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# Demethylation—The Other Side of the Mercury Methylation Coin: A Critical Review

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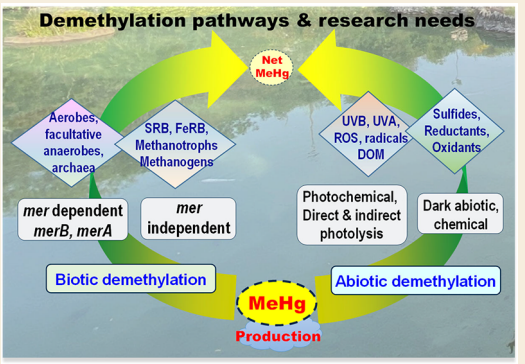
**ABSTRACT:** The public and environmental health consequences of mercury (Hg) methylation have drawn much attention and considerable research to Hg methylation processes and their dynamics in diverse environments and under a multitude of conditions. However, the net methylmercury (MeHg) concentration that accumulates in the environment is equally determined by the rate of MeHg degradation, a complex process mediated by a variety of biotic and abiotic mechanisms, about which our knowledge is limited. Here we review the current knowledge on MeHg degradation and its potential pathways and mechanisms. We describe detoxification by resistant microorganisms that employ the Hg resistance (*mer*) system to reductively break the carbon−mercury (C−Hg) bond producing methane ($CH_4$) and inorganic mercuric Hg(II), which is then reduced by the mercuric reductase to elemental Hg(0). Very recent research has begun to elucidate a mechanism for the long-recognized *mer*-independent oxidative demethylation, likely involving some strains of anaerobic bacteria as well as aerobic methane-oxidizing bacteria, i.e., methanotrophs. In addition, photochemical and chemical demethylation processes are described, including the roles of dissolved organic matter (DOM) and free radicals as well as dark abiotic demethylation in the natural environment about which little is currently known. We focus on mechanisms and processes of demethylation and highlight the uncertainties and known effects of environmental factors leading to MeHg degradation. Finally, we suggest future research directions to further elucidate the chemical and biochemical mechanisms of biotic and abiotic demethylation and their significance in controlling net MeHg production in natural ecosystems.




**KEYWORDS:** *methylmercury, organomercury lyase, reductive and oxidative demethylation, photodemethylation, chemical demethylation, mechanisms and pathways*

## INTRODUCTION

The major focus of our concern with mercury (Hg) as a global environmental pollutant is the potential for the formation of methylmercury (MeHg), a highly potent neurotoxin.[1,2] Mercury enters the environment mostly in its inorganic form, in its three oxidation states [Hg(II), Hg(I), and Hg(0)], and as complexes with various ligands.[3] It is in anoxic environments where inorganic Hg is converted to MeHg by anaerobic microorganisms.[4−6] Methylmercury then enters food chains,[7,8] where it is bioaccumulated and biomagnified to pose a risk to top level feeders such as predatory fish and the humans who consume them.[9] An additional pathway of human exposure to MeHg, affecting billions of consumers, is via the consumption of contaminated rice.[10,11] This paradigm has dominated mercury research for six decades, since our awareness arose by the Hg poisoning incidence in Minamata Bay, Japan, in the mid-1950s.[12,13]

The role of microorganisms in Hg methylation was discovered in the 1960s[4] and, shortly afterward, followed the first reports on the degradation of MeHg, or demethylation.[14,15] These reports documented the disappearance of MeHg from sediment incubations[15] and the isolation of bacterial cultures that degraded MeHg.[16,17] The authors of these early studies concluded that microorganisms in environments where MeHg was formed degraded MeHg, and they suggested that this activity was equally important for the accumulation of MeHg in the environment.[15,16] These competitive, simultaneous methylation and demethylation reactions have since been studied and widely observed in diverse environmental systems, such as freshwater lakes, wetlands, periphyton biofilms, marine water, and sediments.[18−25] Nevertheless, throughout the decades, much more attention has been paid to, and consequently knowledge gained on, methylation as compared to demethylation. A recent Google Scholar search with "mercury methylation" as a

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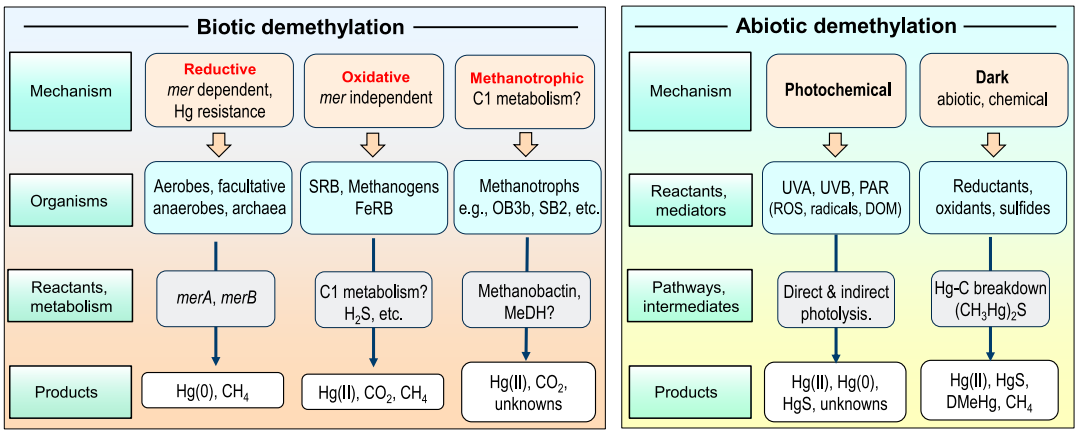


**Figure 1.** Potential mechanisms, microorganisms, reactants, and products of both biotic and abiotic pathways of methylmercury (MeHg) demethylation in the environment. See the main text for details.

query resulted in 50 900 citations and with "methylmercury degradation" in 30 200 citations. Moreover, in spite of early claims to methylation by abiotic processes,[26,27] the current consensus attributes MeHg formation to anaerobic prokaryotes by a process that is specified by the "methylating genes", *hgcA* and *hgcB*.[28] This is not the case for demethylation where multiple biotic and abiotic processes are documented (Figure 1),[23,29−31] and the exact mechanisms of some processes remain obscure.

While several recent review articles have addressed Hg biogeochemical processes,[30−33] there is a scarcity of comprehensive reviews solely focused on MeHg degradation and none focuses on mechanistic aspects. This Review focuses on the mechanisms of MeHg degradation or demethylation, as new processes and mechanistic details have recently emerged for both biotic and abiotic pathways. These processes, the mechanisms driving them, and their environmental significance are the topic of this Review. We summarize recent advances in our understanding of biotic and abiotic demethylation focusing on mechanisms, pathways, and how they are affected by environmental factors. Moreover, we highlight outstanding knowledge gaps pertaining to microbially mediated MeHg degradation with an emphasis on the role of microbial communities in demethylation. We describe controversies regarding oxidative demethylation mechanisms and the roles of reactive oxygen species (ROS), free radicals, and dissolved organic matter (DOM) in photochemical demethylation. Finally, we suggest future research needs that would fill current knowledge gaps toward the integration of demethylation in scientific and regulatory paradigms for the pathways and processes that lead to MeHg bioaccumulation.

## BIOTIC DEMETHYLATION

The initial discovery of demethylation identified methane ($CH_4$) and Hg(0) as the products of microbial degradation both in environmental incubations[15] and with pure cultures isolated from soils[14,17] and sediment and fish incubations.[16] These discoveries coincided with the discovery of the prokaryotic Hg-resistance system, the *mer* operon-mediated functions.[14,34] The *mer* system converts MeHg to $CH_4$ and Hg(0) when the activity of the organomercury lyase, MerB, is combined with that of the mercuric reductase, MerA (see below).[35] It was thus assumed that demethylation was due to the activity of Hg resistant bacteria in environments where MeHg was produced.[36] These initial observations led to the development of an experimental system to measure rates of $^{14}$C-MeHg degradation in environmental samples. The experiment was set up by passing air leaving incubation vessels through a copper oxide combustion tube held at 450 °C to convert all volatile $^{14}$C products to $CO_2$ that was then trapped and counted.[37] This protocol, considered to measure the demethylation potential of environmental incubations, was used in conjunction with a $^{203}$Hg(II) methylation assay[38] to calculate a methylation/demethylation (M/D) ratio. The M/D ratio was broadly used, until the end of the 1990s, to measure MeHg production potentials in the environment (e.g., Korthals and Winfrey;[18] Carroll and Warwick;[39] Vaithiyanathan et al.[40]).

One consequence of measuring demethylation potentials using $^{14}$C-MeHg was the discovery that the gaseous C pool contained both $CH_4$ and $CO_2$,[18] suggesting the existence of additional demethylating mechanism(s) to the one mediated by Hg resistant bacteria.[36] Such mechanism(s) were first explored by Oremland et al.[41] who coined the term oxidative demethylation (OD) to describe the formation of $CO_2$ during demethylation. As OD became established in the Hg biogeochemistry field in subsequent years, the term reductive demethylation (RD) was adopted to describe the production of $CH_4$ during MeHg degradation (e.g., Hines et al.[42]). This term largely refers to the reaction that is mediated by *mer*-carrying resistant microbes (e.g., Schaefer et al.[43]). However, the conversion of MeHg to $CH_4$ involves reduction only when it is coupled to the reduction of Hg(II) to Hg(0), the other product of degradation, by the *mer*-mediated process.[44] For other demethylation processes that produce $CH_4$ but not Hg(0), e.g., the conversion of MeHg to dimethylmercury (DMeHg) and Hg(II) by sulfate reducing bacteria (SRB)[45] or during OD,[46] the term RD is misleading because here the conversion of MeHg to $CH_4$ does not involve a redox change; in both forms, the oxidation state of carbon is −4. It is the oxidation state of Hg which makes the distinction of RD versus OD important. While the *mer*-mediated RD results in volatile Hg(0), OD produces Hg(II), which is subsequently cycled during methylation and demethylation processes.[43]

Demethylation experiments and incubations with $^{14}$C-MeHg have resulted in some recognized patterns of environments and conditions that favor OD or RD. The data that was available until the first half of the 2000s, summarized by Barkay and Wagner-Döbler,[47] pointed to Hg concentration and redox as


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factors constraining the choice between OD and RD. The former, OD, was favored in anoxic environments and low Hg concentrations, and the latter, RD, was favored in oxic and high Hg environments. Data collected since supports these conclusions and adds seasonality as a constraint (Table S1), with RD favored during cooler months in contaminated river sediments[48] and in the sediments of shallow coastal lagoons.[49] Regretfully, studies comparing RD to OD have ceased more than a decade ago with the introduction of Hg stable isotopes to Hg research.[50,51] $^{14}$C-MeHg, which had become increasingly scarce, was replaced with Me$^{xxx}$Hg, where xxx may be any of the seven stable isotopes of Hg. Although the broad use of Hg stable isotopes has immensely benefited Hg biogeochemical research,[21,52,53] we have lost the ability to distinguish degradation pathways and mechanisms by identifying gaseous carbon products.

