Mercury Speciation in Marine Sediments under Sulfate-Limited Conditions

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Sediment profiles of total mercury (Hg) and monomethylmercury (MMHg) were determined from a 30-m drill hole located north of Venice, Italy. While the sediment profile of total Hg concentration was fairly constant between 1 and 10 m, that of the MMHg concentration showed an unexpected peak at a depth of 6 m. Due to the limited sulfate content (<1 mM) at the depth of 6 m, we hypothesized that the methylation of inorganic Hg(II) at this depth is associated with the syntrophic processes occurring between methanogens and sulfidogens. To test this hypothesis, anoxic sediment slurries were prepared using buried Venice Lagoon sediments amended with $HgCl_2$, and we monitored MMHg concentration in sediment slurries over time under two geochemical conditions: high sulfate (1−16 mM) and limited sulfate concentrations (<100 μM). After day 52 and onward from the addition of inorganic Hg(II), the MMHg concentrations were higher in sulfate-limited slurries compared to high sulfate slurries, along with methane production in both slurries. On the basis of these results, we argue that active methylation of inorganic Hg(II) occurs under sulfate-limited conditions possibly by syntrophic processes occurring between methanogens and sulfidogens. The environmental significance of syntrophic Hg(II) methylation should be further studied.

Introduction

Monomethylmercury (MMHg), which accumulates in marine food chains, exhibits potential neurotoxicity to humans and animals, exerted in part through biochemical alterations in protein synthetic activity (*1*). While the main source of MMHg in the oceanic environment is not yet clearly understood (*2, 3*), sediment seems to be one of the major sources of MMHg in coastal environments (*4, 5*). For example, Hollweg et al. (*5*) demonstrated that MMHg flux from coastal sediment is of the same order as that from other sources. Consequently, MMHg production and its distribution in coastal sediments has been widely studied (ref *4* and references therein). Even though the full mechanism of Hg(II) methylation is not yet known, a methyl transfer mechanism associated with enzymatic activities involved in the acetyl-CoA pathway has been proposed for *Desulfovibrio desulfuricans* LS (*6, 7*). In a later study, a number of incomplete oxidizing strains of sulfate-reducing bacteria were found to methylate Hg(II) independently of the acetyl-CoA pathway, suggesting that the Hg(II) methylation mechanism could be a more generic phenomenon associated with carbon metabolism (*8*).

Sulfate concentration in sediment pore water appears to be one of the main factors governing Hg(II) methylation capacity (*9*−*12*). For example, addition of sulfate to sediment stimulated the Hg(II) methylation rate when it also increased sulfate reduction rate and population density of sulfate-reducing bacteria (*13, 14*). Additionally, a positive kinetic relationship between sulfate reduction and Hg(II) methylation rates has demonstrated the existence of a tight coupling between the two processes (*15*). In contrast, an inverse correlation between sulfate reduction and Hg(II) methylation rates was observed in wetland sediments, indicating that sulfide produced from sulfate reduction inhibits microbial Hg(II) methylation (*16*). A solution speciation of Hg, in the form of decreasing neutral Hg-sulfide species with increasing sulfide, was suggested as a reason for the negative effect of sulfide on Hg(II) methylation (*17, 18*). Consequently, sulfate could have a positive or negative effect on the methylation of Hg(II), depending on the geochemical conditions of sediment.

Mercury(II) methylation occurring in sulfate-limited sediments may be attributable to organisms other than sulfidogens. For example, the inhibition of sulfate reduction using molybdate resulted in negligible changes in MMHg production in low sulfate (40−90 μM), anoxic lake sediment slurries (*19*). In the same study, pure cultures of a *Geobacter* sp. strain were able to methylate Hg(II) at an environmentally significant rate that was comparable to that of sulfidogens, suggesting that Hg(II) methylation in sulfate-limited sediments could be driven by Fe(III)-reducing organisms. Another example of Hg(II) methylation in sulfate-limited conditions is given by Pak and Bartha (*20*), who showed that Hg(II) can be methylated at a significant rate when *Desulfovibrio desulfuricans* and *Methanococcus maripaludis* are grown together in a sulfate-free lactate medium. Importantly, neither strain was capable of Hg(II) methylation when grown in isolation under identical conditions. Sulfidogens are well-known to gain energy by oxidizing organic matter to hydrogen and acetate, which in turn is utilized as a substrate by methanogens. The continuous removal of hydrogen by methanogens makes it possible for sulfidogenic bacteria to coexist with methanogenic bacteria (*21*). Although active Hg(II) methylation by interspecies hydrogen transfer has been demonstrated in pure culture systems, the significance of this mechanism in natural sediments has not yet been evaluated.

