

Supplementary Information for

Dimethylmercury Formation Mediated by Inorganic and Organic Reduced Sulfur Surfaces

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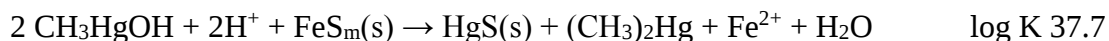
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Supplementary Discussion

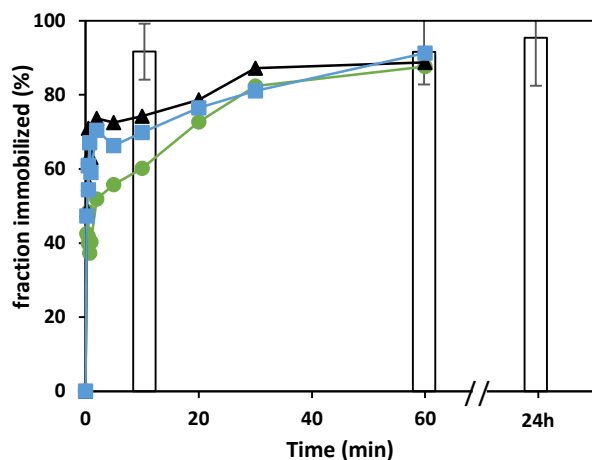
Adsorption of CH₃Hg(aq) onto FeS_m(s). In the first adsorption experiment, we monitored the adsorption of 1, 5.2 and 21 μM of CH₃Hg on 0.26 g L⁻¹ FeS_m(s) for 1 h (Supplementary Fig S1). At all three concentrations, the adsorption was initially rapid with 40-63 % of the added CH₃Hg(aq) immobilized within minutes, followed by a slower and gradual increase in adsorption of the CH₃Hg. At the end of the 1 h experiment the percent CH₃Hg removed from solution was 89, 88 and 91% of added CH₃Hg, corresponding to log K_D values of 4.5, 4.4 and 4.6 L Kg⁻¹, for the test with 1, 5.2 and 21 μM of CH₃Hg respectively. The obtained log K_D values are within range of the typically reported values from aquatic systems¹. A repeated experiment carried out for up to 24 h and using 0.05 μm membrane filters, instead of 0.02 μm syringe filters, showed a similar pattern of immobilization (Supplementary Fig S1; bar graph).

Characterization of synthesized particles. Crystal structures of the synthesized FeS_m(s), CdS(s), and HgS(s) particles was determined using X-ray diffraction (Supplementary Fig S5) and BET, respectively (Supplementary Table S3). The XRD diffractograms were consistent with the diffraction patterns of ordered tetragonal mackinawite, hexatetrahedral hawleyite and cubic metacinnabar, respectively. The FeS_m(s) diffractogram shows broad and less intense peaks compared to those of CdS(s) and HgS(s). This is indicative of small crystallites size and high degree of amorphousness. A high degree of disorder in the synthetic FeS_m(s) is in agreement with existing literature².

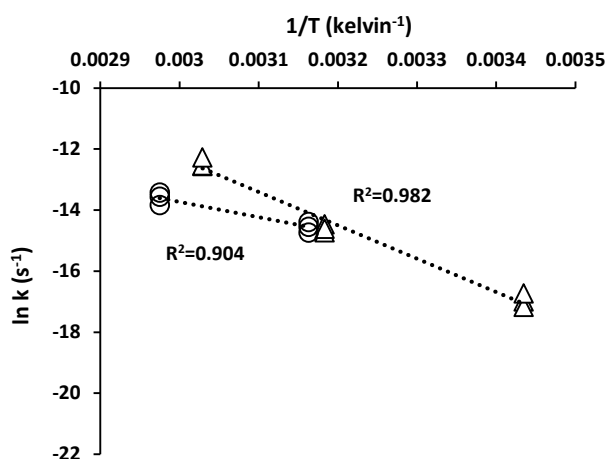
Thermodynamic favorability of the reaction. The equilibrium constant for the overall reaction of CH₃Hg with FeS_m(s) (reaction below) assuming HgS(s) as a final product was calculated from stability constants provided in Stumm and Morgan (1996)³. At the concentrations used in the experiments, and assuming the Fe²⁺ concentration is equivalent to that at equilibrium with the solid phase, at a pH of 7, the overall ΔG for the reaction is $\sim 150 \text{ kJ mol}^{-1}$ at STP. This indicates a highly favorable reaction.



