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Methylation and Demethylation of Mercury Under Controlled Redox, pH, and Salinity Conditions†

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In estuarine sediments, the microbially mediated processes of methylation, demethylation, and volatilization determine the state and overall toxicity of mercury pollutants. The effects of redox potential (E_h) and salinity on the above microbial processes were investigated in reactors constructed to allow for continuous monitoring and adjustment of the pH (6.8) and E_h of freshly collected estuarine sediments. For measurements of methylation and demethylation activity, sediment slurries adjusted to appropriate salinity were spiked with $HgCl_2$ or CH_3HgCl, respectively, and were incubated in the reactors. Methylmercury was measured by gas chromatography. Volatilized elemental mercury (Hg^0) was trapped and determined by cold vapor atomic absorption spectrometry. Volatilization of Hg^0 and CH_3HgCH_3 were found to be minimal. Methylation of Hg^{2+} was favored at E_h −220 mV as compared to +110 mV. At −220 mV, high salinity (2.5%) inhibited methylation, and low salinity (0.4%) favored it. At +110 mV, the salinity effect was less pronounced. Demethylation of CH_3HgCl was favored at +110 mV regardless of the salinity level. Low redox potential under low salinity conditions inhibited demethylation, but high salinity reversed this inhibition. These findings are helpful for interpreting and predicting the behavior of mercury pollutants in estuarine sediments.

Current industrial use of mercury has been estimated to be 12,500 metric tons (1.25×10^7 kg) per year. A substantial part of this and probably a much larger amount from the combustion of fossil fuels are released into the environment (7). Mercury is more insidious than most other heavy metal pollutants because of its propensity to be methylated, yielding the highly neurotoxic and biomagnification-prone monomethyl mercury (7, 13, 19, 23). Methylation of mercuric ions (Hg^{2+}) occurs predominantly in aquatic sediments and to a lesser extent in the water column and in soils (7, 15, 16, 19). Small amounts of methylmercury may be formed by abiotic transmethylation from humic compounds (11, 12), but in aquatic sediments, the resulting methylmercury concentrations are at least an order of magnitude lower than those that result from biomethylation (M. H. Berman and R. Bartha, unpublished data). Strains from wide range of microorganisms, including strict and facultative anaerobes as well as aerobes, have been shown capable of in vitro methylation of Hg^{2+} (7, 19), but whether these are the same organisms that are responsible for biomethylation of Hg^{2+} in sediments is at present only a matter of speculation.

Monomethylmercury is removed from sediments by reduction to volatile elemental mercury and perhaps also by disproportionation to volatile dimethyl mercury by H_2S, although the significance of the latter reaction is yet to be demonstrated under in situ conditions (7, 19). The relative rates of methylation and demethylation processes determine the equilibrium of environmental methylmercury concentrations. It is important to recognize that there is as yet no effective way of separating methylation from demethylation in sediments, and thus, experiments always measure the net result of the two opposing processes rather than one of them alone. Since monomethylmercury is the most hazardous and undesirable form of the pollutant, it is important to know how environmental parameters influence its synthesis and destruction.

On thermodynamic grounds, Wood (22) predicted that methylation of Hg^{2+} by methylcobalamin would be favored at low pH and positive redox potential. Several reviews (9, 19) discuss aerobic and anaerobic methylation of Hg^{2+}, but in none of the cited studies was the redox potential of the incubation system actually measured or controlled. A brief initial report on a reverse correlation of sediment salinity with Hg^{2+} methylation was published by Blum and Bartha (4). Estuarine sediments, according to their location and depth, exhibit wide ranges of salinities and redox potentials. Since the estuaries of the U.S. Atlantic sea coast are subject to various kinds of chemical pollutants, including mercury compounds (14), we undertook to measure the dependence of Hg^{2+} methylation in estuarine sediments on the prevailing redox potential, salinity, and pH.