In oligotrophic fresh water and marine sediments, where energy flow is determined predominantly by methanogens rather than sulfidogens, Hg(II) methylation may be largely

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attributable to a syntrophic association between these two organism types. This association would be particularly conducive to Hg(II) methylation, in contrast to high-sulfate conditions, as a result of the decreased potential for sulfide inhibition (*22*). In the present study, we report on the finding of high MMHg concentrations in the sulfate-limited marine sediments of the Venice Lagoon, Italy. In order to assess the role of methanogens in the production of MMHg, four sets of anoxic sediment slurries were prepared using buried Venice Lagoon sediments (6−7 m depth) amended with $HgCl_2$ (2 μM). Approximately 15 mM of sulfate was added to one set of sediment slurries so that sulfate reduction would represent the major energy transfer process. In contrast, the energy transfer of the other set of slurries (<50 μM sulfate) was considered to mostly depend on methanogens. Monomethylmercury concentrations were then monitored over time in each set of slurries.

Materials and Methods

Drill Hole Sampling. Drill hole SM3 is located to the northwest of Venice, in close proximity to a dredged canal and approximately 3 km east of Site C (Supporting Information, S1). The 30 m-long core was collected in January 2006, sliced at 1 m intervals in the field, and transported to the Thetis SPA laboratory (Venice, Italy) where the shallower top 10 cm of each 1 m core was collected under a N_2-filled glovebox. Each 10 cm sediment sample was centrifuged (5000 rpm for 15 min), the centrifuge bottles were returned to the glovebox, and the supernatant fluids were filtered into Teflon bottles, using 0.45 μm pore size syringe filters (polyethersulfone, PES, membrane). Approximately 10−20 mL of the filtered pore water sample was acidified (0.5% v/v) using trace metal grade HCl solution (12 N) for the analysis of dissolved total Hg (THg) and iron; the remainder was stored for measurements of chloride, alkalinity, sulfate, ammonium, and phosphate. The remaining sediment slices were stored frozen for analyses of sedimentary Hg, MMHg, and other metals.

Pore Water and Mercury Analysis. The analytical methods for pore water geochemistry are listed in the Supporting Information, S2. Analysis of total Hg (THg) in sediment and pore water was carried out using cold vapor atomic fluorescence spectrometry (CVAFS) after appropriate pretreatment of samples (*23, 24*). Analytical details are shown in Supporting Information, S3. Monomethylmercury was extracted from sediments using dichloromethane, as described previously (*25, 26*), and analytical details are presented in Supporting Information, S3. The particle−water distribution coefficients (K_d) of Hg were calculated as follows: K_d = (mass of filter-retained Hg/mass of solids)/(mass of filter-passing Hg/volume of water) = C_s (mol/kg)/C_w (mol/L).

Sediment Microcosm Setup. Preparation methods for anaerobic media are listed in Supporting Information, S4, and Fennell et al. (*27*). Details in slurry preparation methods are also given in Supporting Information, S4. Briefly, four sets of triplicate vials were established for sulfidogenic conditions (S1, S2, S3), sulfidogenic-methanogenic conditions (SM1, SM2, SM3), molybdate added controls (M1, M2, M3), and autoclaved controls. Slurry samples collected every 4 or more days from each vial were centrifuged and the aqueous supernatant phases were filtered through 0.45-μm syringe filters (PES membrane). Filtered supernatants were preserved for the measurement of sulfate, sulfide, THg, and Fe. Dissolved THg and Fe samples were treated with HCl solution (0.5% v/v), and dissolved sulfide samples were treated with Zn-acetate (20 mM). The remaining sediment samples were freeze-dried prior to MMHg analysis. Headspace samples were also collected and stored in crimp-sealed vials to monitor the production of methane, which was measured using an Agilent 3000 Micro GC with helium as the carrier gas. An additional electron donor, 30 mM lactate, was spiked on day 13 and 36 to prevent organic carbon limitation. The solution preparation, sediment slurry incubation, and slurry collection and filtration processes were all carried out in an N_2-filled anaerobic chamber.

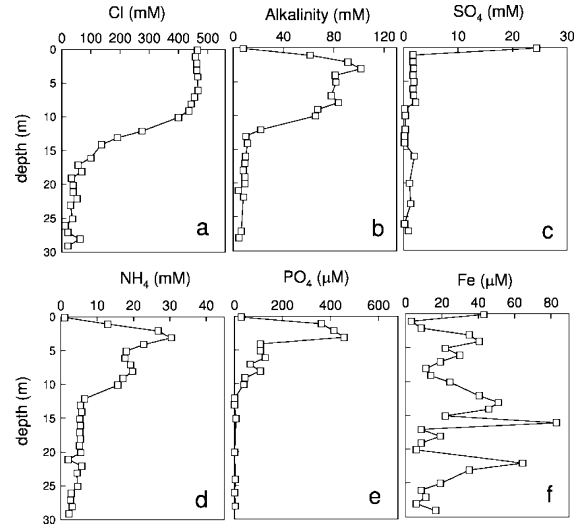

FIGURE 1. Sediment pore water profiles of chloride, alkalinity, sulfate, ammonium, phosphate, and iron in the drill hole SM3.