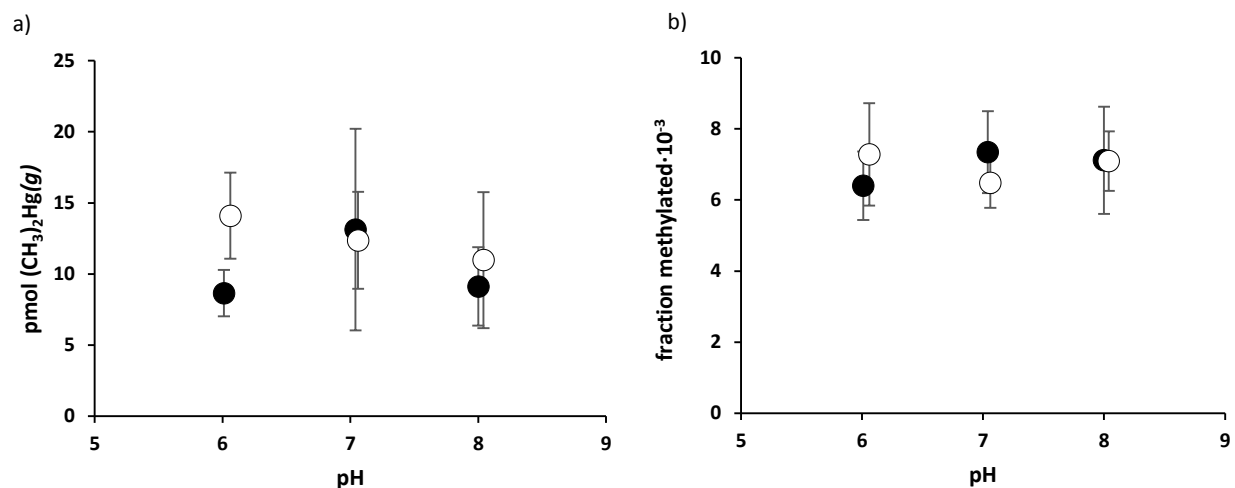
Supplementary Figures



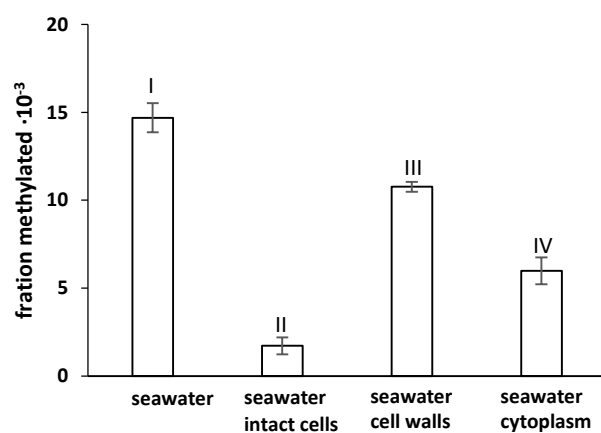
Supplementary Fig. S1. Adsorption of $\text{CH}_3\text{Hg}(\text{aq})$ on $\text{FeS}_\text{m}(\text{s})$. Fraction $\text{CH}_3\text{Hg}(\text{aq})$ immobilized by $\text{FeS}_\text{m}(\text{s})$ as a function of time in an experiment using $0.02 \mu\text{m}$ syringe filters and $\text{CH}_3\text{Hg}:\text{FeS}_\text{m}$ ratios ($\text{nmol } \mu\text{mol}^{-1}$) of 3.9 (blue squares), 1.0 (green circles) and 0.25 (black triangles) or using $0.05 \mu\text{m}$ membrane filters (bar graph) and a $(\text{CH}_3)_2\text{Hg}:\text{FeS}_\text{m}$ ratio of $0.068 \text{ nmol } \mu\text{mol}^{-1}$.