## Reductive Demethylation (RD) by the *mer* System

The *mer* system is broadly distributed among prokaryotes facilitating growth in the presence of elevated Hg concentrations by reducing Hg(II) to Hg(0) that evaporates due to its low aqueous solubility and high vapor pressure. This system consists of MerA and a variety of transport functions and is under fine regulation by MerR, both a repressor and an activator of functional gene expression. The genes encoding for these various functions are colocated in the *mer* operon. A minority of Hg resistant bacteria, the so-called broad-spectrum Hg resistant, specify resistance to organomercury compounds in addition to Hg(II) and encode for the MerB enzyme. For more details about the *mer* system and its functions, please see Barkay and Wagner-Döbler,[47] and for a recent inventory of MerA- and MerB-carrying genomes and metagenomes please see Christakis et al.[54]

**The Organomercury Lyase, MerB.** The initial discovery of demethylation identified $CH_4$ and Hg(0) as the degradation products (see above and Figure 1). Two factors might have played a critical role in this discovery: first, the high concentration of Hg that was added to environmental incubations, 40 $\mu$M (or 8 mg/L),[15] and, second, examining demethylation with cultures that were selected for their resistance to MeHg,[15] phenylmercury acetate (PMA),[55] or Hg(II).[16] These studies set up the stage for two decades of attributing demethylation largely to the reductive process.

Research that progressed simultaneously with environmental studies defined narrow-spectrum Hg resistance (resistance only to inorganic Hg) and broad-spectrum resistance (resistance to organomercury compounds and inorganic Hg).[56−58] This research, driven by the discovery of Hg resistance on R factors in cultures from hospital collections,[59,60] led to the first characterization of MerB using the enzyme encoded by plasmid R831 and the arylmercury PMA as a substrate.[61] The first reports on the purification[35] and biochemical-biophysical characterization of MerB[62] documented its broad substrate specificity and the kinetic superiority of cleaving C−Hg bonds in several arylmercurials as compared to MeHgCl.[35] This observation was confirmed more than two decades later by hybrid density functional theory calculations based on the X-ray crystal structure of the Hg(II)-bound MerB.[63] As most of the research that followed on the mechanism of MerB employed the enzyme encoded by R831 (see below), the pertinence of our current understanding of MerB activity to demethylation in the environment is a relevant question. In addition, environmental surveys of organomercury resistant isolates have often used PMA as a test compound.[64−66]

An $S_E2$ mechanism for a protonolytic cleavage of the Hg−C bond by MerB was proposed by Begley et al.,[62] a model that since has been much refined. Refinements were achieved by genetics[67] and structural studies,[68−70] by the use of model compounds mimicking molecular configurations in the enzyme's active site,[71] and by applying functional theory calculations.[63] Shown by NMR studies[68,69] and X-ray crystallography,[70] the active site consists of three antiparallel $\beta$-sheets surrounded by six $\alpha$-helices[69,70] with an additional $\alpha$-helix reaching from the protein's N terminal to create an enclosed structure in the enzyme's core.[70] A large hydrophobic pocket that may interact with the hydrocarbon moiety of the substrates is located proximal to the catalytic core.[72] Two conserved cysteines, Cys96 and Cys159, and an asparagine, Asp99 (amino acids numbering is of the MerB of R831), are essential for activity; their replacement by site-specific mutagenesis abolished enzymatic activities.[67] Replacement of Asp99 with serine, D99S, resulted in an enzyme that bound Cu(II) through interactions with Cys96 and Cys159.[73] However, a native D99S variant, MerB2 of *Bacillus megaterium* MB1, did not bind Cu(II)[73] and had a much-reduced activity in vivo[74,75] and in vitro (J. Omichinski, personal communication) relative to Asp99 MerB. A third conserved cysteine, Cys160, is not essential for catalysis as it retained 37% of the WT activity when replaced with serine.[67] Cys160 may have a role in the release of Hg(II) from MerB to MerA[68] following catalysis.[63] Cys117, the fourth conserved cysteine in MerB, likely plays a structural role and is buried deep inside the hydrophobic interior of the enzyme.[69]

Based on the breakthrough study of Melnick and Parkin,[71] whereby active sites consisting of three thiol residues led to the protonolysis of methyl- and ethylmercury,[76] and analyses of crystallized MerB,[63,70] the following model for catalysis by MerB has been proposed (Figure 2). Together, Cys96, Cys159, and Asp99 interact with the substrate, R−Hg$^+$, and a water molecule to bring about the cleavage of the Hg−C bond and the release of R−H (in the case of MeHg, $CH_4$). Two possible paths for demethylation were initially proposed. Both assumed that MeHg had formed an initial covalent bond with Cys96 and that Cys159 was protonated. In the first path, Cys159 protonates the leaving group carbon and in the second, Cys159 donates a proton to Asp99 which then protonates the C−Hg bond to form $CH_4$. The most recent data support the second path which is described here (Figure 2).[63,70] MerB initially coordinates R−Hg$^+$ with the thiolate of Cys96 followed by coordination with the thiolate of Cys159 and the concomitant water-aided protonation of Asp99. The two-thiolate coordination with R−Hg weakens the C−Hg bond, rendering it susceptible to protonolysis by a proton that is donated by Asp99. The other product of this reaction, Hg(II), is covalently bound to the two cysteines and coordinated with two oxygens, one of Asp99 and the other of water. Hg(II) in this coordination is likely the substrate for MerA, and it is subsequently reduced to Hg(0). The addition of MerA to a MerB/Hg/dithiothreitol (DTT) complex, where Hg(II) was bound to the two thiolates of DTT, resulted in the removal and reduction of Hg(II). The kinetics of this process could only be explained by direct transfer of Hg(II) from MerB to MerA by substrate channeling.[68] Intriguingly, a MerBA fusion protein where the N terminal of MerA is replaced by the AML domain of MerB is found among soil alphaproteobacteria.[77,78]


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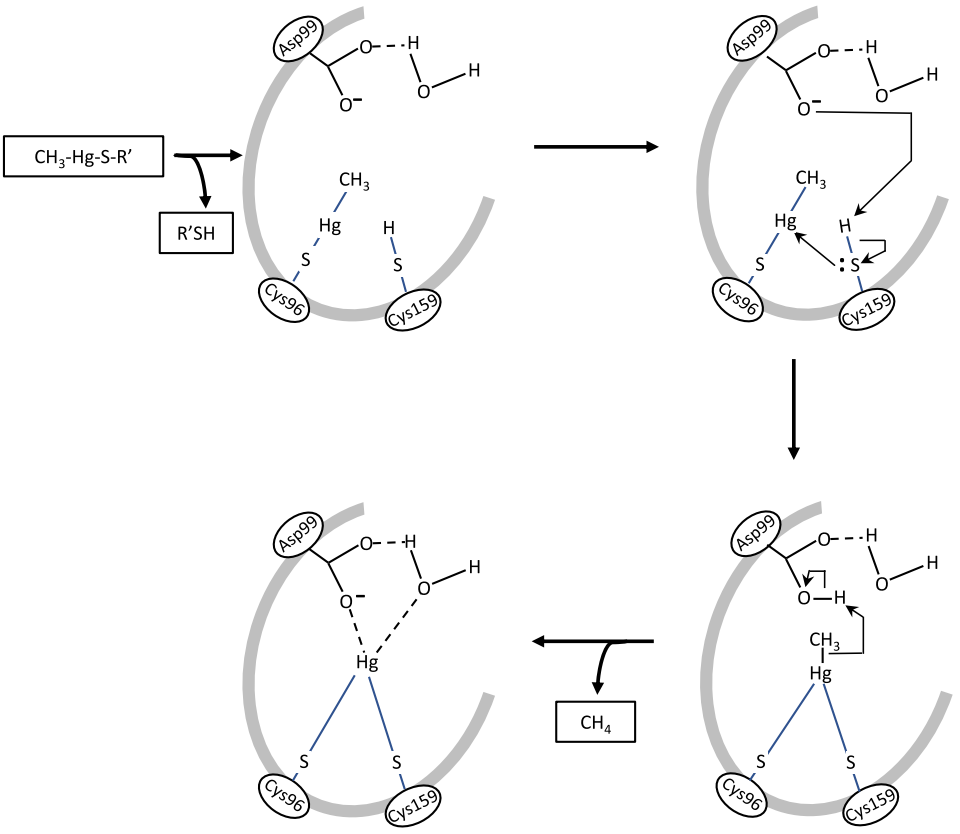


**Figure 2.** Proposed mechanism for demethylation by MerB. The open gray circle depicts the enzyme's active site with conserved residues that are critical for catalysis indicated. Arrows indicate the movement of electrons during the protonolysis process, and solid and dashed lines depict covalent and ionic bonds that are formed during catalysis, respectively. The substrate, MeHg, is depicted in a thiolated form as it is present in the cell cytoplasm. Prepared after Miller,[76] Lafrance-Vannase et al.,[70] and Parks et al.[63]

One of these, *Xantobacter autotrophicus* Py2, was shown to degrade MeHg to Hg(0).[78] In vivo, *Escherichia coli* carrying a broad-spectrum *mer*, in which *merA* was deleted, was phenotypically hypersensitive to organomercury compounds,[79] suggesting enhanced toxicity when the cleaved Hg(II) was not subsequently reduced.

**Evolution and Diversity of MerB.** Unlike MerA where clear relationships to other flavin-dependent disulfide oxidoreductases and an origin among thermoacidophilic Archaea have been documented,[54,77] there is no common ancestry for MerB with other proteins. In this respect, MerB may, at this juncture, be considered an "orphan". The only homology known for MerB is a partial structural homology to NosL, a Cu(I)-binding lipoprotein which is a part of the nitrous reductase system;[80] both enzymes share two "treble-clef"-like structures, typical to TRASH domains, in their active core. This observation led Kaur and Subramanian to suggest the evolution of MerB from zinc fingers and repurposing a structural role to a catalytic one.[81]

While the toxicity of inorganic Hg has likely driven the evolution of MerA,[77,82] the selective pressure for MerB evolution is not clear. Naturally occurring organomercury compounds such as MeHg are present in most environments at ppt to ppb concentration ranges,[83] while the toxicity of MeHg to growing cultures was noted at the ppb to ppm range.[45] This, together with the observation that MerB homologs are largely absent from early evolving prokaryotic lineages,[54,82] have led to the suggestion that MerB was recruited to the *mer* system when man-made organomercury compounds reached contaminated environments at high concentrations.[82,84] By showing a frequent occurrence of MerB in the genomes and metagenomes of anaerobes, Christakis et al.[54] hypothesized that MerB evolution relates to cohabitation with MeHg-forming taxa and, thus, a possible role in detoxification. However, methylation was shown to have no effect on tolerance to Hg among sulfate reducers[85] and, to date, only one MerB-carrying obligate anaerobe, *Geobacter bemidjensis* Bem, has been shown to convert MeHg to Hg(0).[86] Thus, testing of this hypothesis would require more investigation.