Fluorescence in Situ Hybridization. On day 59, one sample from each experimental condition was processed for fluorescence in situ hybridization (FISH) using domain-specific universal probes (EUB338 and Arc915 (*28*)) to distinguish archaea from bacteria. Analytical details are listed in Supporting Information, S5.

Results and Discussion

Pore Water Geochemistry. Figure 1 shows analytical results for chloride, alkalinity, sulfate, ammonium, phosphate, and dissolved iron. Chloride remained constant in the upper 6 m at a concentration of ~470 mM, gradually decreasing thereafter until 9 m (430 mM) and showing a sharp decrease toward 12 m (280 mM; Figure 1a). The sediment at 11 m did not yield pore fluids; thus the sediment layer between 11 and 11.5 m could be considered as a layer that retards diffusion between the upper and lower sediments. Indeed, below 12 m, Cl values tended to decrease to ~50 mM or less, probably because of the presence of several freshwater aquifers. The depletion of sulfate in pore water between 0 and 1 m was accompanied by extensive enrichment in alkalinity, ammonium, and phosphate (Figure 1b−1e), suggesting that the regeneration of these nutrients was mainly driven by organic matter decomposition, which is typical for anoxic sediments rich in organic matter (*29*). We argue that the upper 3−4 m of the sediment column is of fairly recent origin, and is a result of redeposition during dredging operations as confirmed by the presence of trace metals such as Cu and Zn in the sediments (see Supporting Information, S6). The freshwater zone (below 14 m) was clearly characterized by relatively low ammonium and phosphate content. Dissolved iron concentrations in the sediment pore waters were highly variable (Figure 1f). In the upper 10 m, this can best be understood in terms of the potential complexation of reduced iron (Fe^{2+}) by humic substances, which are known in general to have strong binding capacities, leading to increased Fe solubility (*30, 31*).

Total Mercury and Monomethylmercury. The lagoonal section (uppermost 10 m) of the core showed high THg concentrations (890−1440 ng g^{-1}; Figure 2a), much higher than those found in pristine estuarine sediments (*4*). The largest single source of Hg in the Venice Lagoon sediment is known to be the aqueous discharge of Hg that came from

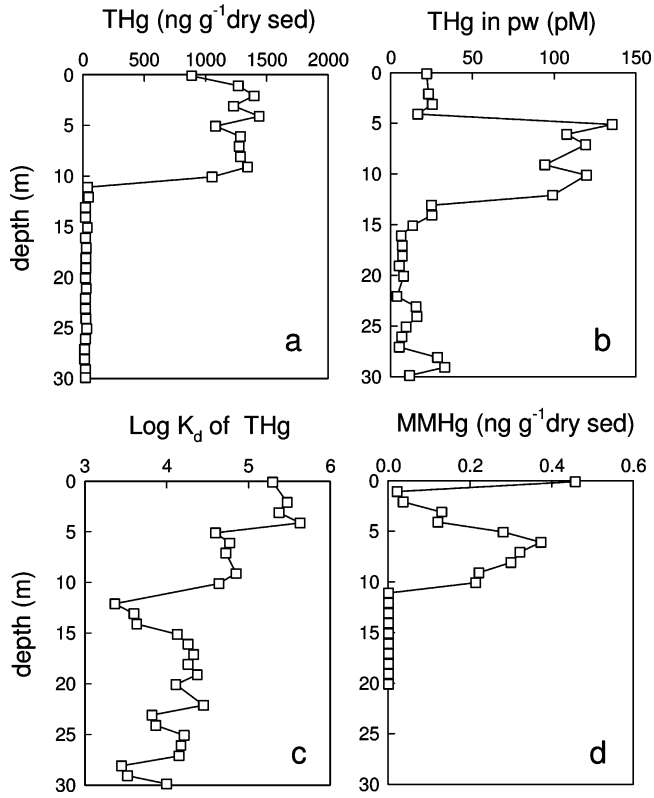

FIGURE 2. Vertical profiles of total Hg (THg) in dry sediment (a), dissolved THg in pore water (b), particle−water partition coefficient, K_d, of THg (c), and monomethylmercury (MMHg) in dry sediment (d).

chloro-alkali plants between the 1950s and 1980s (*32*). Similarly, the concentrations of Zn and Cu were enriched in the upper 4−5 m of the sediment column (Supporting Information, S6), indicating influences of recent industrial inputs since the early 1930s (*33*). The Isonzo River has been one of the largest sources of Hg for the northern Adriatic Sea since the 16th century because of the Hg mining activity in Idrija, Slovenia (*34, 35*). High concentrations of THg were also found in Grado and Marano Lagoon, located along the east coast of the Venice Lagoon (*35*). This mining source might explain the enriched THg found at a depth of 6 to 10 m of the core, if sediments were reworked after mining activity. Thus, we expect that drill hole sediment may have been reworked several times by anthropogenic activities.

