg(CYS)_2^{2-}$	43.9	Starý and Kratzer.[11]

Table SI-4. Summary of experimental variables in Hg methylation assays with *Desulfovibrio desulfuricans* ND132 in the presences of sulfurized DOM samples. DOM isolates were sulfurized as described in the main text, resulting in the S/C ratios reported in the table below. Reported values are means and standard deviations (*n* =3, excepting NLFA experiments, where *n* =2). n.d. = not determined due to lost samples. Cell density is average cell density measured at beginning and end of 3h incubation which typically increased less than 5% over the duration of the experiment.

| DOM Isolate or Control	[DOC] (mg/L)	Measured S/C ratio (mmol S/mol C)	Cell density (x 10^8 cells/mL)	pH	Initial sulfide (μM)	Final sulfide (μM)	Total ^{201}Hg in medium (nM)	Total filterable ^{201}Hg (nM)	Total Me^{201}Hg in medium (pM)
SRHA	9.19	3.42	5.68±0.21	7.27±0.01	2.25±0.05	3.62±0.12	0.30±0.16	0.21±0.01	39.6±4.9
SRHA	9.52	4.12	5.57±0.22	7.31±0.01	2.67±0.26	3.92±0.02	0.30±0.05	0.29±0.04	66.4±1.3
SRHA	9.58	4.73	5.32±0.31	7.25±0.01	3.36±0.08	4.15±0.11	0.32±0.06	0.26±0.04	80.7±1.5
SRHA	9.59	6.12	5.67±0.19	7.20±0.01	4.18±0.24	4.40±0.06	0.31±0.01	0.23±0.004	95.8±1.2
SRHA	9.24	5.83	5.43±0.09	7.33±0.02	3.72±0.17	4.53±0.10	0.39±0.02	0.24±0.01	128.4±1.9
SRFA	10	1.88	4.55±0.82	7.28±0.04	2.33±0.10	3.01±0.29	0.057±0.003	0.028±0.004	24.0±0.6
SRFA	10	3.80	5.75±1.51	7.28±0.04	2.79±0.29	3.22±0.20	0.070±0.008	0.034±0.007	40.5±1.6
SRFA	10	4.22	5.15±0.38	7.29±0.02	2.88±0.10	3.36±0.06	0.083±0.007	0.054±0.014	52.5±2.9
SRFA	10	4.08	5.03±0.52	7.22±0.02	2.87±0.03	3.44±0.14	0.13±0.01	0.088±0.007	86.6±0.2
SRFA	10	5.69	4.74±0.26	7.26±0.02	3.58±0.25	3.76±0.20	0.26±0.001	0.12±0.02	160±8
NLFA	10	3.12	1.44±0.21	7.52±0.12	0.26±0.18	0.95±0.83	0.15±0.07	0.067±0.006	36.9±11.4
NLFA	10	3.98	1.50±0.15	7.74±0.05	0.10±0.04	0.33±0.06	0.099±0.003	0.086±0.006	53.4±13.6
NLFA	10	8.83	1.36±0.18	7.61±0.10	0.05±0.01	0.42±0.31	0.10±0.01	0.090±0.01	49.5±13.4
NLFA	10	12.8	1.66±0.07	7.66±0.04	0.30±0.06	0.42±0.11	0.15±0.01	0.17±0.01	138±9
PLFA	8.3	11.0	4.79±0.23	7.35±0.02	3.99±0.27	6.44±0.78	0.13±0.004	0.018±0.002	39.2±4.0
PLFA	8.3	10.4	5.11±0.45	7.35±0.05	5.13±0.52	6.87±0.47	0.13±0.003	0.036±0.006	64.2±6.3
PLFA	8.3	14.5	4.76±0.33	7.34±0.05	5.13±0.52	7.02±0.28	0.12±0.01	0.038±0.002	53.0±7.0
PLFA	8.3	12.5	5.28±0.37	7.28±0.06	4.78±0.19	6.72±0.54	0.11±0.01	0.030±0.008	62.1±10.7
PLFA	8.3	12.2	5.80±0.89	7.32±0.02	5.13±0.52	6.56±0.26	0.11±0.01	0.049±0.008	57.8±0.8
500 μM L-cysteine control (SRHA)	N/A	N/A	5.74±0.13	7.28±0.00	5.78±0.11	19.3±0.1	0.46±0.02	0.07±0.01	362±6
500 μM L-cysteine control (SRFA)	N/A	N/A	4.04±0.67	7.14±0.00	5.55±0.72	31.1±4.9	0.37±0.02	0.28±0.02	308±11
500 μM L-cysteine control (NLFA)	N/A	N/A	1.43±0.17	7.30±0.06	0.28±0.10	4.08±3.1	0.13±0.02	n.d.	43.4±13.4
500 μM L-cysteine control (PLFA)	N/A	N/A	5.15±0.45	7.23±0.04	5.90±0.88	17.0±0.7	0.31±0.07	0.39±0.01	347±20
No DOM control (SRHA)	N/A	N/A	5.31±0.34	7.40±0.01	3.64±0.13	2.75±0.1	0.28±0.02	0.25±0.01	2.2±0.2
No DOM control (SRFA)	N/A	N/A	3.81±0.10	7.20±0.12	2.74±0.25	3.22±0.53	0.064±0.007	0.007±0.005	6.5±1.2