MATERIALS AND METHODS

Collection and incubation of sediments. Salt marsh sediment was collected from intertidal mud flats in Cheesequake State Park, N.J. at a landward (0.4% salinity) and a seaward (2.5% salinity) site. As determined by ignition, the organic matter content of the oven-dried sediments was 17 to 20%. Salinity was measured with a salinometer (model 33; Yellow Springs Instrument Co., Yellow Springs, Ohio) calibrated by chloride titration (2). The in situ pH and redox potentials of the sediment were measured with a Beckman model G pH meter with a calomel reference electrode against a glass universal electrode for pH and two platinum electrodes (Fisher Scientific Co., Springfield, N.J.) for redox potential. The pH was 6.8 for all samples regardless of depth; the redox potentials were +110 for the upper 2 cm and −220 mV for sediment from a depth of 5 to 20 cm. Sediments from these two depths were collected in plastic core liners (5 by 25 cm) with a hand corer (Wildco Instruments, Saginaw, Mich.). The anaerobic cores were immediately sealed. The sedi-

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ments were used in experiments within 2 h of their collection.

Four reactors were constructed based on a design by Patrick et al. (18) with some modifications. The reactors (Fig. 1) consisted of 2.5-liter glass kettles (Fisher) with electrodes and sampling ports fitted into the covers with silicone stoppers. A calomel electrode served as reference for the glass universal pH electrode and the platinum redox electrodes. The electrodes were connected to a pH-millivolt meter (Accumet 520; Fisher), providing a continuous record of the redox potential. The pH was checked every 6 to 10 h and, if necessary, adjusted by the addition of 1 N NaOH or HCl through the gassing port. Thus, pH was kept within 0.2 units of the in situ value (6.8).

In the laboratory, the collected sediment samples were processed in a manner to preserve their in situ redox potential. Anaerobic samples were immediately placed in a 6500 PACE anaerobic chamber (Lab-Line Instruments, Melrose Park, Ill.), extruded from the core liners, and slurried with water that had been adjusted to the salinity of the collected sediment with a commercially available sea salts mixture (5). The salt solutions were previously deoxygenated by boiling and cooling under nitrogen passed over a hot copper column. Aerobic sediments were slurried in a similar manner except that no attempt was made to avoid exposing them to air. The resulting slurries were introduced into the

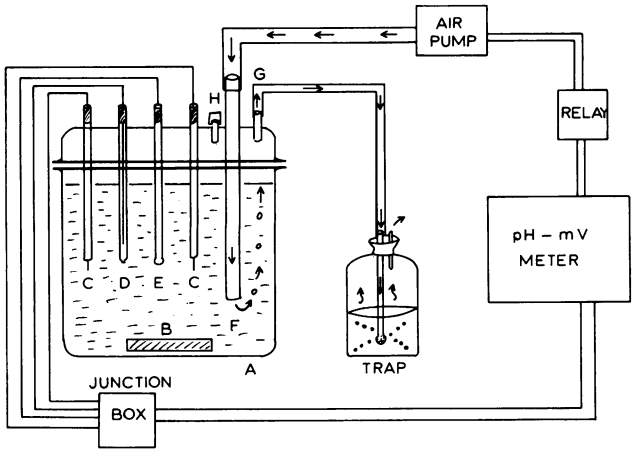

FIG. 1. Reactor assembly, modified from Patrick et al. (18) for measurement of mercury transformations in sediment slurries under controlled redox, pH, and salinity conditions. (A) Glass kettle with removable top, sealed with teflon gasket, polyvinyl tape, and clamp; (B) magnetic stirring bar; (C) platinum electrodes; (D) calomel reference electrode; (E) glass pH electrode; (F) glass standpipe for aeration and sampling of slurry; (G) vent pipe to trap for volatile forms of mercury; and (H) port for gassing with oxygen-free N_2. Operated in the aerobic mode, a drop of the redox potential below +110 mV activated, through a relay switch, an air pump. Air was introduced at a slow rate through a needle valve until the redox potential rose to +110 mV, shutting off the pump. In the anaerobic mode, the in situ redox potential (−220 mV) of the subsurface salt marsh sediment was maintained spontaneously in the sealed reactor under an N_2 atmosphere. The headspace of the reactors was sampled for volatile forms of mercury by flushing them into a trap. Under anaerobic conditions, oxygen-free N_2 was used for this purpose. The slurry was sampled by temporarily sealing the vent pipe and forcing the slurry to rise in the standpipe (F) by gas pressure (air or nitrogen) introduced through the gassing port (H). The sediment slurry was kept homogeneous by continuous magnetic stirring (300 rpm); incubation was at 20°C in the dark.