Dissolved THg showed a different profile from sediment THg, with three zones being distinguished (Figure 2b): a zone from 0 to 4 m with relatively low dissolved THg (17−26 pM); a zone from 5 to 12 m with relatively high dissolved THg (94−136 pM); and a zone below 12 m with very low THg concentrations, often <10 pM. The solubility of Hg in estuarine sediment is mainly controlled by sediment characteristics, such as the content of solid organic matter, acid volatile sulfide (FeS), and pyrite (*36*−*38*). For example, the mobility of Hg was shown to be influenced by Hg adsorption onto amorphous FeS and organosulfide in lake sediments (*36*). The logarithm of the K_d, an average of 5.5 ± 0.14 between 0 and 4 m and 4.7 ± 0.10 between 5 and 10 m, clearly indicates that sediment characteristics are different below a depth of 4 m (Figure 2c). The presence of abundant pyrites and acid volatile sulfide (AVS) between 0 and 4 m may be one reason for this distinction. In fact, the log K_d of 5.5 is similar to the value found at the surface (top 20 cm) of site C (*39*). A large sulfate consumption between the surface and 1 m implies that there is enhanced production of H_2S that reacts with buried iron oxide (*40, 41*). This reaction would initially lead to an AVS, which would subsequently be converted into pyrite (*42*). Between 1 and 4 m, dissolved sulfide produced from the limited sulfate (downward diffusing sulfate (*41*)) may be trapped by Fe and produce AVS and pyrites (*41*). For example, in Black Sea sediment, most of methane-derived sulfide (sulfide produced from a SO_4^{2-}-CH_4 transition zone) was drawn downward to a sulfidization front where it reacted with Fe(III), resulting in the production of a black band of amorphous FeS, which may correspond to a depth of 4 m in our present case (*41*).

The highest MMHg concentration (0.46 ng g^{-1}) was found at the surface with the maximum amount of sulfate, 27 mM (Figure 2d). Monomethylmercury then decreased from the surface down to 1 m (0.02 ng g^{-1}) but, unexpectedly, increased again from 1 to 6 m, (0.37 ng g^{-1}) remaining relatively high (0.21−0.32 ng g^{-1}) until a depth of 10 m. We propose that Hg(II) methylation between 6 and 10 m is attributable to the large availability of dissolved THg (Figure 2b) and syntrophic associations between methanogens and sulfidogens (*21*), since methanogenesis appears to be a main terminal electron accepting process at this depth (5−10 m). This hypothesis was tested, and the results of this test are described in the next section.

Sediment Microcosm Experiment. Figure 3 shows results from sediment slurry incubation experiments designed to understand the role of microbial interactions in the control of MMHg production. Figure 3, parts b and d, shows MMHg concentration in dry sediment (left *y*-axis) and MMHg concentration in dry sediment normalized to dissolved THg concentration (right *y*-axis). The absence of MMHg in autoclaved controls (Supporting Information, S7) provides evidence for microbial MMHg production in S and SM slurries. Although we did not measure the initial rates of methylation and demethylation, we argue that the steady-state MMHg concentration is primarily controlled by the Hg(II) methylation rate. A number of studies have demonstrated that the steady-state MMHg concentration (or %MMHg/THg in sediment) can be used as a proxy for the in situ rate of Hg(II) methylation (*43, 44*).

