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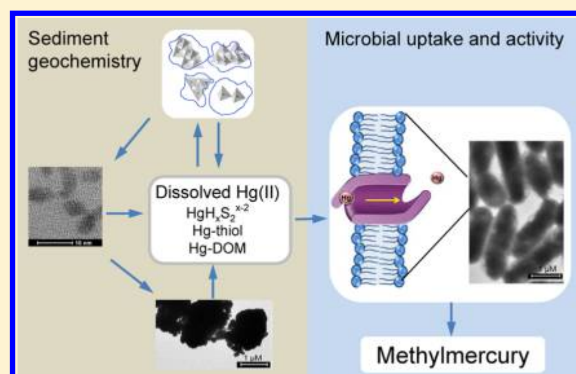
Mechanisms Regulating Mercury Bioavailability for Methylating Microorganisms in the Aquatic Environment: A Critical Review

Heileen Hsu-Kim,* Katarzyna H. Kucharzyk, Tong Zhang, and Marc A. Deshusses

Department of Civil and Environmental Engineering, Duke University, 121 Hudson Hall, Box 90287, Durham, North Carolina 27708, United States

ABSTRACT: Mercury is a potent neurotoxin for humans, particularly if the metal is in the form of methylmercury. Mercury is widely distributed in aquatic ecosystems as a result of anthropogenic activities and natural earth processes. A first step toward bioaccumulation of methylmercury in aquatic food webs is the methylation of inorganic forms of the metal, a process that is primarily mediated by anaerobic bacteria. In this Review, we evaluate the current state of knowledge regarding the mechanisms regulating microbial mercury methylation, including the speciation of mercury in environments where methylation occurs and the processes that control mercury bioavailability to these organisms. Methylmercury production rates are generally related to the presence and productivity of methylating bacteria and also the uptake of inorganic mercury to these microorganisms. Our understanding of the mechanisms behind methylation is limited due to fundamental questions related to the geochemical forms of mercury that persist in anoxic settings, the mode of uptake by methylating bacteria, and the biochemical pathway by which these microorganisms produce and degrade methylmercury. In anoxic sediments and water, the geochemical forms of mercury (and subsequent bioavailability) are largely governed by reactions between Hg(II), inorganic sulfides, and natural organic matter. These interactions result in a mixture of dissolved, nanoparticulate, and larger crystalline particles that cannot be adequately represented by conventional chemical equilibrium models for Hg bioavailability. We discuss recent advances in nanogeochemistry and environmental microbiology that can provide new tools and unique perspectives to help us solve the question of how microorganisms methylate mercury. An understanding of the factors that cause the production and degradation of methylmercury in the environment is ultimately needed to inform policy makers and develop long-term strategies for controlling mercury contamination.



1. INTRODUCTION

Mercury (Hg) is a global pollutant that is released from both natural and anthropogenic sources.[1] Molecules and materials containing this trace element can spread widely in the nature (even in remote areas) through a complex web of transformation and transport processes. In most environmental settings, mercury exists as the elemental form Hg^0, inorganic divalent Hg(II), and organomercury compounds, such as monomethylmercury (MeHg). Each form of mercury can impart health hazards, depending on the dose and route of exposure. MeHg is the species of most concern for humans[2] because of the highly bioaccumulative nature of this organomercurial compound.[3] The neurotoxic effects of MeHg to humans, particularly during early stages of brain development, have been well-documented.[4,5] Moreover, exposure rates to vulnerable portions of the population (maternal age women and newborn children) can be considerable. In the U.S. for example, maternal exposure rates suggest that tens of thousands to hundreds of thousands of children are born each year with in utero MeHg exposures exceeding health guidelines.[5,6] Maternal consumption of fish is believed to be the major route of exposure for newborns. Because of the health risks, millions of river miles and lake acres in the U.S. have been placed under fish consumption advisories,[7] indicating the widespread prevalence and persistence of methylmercury contamination in the environment.

The methylation of mercury in the aquatic environment is a critical step toward accumulation of this toxic metal in the aquatic food chain. MeHg is produced in the environment primarily by anaerobic bacteria that exist in most natural settings. MeHg levels in aquatic systems vary widely and do not necessarily correlate to the total amount of mercury in water or sediments.[8] Instead, mercury methylation rates generally depend on the productivity of the anaerobic microorganisms that can methylate mercury and the bioavailability of inorganic Hg(II) that can be taken up by these bacteria.[9−11]

The processes that result in elevated methylmercury concentrations in the environment have received much attention in the last three decades, yet much is unknown concerning the forms of inorganic mercury that are available for methylation and the biochemical mechanisms by which

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microorganisms mediate this process. This information is needed to determine how methylmercury "hotspots" occur in the environment and to predict the response of ecosystems that are directly or indirectly altered. For example, we have a limited ability to predict how an ecosystem may respond to changes in the source and flux of mercury inputs from atmospheric deposition. Moreover, efforts to remediate contaminated soil and sediment are stymied by our poor understanding of factors controlling methylmercury production. Finally, longer term hydrological and ecological disturbances (such as those induced by climate change) are expected to alter mercury biogeochemistry in ways that remain unknown.

In this analysis, we review our current understanding of the mechanism of microbial mercury methylation and the research needed to address this problem. This review is particularly focused on assessing Hg(II) bioavailability, that is, the geochemical forms of inorganic Hg(II) that can be taken up and methylated by anaerobic microorganisms. In most settings including the water column, aquatic sediments, and extracellular and intracellular matrices, the dissolved aqueous cation Hg^{2+} is a very small portion of total Hg(II).[12,13] Rather, Hg(II) is predominantly coordinated to other molecules (e.g., natural organic matter, chloride, sulfide) or adsorbed to particle surfaces. The species of Hg(II) to which methylating microorganisms are exposed will govern rates of uptake and biotransformation (i.e., methylation). Therefore, in this Review, we evaluate the conventional approach for estimating Hg(II) bioavailability for methylating microorganisms, particularly in light of recent discoveries that point to a different approach. Much progress has been made to delineate the speciation and fractionation of Hg(II) in environments where methylation occurs. This recent work includes studies describing the nanoscale products of reactions involving mercury, sulfide, and dissolved organic matter and the contribution of these species to bioavailability for methylating bacteria.[14−18] Other active research areas include efforts to characterize the diversity of methylating microorganisms and identify the mechanisms of biouptake and methylation. Ultimately, an understanding of the factors influencing mercury methylation potential will inform risk assessments of emission sources and also lead to appropriate strategies for remediating contaminated ecosystems.

2. SOURCES AND TRANSFORMATIONS OF MERCURY IN THE ENVIRONMENT

Mercury is released to the environment from a wide array of sources and cycles through all the compartments of the biosphere (e.g., atmospheric, aquatic, terrestrial), as described in review papers by others.[11,19−21] Natural sources of mercury include volcanic eruptions, forest fires, biomass burning, and low-temperature volatilization.[22] Anthropogenic sources to the biosphere include fossil fuel combustion, mining, waste disposal, and chemical production.[22] All of these sources release mercury to the atmosphere or mobilize the metal from terrestrial settings, leading to deposition or accumulation in aquatic ecosystems.