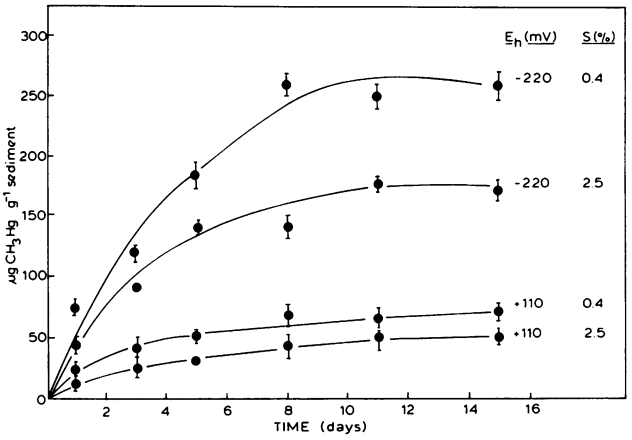

FIG. 2. Methylation of 100 μg of $HgCl_2$ g of salt marsh sediment^{-1} in relation to redox potential and salinity.

reactors, which were then sealed gas-tight, and were incubated at 20°C with continuous magnetic stirring. The in situ redox potential and pH of the sediments were found to remain unchanged during these manipulations.

The incubation of the sediments in the reactors had several advantages. The electrode system allowed a continuous monitoring and adjustment of pH and redox potential. The gas space above the sediment slurry could be sampled for volatile forms of mercury (Hg^0 and CH_3HgCH_3), and the slurry could be sampled without disturbing its redox potential.

Analytical procedure. Monomethylmercuric chloride was purchased from Matheson, Coleman, and Bell (Norwood, Ohio) monomethylmercuric iodide was purchased from Pfalz and Bauer (Flushing, N.Y.), and dimethylmercury was purchased from Eastman Kodak (Rochester, N.Y.). The solvents used were pesticide grade; the acids were certified mercury-free. Monomethylmercury was extracted from sediments by the method of Longbottom et al. (10) and measured by gas chromatography with an EC detector (5). Methylmercuric iodide was used as a standard; the detection limit was 0.02 ng of CH_3HgI μl^{-1}.

Volatile dimethylmercury was flushed from the reactor headspace and trapped in an aqueous solution containing 10% KBr, 1.5% $HgBr_2$, and 0.1% $CuSO_4$. The trap solution was acidified to pH 1.0 and then extracted by the procedure of Longbottom et al. (10), and the resulting monomethylmercury was quantified by gas chromatography (5).

Volatile elemental mercury was flushed from the headspace in separate experiments and trapped in a solution of $KMnO_4$ and H_2SO_4 (8). Mercury in the traps was reduced, volatilized, and quantified by the cold vapor atomic absorption technique (21) with an atomic absorption spectrometer (model 603; Perkin-Elmer, Norwalk, Conn.). All mercury concentrations were calculated per gram of dry sediment. The sediment slurries in the reactors contained ca. 120 mg of dry sediment ml^{-1}, the highest sediment concentration that could be kept homogeneously dispersed by magnetic stirring. All experiments were performed in duplicate and averaged. The error bars in the figures represent the range of duplicate measurements.

RESULTS

Redox potential and salinity. The pH of the salt marsh sediments studied was 6.8 and did not vary with sampling

location or depth profile. For this reason, this parameter was monitored and kept constant but was not adjusted to different values.