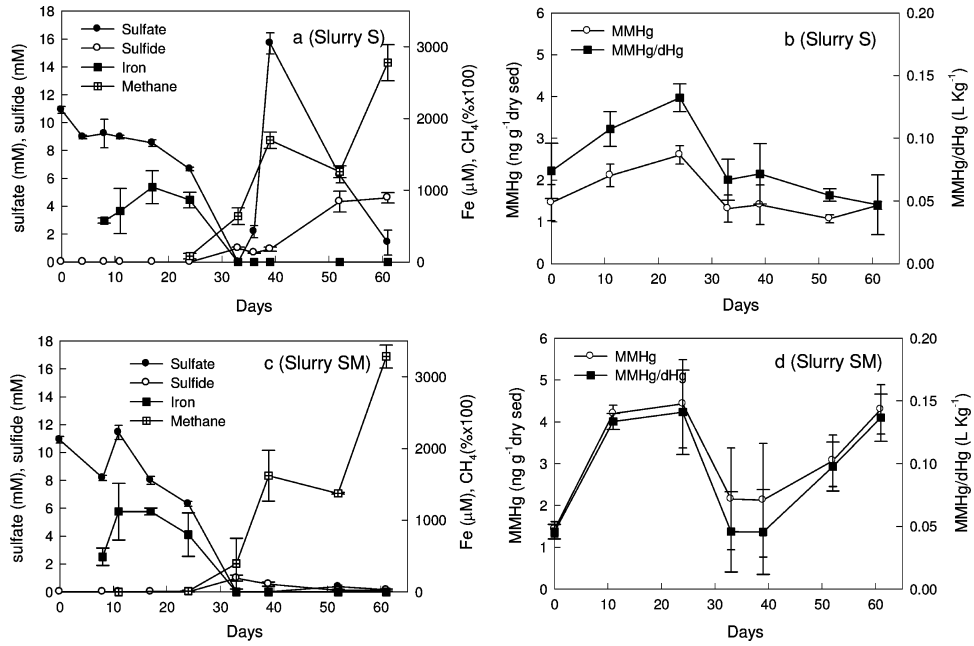

FIGURE 3. Variations of sulfate, sulfide and iron in slurry water, methane in headspace, monomethylmercury (MMHg) in dry sediment, and MMHg in dry sediment normalized to dissolved Hg (dHg) found from the drill hole sediment slurries during the incubation period of day 0 to day 61. In a and b (Slurry S), additional sulfate was added on day 36 and in c and d (Surry SM), additional sulfate was not added.

The MMHg demethylation rate in the S and SM slurries seems to exceed the Hg(II) methylation rate between days 24 and 33 (Figure 3, parts b and d), which corresponds to the largest sulfate consumption period. During the same period, methane began to accumulate, indicating that sulfate might be fully consumed ahead of day 33 so that the terminal electron accepting process switched from sulfate reduction to methanogenesis. Two reasons could explain the decreased MMHg concentration between days 24 and 33: (1) the sulfate reduction process was not occurring during most of this period due to the unavailability of sulfate; or (2) sulfate reduction may have been occurring, but MMHg production was inhibited by accumulated sulfide (*17, 18, 45*). This trend was repeated again between days 40 and 60 in the S slurries (Figure 3b), which supported the plausibility of the second reason, because MMHg did not increase even in the presence of high concentrations of sulfate. From day 33 and onward, sulfate concentration was lower than our detection limit (100 μM) in the SM slurries (Figure 3c). This clearly suggests that net MMHg production could be significant, even without active sulfate reduction, possibly due to syntrophic associations between methanogens and sulfidogens: a geochemical situation analogous to that found at a depth of 5−10 m in drill hole SM3 (Figure 2d).

Enhanced sulfide content decreases availability of dissolved Hg(II) by precipitation of HgS and/or adsorption of Hg onto sulfide minerals (*46*). At the same time, sulfide is known to decrease microbial uptake of Hg(II) (*17, 18*). Therefore, the optimum condition for in situ Hg(II) methylation has been found in sulfide-poor anoxic sediment enriched with biodegradable organic C (*45, 46*). In our case, dissolved THg concentrations in SM slurries after day 33 were not significantly different from those in S slurries ($p = 0.93$, *t*-test), indicating that inhibition of microbial processes rather than unavailability of dissolved THg appears to be a reason for the decreases in MMHg, along with increases in dissolved sulfide. Regarding demethylation rate of MMHg, a consistent demethylation rate has been observed under sulfate- and Fe(III)-reducing conditions, as well as under methanogenic conditions, suggesting that the demethylation rate of MMHg might not be affected by the accumulation of sulfide (*47*).

Microcosm M, the molybdate-added cultures, did not show any methanogenic activity (Supporting Information, S8). This could be because the addition of molybdate not only inhibited the sulfate-reducing bacteria, but also prevented the formation of substrates favorable for the growth of methanogens, such as H_2 and/or acetate, which form during the fermentation of organics (*21*).

We provided additional $HgCl_2$ in aliquots of the S and SM slurries collected on day 68, in order to confirm the Hg(II) methylation capacity under sulfate-limited conditions (see Supporting Information, S9). Monomethylmercury production following this amendment was higher in the SM slurries (from 8.5 to 32 ng g^{-1}) than in the S slurries (from 3.8 to 7.0 ng g^{-1}). Active Hg(II) methylation has been reported for complete-oxidizing sulfate reducers (*6, 7*); therefore, the relatively insignificant MMHg production in slurry S suggests that sulfide inhibits active Hg(II) methylation by complete-oxidizing sulfate reducers.

Fluorescence in Situ Hybridization. Fluorescence in situ hybridization was performed with the day 59 sample using bacterial (EUB338) and archaeal (Arc915) probes (Supporting Information, S10). The presence of archaea in the S cultures is not surprising since, by day 59, copious methane production was observed (c.f., Figure 3a). We also noted the activity of both bacterial and archaeal populations in the SM slurries on day 59, ~26 days after all of the sulfate had been depleted, indicating a population of bacteria that was actively fermenting the added substrate, lactate.