In the gaseous elemental form (Hg^0), mercury is capable of traveling across regional and global distances.[1,23] In the atmosphere, divalent forms of mercury Hg(II) partition more easily to water and particles (compared to Hg^0), resulting in much shorter distances over which Hg(II) travels in the lower troposphere. Thus, oxidative processes in the atmosphere strongly influence overall residence times.[1] In the aquatic environment, the major form of Hg is inorganic Hg(II), and redox reactions in surface waters can result in loss of gaseous elemental Hg^0 to the atmosphere. While MeHg is typically a small proportion of the total Hg in water and sediments, MeHg is the most toxicologically important species in regards to human health risks.[3] MeHg is better retained by higher-level organisms than other Hg species and is the predominant form of mercury that biomagnifies in the aquatic food chain.[24] Previous evidence suggests that nearly all of the mercury (>85%) in the muscle tissue of fish occurs as MeHg.[25−27] Because of the large biomagnification factors of MeHg, fish body burdens for MeHg can be as high as 10^6 times the MeHg concentration in the surrounding water.[2,28]

The accumulation of MeHg in biota is largely dependent on the MeHg concentration in water,[9,11,13] which is controlled by multiple transport and transformation processes involved in the mercury biogeochemical cycle.[19,20] In particular, the balance between MeHg production and degradation, namely, the rate of Hg(II) methylation relative to MeHg demethylation, determines the amount of MeHg in an aquatic system. Methylmercury can be generated from abiotic processes, particularly through pathways involving sunlight.[29,30] Likewise, sunlight degradation is believed to be a major pathway for the decomposition of MeHg at the surface of the water column.[31−34] However, in most freshwater and coastal aquatic settings, anaerobic microorganisms thriving in anoxic zones (such as benthic sediments, saturated soil, stratified water column, periphyton biofilms) are the dominant producers of MeHg. MeHg concentrations in these settings are typically a reflection of production and degradation processes that are occurring simultaneously and are mediated by a variety of microorganisms.

3. MICROBIAL METHYLATION AND DEMETHYLATION OF MERCURY

In low oxygen aquatic settings, the production and degradation of methylmercury is predominantly a microbial process. The biological mechanisms of mercury methylation and demethylation in the environment have been described in recent review papers.[10,35] Thus, this section aims to summarize our current understanding of the microbiology of MeHg production/degradation, particularly in light of advances in the past few years.

3.1. Microbial Production of Methylmercury. *3.1.1. Microbial Methylators.* The methylation of mercury by microorganisms in water, soils, sediments, and even the human intestinal tract has been broadly reported in the literature.[36−41] To date, the isolated environmental strains that are capable of mercury methylation have fallen mostly in the delta-*proteobacteria* classification,[10,42,43] with a few exceptions.[44] The most studied methylators of inorganic Hg(II) for environmental settings belong to sulfate-reducing bacteria (SRB), a group of obligate anaerobes that utilize sulfate as their terminal electron acceptor for energy generation.[45] Methylmercury production by iron-reducing bacteria and methanogens has also been observed in several instances.[46−48] Nevertheless, the dominant role of SRB in Hg(II) methylation is supported by extensive experimental evidence obtained with numerous pure SRB strains isolated from environmental settings[10] and in microcosm experiments with mixed microbial communities derived from sediments, low oxygen regions of the water column, and periphyton.[45,49,50] Evidence supporting the mercury methylating role of SRB in mixed communities

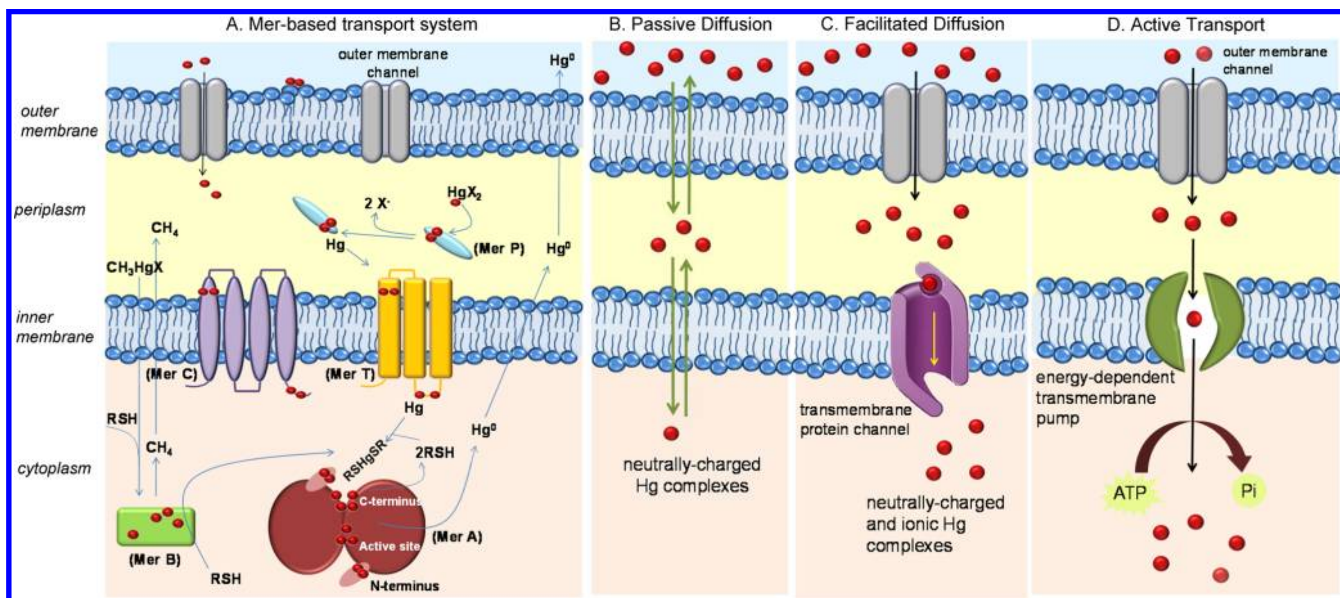

Figure 1. Possible mechanisms of inorganic Hg(II) uptake for Gram negative microorganisms (relevant to most of the methylating strains isolated from aquatic ecosystems). Hg(II) first enters the periplasmic space, likely by passive diffusion of lipophilic Hg(II) species through the outer membrane or by facilitated diffusion of hydrophilic Hg species and other Hg complexes (e.g., Hg-thiols) through outer membrane channels. Transport across the inner membrane could occur through: (A) Mer-based transport system where MerP binds Hg(II) in the periplasm, passes the mercury to MerT, and then transfers the element to MerA for reduction. (Figure 2A is adapted from Barkay et al.[175]) (B) Passive diffusion of lipophilic, neutrally charged complexes (e.g., $HgCl_2$, $Hg(HS)_2$). (C) Facilitated diffusion of neutrally charged or ionic species through a transmembrane protein channel. (D) Active transport of mercury via an energy-dependent transmembrane protein pump. In all cases, dissolved Hg(II) species (indicated by red circles) could comprise of a variety of Hg-ligand complexes, depending on local composition directly outside the outer cell membrane, in the periplasm, and in the cytoplasm. The species of Hg(II) that can be taken up depend on the mode of transport (passive, facilitated, or active) and binding affinities to membrane receptors (for facilitated and active pathways).