In slurries spiked with 100 μg of $HgCl_2$ g of sediment^{-1}, Hg^{2+} methylation showed a strong dependence on redox potential and a somewhat lower dependence on salinity (Fig. 2). Of the variables tested, the combination of low (−220 mV) redox potential and low (0.4%) salinity was the most conducive to methylmercury synthesis, as evidenced both by the highest net rate of methylation and by the relatively high equilibrium methylmercury concentration attained. At high salinity (2.5%) and low redox potential, methylation activity was lower by 38%. In comparison, at a positive redox potential (+110 mV) and low salinity, methylation of Hg^{2+} was strongly repressed. High salinity at +110 mV appeared to cause a further reduction in methylation, but because of the low activities involved, the significance of the latter effect was less certain. Concentrations of methylmercury approached a steady state after 8 to 12 days of incubation. At this point, the addition of a second identical $HgCl_2$ spike resulted in renewed methylmercury synthesis at the previous rate, approximately doubling the steady-state concentrations in each case (data not shown).

The effect of salinity on Hg^{2+} methylation, reported by Blum and Bartha (4) and confirmed in this study, required some interpretation as to its mechanism. The anionic components of sea salts have an effect on the speciation of mercury in aquatic systems, influencing the transfer of methyl groups from methylcobalamin (5). They also affect the microbial community that interacts with mercury. Since preliminary experiments (unpublished data) implicated the sulfate reduction process as being chiefly responsible for the inhibition of Hg^{2+}-methylating activity at high-salinity and low-redox-potential conditions, in a separate experiment, we compared the inhibitory effect of total sea salts to that by sulfate alone. The concentration of sulfate in full-strength sea water is 2.7 g liter^{-1} (20). At 2.5% salinity, the corresponding sulfate concentration is 2.1 g liter^{-1}. Added at this level to low-salinity sediment (Fig. 3) and incubated at low redox potential, sulfate alone inhibited the methylation of Hg^{2+} in the same manner as did total sea salts. At high redox potential, SO_4^{2-} (2.1 g liter^{-1}) did not inhibit Hg^{2+} methylation (data not shown).

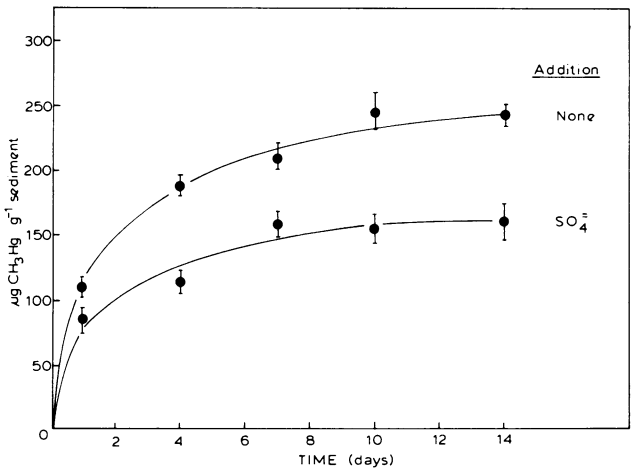

FIG. 3. Methylation of 100 μg of $HgCl_2$ g of saltmarsh sediment^{-1} at E_h −220 and 0.4% salinity, with or without SO_4^{2-} (2.1 mg ml^{-1}).

TABLE 1. Cumulative volatilization of Hg^0 and CH_3HgCH_3 from low-salinity (0.4%) estuarine sediments spiked with 100 μg of $HgCl_2$ g of sediment^{-1}

Day	Volatilization[a]			
	Hg^0		CH_3HgCH_3	
	+110 mV	−220 mV	+110 mV	−220 mV
0.1	26.0	15.0		
0.5	28.5	15.8		
1	29.2	16.1	ND	ND
2	29.6	16.3	ND	ND
4	29.9	16.3		
7	30.5	16.3	ND	5.9
10			ND	15.3

[a] All data are in nanograms per gram of sediment. ND, Not detected.