We have evidence that the bacterial population enriched in the S slurries was different from that in the SM cultures: The "S cultures" showed numerous rod-like bacteria, suggesting the dominance of the complete oxidizing group of sulfate-reducing bacteria, such as the *Desulfobacter* spp.; however, the "SM cultures" showed numerous vibrio-shaped bacteria instead, suggesting the dominance of incomplete oxidizers such as *Desulfovibrio vulgaris* (*48, 49*). This observation deserves further quantification; yet, assuming it is true, the dominance of the incomplete oxidizer in the SM slurries would support our hypothesis of syntrophic Hg(II) methylation under sulfate-limited conditions. It is well-

known that incomplete oxidizers actively produce acetate and hydrogen, which are substrates for methanogens (*21*).

Environmental Implications. The results of the slurry experiments successfully showed that active Hg(II) methylation occurs under sulfate-limited conditions, possibly by interspecies hydrogen transfer processes between methanogens and sulfidogens. However, we cannot rule out the possible Hg(II) methylation by methanogens, since our methanogenic culture was inconclusive. Nevertheless, it is generally accepted that methanogens do not aid in MMHg production (*43*). For example, the addition of bromoethanesulfonic acid (BES), an inhibitor of methanogenesis, in anoxic Everglade sediments showed insignificant inhibition of Hg(II) methylation (*16*). A similar result has been reported using pure cultures of the methanogenic bacterium spiked with inorganic Hg(II): *Methanococcus maripaludis* was unable to methylate Hg(II) but was able to demethylate MMHg at a rate comparable to that of a sulfidogen (*43*).

In summary, a high concentration of MMHg was found in deeply buried sediments of the Venice Lagoon, where hydrogen production and consumption may occur from the syntrophic association of sulfidogens and methanogens. It appears that this mechanism is more favorable for MMHg production than active sulfate reduction mechanism, due to a decreased potential of sulfide inhibition. Further investigations are needed to understand the ecological and environmental significance of syntrophic Hg(II) methylation under in situ conditions.

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Supporting Information Available

Six figures and four texts. This information is available free of charge via the Internet at http://pubs.acs.org/.

Literature Cited

(1) Cheung, M. K.; Verity, M. A. Experimental methyl mercury neurotoxicity—locus of mercurial inhibition of brain protein-synthesis in vivo and in vitro. *J. Neurochem.* **1985**, *44* (6), 1799–1808.

(2) Monperrus, M.; Tessier, E.; Amouroux, D.; Leynaert, A.; Huonnic, P.; Donard, O. F. X. Mercury methylation, demethylation and reduction rates in coastal and marine surface waters of the Mediterranean Sea. *Mar. Chem.* **2007**, *107* (1), 49–63.

(3) Cossa, D.; Averty, B.; Pirrone, N. The origin of methylmercury in open Mediterranean waters. *Limnol. Oceanogr.* **2009**, *54* (3), 837–844.

(4) Fitzgerald, W. F.; Lamborg, C. H.; Hammerschmidt, C. R. Marine biogeochemical cycling of mercury. *Chem. Rev.* **2007**, *107* (2), 641–662.

(5) Hollweg, T. A.; Gilmour, C. C.; Mason, R. P. Methylmercury production in sediments of Chesapeake Bay and the mid-Atlantic continental margin. *Mar. Chem.* **2009**, *114*, 86–101.

(6) Choi, S. C.; Chase, T.; Bartha, R. Enzymatic catalysis of mercury methylation by *Desulfovibrio desulfuricans* LS. *Appl. Environ. Microbiol.* **1994**, *60* (4), 1342–1346.

(7) Choi, S. C.; Chase, T.; Bartha, R. Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfuricans* LS. *Appl. Environ. Microbiol.* **1994**, *60* (11), 4072–4077.

(8) Ekstrom, E. B.; Morel, F. M. M.; Benoit, J. M. Mercury methylation independent of the acetyl-coenzyme pathway in sulfate-reducing bacteria. *Appl. Environ. Microbiol.* **2003**, *69* (9), 5414–5422.

(9) King, J. K.; Saunders, F. M.; Lee, R. F.; Jahnke, R. A. Coupling mercury methylation rates to sulfate reduction rates in marine sediments. *Environ. Toxicol. Chem.* **1999**, *18* (7), 1362–1369.

(10) King, J. K.; Kostka, J. E.; Frischer, M. E.; Saunders, F. M. Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments. *Appl. Environ. Microbiol.* **2000**, *66* (6), 2430–2437.

(11) Harmon, S. M.; King, J. K.; Gladden, J. B.; Chandler, G. T.; Newman, L. A. Methylmercury formation in a wetland mesocosm amended with sulfate. *Environ. Sci. Technol.* **2004**, *38* (2), 650–656.