generally stem from experiments, where the addition of sulfate resulted in enhanced MeHg production (in sulfur-limited settings[51]), and the addition of molybdate, a selective inhibitor of sulfate reduction in SRB, suppressed Hg(II) methylation.[45,50] However, the ability of mercury methylation does not appear to correspond with the phylogeny of SRB. For example, not all SRB can methylate mercury.[30] Also, the capacity to generate MeHg among SRB was found to depend on the strain rather than species or genus.[43]

Among the non-SRB strains that can produce MeHg, researchers have isolated dissimilatory iron-reducing *Geobacter* spp. which are phylogenetically close to some methylating SRB within the class of delta-proteobacteria.[46,48] The ability to methylate Hg is not common to all iron-reducing bacteria, as indicated by the absence of mercury methylation capabilities by several *Shewanella* spp.[48] Methanogenic activity in macrophytic periphyton has also recently been linked to the methylation of mercury.[47] While the researchers of this study were not able to identify the individual methanogens responsible for mercury methylation,[47] the researchers did identify sequences of methanogens among the active microorganisms in the biofilms, including those in the archeal orders *Methanococcales*, *Methanobacteriales*, and *Methanosarcinales*.[47] The very recent discovery and characterization of a two-gene cluster, *hgcA* and *hgcB*, that correlates with the ability of bacteria to methylate mercury[52] will certainly open new possibilities for the identification of methylating organisms in complex environments.

3.1.2. Pathways of Mercury Biouptake. Microbial methylation of mercury is likely to be an intracellular reaction.[42,52−55] Thus, transport of inorganic mercury from the microorganism's extracellular surroundings and through the outer and inner membranes is an important step leading to its biomethylation.[42,55−57] A few possibilities exist for membrane transport of Hg(II) in microorganisms (Figure 1). For those with the *mer*-resistance system, the uptake of divalent inorganic Hg(II) is believed to be mediated by transport proteins, among which MerC, MerP, and MerT play an important role (Figure 1A).

In addition to the Mer-based transporters, alternative mercury transport pathways must exist since the known bacterial isolates that produce MeHg, including all obligatory anaerobic microorganisms, do not have the *mer* sequence in their genomes.[10] Most of the isolates capable of mercury methylation are Gram negative bacteria.[42,43] Thus in Figure 1B-D, we summarize the possible inorganic Hg(II) uptake mechanisms for these types of microorganisms. For Gram positive microorganisms, the possible uptake pathways would be similar except that these microorganisms lack an outer membrane lipid bilayer and possess a thicker peptidoglycan layer outside the cytoplasmic membrane.

One possible transport pathway is passive diffusion of lipophilic, neutrally charged complexes of Hg(II) across the cell membrane (Figure 1B). Evidence for a passive diffusion-based transport mechanism is generally limited to studies[58−60] with aerobic microorganisms that are not known to produce MeHg. In these studies, the organisms appeared to take up neutrally charged Hg-chloride complexes, forms of Hg(II) that are not expected in high abundance in anaerobic and organic matter-rich settings (described further in section 4). The concept of passive uptake of neutrally charged Hg(II) complexes has been applied to SRB in anaerobic settings,[61−63] but as we discuss later, assumptions regarding the geochemistry of Hg(II)-sulfides must be made for this approach to work.

More recent studies have directly focused on methylating microorganisms in identifying the rates of Hg(II) associations with cells and mechanisms of biouptake.[55,56,64,65] While it is difficult to distinguish between Hg adsorption to cells and transmembrane uptake, some of these studies[49,50] indicated that Hg(II) uptake did not occur through passive diffusion, but rather through another process, such as facilitated or active uptake with membrane transport proteins (Figure 1C and 1D). This body of work demonstrated that mercury added to cultures as Hg-complexes with low molecular weight-thiols resulted in association of Hg with the cellular fraction in the cultures and subsequent production of methylmercury.[55,56] While one could conclude that the Hg-thiol complexes were directly taken up by methylating microorganisms, an alternative explanation could be that the thiols prevented the formation or adsorption of Hg(II) to suspended particles in anaerobic culture media.[17,18,42] Other evidence also points to an active transport mechanism for Hg uptake, particularly for the iron-reducing strain *G. sulfurreducens* where the disruption of microbial metabolism resulted in decreased Hg uptake and methylation.[55] The involvement of an active uptake mechanism is less clear for SRB.[55,64] In experiments with the same SRB strain, methylation rates and microbial metabolism was demonstrated to be linked in one study[55] and poorly correlated in another.[64] If facilitated and active transport processes are indeed the major pathways for Hg uptake, it is likely that the membrane transporters are intended for a nonspecific function (e.g., trace metal uptake) and fortuitously mediate Hg(II) uptake.[55,66]

3.1.3. Biochemical Mechanism of Methlyation. Until very recently, little was known regarding the biochemical pathway of methylation after Hg(II) crosses the cytoplasmic membrane.[10,35] The enduring absence of identified genetic systems[36−41] for mercury methylation and lack of clear correlations of taxonomy of methylating microorganisms and methylation rates[43,45−48,67] have long been major obstacles for advancing our knowledge of this phenomenon. However, at the time this Review went to press, a two-gene cluster *hgcA* and *hgcB* was reported to be required for mercury methylation in *Desulfovibrio desulfuricans* ND132 and *Geobacter sulfurreducens* PCA. The gene cluster encodes a putative corrinoid protein facilitating methyl transfer and a ferredoxin carring out corrinoid reduction, resulting in mercury methylation[52] consistent with a pathway proposed earlier.[54] It is not yet known whether this gene cluster is universal to all mercury methylators. In any case, there is consenus that the biochemical reactions causing methylation of mercury are strictly intracellular, followed by a rapid transport or diffusion of MeHg outside the cell.[42,55,56,64] The ability to produce MeHg is constitutive rather than induced by exposure to mercury,[42] and as mentioned above, it appears to be closely linked to the *hgcA* and *hgcB* two-gene cluster. Since MeHg production is primarily associated with the activity of sulfate-reducing organisms, it has been proposed that the organism's ability to methylate mercury is most likely associated with substrate specificity of its enzymes.[54] Prior to the recent identification of the *hgcAB* system, microbiologists have postulated that methylmercury production could be linked to a specific methyl-transferase pathway, to a Hg-specific uptake pathway, or to the biochemistry of Hg binding within the cell.[10,42,68,69]

It now appears likely that one mechanism of mercury methylation follows one that was first described for the sulfate-reducing bacterium *Desulfovibrio desulfuricans* LS, a dissimilatory incomplete oxidizer of short-chain fatty acids.[38,67,54] Methylation of inorganic Hg(II) occurred through methylcobalamin compounds and the acetyl-coenzyme A (acetyl-CoA) pathway. This biochemical pathway for mercury methylation is largely consistent with the recent report[52] on the genetic basis for bacterial methylation of mercury. This mechanism is likely to be relevant for other SRB strains that utilize the acetyl-CoA pathway for major carbon metabolism.[68,69] However, several SRB strains have been observed to methylate mercury even though they either lacked detectable activities of acetyl-CoA enzymes or were exposed to acetyl-CoA inhibitors that blocked MeHg production in complete oxidizers.[68,69] Therefore, more than one biochemical pathway of Hg methylation may exist in SRB.