A MerB phylogeny constructed mostly using sequences with a demonstrated MerB activity and including recently identified archaeal homologs[54] is presented in Figure 3. The archaeal sequences were included even though MerB activities have never been demonstrated in an archaeon due to the novelty of their discovery. The phylogeny shows two major clusters; basal to both are archaeal sequences, one with four bacterial MerB and the other with eight sequences with a documented activity. When one proteome contains two or more MerB, such as in the case of *Bacillus* spp. and *Pseudomonas* sp. K62, these are divided between the two clusters, attesting to the mosaic nature of *mer* systems.[82] We used the NosL sequence as an outgroup in this phylogeny, although this sequence is a structural homologue of MerB[80] but not a true paralogue. However, the branching order still deciphers possible evolutionary relationships and order among the included MerB. In this tree, the most dissimilar sequences, those of the *E. coli* plasmid R831, *Pseudomonas stutzeri* OX, and *Pseudomonas* sp. K62 MerB1, share only about 27% identity (pairwise alignment


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**Table 1. Organomercury Lyase (MerB) Functional Diversity**

| organism | MerB variant | substrate specificity | comments | ref |
|---|---|---|---|---|
| *Pseudomonas* sp. K62[a] | S-1 | PMA,[b] PCMB, MeHg | purified enzyme; lowest $K_m$ and highest $V_{max}$ achieved with MeHg | 91 |
| | S-2 | PMA, PCMB | purified enzyme; no activity with MeHg and higher $K_m$ and $V_{max}$ with PMA and PCMB than for S-1 | 92 |
| *Escherichia coli* J53-1 | R831 | PMA, MeHg, EthHg | partially purified; kinetic analysis showed $2K_m$ for PMA degradation and a single one for alkylmercury compounds | 61 |
| | | variety of substrates | purified enzyme; kinetic parameters showing orders of magnitude faster reaction with arylmercurials relative to MeHg | 62 |
| *Staphylococcus* spp. | e.g., pI258 | PMA, MeHg, (EthHg)[c] | resting cell assays | 57, 74 |
| *Pseudomonas* spp.[d] | e.g., pVS2 | PMA, MeHg, EthHg | resting cell assays | 58 |
| *Bacillus megaterium* MB1 | MerB3 | PMA, PHMB, MeHg, EthHg | growing cultures MIC assays using individual *merB* cloned in *E. coli* DH5α | 74, 75, 93 |
| | MerB2 | EthHg, PMA, PHMB[e] | | |
| | MerB1 | PMA, MeHg, EthHg | | |
| *Pseudomonas stutzeri* PB | pPB | PMA, MeHg, EthHg | growing cultures MIC and reductase assays | 94 |

[a]Strain was isolated from a PMA contaminated soil.[84] [b]PMA, phenylmercuric acetate; PCMB, *p*-chloromercuric benzoate; EthHg, ethylmercury; PHMB, *p*-hydroxymercury benzoate. [c]Tested with thimerosal; no resistance and ±volatilization observed. [d]10 plasmids from various pseudomonads tested in *P. aeruginosa* PAO and *P. putida* AC10; rates of volatilization were higher with PMA then either organomercurial as substrates.[58] [e]Very low activities; none with MeHg.

by https://blast.ncbi.nlm.nih.gov/Blast.cgi). In comparison, the most dissimilar MerA sequences, those of an early lineage, *Solfulubos solfataricus* P2,[87] and a most evolved sequence, that of the proteobacterial Tn501, are 35.28% identical. Thus, proteins with MerB activity have a high sequence diversity and at present lack a clear origin and an evolutionary path.

**Functional Diversity of MerB.** Linking specific microbially mediated pathways to a biogeochemical process as it occurs within environmental complexity depends heavily on the use of molecular tools to identify genes, transcripts, proteins, and metabolites in environmental "meta-omics".[95,96] It requires that much is known about the genetic and functional diversity of the investigated process, and it is in this respect that connecting MerB with environmental demethylation is difficult. First, MerB has a broad substrate specificity, spanning the naturally occurring organomercurials MeHg and ethylmercury (EthHg) and a variety of largely industrial products used as fungicides (e.g., PMA),[97] preservatives (e.g., thimerosal),[98] or antiseptics (e.g., merbromin).[99] Second, the nucleic and amino acid sequence diversity of *merB* and MerB, respectively, is very broad (see above and Barkay and Wagner-Döbler[47]), and since the identification of genes, transcripts, and proteins in environmental metagenomes is largely based on sequence similarity, a true MerB cannot be identified with a high degree of confidence. The only study we are aware of that assessed the role of *mer*-mediated functions in controlling MeHg accumulation in different watersheds[43] used *merA* probes as a proxy for *merB*. MerB was implicated in demethylation in a highly contaminated system when rates of MeHg degradation were higher, $^{14}CH_4$ was the primary degradation product of $^{14}$C-MeHg, and *merA* was more abundant as compared to a less contaminated site where $^{14}CO_2$ was the major demethylation product.[43]

One approach to begin to develop MerB-targeted molecular tools may be an examination of MerB whose relative efficiency with different substrates has been determined, considering whether unique molecular or structural characteristics can be related to their substrate preference. This approach could possibly identify "MeHg-degrading MerB" leading to the development of molecular probes and primers for biogeochemical research. A few studies have examined the relative efficiency of MerB with various substrates (Table 1). Early comparisons of MerB of R831 using partially[61] and fully purified[35] enzyme preparations suggested higher catalytic potentials with arylmercury as compared to MeHg as revealed by kinetic parameters ($K_m$, $V_{max}$, turnover rate). Tezuka and Tonomura reported the purification of two proteins with MerB activity, termed S-1[91] and S-2,[92] from the soil bacterium *Pseudomonas* sp. K62. Intriguingly, while S-1 was active with the arylmercurials PMA and *p*-hydroxymercury benzoate (PHMB) and with MeHg, S-2 was only active with the arylmercury compounds. In follow up work, Kiyono et al.[79] reported two Hg resistance plasmids, pMR26 and pMR68, in strain K62. The pMR26 *mer* system specified degradation of arylmercury and MeHg,[79] though with different efficiencies.[74] An *E. coli* clone with the *mer* system of pMR68 more readily volatilized Hg from PMA than the clone with pMR26's *mer* system (85% vs 65% removal in 16 h of growth, respectively).[100] Regretfully, the authors did not report volatilization from MeHg by the two *mer* systems, leaving the question of which one of them encodes for the S-1 and S-2 enzymes unanswered.

The most thorough attempt to relate the diversity of MerB to their substrate specificity has been carried out by the group of Endo,[54,75] who examined three MerB loci in a plasmid carried by *B. megaterium* MB1; the presence of multiple MerB loci among bacilli is quite common.[54] To identify the MerB that was best suited for bioremediation of organomercury contamination, investigators measured the degradation of different organomercury substrates by strains with partial deletions of *mer*,[93] cloned individual loci in *E. coli*,[75] and by purified enzyme extracts.[75] MerB1 and MerB3 were very similar in their activities; both degraded alkylmercurials slightly more readily than PMA, though MerB3 was particularly active with PHMB.[75] MerB2, on the other hand, with a D99S substitution (see Figure 2 and above), did not degrade MeHg and had limited activities with all other substrates.


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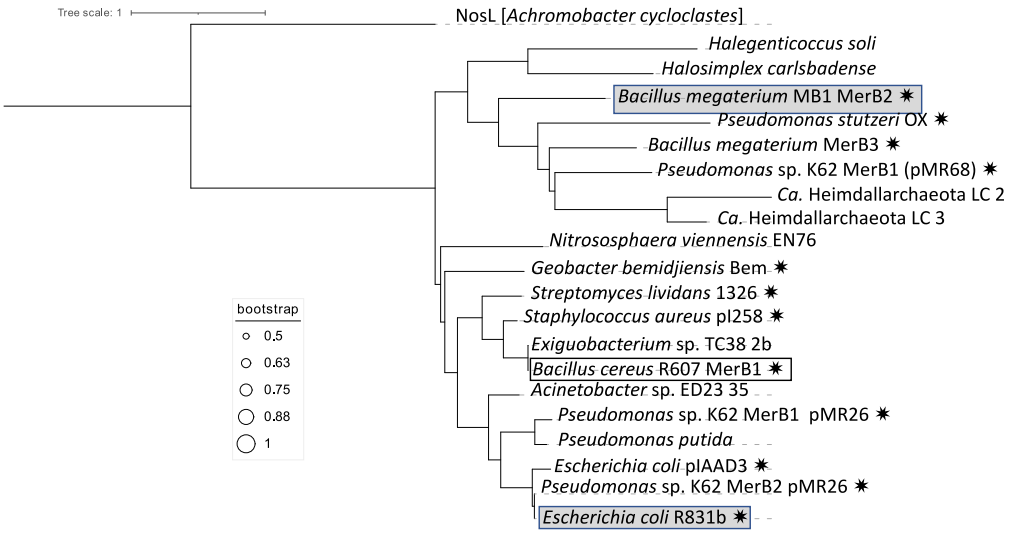


**Figure 3.** Phylogeny of MerB (maximum likelihood) emphasizing loci for which activity has been demonstrated (stars) and including newly identified Archaea MerB homologs;[54] MerB activity has never been reported in the archaea. The tree is outgrouped using the sequence of NosL, a 20 kDa Cu(I)-binding lipoprotein which is a part of the multiprotein assembly of the nitrous oxide reductase. NosL is the only known protein to share structural similarity with MerB.[80] Lineages appearing in gray boxes are those for which a preference to arylmercury substrates has been documented, and the one entry boxed in white indicates a preference for alkylmercury substrates (see text). To construct the phylogeny, selected sequences were aligned using MUSCLE version 3.8.31[88] under default settings and trimmed using TrimAl version 1.2[89] in an automated mode (-automated1). Columns with gaps (in ≥50% sequences) were removed. The resulting alignments (length ≥ 80 amino acids) were used to build phylogenetic trees using FastTree[90] under the "WAG + CAT" model. To achieve a higher level of accuracy, we used four rounds of minimum-evolution SPR moves (-spr 4) and made ML nearest-neighbor interchanges more exhaustive (-mlacc 2 -slownni).