(12) Mitchell, C. P. J.; Branfireun, B. A.; Kolka, R. K. Assessing sulfate and carbon controls on net methylmercury production in peatlands: An in situ mesocosm approach. *Appl. Geochem.* **2008**, *23* (3), 503–518.

(13) Gilmour, C. C.; Henry, E. A.; Mitchell, R. Sulfate stimulation of mercury methylation in freshwater sediments. *Environ. Sci. Technol.* **1992**, *26*, 2281–2287.

(14) Branfireun, B. A.; Roulet, N. T.; Kelly, C. A.; Rudd, J. W. M. In situ sulphate stimulation of mercury methylation in a boreal peatland: Toward a link between acid rain and methylmercury contamination in remote environments. *Global Biogeochem. Cycles* **1999**, *13* (3), 743–750.

(15) King, J. K.; Harmon, S. M.; Fu, T. T.; Gladden, J. B. Mercury removal, methylmercury formation, and sulfate-reducing bacteria profiles in wetland mesocosms. *Chemosphere* **2002**, *46*, 859–870.

(16) Gilmour, C. C.; Riedel, G. S.; Ederington, M. C.; Bell, J. T.; Benoit, J. M.; Gill, G. A.; Stordal, M. C. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. *Biogeochemistry* **1998**, *40*, 327–345.

(17) Benoit, J. M.; Gilmour, C. C.; Mason, R. P.; Heyes, A. Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters. *Environ. Sci. Technol.* **1999**, *33* (6), 951–957.

(18) Benoit, J. M.; Gilmour, C. C.; Mason, R. P. The influence of sulfide on solid phase mercury bioavailability for methylation by pure cultures of *Desulfobulbus propionicus* (1pr3). *Environ. Sci. Technol.* **2001**, *35* (1), 127–132.

(19) Fleming, E. J.; Mack, E. E.; Green, P. G.; Nelson, D. C. Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium. *Appl. Environ. Microbiol.* **2006**, *72* (1), 457–464.

(20) Pak, K. R.; Bartha, R. Mercury methylation by interspecies hydrogen and acetate transfer between sulfidogens and methanogens. *Appl. Environ. Microbiol.* 199864 (6), 1987–1990.

(21) Phelps, T. J.; Conrad, R.; Zeikus, J. G. Sulfate-dependent interspecies H_2 transfer between *Methanosarcina barkeri* and *Desulfovibrio vulgaris* during coculture metabolism of acetate or methanol. *Appl. Environ. Microbiol.* **1985**, *50* (3), 589–594.

(22) Drott, A.; Lambertsson, L.; Bjorn, E.; Skyllberg, U. Importance of dissolved neutral mercury sulfides for methyl mercury production in contaminated sediments. *Environ. Sci. Technol.* **2007**, *41* (7), 2270–2276.

(23) Gill, G. A.; Bruland, K. W. Mercury speciation in surface freshwater systems in California and other areas. *Environ. Sci. Technol.* **1990**, *24* (9), 1392–1400.

(24) Choe, K. Y.; Gill, G. A.; Lehman, R. D.; Han, S.; Heim, W. A.; Coale, K. H. Sediment-water exchange of total mercury and monomethyl mercury in the San Francisco Bay-Delta. *Limnol. Oceanograph.* **2004**, *49* (5), 1512–1527.

(25) Bloom, N. S.; Gill, G. A.; Cappellino, S.; Dobbs, C.; McShea, L.; Driscoll, C.; Mason, R.; Rudd, J. Speciation and cycling of mercury in Lavaca Bay, Texas, sediments. *Environ. Sci. Technol.* **1999**, *33* (1), 7–13.

(26) Liang, L.; Horvat, M.; Bloom, N. S. An improved speciation method for mercury by GC CVAFS after aqueous-phase ethylation and room-temperature precollection. *Talanta* **1994**, *41* (3), 371–379.

(27) Fennell, D. E.; Rhee, S. K.; Ahn, Y. B.; Haggblom, M. M.; Kerkhof, L. J. Detection and characterization of a dehalogenating microorganism by terminal restriction fragment length polymorphism fingerprinting of 16S rRNA in a sulfidogenic, 2-bromophenol-utilizing enrichment. *Appl. Environ. Microbiol.* **2004**, *70* (2), 1169–1175.