Future studies on the biochemistry of mercury methylation will build on the recent discovery of the *hgcAB* system, enabling mechanistic studies that could not be imagined before. The search for other possible biochemical pathways could perhaps target other enzymatic pathways that involve methyl transfer steps. An example is the synthesis of methionine, a process that is well-characterized for the fungus *Neurospora crassa*[70] and that likely occurs within most microorganisms.[71] Another possibility introduced by Larose et al.[72] is the biological degradation of dimethylsulfoniopropionate (DMSP), an organosulfur compound that is especially abundant in marine microorganisms and is best known as a protection agent against osmotic stress.[73] Decomposition of DMSP results in the generation of methyl donors that could be relevant for mercury methylation.[72,74] One could propose many other metabolic functions generating methyl donors, and more in-depth research is needed to determine their potential roles for MeHg production. Since these proposed pathways could occur in a wide variety of microorganisms, including both aerobes and anaerobes,[73] future work would also need to address why the methylation of mercury seems to occur only with anaerobic microorganisms and mainly certain sulfate reducers in the aquatic environment.

3.2. Microbial Degradation of MeHg. Biological demethylation is a major pathway of methylmercury degradation below the photic zone in the aquatic environment. A vast majority of the microorganisms identified as Hg methylators also have the ability to degrade MeHg.[57] Thus, microbial demethylation of MeHg should be considered in the overall assessment of mercury methylation potential in anaerobic settings. Compared to the progress made in identifying microorganisms that can methylate Hg(II), less work has been done to identify microorganisms that demethylate MeHg. Nevertheless, the capability for mercury demethylation does appear to be a widespread attribute among microbial communities in anaerobic settings.[75]

Microbiologists have described two pathways by which microorganisms degrade methylmercury.[75] The first is reductive demethylation mediated by the *mer*-operon system leading to the formation of Hg^0 and CH_4. The second is oxidative demethylation in which MeHg is degraded to inorganic Hg(II), CO_2, and small amounts of CH_4 as a cometabolic byproduct of methylotrophic metabolism.[75] Oxidative demethylation is mediated by anaerobic bacteria and may be somewhat analogous to monomethylamine degradation by methanogens or to acetate oxidation by sulfate-reducing bacteria.[35,75]

The specific biodegradation pathway for methylmercury in anaerobic settings has relevance to the global mercury cycle since reductive demethylation to elemental Hg^0 can result in evasion of gaseous mercury from water, soil, and sediments.[10,35]

In contrast, inorganic Hg(II) as the product of the oxidative demethylation can be available for methylation within the anaerobic microbial community. Thus, a cycle of methylmercury production and degradation may exist among anaerobic communities that do not have the *mer* operon, such as those identified microorganisms that can both methylate and demethylate mercury.[57]

4. GEOCHEMICAL FACTORS AFFECTING NET PRODUCTION OF METHYLMERCURY

Numerous microbial studies on mercury methylation have indicated that cellular uptake is a limiting step for MeHg production.[8,17,55] The uptake of Hg(II) may involve specific Hg(II) complexes or forms of mercury that can bind to a nonspecific transmembrane transport system[55] as shown in Figure 1. Therefore, the geochemical speciation of mercury in the environment will be critical toward determining the bioavailability of mercury for methylating microorganisms. As discussed in the previous section, the precise mechanisms of uptake remain largely unknown, and a few possibilities exist. Therefore, our ability to directly relate geochemical speciation and bioavailability remains limited.

4.1. Geochemical Speciation of Inorganic Hg(II) in the Aquatic Environment. The determination of the bioavailable forms of mercury for methylating microorganisms first requires an understanding of the forms of inorganic Hg(II) to which the methylators are exposed. A wide variety of Hg compounds exists in anaerobic settings, yet only a small portion of the total inorganic mercury is likely to be available for cellular uptake. In natural waters, inorganic divalent mercury generally persists in the form of aqueous mercury−ligand complexes (e.g., Hg^{2+} complexes with chloride, inorganic sulfide, or dissolved organic matter) or Hg(II) associated with particles (mercury-bearing minerals or Hg^{2+} adsorbed to particle surfaces). The relative partitioning of inorganic Hg(II) in various dissolved and particulate forms will govern the overall mobility of Hg in aquatic systems and the bioavailability of Hg to methylating microorganisms in anaerobic settings. One can deduce the partitioning of Hg(II) into different chemical forms based on experimental assays, such as size separation (i.e., filtration with a particular pore size or molecular weight cutoff) or metal−ligand complexation from experimentally determined thermodynamic binding strengths of "dissolved" Hg complexes.

Size fractionation of Hg(II) generally involves filtration of aqueous samples with filters of various pore size. While dissolved Hg(II) is often defined by the amount of the metal that can pass through a 0.2 or 0.45 μm filter, 20−80% of this fraction may comprise colloidal-bound Hg(II).[76−78] The lower end of this range generally occurs in saline water, while the higher proportion of colloidal Hg occurs in freshwater,[76−78] consistent with the flocculation of colloids in saline water. In the water of anoxic settings where methylation occurs (e.g., sediments, bottom waters, periphyton), the proportion of mercury in the colloidal fraction is not as well documented. However, the presence of colloidal Hg could be expected in light of evidence showing that nanoparticulate forms of Hg can persist as byproducts of metal sulfide precipitation occurring in the presence of dissolved organic matter (DOM).[14−16,79]

The size ranges that define dissolved, colloidal, and particulate mercury are based on the pore size or the molecular weight cutoff of filtration units employed in the experiment. The size distinctions are nominal, indeed, as there is no natural cutoff to distinguish between dissolved molecules, nanoparticles, and larger particles.[80] Furthermore, dissolved Hg(II) (with its sticky tendencies) can adsorb to filters, resulting in fractionation data that is difficult to decipher. To avoid potential artifacts, researchers must employ proper controls, such as filtration of a simulated water or an ultrafiltered natural water sample that has been spiked with dissolved Hg(II) to mimic the dissolved forms of mercury at the study site.[81] Ultrafiltration is often presumed to capture all forms of particles.[81,82] However, colloids that comprise of aggregates of smaller particles can potentially pass through filter membranes (especially under high pressure[83]) as a result of disaggregation at the membrane surface and reaggregation after the primary particles are forced through the membrane pores. Moreover, nanostructured particles (e.g., dendritic aggregates of nanoparticles) are likely to have different levels of reactivity toward dissolution in comparison to larger crystalline particles. The subtleties of these experimental artifacts are often overlooked and further complicate interpretation of size fractionation data.