While these studies clearly suggest that MerB variants differ in their catalytic efficiency with different substrates, a true comparison is impossible due to different experimental approaches and conditions that have been employed by different investigators. Moreover, there is little relationship between the amino acid sequence similarity of MerB and its substrate specificity. The two MerBs with preference to arylmercury, MerB2 of R831 and MerB2 of *B. megaterium*, are only 27.81% identical, while the sequences of MerB1 of R831 and *B. cereus* R607, the latter with a slight preference to alkylmercury (Table 1 and Figure 3), are 41.92% identical. Current interest in the role of this enzyme in controlling MeHg concentrations in contaminated environments calls for targeted studies, such as that recently described by Pathak et al.,[101] that would identify and characterize MeHg-specific MerB.

**MerB in Bioremediation.** Attempts to use the *mer* system in the remediation of mercury contamination were initiated in the 1990s and used common approaches such as bioaugmentation with natural isolates collected in contaminated sites,[102] optimization of performance by genetic engineering,[103] and construction of bioreactors designed to reduce industrial contamination.[104] For a comprehensive summary on the deployment of the *mer* system in bioremediation up until the early 2000s, please see Barkay and Wagner- Döbler.[47] The most renowned effort for MerB-based remediation has been the construction of transgenic plants for organic mercury contaminated soils.[105,106] In such plants, the *merAB* genes, modified to optimize expression in plants,[107] have been cloned into nucleus or plastid genomes. Cloning was in genomic locations that directed MerAB to the periplasm, the endoplasmic reticulum, the plastid, or the cell wall. In the greenhouse, such plants had increased tolerance to PMA and MeHg, accumulated Hg many folds over the concentration in the soil, and converted organomercury to Hg(0).[108] Moreover, evidence suggests growth stimulation of *mer*-transgenic plants in Hg-contaminated soils.[106] To the best of our knowledge, this promising technology has not been implemented in remediation efforts of contaminated soils possibly due to the remaining limitations.[105] Such limitations include knowledge gaps on the tolerance of plants to Hg, transport from soils to roots, in-plant translocation processes, plant compartments as Hg sinks, and impact of Hg chemical speciation as affected by complex environmental conditions.[105,106,109] Other MerB-based remediation approaches have been described including optimized microbial organomercury degraders (e.g., Chien et al.[75]) as well as construction of bacterial biosensors for the detection of organomercury.[110]

An interesting new development is based on the MerB active site mimics such as those constructed and tested by Melnick and Parkin.[71] Their research proposed a mechanism for the protonolytic cleavage of the C−Hg bond in R−Hg−X by three Cys residues (see above), raising the possibility of using such mimics in waste remediation. Strasdeit,[72] in a commentary on Melnick and Parkin's discovery, suggested that if such mimics can be anchored to a solid surface, MeHg could be removed from contaminated waste streams. Indeed, a mimic of MerB's active site, a synthetic imidazole-based selone with a $N(CH_2)_3$−SH substitution, was recently shown to protonolytically cleave the C−Hg bond in MeHg and EthHg at 21 °C without an external proton source.[111] The feasibility of this approach in remediation remains to be demonstrated, however.

## Oxidative Demethylation (OD)

Compared to *mer*-mediated RD in oxic environments, relatively few studies have examined OD, which is a nonspecific cometabolic process not related to Hg detox-


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ification and commonly observed in anoxic environments.[41,42,46,49,112,113] This is in part due to the lack of isolated pure cultures to perform OD experiments and to the scarcity of $^{14}CH_3Hg$ to facilitate the distinction of $^{14}CH_4$ from $^{14}CO_2$. Until recently, SRB and methanogens were considered as major players in OD, which can take place at relatively low Hg concentrations (e.g., picomolar to nanomolar), with Hg(II), $CO_2$, and $CH_4$ identified as the major reaction products.[41,46,112,113] Since many of these studies are carried out under anoxic conditions and are also net MeHg sources, it remains challenging to tease out the mechanisms and specific microbial strains supporting MeHg degradation. Furthermore, some recent studies cast doubt about OD by SRB and other obligate anaerobes,[114−116] as MeHg readily reacts with $H_2S$, the product of sulfate reduction, via the formation of $(CH_3Hg)_2S$ intermediates in anoxic environments. This indirect demethylation process can occur in solution as well as on mineral surfaces and may thus play a dominant role in sulfidogenic environments.

**Microbial Communities.** Early studies through incubation experiments with estuarine sediments by Compeau and Bartha[117] suggested that a demethylation process different from RD might exist. The study reported similar demethylation rates in a high-salinity saltmarsh soil under anaerobic conditions with low redox potential (−220 mV) as those observed in a low salinity soil under oxic conditions.[117] Similarly, using radioisotopically labeled $^{203}Hg(II)$ and $^{14}CH_3HgI$ tracers, Korthals and Winfrey[18] examined simultaneous Hg methylation and demethylation in water and sediments from an oligotrophic northern Wisconsin seepage lake. Demethylation was found to be the greatest in the surficial sediments but decreased slightly with sediment depth under anoxic conditions. This is among the first studies which have monitored the products of MeHg degradation ($^{14}CO_2$ and $^{14}CH_4$), although regretfully no monitoring data were reported and the mechanism of MeHg degradation was not explained. Nonetheless, the discovery of MeHg degradation in anoxic water and sediments revealed another biological mechanism besides the Hg resistance pathway for demethylation.

Subsequent studies using $^{14}CH_3HgI$ reported that demethylation was related to anaerobic microorganisms that do not contain *mer* operons, such as methanogens and SRB.[41] These authors first titled this degradation pathway as OD, since the final products of demethylation were Hg(II), $CO_2$, and a small amount of $CH_4$. Demethylation assays with metabolic inhibitors, such as molybdate for SRB and 2-bromoethanesulfonate (BES) for methanogens, and specific substrate amendments (e.g., trimethylamine, methanol, and dimethylsulfide) have attributed OD to both methanogens and SRB.[41,46,112,118−120] Experiments with pure cultures of the methanogen *Methanococcus maripaludis* and *Desulfovibrio desulfuricans* strains LS and ND132 also indicated MeHg degradation under strict anaerobic conditions.[121] Pak and Bartha[121] observed the production of $^{14}CH_4$, but not of $^{14}CO_2$, during demethylation with $^{14}CH_3HgI$. These authors proposed that MeHg is decomposed by SRB and methanogens that release $CH_4$, which is then oxidized by methanotrophs to $CO_2$.[118,122] Additional studies by Pak and Bartha[118] confirmed the involvement of SRB and methanogens in MeHg degradation both in pure cultures and freshwater sediments and suggested that the production of $CH_4$ and $CO_2$ from MeHg may represent enzymatically catalyzed Hg methylation in reverse. However, when this hypothesis was tested by following the products of $^{14}CH_3Hg^+$ degradation by two methylating *D. desulfuricans* strains LS and ND132 as well as by *M. marialudis*, the only gaseous C product was found to be $CH_4$.[121] Similarly, Bridou et al.[122] have tested more than a dozen of SRB strains for demethylation; all of them were found to degrade MeHg although the observed rates and extent of demethylation were not corrected by abiotic controls. These observations nevertheless supported that SRB strains contributed to MeHg degradation under anoxic conditions and Hg(II), rather than Hg(0), was the product of the degradation consistent with the OD pathway.

Several recent studies with freshwater and estuarine sediments, as well as paddy soils, also confirmed that the principal microorganisms involved in OD were anaerobes, SRB, and methanogens.[119,120,123] However, as noted earlier, it remains challenging to isolate and tease out specific microbial strains supporting OD as the incubation systems are net MeHg sources under anoxic conditions. Most studies relied on the use of inhibitors to differentiate a role to specific microbial guilds in OD. For example, in a recent microcosm study of profundal sediment and bottom water from a sulfate-rich, hypereutrophic reservoir, Fuhrmann et al.[120] reported that the addition of molybdate, an SRB inhibitor, generally decreased the MeHg concentration due to inhibited MeHg production or stimulated MeHg degradation by SRB. However, the addition of the methanogenic inhibitor BES substantially increased MeHg concentration 2−4-fold, suggesting that methanogens were potent demethylators. One study attempted to isolate and differentiate microbial communities responsible for demethylation according to their dependence on oxygen into three groups: aerobic, anaerobic, and SRB microbial communities.[123] Significant demethylation was observed with both aerobic and anaerobic microbial communities in oxic and anoxic environments. However, the study was unable to pinpoint specific microbes responsible for MeHg degradation, as many complex environmental factors are known to affect the dynamics of Hg cycling and transformation in the environment.[124]

**OD Demethylation Mechanisms.** OD was named since the final products of demethylation were the oxidized forms Hg(II) and $CO_2$ and a small amount of $CH_4$ (Figure 1).[41,118] The difference of OD between SRB and methanogens is that the former can produce $H_2S$ and $CO_2$ (eq 1) while the latter produces $CH_4$ (eq 2).[41,46] It was later speculated that OD is probably related to the one-carbon (C1) metabolism of SRB and methanogens, since the addition of C1 compounds such as methanol, methylamine, and methylsulfide significantly inhibited demethylation.[41,47] Unlike the active detoxification mechanism of MerB-carrying microorganisms, OD is proposed as part of microbial metabolisms, with MeHg likely acting as the electron donor.[41,46] It was hypothesized that the mechanisms for degradation of MeHg by SRB is similar to that of acetate oxidation (eq 1) while the co-metabolism of MeHg by methanogens proceeds by an OD pathway analogous to monomethylamine degradation (eq 2):[46]

$$SO_4^{2-} + CH_3Hg^+ + 3H^+ \rightarrow H_2S + CO_2 + Hg^{2+} + 2H_2O \quad (1)$$

$$4CH_3Hg^+ + 2H_2O + 4H^+ \rightarrow 3CH_4 + CO_2 + 4Hg^{2+} + 4H_2 \quad (2)$$



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Therefore, an increase in the $^{14}CO_2/^{14}CH_4$ ratio in anoxic incubations may reflect a shift from methanogen to SRB dominated demethylation, which does not produce $CH_4$ (eq 1). However, we again emphasize that, while these hypothesized OD pathways are sound, they have not been experimentally validated. Bacterial strains that oxidatively degrade MeHg in water and sediments have not been isolated or characterized, and the pathways leading to OD remain to be investigated.[44,47,118]