In our reactor studies, volatilization of elemental (Hg^0) and dimethylmercury (CH_3HgCH_3) from low-salinity sediments was minimal (Table 1). Slightly more Hg^0 was released at +110 mV than at −220 mV. The bulk of the volatilized Hg^0 was trapped during the first 3 h of the experiment, indicating either that the reduction was nonbiological or that Hg^{2+} was rapidly converted to a form that was no longer susceptible to reduction. Production of dimethylmercury was detectable only under anaerobic conditions and only in extremely low amounts.

Hg^{2+} concentration. In the relatively unpolluted Cheesequake salt marsh sediment, methylmercury before spiking was below the detection limit. An experiment was conducted to determine the effect of various levels of Hg^{2+} spiking on methylmercury synthesis (data not shown). In low-salinity anaerobic sediments, concentrations of $HgCl_2$ from 1 to 300 μg g of sediment^{-1} had no perceivable inhibitory effect on the methylation process. The absolute concentrations of methylmercury formed by day 14 increased from 0.020 to 0.700 μg g of sediment^{-1}, but the relative amount converted to methylmercury declined from 2.60 to 0.23% with increasing Hg^{2+} concentrations. A steady-state concentration of methylmercury was not attained at the highest level of Hg^{2+} addition before the termination of the experiment.

Demethylation. Microbial demethylation activity obviously influences the equilibrium methylmercury concentrations attained in estuarine sediments, and the effects of redox potential and salinity on this process were also measured (Fig. 4). When spiked with monomethylmercuric chloride (CH_3HgCl) at the level of 1 μg g of sediment^{-1} and incubated

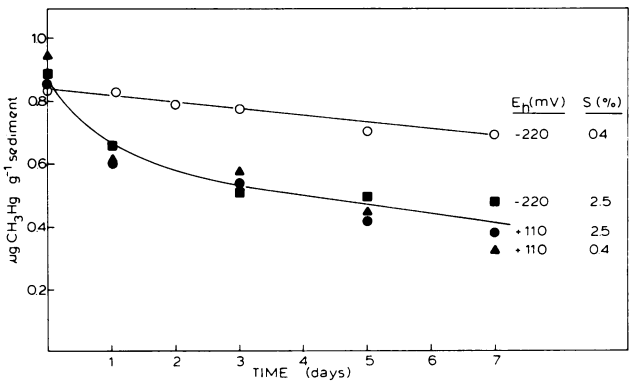

FIG. 4. Demethylation of 1 μg of CH_3HgCl_2 g of saltmarsh sediment^{-1} in relation to redox potential and salinity.

for 7 days, only 15% of the added methylmercury was removed at −220 mV and 0.4% salinity. All other combinations resulted in the removal of 55 to 65% of the added monomethylmercury.

DISCUSSION

Reactors. The performance of the reactors was highly consistent. Whenever repeated with newly collected sediment batches, the described results were accurately reproduced, in respect to both time course and equilibrium methylmercury concentrations. In earlier experiments performed in our laboratory, the methylmercury concentrations went through a maximum between days 8 and 12 of incubation and subsequently declined (4). The same phenomenon was reported by other authors (17). We have suspected this to be an artifact of sample preparation that exposed the previously anaerobic sediment to some oxygen in spite of certain precautions (4). The practices of collecting and transporting the sediments in sealed core liners and performing the slurry preparation steps in an anaerobic chamber consistently eliminated the decline in methylmercury concentration after a maximum value was attained. Therefore, it appears that the previously observed decline in concentration was an artifact of sample preparation rather than a successional phenomenon in response to the accumulation of methylmercury.