Diffusive gradient thin film (DGT) passive sampling devices are another approach for fractionating inorganic Hg(II) species in anaerobic water and sediments.[84−88] The DGT device consists of a membrane or gel layered over a functionalized resin. When deployed in water or sediments, Hg(II) compounds (presumably only aqueous dissolved complexes of Hg) diffuse through this membrane/gel layer and accumulate on the resin through direct chelation of functional groups (e.g., thiolate ligands). This technique is typically used to estimate the "chemically labile" Hg(II) concentration, presumed to be dissolved species, over a specific deployment time. However, the approach requires assumptions regarding the diffusional properties of Hg(II) compounds into the sampler: the diffusion coefficients for low molecular weight species (e.g., $HgCl_2$ complexes) can be two times greater than coefficients for Hg(II)−DOM complexes.[87] Diffusion coefficients are also related to the stability of dissolved Hg−ligand complexes.[87] Therefore, further development of these passive samplers will need to address how Hg flux is altered by multiple Hg-binding site affinities on DOM and the wide range of molecular weight and aggregation states that can occur for Hg−DOM and polynuclear Hg−sulfide compounds. It is also unclear if DGT samplers are capturing Hg(II) originating from particles in the sampling matrix (i.e., through dissolution or desorption reactions).

The chemical species of Hg(II) in the aquatic environment can also be deduced based on binding strength of distinct dissolved Hg(II)−ligand complexes. Trace metal complexation has been studied extensively in the past using a wide variety of methods that include electrochemical, competitive ligand exchange, and chromatographic approaches.[89] Thiol-functionalized DGT resins could also be interpreted as a form of in situ competitive ligand exchange. Complexation of dissolved Hg(II) compounds has been quantified by competitive ligand exchange with a Hg^{2+}-binding ligand, typically a chelating agent or a low molecular weight thiol.[90−96] Hg(II) complexes with these competing ligands are then separated from the sample (e.g., with an ion exchange or hydrophobic resin, or via dialysis) and quantified. In general the competitive ligand exchange experiment involves a titration of the sample with either dissolved Hg(II) or the competing ligand. From the titration data, researchers then calculate the thermodynamic stability of the Hg−ligand complex that is native to the sample (often modeled as a single homogeneous ligand binding site). Hg−ligand binding strength has also been quantified based on reactivity

toward a reductant (i.e., stannous chloride), a method that is analogous to voltammetric techniques for other trace metals such as copper.

When all of these dissolved Hg(II) complexation techniques were applied to streams, rivers, estuaries, and municipal wastewater effluent,[90,91,95,97] the results generally demonstrated that the stability constants for Hg–ligand complexes resembled those for Hg–sulfhydryl (i.e., thiol) complexes, consistent with spectroscopic studies of Hg coordination to DOM isolates.[98−101] It is important to note that these Hg(II) complexation studies assume that only dissolved forms of Hg(II) are being probed in the experiments. This presumption could be particularly erroneous in settings such as municipal wastewater effluent that contain nanoscale metal sulfides as potential binding "ligands" for Hg(II).[90,102]

Overall, previous assessments of mercury geochemistry demonstrated that in most natural waters, Hg(II) exists as a mixture of dissolved, colloidal, and particulate phases. Furthermore, dissolved (and possibly colloidal) forms of mercury are associated with natural organic matter (NOM), particularly via specific binding with sulfhydryl functional groups on the NOM. In certain settings located near a source of sulfide, this Hg(II) can also be complexed by inorganic sulfides, such as dissolved or nanoparticulate entities.

4.2. Predicting Hg Methylation Potential: Chemical Equilibrium Speciation. Methylmercury production rates in water and sediments do not always correlate with the amount of total mercury (in filtered or unfiltered water).[20,63,103−105] Moreover, researchers have hypothesized that only a small proportion of the inorganic mercury in anaerobic settings is available for uptake by methylating bacteria.[8,19,20] To that end, bioavailability models have been devised to link the geochemical speciation of inorganic mercury to methylmercury production in anaerobic settings. The most established approach for modeling mercury bioavailability assumes that biouptake occurs through a passive diffusion mechanism.[61,106] In this case, one would presume that lipophilic mercury species, such as small, neutrally charged dissolved Hg(II) complexes, can be taken up by methylating microorganisms (Figure 2). From these assumptions, the concentration of bioavailable forms of Hg(II) (i.e., neutrally charged Hg–sulfide complexes) are subsequently estimated from thermodynamic equilibrium models of Hg(II) complexes.[61,106]

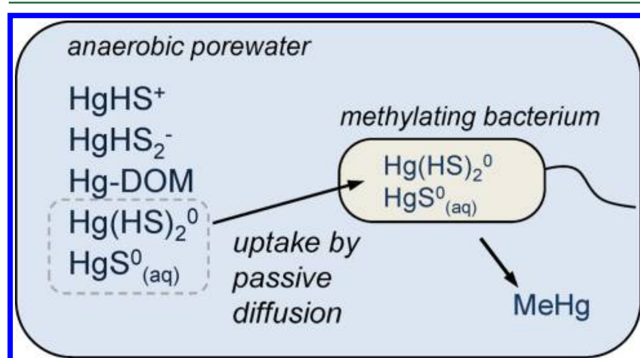

Figure 2. Neutral mercury-sulfide bioavailability model postulates that only the neutrally charged forms of Hg(II) are able to passively diffuse into methylating bacteria. The model also presumes that the speciation of dissolved inorganic mercury in porewater has reached chemical equilibrium. From this basis, equilibrium chemistry predicts that aqueous mercury-sulfide complexes are the predominant form of dissolved mercury in porewater, and the net production of methylmercury (MeHg) is related to the concentration of neutrally charged Hg–sulfide complexes. (Originally postulated by Benoit et al.[61])

In this modeling approach, the inputs are the concentrations of dissolved Hg(II), the concentrations of other aqueous constituents that can potentially complex Hg^{2+}, and the thermodynamic stability constants for the formation of these complexes (Table 1). This approach has been utilized by several others in attempts to draw correlations between observed MeHg concentrations in environmental samples and the calculated concentrations of neutrally charged forms of dissolved Hg(II).[63,104,107−110] The use of equilibrium speciation to predict mercury bioavailability relies on assumptions that the input parameters for the model (i.e., the stability constants) are accurate, that a clear distinction can be made between fully dissolved and particulate Hg(II) concentrations in the model system (e.g., with filtration or DGT techniques), and that the partitioning of mercury between various chemical species can be represented by equilibrium chemistry. Much work has been performed to address the first of these assumptions through studies that seek to improve the accuracy of Hg-ligand binding constants. The latter two assumptions bring many uncertainties for this approach. As discussed earlier, colloidal phases of Hg(II) could be an important fraction of mercury in 0.2-μm filtered water. The question of whether or not chemical equilibrium is reached under environmentally relevant conditions has not yet been firmly answered.

The general consensus emerging from this thermodynamic equilibrium approach is that dissolved mercury is mainly complexed to reduced sulfur-containing ligands such as inorganic sulfides (H_2S/HS^-, polysulfides) and organic sulfhydryls (e.g., dissolved organic matter) in anaerobic settings relevant to biomethylation. Much effort has been devoted toward quantifying stability constants for Hg–DOM complexes.[90−96] The values for stability constants vary widely and depend on empirical factors such as the reaction stoichiometry used to model the Hg–DOM interaction, the type of DOM, the method used to measure Hg–DOM constants, and the composition of the sample used to perform the measurement (e.g., Hg/DOM concentration ratio). Overall, most agree that at environmentally relevant dissolved Hg(II) concentrations (typically less than 1 nM), complexation of Hg^{2+} by DOM involves reduced-S functional groups.