**Oxidative Demethylation by SRB Revisited.** While most studies so far clearly showed the important role of SRB in OD, demethylation by SRB could be indirectly affected by the production of $H_2S$ during anaerobic respiration of sulfate, as reported in several early studies.[45,125,126] Abiotic reactions between MeHg and $H_2S$ can form relatively insoluble dimethylmercury sulfide [$(CH_3Hg)_2S$], which is then converted to HgS, DMeHg, and $CH_4$.[45,115,125,126] Baldi et al.[45] studied the response of two sulfate reducers, one methylator (*D. desulfuricans* LS),[127] and another nonmethylator and showed a high tolerance to MeHg that was 10-fold higher than that afforded to the aerobe by a broad-spectrum *mer* system. By carefully accounting for Hg speciation in their incubations and following the formation of various metabolites, the authors observed a spontaneous precipitation of $CH_3Hg^+$ as $(CH_3Hg)_2S$ with biogenic $H_2S$ which was then converted to $(CH_3)_2Hg$ or DMeHg and metacinnabar (HgS) in the $H_2S$-acidified growth medium. DMeHg was further converted to $CH_4$ and $CH_3Hg^+$. Overall, two molecules of $CH_3Hg^+$ were converted to HgS, $CH_3Hg^+$, and $CH_4$. This is, therefore, an example for $CH_4$ formation during demethylation that is not associated with reductive or oxidative demethylation. This process explained a previously described plasmid-encoded resistance to MeHg in another anaerobe, *Clostridium cochlearium*,[128] pointing to the generality of this resistance mechanism among anaerobes.[45]

A recent study thus calls for careful reexamination of reported OD of MeHg by SRB and perhaps other obligate anaerobes.[115] Experimentally determined demethylation rates could depend largely on experimental conditions, and there are many factors that can influence the reactions and measurements of MeHg degradation. For example, simultaneous production and degradation of MeHg were reported initially with the SRB strain *D. desulfuricans* ND132,[85] now reclassified as *Pseudodesulfovibrio mercurii*,[129] but subsequent studies found no significant demethylation by this strain, at least within a short term (up to 24 h).[130,131] Graham et al.[130] also tested the hypothesis that all *Desulfovibrio* species were capable of Hg(II) methylation but that rapid demethylation would mask its production. Their results indicate that demethylation is not observed in every *Desulfovibrio* strain tested. Moreover, in a recent study of Hg(II) methylation and demethylation by the SRB *Pseudodesulfovibrio hydrargyri*, Isaure et al. reported that demethylation did not require biological activity.[132] Zhou et al.[133] observed significant demethylation in water-saturated paddy soils, but the addition of molybdate and BES, as the respective inhibitors of SRB and methanogens, showed insignificant effects on MeHg degradation. These and other studies therefore underscore the need for additional detailed, thorough investigations of specific microbial species and the mechanisms of OD both in pure cultures and in natural water and sediments.

## Methanotrophic and Other Biotic Demethylation Pathways

While both RD and OD pathways have received extensive attention, two early studies and some recent work have shown possible involvement of iron-reducing bacteria (FeRB)[41,86,134] and methanotrophs[135,136] in MeHg degradation. Studies by Oremland et al.[41] found that demethylation of $^{14}CH_3HgI$ by freshwater methanogens accounted for only ∼50% of the $^{14}CH_4$ production and a minor amount of $^{14}CO_2$ production, implying that other microbes, in addition to methanogens and SRB, also contributed to MeHg demethylation. Similarly, in a study of microbial methylation and demethylation in anoxic sediments under iron-reducing conditions, MeHg degradation was consistently observed at rates comparable to those observed in sulfidogenic and methanogenic wetland sediments.[134] Both of these studies suggest that FeRB could be at least partially responsible for demethylation in anoxic sediments, although no additional details or follow-up investigations were carried out to characterize the microbial communities involved. Recent studies using sequencing and metatranscriptomic analyses suggest that some microbial taxa, such as *Catenulisporaceae*, *Frankiaceae*, *Mycobacteriaceae*, and *Thermomonosporacea*, may be directly or indirectly associated with MeHg degradation in rice paddy soils.[133]

The role of FeRB in MeHg degradation has been shown in one pure culture study, in which *Geobacter bemidjiensis* Bem was found to both methylate Hg(II) and demethylate MeHg at low Hg concentrations (i.e., picomolar to nanomolar) in anoxic incubations.[86] Although the exact mechanism of MeHg degradation by this organism is not fully understood, it is speculated that *G. bemidjiensis* Bem uses a reductive demethylation pathway to degrade MeHg anaerobically, with elemental Hg(0) as one of the major degradation products. Nearly 100% of MeHg could be converted to Hg(0) in 48 h, although the production of $CH_4$ and $CO_2$ was not determined or discerned due to the low concentrations of MeHg used in the study. The *G. bemidjiensis* Bem strain does contain a *merA* gene encoding a mercuric reductase and could thus have the potential to reduce Hg(II) to Hg(0).[86] Homologs of the alkylmercury lyase MerB are also found in *G. bemidjiensis* Bem, but the role of MerA and MerB in the degradation of MeHg by *G. bemidjiensis* requires further investigation. If proven, this demethylation pathway could be important in the natural environment as bacterial species closely related to the *G. bemidjiensis* Bem strain are widely observed in subsurface sediments and permafrost soils,[137−140] where MeHg is readily formed.

Some early studies also suggested the potential involvement of methanotrophs leading to OD,[41] but no further investigations were pursued due to the assumption that methanotrophy would otherwise produce copious amounts of $CO_2$ from $CH_4$ oxidation and thus dilute $^{14}CO_2$. Recent studies with pure cultures, however, indicate that some strains of methanotrophs (e.g., *Methylosinus trichosporium* OB3b and *Methylocystis* sp. SB2) can not only take up but also degrade MeHg at low picomolar to nanomolar concentrations (Figure 4).[135,141] Other methanotrophs, such as *Methylococcus capsulatus* Bath, *Methylocystis parvus* OBBP, and *Methylomicrobium album* BG8, can take up but not degrade MeHg (Figure 4A). Demethylation appears to be facilitated by a metal-binding chalkophore, methanobactin, synthesized by methanotrophs such as *M. trichosporium* OB3b and *Methyl-*


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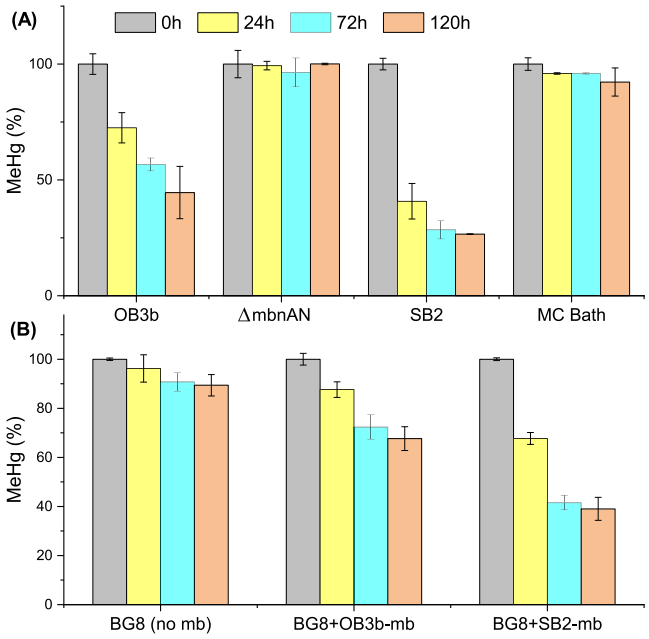


**Figure 4.** (A) Methylmercury (MeHg) degradation by different methanotrophs, *M. trichosporium* OB3b and its methanobactin (mb)-deficient mutant Δ*mbnAN*, *Methylocystis* sp. SB2, and *M. capsulatus* Bath in MOPS buffer (5 mM).[135] (B) Degradation of MeHg by *M. album* BG8 in MOPS in the absence or presence of 45 μM mb isolated from either *M. trichosporium* OB3b or *Methylocystis* sp. SB2.[141] Modified from Lu et al.[135] (Copyright Science Advances) and from Kang et al.[141] (Copyright Springer Nature).

*ocystis* sp. SB2. This was evidenced by no demethylation activities in the OB3b mutant Δ*mbn*AN deficient in the synthesis of methanobactin (Figure 4A). However, demethylation was not observed with the isolated methanobactin alone, indicating that methanobactin likely served as a binding agent or a mediator that enables methanotrophs to degrade MeHg.[135] Methanobactin is therefore required for methanotrophic-mediated demethylation, but it is not sufficient. It is hypothesized that demethylation by methanotrophs may involve the initial binding of MeHg by methanobactin followed by cleavage of the C−Hg bond in MeHg by methanol dehydrogenase (MeDH),[135] since the addition of methanol (>5 mM) inhibited MeHg degradation. In an early study of MeHg degradation in freshwater sediments, Oremland et al.[41] also noted an inhibitory effect of methanol and suggested that metabolic pathways for methanol utilization may be involved in demethylation of MeHg. However, this proposed mechanism has yet to be experimentally validated,[135] although deletion of methanol dehydrogenase genes may be lethal for methanotrophs as they are essential for metabolism.

The findings of methanotrophic demethylation suggest a potentially significant pathway of MeHg degradation in natural water and sediments, as these microbes are ubiquitous, particularly at the anoxic−oxic interface where $CH_4$ and MeHg are commonly observed. Methanotrophs are found in diverse locations, such as freshwater and marine sediments, bogs, forest and agricultural soils, and volcanic soils,[142,143] although questions remain as to what extent these microbes may take up or degrade MeHg in the environment. Demethylation could be potentially overwhelmed by the large availability of other reduced C1 compounds (e.g., $CH_4$) in the environment where methanotrophs are active. However, all methanotrophs tested to date ($n$ = 10) were found to take up substantial amounts of MeHg, regardless of whether they can degrade it.[135] More importantly, recent studies by Kang et al.[141] indicate that methanotrophic-mediated MeHg degradation may be more widespread than previously thought, as some methanotrophs (e.g., *M. album* BG8) that cannot produce methanobactin could "steal" or take up methanobactin made by others, allowing them to degrade MeHg (Figure 4B). Demethylation could be substantially enhanced with the addition of methanobactin isolated from either *M. trichosporium* OB3b or *Methylocystis* sp. SB2. While additional studies are warranted, these results suggest a possible broader involvement of C1-metabolizing aerobes and important roles of methanotrophs in the degradation of MeHg.[135,141] It is thus important to consider these microbes in assessing the relative rates of methylation and demethylation as an indication of the potential for MeHg accumulation in the environment.