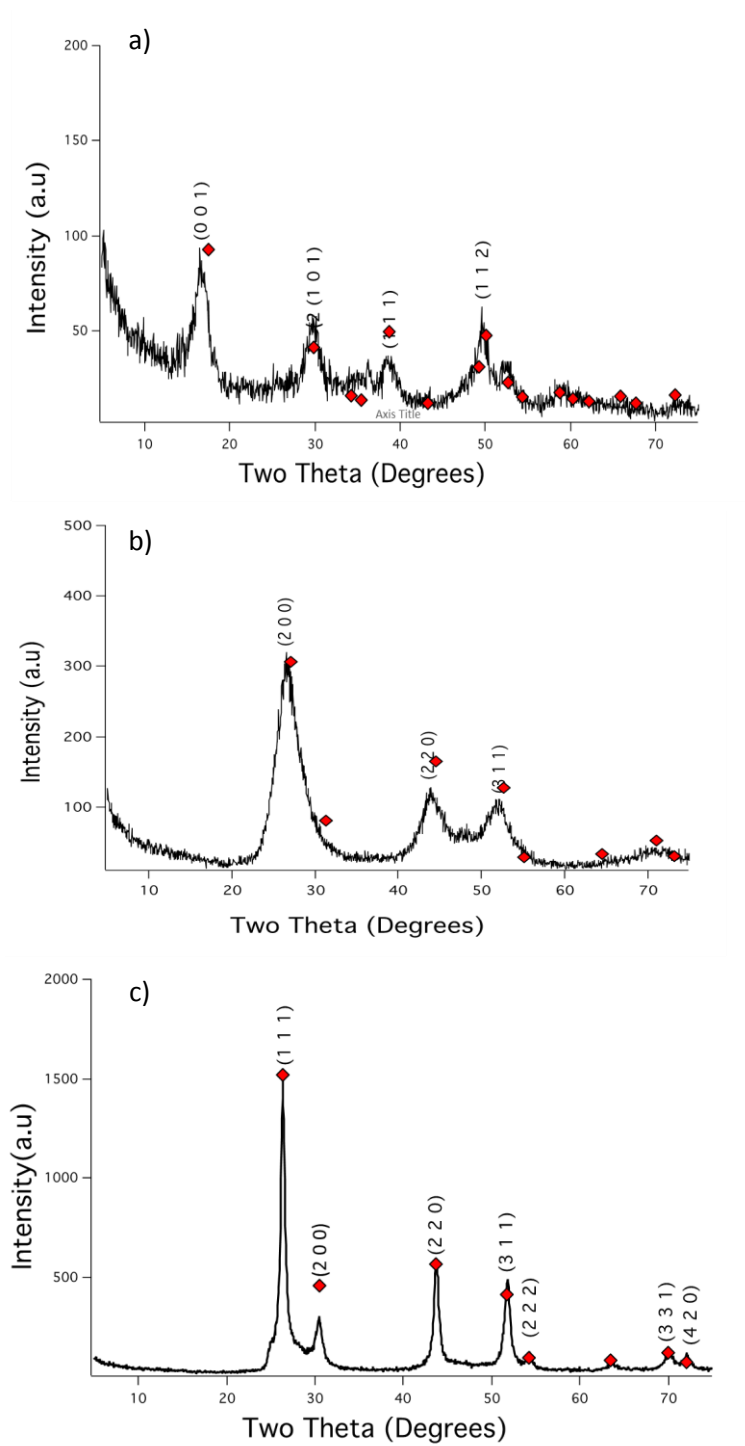
Supplementary Fig. S2. Methylation of CH_3Hg on $\text{FeS}_\text{m}(\text{s})$ at different temperatures. Effect of temperature ($1/T$ (kelvin^{-1})) on the reaction rate constant (k , s^{-1}) for $7.5 \text{ nmol } \text{CH}_3\text{Hg}$ added to $150 \mu\text{mol } \text{FeS}_\text{m}(\text{s})$ (triangles) and $390 \text{ nmol } \text{CH}_3\text{Hg}(\text{aq})$ reacted with $380 \text{ nmol } \text{S}^{2-}(\text{aq})$ (circles). Both regression models were statistically significant (linear regression, ANOVA, $p < 0.05$). Activation energy, E_a (kJ/mol), for the formation of $(\text{CH}_3)_2\text{Hg}$ was determined assuming a pseudo first order reaction and using the Arrhenius Equation ($\ln k = \ln A_e - E_a/RT$; rate constant (k), frequency factor (A_e), activation energy (E_a), gas constant (R), temperature (T ; in kelvin)). The activation energy (including standard deviation) was calculated from the slope of $\ln k$ vs. $1/T$ (slope = $-E_a/R$).