While much of the focus in the past decade has been on elucidating the Hg–DOM interaction, thermodynamic predictions of dissolved Hg(II) complexation by inorganic sulfides are equally challenging. The difficulty lies with large discrepancies in binding constants and the identity of the major forms of Hg–sulfides. For example, there is much uncertainty concerning the dissolved $HgS^0_{(aq)}$ (or $HgOH\text{-}SH_{(aq)}$) complex, a species that was incorporated into a bioavailability model by Benoit et al.[61] (summarized in Figure 2). In developing this model, the researchers needed to include the neutrally charged $HgS^0_{(aq)}$ molecule as a form of dissolved Hg(II) to fit field data that included MeHg concentrations observed over a large sulfide concentration gradient. In the original paper citing the $HgS^0_{(aq)}$ compound,[111] the authors Dryssen and Wedborg extrapolated the intrinsic solubility of $HgS^0_{(aq)}$ ($K_{sp1} = 10^{-10}$ for the reaction: $HgS_{(s)} \Leftrightarrow HgS^0_{(aq)}$) (Table 1) from data on Zn- and Cd-sulfides. Dryssen and Wedborg also discussed the uncertainty of this K value and noted that this form of mercuric sulfide was probably colloidal HgS rather than a mononuclear aqueous complex. Exper-

Table 1. Stability Constants (I = 0 M, 25 °C) for $HgS_{(s)}$ Solubility and Hg(+II)−Ligand Complexation Reactions Relevant to Natural Waters

	log K	ref
β-$HgS_{(s)} + H^+ \Leftrightarrow Hg^{2+} + HS^-$	log $K_{s0} = -38.7 \pm 2$	117
$HgS_{(s)} \Leftrightarrow HgS^0{}_{(aq)}$	log $K_{s1} = -10$ or -22.3	111
$HgS_{(s)} + (n-1)S^0{}_{(s)} + HS^- \Leftrightarrow Hg(S_n)HS^-$	-3.97 ± 0.17	176
$Hg^{2+} + HS^- \Leftrightarrow HgSH^+$	30.2	61
$Hg^{2+} + 2HS^- \Leftrightarrow Hg(SH)_2{}^0$	37.7	111
$Hg^{2+} + 2HS^- \Leftrightarrow HgHS_2{}^- + H^+$	31.5	111
$Hg^{2+} + 2HS^- \Leftrightarrow HgS_2{}^{2-} + 2H^+$	23.2	111
$Hg^{2+} + HS^- \Leftrightarrow HgS_{(aq)}{}^0 + H^+$	28.7 ± 2[a]	
	16.4 ± 2[b]	
$Hg^{2+} + RS_2{}^{2-} \Leftrightarrow Hg(RS_2)$	38.3[c]	94
	28.7[d]	177
$RS_2{}^{2-} + H^+ \Leftrightarrow RS_2H^-$	8.4	94
$RS_2H^- + H^+ \Leftrightarrow RS_2H_2$	8.4	94
$Hg^{2+} + H_2O \Leftrightarrow HgOH^+ + H^+$	−3.4	117
$Hg^{2+} + 2H_2O \Leftrightarrow Hg(OH)_2{}^0 + 2H^+$	−6.2	117
$Hg^{2+} + 3H_2O \Leftrightarrow Hg(OH)_3{}^- + 3H^+$	−21.1	117
$Hg^{2+} + Cl^- \Leftrightarrow HgCl^+$	7.3	117
$Hg^{2+} + 2Cl^- \Leftrightarrow Hg(Cl)_2{}^0$	14.0	117
$Hg^{2+} + 3Cl^- \Leftrightarrow Hg(Cl)_3{}^-$	15.0	117
$Hg^{2+} + Cl^- + H_2O \Leftrightarrow HgOHCl^0 + H^+$	4.2	117
$H_2S \Leftrightarrow HS^- + H^+$	7.0	117

[a] log $K_{HgS(aq)}$ = log K_{s1} − log K_{s0}, where log K_{s1}= −10. [b] log $K_{HgS(aq)}$ = log K_{s1} − log K_{s0}, where log K_{s1}= −22.3. [c] log K_{Hg-DOM} for a peat humic acid. [d] log K_{Hg-DOM} for an aquatic humic acid.

imental measurements with photon scattering seemed to indicate that this HgS^0 'aqueous'complex, which was originally presumed to be lipophilic based on partitioning into octanol,[62] was more likely to be nanoparticles of HgS that could also partition into octanol.[14] Likewise, Skyllberg[112] postulated that the formation of $HgS^0{}_{(aq)}$ from $HgS_{(s)}$ should be represented by the smaller value estimated by Dryssen and Wedborg (K_{sp1} = $10^{-22.3}$).

The decision to incorporate $HgS^0{}_{(aq)}$ (and the appropriate stability constant) into equilibrium models for mercury speciation dramatically alters the outcome of the calculation and the predicted concentration of neutrally charged mercury species. For example, we calculated the equilibrium speciation of dissolved Hg(II) in a solution that would be representative of 0.2 μm-filtered water from an anaerobic setting (Figure 3). In calculations that utilized the Hg−DOM binding constant corresponding to aquatic humic acid (Figure 3A and 3B), the result indicated that the use of the larger intrinsic solubility K_{sp1} of 10^{-10} leads to an estimation that $HgS^0{}_{(aq)}$ is the dominant form of dissolved Hg(II) in anaerobic porewater at relatively low sulfide concentrations (<10^{-4} M total sulfide) and that other forms of dissolved mercury such as $HgS(HS)^-$ are important only at high sulfide levels (i.e., >10^{-4} M, Figure 3A). This transition coincided with observations that net MeHg production and Hg partitioning into octanol was reduced at high sulfide levels, leading researchers to believe that mercury bioavailability is related to passive diffusive transport of lipophilic Hg(II) complexes.[61,62] In contrast, if the smaller solubility constant for $HgS^0{}_{(aq)}$ is used in the speciation calculation ($K_{sp1} = 10^{-22.3}$), $HgS^0{}_{(aq)}$ concentration is negligible, and $HgS(HS)^-$ is the major form of dissolved mercury, regardless of sulfide concentration (Figure 3B). The need to fit a bioavailability model is a somewhat unsatisfactory basis for the choice of one stability constant over the other. In this case, the assumptions for the neutral mercury sulfide bioavailability theory need to be reexamined.