Table SI-5. Predicted equilibrium speciation of inorganic Hg(II) based on measured total ^{201}Hg in medium, pH, sulfide, DOC, and S/C ratio of DOM. Hg(SR)$_2$ is a two-coordinate complex of Hg(II)$_i$ with organic thiols; Hg(SH)$_2$ is the equivalent complex with inorganic sulfide.

DOM Isolate or Control	[RSH]$_T$ (μM)	[Meta-cinnabar] (M)	[Hg(SR)$_2$] (M)	[Hg(SH)$_2$] (M)	[HgS$_2$H$^-$] + [HgS$_2$$^{2-}$] (M)	Total dissolved Hg (M)
SRHA	0.62	1.77×10^{-10}	7.28×10^{-15}	1.65×10^{-11}	1.06×10^{-10}	1.23×10^{-10}
SRHA	0.77	1.60×10^{-10}	1.08×10^{-14}	1.74×10^{-11}	1.23×10^{-10}	1.40×10^{-10}
SRHA	0.89	1.65×10^{-10}	1.15×10^{-14}	2.18×10^{-11}	1.33×10^{-10}	1.55×10^{-10}
SRHA	1.15	1.36×10^{-10}	1.55×10^{-14}	2.69×10^{-11}	1.47×10^{-10}	1.74×10^{-10}
SRHA	1.06	2.14×10^{-10}	1.69×10^{-14}	2.10×10^{-11}	1.55×10^{-10}	1.76×10^{-10}
SRFA	0.40	undersaturated	1.70×10^{-15}	7.50×10^{-12}	4.94×10^{-11}	5.69×10^{-11}
SRFA	0.80	undersaturated	6.44×10^{-15}	9.24×10^{-12}	6.09×10^{-11}	7.01×10^{-11}
SRFA	0.88	undersaturated	9.12×10^{-15}	1.07×10^{-11}	7.18×10^{-11}	8.25×10^{-11}
SRFA	0.85	undersaturated	1.19×10^{-14}	1.90×10^{-11}	1.09×10^{-10}	1.28×10^{-10}
SRFA	1.19	1.02×10^{-10}	2.13×10^{-14}	2.10×10^{-11}	1.32×10^{-10}	1.53×10^{-10}
NLFA	0.84	1.21×10^{-10}	1.00×10^{-13}	2.18×10^{-12}	2.54×10^{-11}	2.77×10^{-11}
NLFA	1.06	8.73×10^{-11}	6.80×10^{-13}	5.20×10^{-13}	1.03×10^{-11}	1.15×10^{-11}
NLFA	2.36	9.30×10^{-11}	3.00×10^{-12}	5.81×10^{-13}	8.42×10^{-12}	1.20×10^{-11}
NLFA	3.42	1.32×10^{-10}	3.77×10^{-12}	9.70×10^{-13}	1.59×10^{-11}	2.06×10^{-11}
PLFA	3.55	undersaturated	9.18×10^{-14}	1.53×10^{-11}	1.19×10^{-10}	1.34×10^{-10}
PLFA	3.39	undersaturated	6.34×10^{-14}	1.53×10^{-11}	1.19×10^{-10}	1.35×10^{-10}