Supplementary Fig. S3. Methylation of CH₃Hg on FeS_m(s) at different pH and ionic strengths. Formation of (CH₃)₂Hg when adsorbing CH₃Hg on FeS_m(s) at pH of 6, 7 or 8 and ionic strength of 0.017 (white circles) or 0.20 M (black circles) in a) short term and b) long term experiments. No significant differences (two-way ANOVA, $p > 0.05$) were found for the amount of (CH₃)₂Hg formed in a) or b).



Supplementary Fig. S4. Methylation of CH₃Hg on FeS_m(s) in sea water in the presence of organic material. Fraction of CH₃Hg methylated when adsorbing CH₃Hg on FeS_m(s) in artificial sea water, and in artificial sea water with whole cells, cell walls (pellet obtained at 754 G for 15 min) or the cytoplasm (remaining in solution after centrifugation at 754 G, 15 min) of $1.2 \cdot 10^6$ cells of *Thalassiosira weissflogii*. Roman letters indicate significant differences ($p < 0.05$, ANOVA followed by Tukey's post-hoc test).



Supplementary Fig. S5. Characterization of crystal structure. X-ray diffractograms of synthesized a) FeS_m(s), b) CdS(s) and c) HgS(s) with diffraction patterns (red diamonds) for ordered tetragonal mackinawite⁴, hexatetrahedral Hawleyite⁵ and cubic metacinnabar⁴, respectively.

Supplementary Tables

Supplementary Table S1. Production of $(\text{CH}_3)_2\text{Hg}(g)$ from $\text{CH}_3\text{Hg}(aq)$ added to filtered and unfiltered $\text{FeS}_m(s)$ slurries. Amount of $(\text{CH}_3)_2\text{Hg}(g)$ (pmol) formed from 2.3 nmol of $\text{CH}_3\text{Hg}(aq)$ added to 0.2 μm filtrated and unfiltered $\text{FeS}_m(s)$ slurries. The unfiltered $\text{FeS}_m(s)$ slurry contained 5.6 μmol $\text{FeS}_m(s)$ giving a $\text{CH}_3\text{Hg}:\text{FeS}_m(s)$ ratio of 0.41 ($\text{nmol } \mu\text{mol}^{-1}$).

Sample	Purging interval (min)	$n(\text{CH}_3)_2\text{Hg}(g)$ (pmol)
Filtrated slurry	0-20	<0.038
$\text{FeS}_m(s)$	0-20	0.37 $\pm$ 0.08
	20-40	0.21 $\pm$ 0.07
	40-60	0.16 $\pm$ 0.07

Supplementary Table S2. Methylation of CH_3Hg on $\text{FeS}_m(s)$, $\text{CdS}(s)$ and $\text{HgS}(s)$. Fraction of CH_3Hg methylated on $\text{CdS}(s)$, $\text{FeS}(s)$ and $\text{HgS}(s)$ at equal CH_3Hg to specific surface area ratios (9.6 nmol CH_3Hg , 970 nmol $\text{CH}_3\text{Hg m}^{-2}$ mineral surface). No significant differences were found for the methylation on the sulfide minerals tested (ANOVA, $p>0.05$).