The choice of the Hg−DOM stability constant is another source of uncertainty for speciation models. The calculations in Figure 3A and 3B are based on Hg binding with aquatic humic substances ($K_{HgDOM} = 10^{28.7}$), which we believe is a better representation of DOM in aquatic settings occupied by methylating microorganisms.[14] If the larger Hg−DOM binding constant $K_{HgDOM} = 10^{38.3}$, which is derived from a soil organic matter, is used in the calculation, then Hg−DOM species are predicted to control Hg speciation for dissolved sulfide concentration less than 0.1 mM. The merits of one Hg−DOM binding constant over another have been extensively reviewed in previous papers,[12,14,92,112,113] and we defer to these for detailed analysis. A key point to recognize, though, is that Hg−DOM ligand complexation reactions shown in Table 1 are vastly simplified representations of Hg interactions with DOM. The binding of Hg^{2+} ions to the "strong" ligands in DOM appears to be slow (e.g., ∼1 day or longer[114]), suggesting that perhaps the kinetics of Hg−DOM interactions, rather than stability at a presumed equilibrium state, need greater consideration. The identity of these strong binding sites is further complicated by evidence for polynuclear metal-sulfide clusters as part of the reduced-S pool in natural organic matter isolates.[115,116] These types of S(-II) groups could be expected to have a very high affinity for Hg(II) through metal exchange reactions, ultimately resulting in Hg(II) species that better resemble mixed metal−sulfide clusters infused within the organic matter matrix rather than Hg-sulfhydryl coordination.[98,116]

In addition to the binding interactions between dissolved Hg−sulfides and Hg−organic matter, a third source of uncertainty for the speciation model is the solubility products K_{s0} for minerals such as metacinnabar and cinnabar, which vary by orders of magnitude in the NIST database for critically

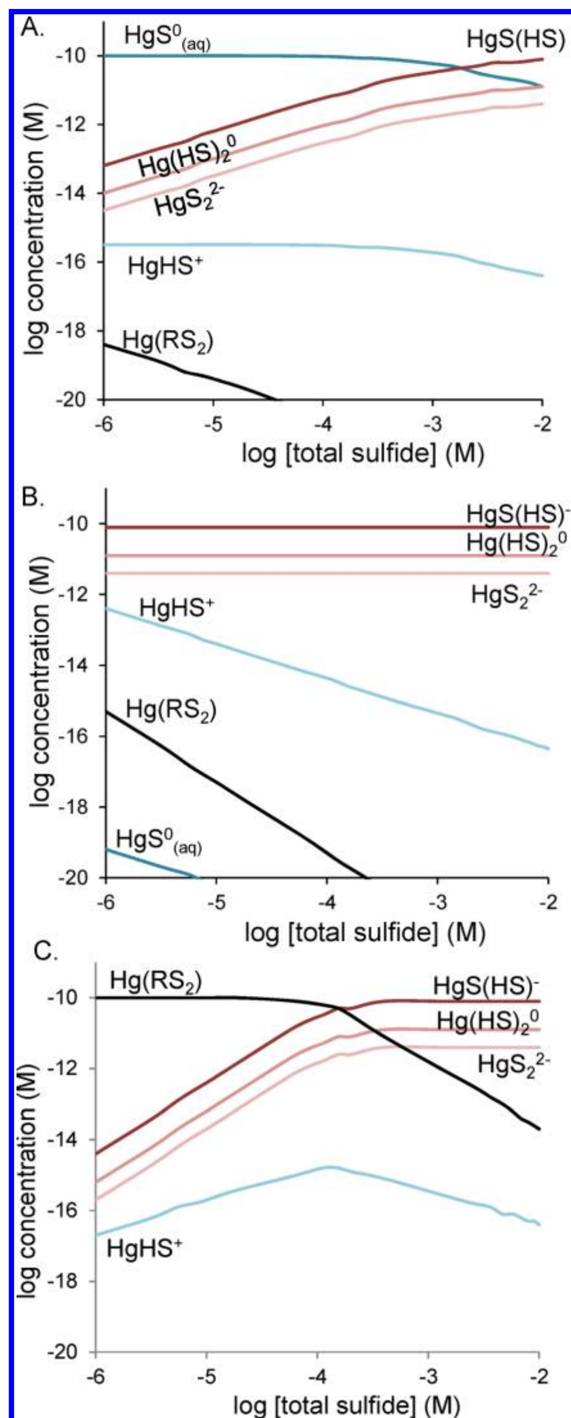

Figure 3. Predicted equilibrium speciation of dissolved Hg(II) in a solution representative of filtered anaerobic water: 10^{-10} M dissolved Hg(II), 10^{-6} M sulfhydryl concentration associated with DOM, 0.5 M Cl^-, pH 7. Calculations were performed used the stability constants listed in Table 1, assuming no precipitation of $HgS_{(s)}$ and two different stability constants for the formation of Hg−DOM and $HgS^0_{(aq)}$ complexes: (A) $K_{Hg-DOM} = 10^{28.7}$, $K_{HgS(aq)} = 10^{28.7}$; (B) $K_{Hg-DOM} = 10^{28.7}$, $K_{HgS(aq)} = 10^{16.4}$; and (C) $K_{Hg-DOM} = 10^{38.3}$, $K_{HgS(aq)} = 10^{16.4}$.

selected stability constants[117] (Table 1). Depending on the value used for the solubility of metacinnabar (K_{s0} for β-$HgS_{(s)}$), the saturation state of this mineral is near, below, or above saturation in our calculations shown in Figure 3. Whether metacinnabar is undersaturated or oversaturated particularly depends on the solubility product for $HgS_{(s)}$. The development of the neutral mercury-sulfide bioavailability model required the selection of a relatively large $HgS_{(s)}$ solubility constant (K_{s0}= $10^{-36.5}$, Table 1) in conjunction with a large formation of $HgS^0_{(aq)}$ so that the transition of the predominant dissolved Hg−sulfide species (e.g., $HgS^0_{(aq)}$ versus $HgS(HS)^-$ in Figure 3A)) could be matched to field data showing a decrease of MeHg with an increase of sulfide concentration.[61] The selection of this relatively large K_{s0} value caused the equilibrium calculations to predict undersaturation (with respect to metacinnabar $HgS_{(s)}$,) for dissolved Hg concentration less than 10^{-10} M. Because of these assumption, one could conclude that the formation of Hg−sulfide particles is not thermodynamically favored in most anaerobic settings,[63,82] a notion that conflicts with direct observations of $HgS_{(s)}$ in soil and sediments.[118,119]

4.3. Hg−Sulfide−Organic Matter Speciation at Nonequilibrium. The Benoit et al.[61,106] approach to estimating mercury bioavailability heavily relies on the assumption that Hg speciation in anaerobic waters can be represented by chemical equilibrium, and perhaps it is this assumption that should be given greatest consideration. Previous measurements to deduce the forms of mercury in environmental samples (whether the characterization involves metal−ligand stability or fractionation of particulate vs dissolved) are difficult to interpret because of the heterogeneity of mercury-containing compounds in natural waters, particularly in anaerobic settings. The constituents that comprise an anoxic surface water, sediment porewater or biofilm extracellular matrix include a continuum species: from dissolved molecules to polynuclear clusters, amorphous nanoparticles, and larger (perhaps crystalline) particles (Figure 4). This mixture of compounds would not be predicted from chemical equilibrium (with or without the incorporation of mineral phases) and likely represent intermediates of metal−ligand complexation, mineral precipitation and dissolution processes at nonequilibrium. Several studies have pointed to the importance of rate-limited processes (e.g., $HgS_{(s)}$ dissolution, precipitation, mass transfer across depth) for influencing Hg geochemistry in sulfidic settings.[17,18,79,107,120−122]