Redox potential. Wood predicted that aquatic environments with positive redox potential and low pH would favor Hg^{2+} methylation (22). Bisogni and Lawrence (3), using reactors that simulated aerobic and anaerobic sewage treatment, found rates of methylmercury synthesis under aerobic conditions approximately twice as high as those under anaerobic conditions. On the other hand, Fagerstrom and Jernelov (6), working on freshwater sediments, and Olson and Cooper working on marine sediments of San Francisco Bay (17), found higher Hg^{2+} methylation activity and higher persistence of methylmercury under anaerobic conditions than under aerobic incubation conditions. Preliminary experiments on Hg^{2+} methylation in our laboratory (J. Blum and R. Bartha and G. Compeau and R. Bartha, unpublished data), in which estuarine sediment samples were used under aerobic and anaerobic incubation conditions but without an actual measurement of the redox potential, pointed in the same direction. Our present results, obtained at precisely controlled redox potentials, leave no doubt that in estuarine sediments methylation of Hg^{2+} and persistence of the methylmercury formed are both favored at −220 mV as compared to +110 mV. The negative redox potential appears to favor Hg^{2+} methylating versus demethylating microbial populations and this factor seems to override any negative effect this redox potential may have on the availability of Hg^{2+} as a recipient for carbanion from methylcobalamin (22).

Salinity. Our results confirm and extend an earlier report on the inverse correlation of salinity with Hg^{2+} methylation (4). They also lend further support to the theory that H_2S produced from sulfate in low redox potential environments represents the principal mechanism by which salinity interferes with Hg^{2+} methylation. Added methylmercury was less stable under high-salinity than under low-salinity conditions. The mechanisms of monomethylmercury depletion were not investigated in this experiment.

Hg^{2+} concentrations. Our Hg^{2+} spiking levels (10 to 300 μg of $HgCl_2$ g of sediment^{-1}) appear to be relatively high. It should be noted, however, that the sediment was diluted in the slurry (120 mg of sediment ml of slurry^{-1}) and, therefore, the actual per volume $HgCl_2$ concentrations were considerably lower (0.12 to 36 μg of $HgCl_2$ ml of slurry^{-1}). In sediments, Hg^{2+} binds competitively to microorganisms, organic matter, and clay (7, 19), and thus, Hg^{2+} toxicity in the presence of sediment is greatly diminished. Enrichment cultures and isolates originating from sediment rarely grew above 50 μg of $HgCl_2$ ml^{-1} if sediment was not present, and Hg^{2+}-methylating activity was inhibited above 10 μg of $HgCl_2$ ml^{-1} (unpublished data). Up to the highest $HgCl_2$ concentration tested, no inhibition of methylation was evident in the sediment slurry. The percentage of the total mercury converted to monomethylmercury declined with increasing spiking levels. This phenomenon was noted also by other authors (1, 7, 9, 19).

Environmental implications. Our experiments demonstrate that in estuarine sediments, the synthesis and stability of monomethylmercury are enhanced under anaerobic, low-salinity conditions. Conversely, aerobic, high-salinity conditions are either less favorable for monomethylmercury synthesis or are more conducive to its destruction. The mechanisms behind the observed effects are only partially known. The redox effects are probably linked to the types of microbial metabolism they permit. The observed salinity effects (4) are largely explainable with the reduction of sulfate to sulfide and the very limited ability of the resulting HgS to serve as methyl group acceptor (7, 9, 19). To a much lesser extent, the carbonate-bicarbonate anions of seawater also interfere with Hg^{2+} methylation (5). Instability of monomethylmercury under anaerobic, high-salinity conditions may be due either to disproportionation by H_2S to volatile dimethylmercury or to reduction to Hg^0 (7, 19). In the presence of H_2S, Hg^0 would precipitate as HgS rather than volatilize. Our previous findings (5) on the stability of monomethylmercury in presence of H_2S is not conclusive in this respect, since any dimethylmercury formed in our closed test system would have been converted back to monomethylmercury by our analytical procedure. High sulfide levels not only occur in reduced marine sediments but are frequently the result of industrial or domestic wastewater discharges. In such a case, Hg^{2+} methylation was almost completely supressed, although the salinity of this anaerobic sediment was low (Berman and Bartha, unpublished data). Microbiota in aquatic sediments that are reducing but low in sulfide seem to be most prone to convert inorganic mercury pollutants to the more dangerous monomethylmercury form.

ACKNOWLEDGMENTS

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