	Fraction methylated $\cdot 10^{-3}$	$\text{CH}_3\text{Hg}:(\text{Fe/Cd/Hg})\text{S}(s)$ ratio	
		nmol μmol^{-1}	nmol surface area (m^2) $^{-1}$
$\text{FeS}_m(s)^4$	8.3 $\pm$ 3.6	4.6	970
$\text{CdS}(s)^4$	2.2 $\pm$ 1.3	10	970
$\text{CdS}(s)^5$	6.3 $\pm$ 4.3	18	970
$\text{HgS}(s)^4$	4.0 $\pm$ 1.9	4.3	970

¹⁾ Precipitated at equimolar amounts of metal and sulfide

²⁾ Precipitated at a Cd:S ratio of 2:1

Supplementary Table S3. Methylation of CH_3Hg on $\text{FeS}_m(s)$ of different age. Fraction of CH_3Hg methylated when the reaction was mediated by $\text{FeS}_m(s)$, aged for 1hour, 1day or 7 days. The amounts of CH_3Hg and $\text{FeS}_m(s)$ reacted was 9.6 nmol and 2.8 μmol respectively, giving a final $\text{CH}_3\text{Hg}:\text{FeS}_m(s)$ ratio of 3.4 nmol μmol^{-1} . No statistical differences between the reactions with the $\text{FeS}_m(s)$ of different ages ($p>0.05$) was observed. Change in the internal structure of synthetic $\text{FeS}_m(s)$ has previously been demonstrated to occur within hours to days⁶.

	Fraction methylated $\cdot 10^{-3}$
$\text{FeS}_m(s)$ aged 1h	16 $\pm$ 4.6
$\text{FeS}_m(s)$ aged 1d	17 $\pm$ 14
$\text{FeS}_m(s)$ aged 7d	12 $\pm$ 3.3

Supplementary Table S4. Surface area of $\text{FeS}_m(s)$, $\text{CdS}(s)$ and $\text{HgS}(s)$. Specific surface areas of precipitate crystals determined using BET.

	Specific surface area ($\text{m}^2 \text{g}^{-1}$)
$\text{FeS}_m(s)^{1,2}$	34.9
$\text{FeS}_m(s)^1$	55.3
$\text{CdS}(s)^1$	73.9
$\text{CdS}(s)^3$	126
$\text{HgS}(s)^1$	19.29

¹⁾ Precipitated with equimolar amounts of metal and sulfide

²⁾ $\text{FeS}_m(s)$ batch used for all experiments except data presented in Supplementary Table S2.

³⁾ Precipitated with a Cd:S ratio of 2:1

Supplementary Table S5. Summary of experimental details. Form and amount of sulfide used to mediate the methylation, the initial amount of CH₃Hg and the final total volume of the aqueous solution used.