PLFA	4.70	undersaturated	1.08×10^{-13}	1.44×10^{-11}	1.09×10^{-10}	1.24×10^{-10}
PLFA	5.75	undersaturated	7.14×10^{-14}	1.38×10^{-11}	9.11×10^{-11}	1.05×10^{-10}
PLFA	5.85	undersaturated	7.21×10^{-14}	1.35×10^{-11}	9.74×10^{-11}	1.11×10^{-10}
500 μM L-cysteine control (SRHA)	500	undersaturated	4.60×10^{-10}	2.43×10^{-19}	1.68×10^{-14}	4.60×10^{-10}
500 μM L-cysteine control (SRFA)	500	undersaturated	1.27×10^{-10}	7.12×10^{-17}	9.94×10^{-15}	1.27×10^{-10}
500 μM L-cysteine control (NLFA)	500	undersaturated	3.09×10^{-10}	6.09×10^{-17}	3.56×10^{-16}	3.09×10^{-10}
500 μM L-cysteine control (PLFA)	500	undersaturated	3.71×10^{-10}	2.59×10^{-15}	1.23×10^{-14}	3.71×10^{-10}
No DOM control (SRHA)	0	1.33×10^{-10}	0	1.44×10^{-11}	1.26×10^{-10}	1.40×10^{-10}
No DOM control (SRFA)	0	undersaturated	0	9.91×10^{-12}	5.41×10^{-11}	6.40×10^{-11}
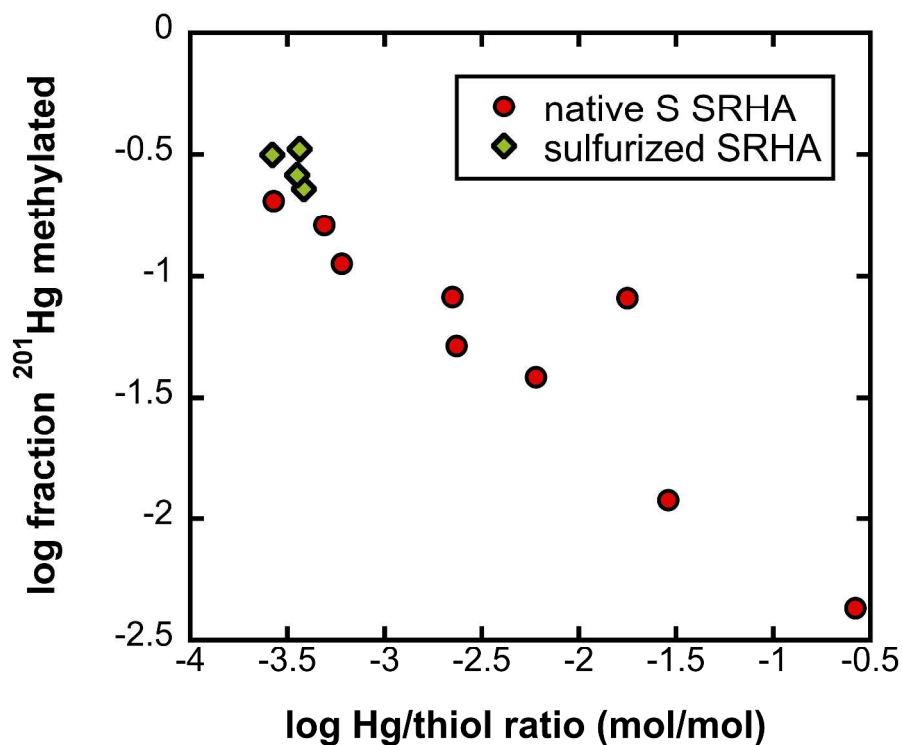
Figure SI-1. Relationship between log Hg/thiol ratio and log fraction [201]Hg methylated in solutions containing Suwannee River humic acid (SRHA), sulfide, and [201]HgCl$_2$. Data for native SRHA include data from this study and from Graham et al.[12] Data for sulfurized SRHA from this study only. Thiol concentrations were estimated based on measured S/C ratio for SRHA samples and the assumption that 70% of total DOM S was thiols.
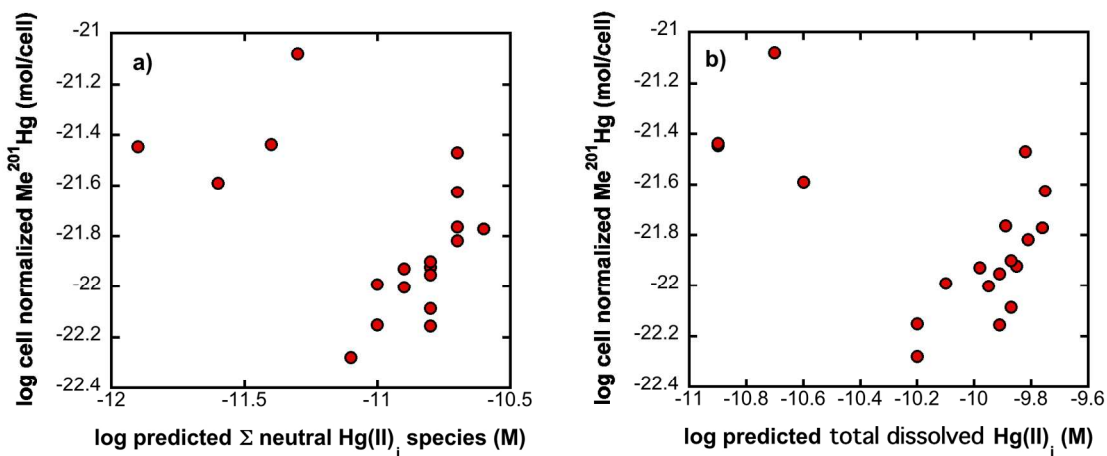
Figure SI-2. Correlations between the sum of neutral Hg(II)$_i$ species (**panel a**) or total dissolved Hg(II)$_i$ (**panel b**) and cell-normalized MeHg production. MeHg production cell-normalized due to significant differences in cell density between experiments. Data log-transformed due to non-normal distributions.

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