Finally, we note that biotic demethylation is not limited to microorganisms but may also involve higher plants, phytoplankton, animals, and wildlife in environmental systems. Phytoplankton is known to take up and concentrate MeHg, and eutrophication and algal blooms are found to reduce MeHg levels,[144−146] which is often referred to as an algal dilution effect, resulting in decreased bioaccumulation through trophic transfer.[145,147] This process could be important, especially considering global environmental changes such as the increased frequency, magnitude, and duration of phytoplankton blooms.[32,148] However, whether phytoplankton can degrade MeHg is an open question. While unproven, several recent studies suggested the possible MeHg degradation by phytoplankton, including the marine alga *Isochrysis galbana* under solar irradiation[149] and the pico- and nanoplankton,[150] although the pathways involved in demethylation were unclear as these reaction systems were not axenic. Demethylation in rice plants has also been documented.[151,152] It was found that, when exposed to MeHg, the percentages of inorganic Hg in rice roots and shoots increased while MeHg decreased.[151] Strickman and Mitchell[152] noted significant losses of MeHg from rice plant tissues between flowering and maturity in a greenhouse experiment. However, these experiments were not performed under axenic conditions either and the pathways and mechanisms remain unclear. Several studies also reported demethylation occurring in mouse liver, waterbirds, fish, and marine mammals.[153−157] One recent study found in vivo detoxification of MeHg-cysteinate (MeHgCys) in multiple animals (e.g., waterbirds, freshwater fish, and earthworms),[158] although the mechanism appeared to result from abiotic transformation(s) of MeHgCys into selenocysteinate [$Hg(Sec)_4$] complexes (described below). Others attributed demethylation to microorganisms, such as those isolated from yellowfin tuna.[157]

## ABIOTIC DEMETHYLATION

Biological demethylation is clearly important in MeHg degradation in water and sediments, particularly below the photic zone. However, abiotic demethylation has long been recognized (Figure 1), although most studies so far have focused on photochemical demethylation, which is thought to be responsible for as much as 80% of MeHg degradation in some freshwater lakes.[159−162] A recent study suggests that dark abiotic demethylation may contribute equally to biological demethylation in some swamps in Sweden.[163] Additionally, MeHg is shown to be degraded through the formation and


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decomposition of the binuclear bis(methylmercuric(II)) sulfide complex, $(CH_3Hg)_2S$, in sulfide-rich environments.[45,114,115,125,126] The presence or absence of sulfide or sulfide minerals is thus considered as a key factor behind the variability in rates of MeHg formation and demethylation reported for different environmental settings.[164] All these demethylation processes, in combination with Hg methylation, determine the net MeHg levels in the environment and are thus critically important in assessing the bioaccumulation and health impact of MeHg.

### Photochemical Demethylation

MeHg can be degraded either directly or indirectly by light through photolysis or photochemical reactions, a mechanism so-called photodemethylation (Figure 5). Photodemethylation

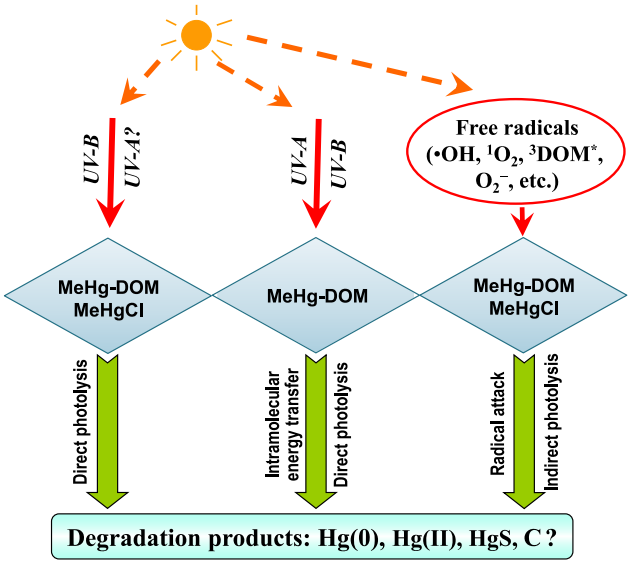


**Figure 5.** Proposed photodemethylation pathways of methylmercury (MeHg) predominantly in the form of either MeHg−DOM or MeHgCl complexes in freshwater or seawater.[178,180] Direct photolysis of the C−Hg bond in MeHg (left), photolysis by photoexcited DOM-MeHg (i.e., organic ligand-MeHg) complexes resulting in direct energy transfer and breakdown of the C−Hg bond (middle), and indirect photolysis by photochemically produced free radicals and reactive oxygen species (right). UV-B irradiation (280−320 nm) is the most effective in causing direct photolysis of MeHg, although it is a minor component of solar irradiation reaching the earth's surface.[33,181]

is obviously only significant in surface water due to rapid attenuation of light in the water column, especially for ultraviolet (UV) radiation.[159,160,165] Additionally, the rate and efficiency of photodemethylation strongly depend on the radiation intensity and wavelength; shorter waveband UV-B light (280−320 nm) is far more effective in degrading MeHg than longer waveband UV-A light (320−400 nm) and the visible or photosynthetic active radiation (PAR) (400−700 nm).[33,160,161,166−170] For example, in a study of photodemethylation along a lake-wetland gradient in a boreal coniferous forest, the relative ratio of demethylation rate constants with UV-B, UV-A, and PAR was found to be 3100:43:1 in surface waters.[167] Similarly, Black et al.[161] reported that photodemethylation rate constants were at least 400-fold greater for UV-B and 37-fold greater for UV-A than PAR in water. In a study of the effect of natural solar spectrum and UV radiation on the extent and signature of the Hg isotope MIF, Rose et al.[171] found that UV-B radiation was mostly responsible for the MIF with minor contributions from UV-A during photodemethylation. The strong relationship observed between the Hg MIF and energy of incident radiation suggests that MIF signatures may be used as a tool for quantifying photochemical cycling of Hg. Indeed, MIF has been demonstrated in tracing the source and degradation of MeHg in marine biota,[149,156,162,172−177] and it is estimated that about 56−80% of MeHg could be photodegraded prior to entering the food web.[162] MIF has also been used in studying the effects of DOM and its chemical-structural properties on photodemethylation of MeHg,[171] but additional studies are warranted to understand the links between MIF and photoreduction and photodemethylation before this tool can be fully utilized.

**Role of DOM in Photodemethylation.** While photodemethylation is clearly waveband specific, environmental factors, such as the presence or absence of DOM, reactive oxygen species (ROS), and free radicals, are known to influence the rates and extent of photodemethylation. DOM forms strong complexes with MeHg[178,179] and can thus have significant influences on MeHg chemical speciation and its subsequent photochemical transformation in water. MeHg in natural water is unlikely present as the free $CH_3Hg^+$ ion, but rather it is complexed with DOM, especially in freshwater, where DOM concentrations are usually many orders of magnitude higher than MeHg concentrations. Therefore, different rate constants observed with UV-B, UV-A, and PAR under different environmental conditions[33,160,161,166,167] may be explained partially by the variations in water chemistry, such as the quantity and quality of DOM. DOM can influence photodegradation rates of MeHg in multiple ways, either by increasing the rates of demethylation through the formation of MeHg−DOM complexes and photochemically produced free radicals (discussed below) or by decreasing the rates by quenching free radicals or through the attenuation of solar radiation that causes photodemethylation.

Since DOM contains abundant aromatic compounds or chromophores,[182,183] it strongly absorbs the available radiation, particularly at those shorter wavebands involving MeHg photodegradation. It is therefore not surprising that, in surface waters containing relatively high contents of DOM, much of the light could be absorbed by DOM and a negative correlation is observed between DOM contents and photodemethylation rates.[30,181,184,185] Competition for photons within DOM structures may also reduce the potential for MeHg photodegradation in high DOM waters. For example, Li et al.[166] observed that about 31.4% of MeHg was removed by photodegradation in Florida Everglades water and this percentage of photodemethylation was much lower than that reported for other ecosystems,[159,160] likely resulting from the higher concentration of DOM in the Everglades water. Additionally, studies by Fleck et al.[186] suggest that the optical signatures or the quality of DOM were important in affecting MeHg photodegradation. Relationships between MeHg loss and DOM optical properties (or absorption bands) indicate that aromatic and quinoid structures within DOM are important contributors to MeHg photodegradation. However, once corrected for light attenuation caused by DOM absorbing components in different water samples, Fernandez-Gomez et el.[167] found that all experimental results converged to a common photodemethylation rate constant at a given


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irradiation wavelength. Motta et al.[162] suggested that there might be a common mechanism for photodegradation of MeHg in surface waters.

What remains controversial are the mechanisms and pathways by which DOM enhances photodemethylation. There is a lack of consensus regarding the role of DOM in the photodegradation of MeHg.[30,161,167,181,185,187−191] Some studies observed a positive correlation between MeHg photodegradation and DOM concentration,[160,167,181,187,191,192] but others found no significant influence or negative correlations between DOM and MeHg photodegradation.[161,185,186,188,190,193] This discrepancy or the lack of DOM-concentration dependent effects on photodemethylation may be explained by the multicomponent, multifunctional properties of DOM[194,195] and the use of relatively high DOM concentrations or high DOM-to-MeHg ratios in various experiments. As noted earlier, a high DOM could in fact decrease photodemethylation rates as DOM contains abundant chromophores, which can attenuate the radiation energy and quench free radicals. In natural water, MeHg concentrations are extremely low (in the pM range), but DOM concentrations are orders of magnitude higher, usually at micromolar to millimolar DOC ranges.[178] Chemical speciation analysis indicates that nearly 100% of MeHg is complexed with thiols at a thiol-to-MeHg ratio of $\sim 10^2$ so that a further increase in the thiol-to-MeHg ratio would be expected to show little influence or even inhibit the photodegradation of MeHg.[30,181,185] On the other hand, positive correlations between MeHg photodegradation and DOM concentration could be expected in experiments with low DOM concentrations or low DOM-thiol-to-MeHg ratios (e.g., 0.006−50).[181,187,189,191] These findings are counterintuitive, as it suggests that as long as there exist excess amounts of DOM (or DOM-thiols) for MeHg binding, the nature and concentration of DOM may not be important to the rate of MeHg photodegradation. However, the extent and signature of Hg MIF could still be sensitive to different DOM ligands that bind MeHg.[196] Therefore, a negative or no correlation between DOM concentrations and MeHg photodegradation by no means indicates that DOM is not involved in demethylation but may be masked due to the presence of excess amounts of DOM.