(28) Amann, R. I.; Krumholz, L.; Stahl, D. A. Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental-studies in microbiology. *J. Bacteriol.* **1990**, *172* (2), 762–770.
(29) Barbanti, A.; Bergamini, M. C.; Frascari, E.; Miserocchi, S.; Ratta, M.; Rosso, G. Diagenetic processes and nutrient fluxes at the sediment-water interface, northern Adriatic Sea, Italy. *Mar. Freshwater Res.* **1995**, *46* (1), 55–67.
(30) Yang, R. J.; van den Berg, C. M. G. Metal complexation by humic substances in seawater. *Environ. Sci. Technol.* **2009**, *43* (19), 7192–7197.
(31) Brumsack, H. J.; Gieskes, J. M. Interstitial water trace-metal chemistry of laminated sediments from the Gulf of California, Mexico. *Mar. Chem.* **1983**, *14* (1), 89–106.
(32) Bloom, N. S.; Moretto, L. M.; Scopece, P.; Ugo, P. Seasonal cycling of mercury and monomethyl mercury in the Venice Lagoon (Italy). *Mar. Chem.* **2004**, *91*, 85–99.
(33) Frignani, M.; Bellucci, L. G.; Langone, L.; Muntau, H. Metal fluxes to the sediments of the northern Venice Lagoon. *Mar. Chem.* **1997**, *58*, 275–292.
(34) Covelli, S.; Faganeli, J.; Horvat, M.; Brambati, A. Mercury contamination of coastal sediments as the result of long-term cinnabar mining activity (Gulf of Trieste, northern Adriatic sea). *Appl. Geochem.* **2001**, *16* (5), 541–558.
(35) Piani, R.; Covelli, S.; Biester, H. Mercury contamination in Marano Lagoon (Northern Adriatic sea, Italy): Source identification by analyses of Hg phases. *Appl. Geochem.* **2005**, *20* (8), 1546–1559.
(36) Belzile, N.; Lang, C. Y.; Chen, Y. W.; Wang, M. The competitive role of organic carbon and dissolved sulfide in controlling the distribution of mercury in freshwater lake sediments. *Sci. Total Environ.* **2008**, *405*, 226–238.
(37) Hammerschmidt, C. R.; Fitzgerald, W. F. Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ. Sci. Technol.* **2004**, *38* (5), 1487–1495.
(38) Niessen, S.; Foucher, D.; Clarisse, O.; Fischer, J. C.; Mikac, N.; Kwokal, Z.; Fajon, V.; Horvat, M. Influence of sulphur cycle on mercury methylation in estuarine sediment (Seine estuary, France). *J. Phys. IV* **2003**, *107*, 953–956.
(39) Han, S.; Obraztsova, A.; Pretto, P.; Choe, K. Y.; Gieskes, J.; Deheyn, D. D.; Tebo, B. M. Biogeochemical factors affecting mercury methylation in sediments of the Venice Lagoon, Italy. *Environ. Toxicol. Chem.* **2007**, *26*, 655–663.
(40) Thamdrup, B.; Fossing, H.; Jorgensen, B. B. Manganese, iron, and sulfur cycling in a coastal marine sediment, Aarhus Bay. *Geochim. Cosmochim. Acta* **1994**, *58* (23), 5115–5129.
(41) Jorgensen, B. B.; Bottcher, M. E.; Luschen, H.; Neretin, L. N.; Volkov, I. I. Anaerobic methane oxidation and a deep H_2S sink generate isotopically heavy sulfides in Black Sea sediments. *Geochim. Cosmochim. Acta* **2004**, *68* (9), 2095–2118.
(42) Rickard, D.; Luther, G. W. Kinetics of pyrite formation by the H_2S oxidation of iron(II) monosulfide in aqueous solutions between 25 and 125 degrees C: The mechanism. *Geochim. Cosmochim. Acta* **1997**, *61* (1), 135–147.
(43) Pak, K. R.; Bartha, R. Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. *Appl. Environ. Microbiol.* **1998**, *64* (3), 1013–1017.
(44) Heyes, A.; Mason, R. P.; Kim, E. H.; Sunderland, E. Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes. *Mar. Chem.* **2006**, *102*, 134–147.
(45) Gagnon, C.; Pelletier, E.; Mucci, A.; Fitzgerald, W. F. Diagenetic behavior of methylmercury in organic-rich coastal sediments. *Limnol. Oceanogr.* **1996**, *41* (3), 428–434.
(46) Gagnon, C.; Pelletier, E.; Mucci, A. Behaviour of anthropogenic mercury in coastal marine sediments. *Mar. Chem.* **1997**, *59*, 159–176.
(47) Warner, K. A.; Roden, E. E.; Bonzongo, J. C. Microbial mercury transformation in anoxic freshwater sediments under iron-reducing and other electron-accepting conditions. *Environ. Sci. Technol.* **2003**, *37* (10), 2159–2165.
(48) Postgate, J. R.; Campbell, L. L. Classification of *Desulfovibrio* species, the nonsporulating sulfate-reducing bacteria. *Bacteriol. Rev.* **1966**, *30* (4), 732–738.
(49) Widdel, F.; Pfennig, N. Studies on dissimilatory sulfate-reducing bacteria that decompose fatty-acids. 1. Isolation of new sulfate-reducing bacteria enriched with acetate from saline environments-description of *Desulfobacter postgatei* gen. nov., sp. nov. *Arch. Microbiol.* **1981**, *129* (5), 395–400.

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