Test ¹⁾	Sulfide form		CH ₃ Hg	CH ₃ Hg:sulfide form	Volume
	name	(μmol)	(nmol)	(nmol μmol ⁻¹)	(ml)
Initial test (text)	FeS _m (s)	5.6	2.3	0.41	1.1
Varying CH ₃ Hg (fig 1)	FeS _m (s)	2.8	0.7, 2.8, 11	0.25, 1.0, 3.9	0.6
Varying FeS _m (s) (fig 2)	FeS _m (s)	0.0028 to 280	9.6	3400 to 0.034	0.6
FeS _m (s) and S ²⁻ (aq) (fig 4)	FeS _m (s)	2.8	12	4.3	0.6
	S ²⁻ (aq)	2.8, 0.13, 0.013,	12	4.3, 92, 920,	0.6
		0.0063		1900	
Test of thiols ²⁾ (fig 5)	FeS _m (s)	2.8	9.6	3.4	0.6
	Cysteine	0.2	200	1000	0.6
	3-mercaptopropionic acid	0.2	200	1000	0.6
	1,2-ethanedithiol	0.1	200	2000	0.6
Temperature effect ³⁾ (fig S2)	FeS _m (s)	2.8	7.5	2.7	0.6
pH and ionic strength ⁴⁾ (fig S3)	S ²⁻ (aq)	0.38	390	1000	0.6
	FeS _m (s)	2.8	8	2.9	0.6
Artificial sea water and t.w. ⁵⁾ (fig S4)	FeS _m (s)	2.8	9.6	3.4	2.5
Different sulfide minerals ⁶⁾ (table S2)	CdS(s)	0.93	9.6	10	0.6
FeS _m (s) aging (table S3)	CdS(s) ⁷⁾	0.54	9.6	17	0.6
	FeS _m (s)	2.0	9.6	4.8	0.6
	HgS(s)	2.2	9.6	4.4	0.6
	FeS _m (s) (aged 1h, 1d, 7d)	2.8	9.6	3.4	0.6

¹⁾ figure or table where the data is presented is given in brackets.

²⁾ tests performed in 500 μL 0.8% (v:v) acetone in degassed MQ water

³⁾ Sealed experimental vessels were incubated for 30 min in a water bath at a temperature of 0, 18, 40 and 60 °C (n=3). The reaction vessels were cooled on ice for 30 minutes and before (CH₃)₂Hg(g) was sampled from the headspace.

⁴⁾ prepared in 0.002 M phosphate buffer at pH of 6, 7 or 8 and ionic strength of 0.2 or 0.016 M (set with NaCl)

⁵⁾ Artificial sea water was prepared as described by Kester et al.⁷. Axenic cultures of the diatom *Thalassiosira weissflogii* (t.w; culture 1.26-15, National Oceanic and Atmospheric Administration, Milford, US) were grown at f/4 for trace metals and f/2 for other nutrients⁸. The culture was purged with N₂ to remove the excess oxygen (“sea water intact cells”) and a subsample was then sonicated at cycles of 3 seconds ON:OFF for 2 minutes while cooling on ice. The cell wall material and organelles (i.e. nuclei and mitochondria) was then isolated from the cytoplasm (“sea water cytoplasm”) by centrifugation at 754 G⁹ for 15 min and resuspended in artificial sea water (“sea water cell walls”). Formation of (CH₃)₂Hg from CH₃Hg and FeS_m(s) was tested by incubating 100 μL of a CH₃Hg(aq) solution with 500 μL of a FeS_m(s) slurry in 2 ml of artificial sea water or the “sea water intact cells”, “sea water cell wall” or “sea water cytoplasm” media.

⁶⁾ the amount of the minerals was adjusted to give a surface area of 9.9·10⁻³ m²

⁷⁾ CdS(s) precipitated with excess of Cd (Cd:S ratio of 2:1)

Supplementary Methods

Synthesis of $(\text{CH}_3)_2^{200}\text{Hg}(\text{aq})$. The $(\text{CH}_3)_2^{200}\text{Hg}$ standard was manufactured in house from $^{200}\text{HgCl}_2$ and 3M methyl magnesium chloride in tetrahydrofuran (Alfa Aesar)¹⁰. A $^{200}\text{HgCl}_2(\text{aq})$ solution was heated until dryness in a glass tube. The tube was then put in an ice bath, 3M methyl magnesium chloride in tetrahydrofuran was added and the reaction was left overnight at room temperature. The reaction was cooled on ice before quenching the reaction by addition of water saturated with NaCl (exothermic reaction, added dropwise). The organic layer (tetrahydrofuran containing $(\text{CH}_3)_2^{200}\text{Hg}$) was then extracted using a Pasteur pipette and transferred to a new tube which was stored at -4°C . Working solutions of $(\text{CH}_3)_2^{200}\text{Hg}(\text{aq})$ were prepared by dilution of the stock solution in purified MQ.

Supplementary References

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