**Photodemethylation Mechanisms.** Direct and indirect photolysis are the two major pathways responsible for photodemethylation (Figure 5). Direct photolysis can occur through the absorption of light by the C−Hg bond of MeHg[33,181] or the Hg−S bond of MeHg−DOM complexes, leading to energy transfer to and breakdown of the C−Hg bond.[187,189] Rapid, direct photolysis of MeHg in deionized water was observed under artificial UV-B radiation,[33] but little or no direct photolysis occurred under UV-A or PAR radiation,[33,181] again indicating that the rates and extent of photodemethylation are strongly waveband dependent. The result suggests that, in purified water, only UV-B may play a role in direct photolysis of MeHg. Rose et al.[171] also found that UV-B radiation under the solar spectrum was mostly responsible for the MIF observed during photodemethylation, although UV-B makes up only less than 0.25% of the total solar radiation that reaches Earth's surface.[167,197,198] While UV-A makes up about 5% of the total solar radiation reaching Earth's surface,[167,197,198] it seems ineffective in causing direct photolysis of MeHg in purified water.[33,181] However, natural water or even purified water always contains some levels of DOM (e.g., >0.2 mg $L^{-1}$), which are usually in a large excess compared to typical picomolar concentrations of MeHg in water. Therefore, MeHg in natural water is mostly complexed with DOM via the Hg−S bond[178,179] and light absorption by the MeHg−DOM complexes leads to direct or indirect energy transfer to and breakdown of the C−Hg bond. As such, UV-A is thought to play a major role in degrading MeHg, as illustrated by photodemethylation rates being about 10-fold higher in lake water samples exposed to full spectrum solar radiation than those exposed only to visible light (400−700 nm).[160] In the high DOM waters of the Florida Everglades, the relative contributions of solar UV-A, UV-B, and visible light to photodemethylation were found to be about 85, 15, and 0%, respectively.[166]

In simulated laboratory photodegradation experiments, the addition of DOM was found to increase both the rates and extent of MeHg photodemethylation.[33,181,189,192] Additionally, it was found that some compounds (e.g., thiosalicylate and reduced DOM) containing both thiols and aromatic moieties within the same molecule increased the rate of MeHg photodegradation by far more than those containing only aromatics or thiols (e.g., salicylate or glutathione, or their combinations). The enhancement mechanism is attributed to a combination of (1) strong binding between MeHg and thiolate functional groups and (2) a photoexcited triplet state of the aromatics on the same DOM molecule, resulting in direct energy transfer and breakdown of the C−Hg bond in MeHg (Figure 5). MeHg binding to the reduced S group pulls electrons toward S, increasing the electronegativity of C and thereby weakening the C−Hg bond and leading to its breakdown by the excited triplet state.[189] These findings corroborated with the observation of aromatic and quinoid structures within the sphere of the Hg(II)−DOM bond being mostly responsible for MeHg photodegradation.[186] In a systematic study of the photodegradation of MeHg bound to a variety of organic ligands or DOM analogues with different functional groups, such as thiosalicylate, thiophenol, and thioaniline, Zhang et al.[192] also showed that thiol and phenyl functional groups together play a critical role in governing the photodegradation of MeHg. Similarly, Fernandez-Gomez et el.[167] conducted experiments spanning a range of MeHg-to-DOM ratios, whereby at high ratios MeHg is forced to bind to O and N functional groups in DOM due to saturation of the more favorable reduced S binding sites. Their results indicated that degradation rates decreased as MeHg-to-DOM ratios increased, thereby supporting the above hypothesis that binding of MeHg to reduced S in DOM is essential to photodemethylation. Tai et al.[187] also concluded that direct photolysis of MeHg−DOM complexes via intramolecular electron transfer is likely the dominant mechanism for demethylation in the surface water of the Florida Everglades whereas ROS played only a minor role in MeHg photodegradation.

The indirect mechanism of photodemethylation is generally attributed to photochemically induced ROS and free radicals, such as hydrogen peroxide ($H_2O_2$), hydroxide radical (·OH), singlet oxygen ($^1O_2$), superoxide ($O_2^-$), and the excited triplet state of DOM ($^3$DOM*) (Figure 5). These ROS and radicals widely exist in nature, and, in particular, light absorbing chromophoric groups in DOM (e.g., quinones or semiquinones, phenols, peroxyls, and carbonyls) are known to produce an array of ROS, including $H_2O_2$, $O_2^-$, $^1O_2$, ·OH, and $^3$DOM*.[189,199−203] Regretfully, results so far remain con-


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troversial regarding the role of ROS and free radicals on MeHg photodegradation.[161,167,187−191,204] For example, Hammerschmidt and Fitzgerald[190] reported that iron and ·OH radicals, not DOM, are primarily responsible for MeHg photodegradation. Other studies, however, found that DOM could inhibit photodemethylation by complexing iron and/or scavenging ·OH radicals.[169,181] An early study of MeHg and EthHg degradation under UV and visible light suggested that $^1O_2$ was mainly responsible for photodemethylation in seawater.[204] Similarly, studies by Zhang and Hsu-Kim[191] and Sheng et al.[203] showed that $^1O_2$ produced by DOM or particulate soil organic matter after absorbing light leads to breakage of the C−Hg bond that has been weakened due to MeHg binding to a thiolate group on DOM. These authors argued that ·OH radicals played only a minor role in photodemethylation. However, studies by Fernandez-Gomez et al.[167] suggest that the spectrum dependence of MeHg photolysis is not caused by a single $^1O_2$ or ·OH radical but by many different organic radicals and/or ROS that are present over a wide spectral range. Black et al.[161] and Tai et al.[187] found that none of these reactants alone ($^1O_2$, ·OH, thiols, and iron) are important in MeHg photodegradation. These findings corroborate with those observed by Qian et al.[189] and Jeremiason et al.[188] It is thus suggested that multiple pathways[161] or direct photolysis of MeHg−DOM complexes may be responsible.[187,189] It is important to note, however, that most studies to date have relied on the use of scavengers to assess relative contributions of free radicals and ROS in photodemethylation. A recent study indicates that the choice of appropriate scavengers could be critically important in assessing photodemethylation pathways.[205] Among 20 commonly used radical scavengers, only 9 were found to be suitable in determining photodemethylation, as these reactions depended not only on the irradiation conditions but also on water chemical properties.[205] These findings may thus partially explain the observed discrepancies regarding the roles and mechanisms of different radicals and ROS in causing photodemethylation.

**Photodegradation Products of MeHg.** Most studies so far have focused on the rates and mechanisms of photodemethylation, but few studies have examined photodegradation products. Several studies found mercuric Hg(II) as the main product of MeHg photodemethylation,[191,206] whereas others reported that elemental Hg(0) is the main product.[170,172,192,207] In addition to Hg(0) and Hg(II), one study reported the formation of chloroform and formaldehyde via ·OH radical-induced degradation of MeHg during nitrate photolysis.[207] Identification of these reaction products is complicated by the fact that both Hg(0) and Hg(II) or Hg(II)−DOM complexes from photodemethylation are all photoreactive; they could be either photochemically reduced or oxidized, depending on water chemistry and experimental conditions.[208−213] Irradiation of Hg(II)−DOM complexes could lead to the formation of HgS,[213] and direct transfer of electrons from photosensitized DOM to Hg(II) within Hg(II)−DOM complexes was proposed as a pathway for Hg(II) photoreduction.[208] However, reactions between Hg(0) and photochemically produced ROS or DOM itself could also oxidize Hg(0) to Hg(II) species even in the dark.[211,212,214] It is thus expected that the pathways and reaction products of MeHg photodegradation may vary greatly in different aquatic systems with varying chemical compositions and DOM concentrations, making the relative importance of many factors dependent on experimental conditions. Therefore, the rates, products, and controls on photodemethylation warrant further investigation across diverse aquatic ecosystems, particularly those containing high concentrations of photoreactive dissolved species, such as DOM and Fe(II)/Fe(III). Toward this end, advanced molecular tools, such as high resolution Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) and electron paramagnetic resonance (EPR) spectroscopy,[195,211,213] could be useful in elucidating specific DOM or Hg(II)−DOM species, free radicals, and their transformations before and after light exposure.

## Chemical Demethylation Pathways

While most studies of MeHg degradation have focused on photochemical and microbial demethylation in the aquatic environment, chemical demethylation is known to occur despite the kinetic and thermodynamic stability of MeHg. Strong reductants (e.g., borohydride) or oxidants (e.g., bromine chloride) are commonly used in the laboratory to convert MeHg and other organo-Hg forms to inorganic Hg species even though these strong reductants or oxidants are usually absent in the natural environment. However, there are two known chemical demethylation pathways which have been demonstrated in laboratory studies: (1) MeHg reacts with $H_2S$ or sulfide minerals and degrades to form $HgS_{(s)}$ and DMeHg,[45,114,115,125,126,215] whereas DMeHg could also degrade to MeHg in the presence of dissolved sulfide and mackinawite (FeS);[116] (2) MeHg reacts with selenoamino acids leading to the formation of $HgSe_{(s)}$ as the final product.[216,217] While the latter reaction is observed at relatively high levels of selenium in laboratory experiments, the beneficial effect of Se on MeHg toxicity in mammals has been known for decades.[218−220] Additionally, limited studies have shown that iron- and manganese-bearing minerals, such as annite, birnessite, and amorphous $MnO_2$, could degrade or remove MeHg,[221−224] although sorption of MeHg on $MnO_2$ was identified as the dominant mechanism of its removal.[224] Hydroxyl radicals are thought to be responsible for MeHg degradation by annite, especially in the presence of Cu under oxic conditions,[221,222] but these observations were made with high concentrations of MeHg and annite. It remains questionable whether such reactions occur under realistic environmental conditions, particularly considering that minerals are often coated with natural organic matter and MeHg could be sorbed or tightly associated with organic matter and minerals in soil.

Rowland et al.[126] are among the first who observed significant loss or degradation of radio-labeled $Me^{203}Hg$ when reacted with $H_2S$ in laboratory incubations. The loss of $Me^{203}Hg$ increased linearly with its concentration, but the reaction products were not identified in this study. Subsequent studies by Craig and co-workers[125,215] confirmed a rapid loss of MeHg during reactions with $H_2S$. The mechanism of MeHg loss was attributed to the formation of $(CH_3Hg)_2S$, which then slowly decomposes to form HgS and volatile DMeHg. These early studies therefore pointed to a potential mechanism for the degradation of MeHg in the environment since $H_2S$ is known to be formed by SRB in anoxic water and sediments. Indeed, Baldi et al.[45] demonstrated that insoluble, decomposable, white $(CH_3Hg)_2S$ solids formed instantly in the reaction of MeHg with $H_2S$ formed by *D. desulfuricans* strains. This organomercurial $(CH_3Hg)_2S$ was extracted with chloroform and identified by gas chromatography equipped with mass spectrometry. These authors also found that the


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disappearance of MeHg was correlated to the production of $H_2S$ and thus rationalized that the *D. desulfuricans* were resistant to high concentrations of MeHg by producing insoluble $(CH_3Hg)_2S$, which ultimately decomposed to metacinnabar and DMeHg under anaerobic conditions.

Recent studies extended these findings by demonstrating the formation of DMeHg from MeHg adsorbed onto sulfide mineral surfaces or organic dithiols.[114,115] The reaction mechanism was proposed initially to involve neighboring MeHg moieties bound to sulfide sites on a mineral surface through an $SN_2$-type reaction mechanism to form DMeHg, where the remaining Hg atoms then incorporate onto the mineral surface.[114] However, subsequent studies using a combined experimental and computational density functional theory (DFT) calculation approach suggest that coordination of Hg(II) by multiple S atoms on the sulfide mineral provides transition state stabilization and activates a C−Hg bond for methyl transfer or DMeHg formation.[115,225,226] The reaction between MeHg and $H_2S$ proceeds through a novel mechanism involving rearrangement of the $(CH_3Hg)_2S$ complex or direct transmethylation from one MeHg substituent to another facilitated by strong Hg−Hg interactions that activate a methyl group for intramolecular transfer to form DMeHg.[225] These findings are important and suggest that reduced sulfur groups on mineral surfaces could mediate the degradation of MeHg concomitant with formation of DMeHg in the environment since a significant fraction of MeHg (up to 90%) in natural waters could be adsorbed on sulfide mineral or organic surfaces.[114,115] Moreover, an additional product of this process is $CH_4$ at low pH.[45] These observations cast doubts on reported oxidative demethylation of MeHg by SRB, as described earlier, since the binuclear $(CH_3Hg)_2S$ complex could be the dominant MeHg species under high MeHg concentrations typically used in laboratory investigations of MeHg production or degradation by SRB.[115]

Similar to the sulfide-mediated demethylation pathway, seleno-mediated demethylation is thought to involve reactions between MeHg and selenoamino acids via the formation of bis(methylmercuric)selenide $[(CH_3Hg)_2Se]$ and DMeHg as intermediates.[216,217] The final degradation product is $HgSe_{(s)}$. Importantly we note that these reactions are observed at relatively high levels of Se and only limited studies are currently available. However, this mechanism of MeHg demethylation is known to occur in vivo in humans[227,228] and in the liver and intestine of marine mammals,[218−220,227] as Se has long been recognized for its mitigating effect on Hg toxicity. Elevated cortical selenium with significant proportions of nanoparticulate $HgSe_{(s)}$ was observed in human brain tissue of individuals poisoned with high levels of MeHg.[227] Studies have also shown that a large portion of MeHg could be demethylated to inorganic Hg(II) species primarily in the liver and kidneys of waterbirds as $HgSe_4$ and minor Hg-dithiolate $[Hg(SR)_2]$ complexes.[229,230] Together, these findings suggest that Se could transform MeHg into inorganic Hg, potentially decreasing its bioavailability and bioaccumulation.

Future studies, however, are warranted to validate whether or not the sulfide- or seleno-mediated demethylation processes are occurring and what roles soil minerals and organic matter may play in natural systems at low MeHg concentrations. Several studies reported unexplained abiotic demethylation in natural sediments.[163,231] For example, using inhibitor addition and autoclaved control experiments, Kronberg et al.[163] found that the addition of molybdate (to inhibit SRB) had little effect on MeHg demethylation but abiotic demethylation dominated at one of the swamps they studied. Although the demethylation mechanism was unknown, these authors suggested that abiotic demethylation may be widely distributed with an equal contribution as biological demethylation. In a microcosm study of the interactions between $H_2S$ and MeHg in River Mersey (England) intertidal estuary sediments, Craig and Moreton[215] estimated that the formation and transport of DMeHg to the atmosphere could account for a loss of about 12% of MeHg annually from sediments and may thus be a substantive part of MeHg degradation contributing to the biogeochemical cycling of Hg in the environment.

## CONCLUSIONS AND RESEARCH NEEDS

(i) MeHg demethylation is just as important as methylation in determining how much MeHg is available for bioaccumulation in the food chain. Our current understanding is that demethylation is the consequence of several biological and abiotic processes that are impacted by a complexity of environmental factors. Microbial demethylation is a process that appears more widespread across the microbial genera than Hg methylation, which is largely attributed to the activities of anaerobic microbes, although this may be an oversimplification. Therefore, for a full understanding of net MeHg production in the environment more research is needed on the degradation of MeHg as influenced by multitude, complex environmental factors and dynamics.

(ii) *mer*-Mediated reductive demethylation (RD) largely occurs in sedimentary environments under oxic conditions and at relatively high Hg levels. The role of the microbial MerB in MeHg degradation in the environment is not clear. This knowledge gap is due to the lack of molecular tools needed to identify specific microbial processes within the complexity of the interactions that occur when microbial communities are functioning in their native habitats. The high diversity of MerB, its broad substrate-specificity range, and its unknown evolutionary origin and path challenge the design and application of reliable molecular probes. Isolation and characterization of "MeHg-specific MerB" would support the development of missing understanding and molecular tools to study this enzyme's role in biotic degradation of MeHg.

(iii) The term RD is only accurate to describe *mer*-mediated demethylation where the reduction of Hg(II) to Hg(0) follows the breakage of the Hg−C bond. RD as a general term describing processes that result in $CH_4$ formation is misleading, as we now know that other demethylation processes, both biotic and abiotic, may lead to the production of $CH_4$ during demethylation without changing the redox state of the C moiety.

(iv) The OD process involving SRB and methanogens has been proposed and studied for decades but, regretfully, no bacterial strains responsible for OD have been isolated from environmental samples, limiting mechanistic studies and understanding of this process. Moreover, several recent studies have casted doubt about OD by SRB and other obligate anaerobes, as MeHg readily reacts with $H_2S$, the product of sulfate reduction. That reaction results in the formation of $(CH_3Hg)_2S$ intermediates in anoxic environments, leading to the production of HgS, DMeHg, and $CH_4$. This indirect demethylation process occurs in solution as well as on mineral surfaces and may be a major pathway for demethylation in sulfidogenic environments.


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(v) Recent studies suggest that methanotroph-mediated demethylation likely plays an important role in MeHg degradation, as methanotrophs are observed in diverse environments, particularly at the oxic and anoxic interface where $CH_4$ and MeHg are produced. Current studies, however, have focused on pure culture incubations addressing the degradation mechanisms, which remain to be explored. Toward this end, detailed studies of the reaction pathways and products (C in particular) are needed. Isolation of methanotrophic strains and experiments involving the deletion of genes that are involved in demethylation will be essential in understanding and assessing the extent and significance of methanotrophic demethylation in the environment.

(vi) Photochemical demethylation is a dominant pathway in surface waters (e.g., lakes, oceans, shallow water bodies) leading to degradation of MeHg prior to uptake by the food web. The rates and extent of MeHg photodemethylation, however, strongly depend on the irradiation wavelength and intensity; shorter UV wavebands are much more effective than longer wavebands, resulting in direct photolysis of MeHg. Mechanisms of indirect photolysis by free radicals and ROS remain elusive, as the process is complicated by a multitude of environmental factors, the water chemistry, the DOM content, and the choice of scavengers. Complex interactions among various water constituents, free radicals, scavengers, and MeHg could result in either increased or decreased photodemethylation rates and should thus be considered fully in future investigations.

(vii) The multicompositions and multifunctional roles of DOM in photodemethylation are likely the culprit of discrepancies and controversies regarding the effects of DOM on MeHg photodegradation. DOM consists of thousands of molecular components and chromophores and can form strong complexes with MeHg and other metal ions [e.g., Fe(III)/Fe(II)], absorb or attenuate light, and quench or generate an array of photochemically induced free radicals. These complex reactions and processes could occur concurrently and may thus increase or decrease the rates and extent of photodemethylation, depending on specific environmental conditions and water chemistry. As such, advanced molecular tools and techniques, such as Hg MIF, FTICR-MS, and EPR spectroscopy, are needed to further explore how DOM chemical-structural properties influence MeHg photodegradation and whether a common mechanism is responsible.

(viii) Finally, while multiple pathways of MeHg degradation exist in the environment, specific environmental conditions and physicochemical boundaries determine which mechanism(s), for example, photochemical versus microbial demethylation, may dominate. It remains challenging to tease out what is the environmental significance of each of the demethylation processes, what microbes are responsible, and how they are modulated by environmental factors. Most studies so far have been conducted in controlled laboratory conditions necessary to elucidate mechanisms, but studies under realistic environmental conditions (e.g., natural water and sediments at low Hg or MeHg concentrations) are critically needed. The best way to link the transformations of Hg species and composition of the microbial community in environmental samples may be to isolate representative strains, characterize their demethylation activities, and apply this knowledge back to activities in the environment. A key to these studies is the use of Hg stable isotopes as well as $^{13}C$- or $^{14}C$-labeled MeHg to facilitate the identification of degradation intermediates and products. This approach, when employed at natural concentrations, has been used in pure culture incubations, sediment slurries, and other environmental samples and has shown a high potential in terms of sensitivity and precision.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsenvironau.1c00022.

Effect of redox, mercury concentration, and seasonality on the demethylation pathway in mercury-impacted ecosystems (PDF)

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### Notes

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## ■ DEDICATION

This Review is dedicated to the memory of Dr. Ron Oremland who made immeasurable contributions to our understanding of the biogeochemistry of many elements and of methylmercury demethylation in particular.

## ■ LIST OF ABBREVIATIONS

Asp - Asparagine
BES - 2-Bromoethanesulfonate
$CH_4$ - Methane
$CO_2$ - Carbon dioxide
Cys - Cysteine

DFT - Density functional theory
DMeHg - Dimethylmercury
DOC - Dissolved organic carbon
DOM - Dissolved organic matter
DTT - Dithiothreitol
EPR - Electron paramagnetic resonance
EthHg - Ethylmercury
FeRB - Iron-reducing bacteria
FTICR-MS - Fourier transform ion cyclotron resonance - mass spectrometry
Hg - Mercury
mb - Methanobactin
MeDH - Methanol dehydrogenase
MeHg - Methylmercury
MerA - Mercuric reductase
MerB - Organomercury Lyase
MIF - Mass independent fractionation
MOPS - Morpholino-propane sulfonic acid
NMR - Nuclear magnetic resonance
OD - Oxidative demethylation
OH - Hydroxyl
ORNL - Oak Ridge National Laboratory
PCMB - *p*-Chloromercuric benzoate
PHMB - *p*-Hydroxymercuric benzoate
PMA - Phenylmercuric acetate
RD - Reductive demethylation
ROS - reactive oxygen species
SRB - Sulfate-reducing bacteria
UV - Ultraviolet

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