As more studies are emerging to highlight the importance and unique reactivities of colloidal or nanoscale HgS, the use of $HgS^0_{(aq)}$ to represent a single bioavailable form of mercury presents a few problems. First, the basis for the neutral mercury bioavailability model is that particles have no bioavailability (i.e., they cannot be directly taken up by cells). Thus, these nanoscale materials are supposed to provide the same contribution of bioavailable Hg as macrocrystalline $HgS_{(s)}$ (via dissolution or desorption), even as experiments show differences in methylation between microorganisms exposed to nanoparticulate and microparticulate HgS[18]. Second, HgS and other metal nanoparticles themselves can vary widely in terms of their degree of crystallinity, aggregation state, and composition. Thus, one term to represent colloidal HgS is inadequate for describing a complex array of compounds that are changing in composition and structure over time. Moreover, recent evidence has demonstrated that the primary mode of mercury biouptake is not a passive diffusion mechanism, but rather involves a facilitated or active transport mechanism.[55,56]

Improvements to models of methylation potential will need to consider the contribution of natural organic matter. Equilibrium speciation calculations indicate that the concentration of Hg(II)−DOM complexes are negligible for sulfidic settings (Figure 3A and 3B).[14,17] However, field and

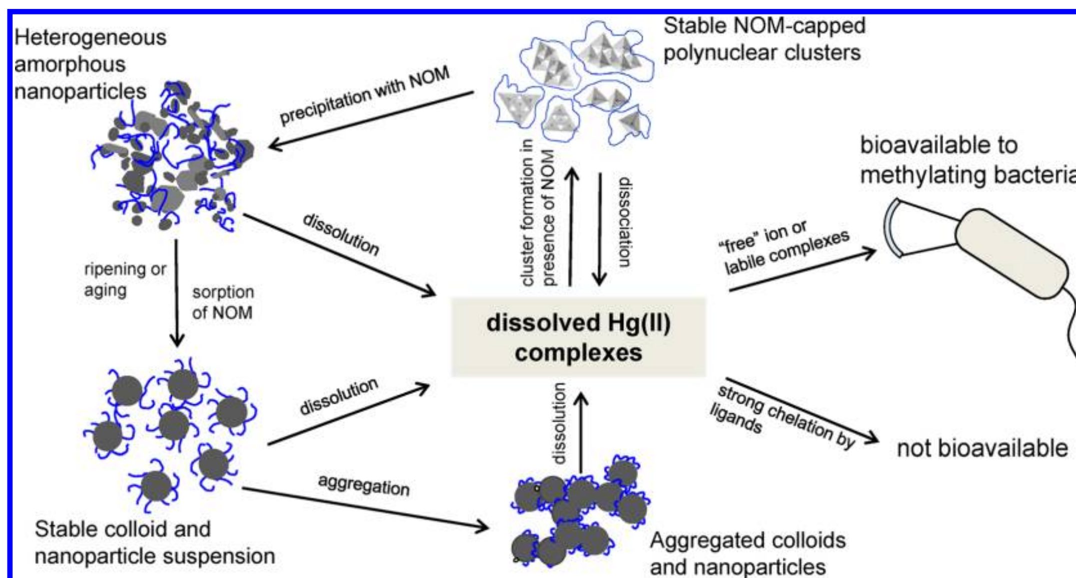

Figure 4. The transformations of mercuric sulfides in anoxic settings involve a diverse collection of species, many of which are intermediates of metal−ligand complexation reactions and precipitation and dissolution of $HgS_{(s)}$. Natural organic matter (NOM) is expected to play an important role in modifying reaction rates and the composition of metastable intermediates. If uptake of Hg(II) involves a facilitated mechanism (e.g., via metal transporter), then bioavailability will be governed by the propensity of Hg(II) to bind to a receptor site. Nanoscale phases could also cross membrane boundaries, but these would presumably need to dissolve or dissociate inside the organism prior to methylation. Modified from Aiken et al.[80].

experimental data have demonstrated correlations between organic carbon concentration and MeHg production.[9,17,123,124] NOM could contribute to Hg bioavailability and methylation potential in two ways. First, complexation of Hg(II) by DOM had been hypothesized to decrease the amount of Hg(II) available to the methylating bacteria due to the difficulty for the large macromolecular and hydrophilic Hg−DOM complexes to diffuse through the cell membranes.[125,126] On the other hand, in most settings MeHg concentration was observed to increase with organic carbon content in sediments.[124,127,128] This positive correlation was typically attributed to a stimulating effect of the labile carbon on microbial growth. Neither of these two theories fully captures the inter-related roles of natural organic matter and sulfide for inorganic Hg(II) bioavailability and methylation. Exceptions to the correlation between MeHg and organic matter have been reported[129] in which the coexistence of sulfide and DOM appeared to yield a favorable geochemical environment for microbial Hg(II) uptake.[17,129] Therefore, the explicit mechanism through which DOM influences mercury methylation needs to be investigated in conjunction with other environmental variables, especially sulfide.

While Hg^{2+} preferentially coordinates to inorganic sulfides over organic thiols associated with DOM, organic matter can influence Hg(II) speciation in other ways, particularly if the coordination reactions involving Hg are at a nonequilibrium status. DOM is known to enhance the dissolution rate of cinnabar and inhibit the precipitation rate of metacinnabar.[79,120,130] Moreover, macromolecular characteristics of the organic matter such as molecular weight and aromatic carbon content correlate with precipitation rates of metal sulfides[16,131] and possibly the bioavailability of mercury for methylating bacteria.[17,103] The occurrence of an aqueous ternary DOM-Hg−sulfide complex is a possible explanation.[82] However, more recent studies have demonstrated that organic matter plays a significant role in slowing the growth and aggregation of HgS nanoparticles as they precipitate in aqueous suspension.[14,15] These nanoparticles are likely to consist of a metacinnabar-like material (in terms of Hg−S coordination structure) that result in amorphous or nanocrystalline Hg−S−DOM nanoparticles.[15,16]

These recent findings on the chemistry of mercury, sulfide, and organic matter highlight the inadequacies of an equilibrium-based approach. Perhaps a rate-based approach is needed to model mercury speciation and bioavailability, just as a rate-based approach is used to model microbial growth and biotransformations. The main challenge with a kinetics-based approach is that an understanding of chemical reaction mechanisms is needed, particularly in systems involving heterogeneous materials (dissolved, colloidal, and particulate). In this case, recent advances in the nanogeosciences may provide a path forward toward improving assessments of mercury speciation and bioavailability.

4.4. Nanogeochemistry of Mercury. In the past decade, geochemists and aquatic chemists have realized that nanoscale particles are ubiquitous in the environment.[132−134] Much of this work to document the presence of naturally occurring nanoparticles has involved metal elements that are much more abundant than mercury (e.g., iron, aluminum, manganese, titanium, zinc). Nanoparticles and polynuclear clusters of metal sulfides such as ZnS and CuS have been observed in settings such as the biofilms of sulfate-reducing (and sulfide-generating) bacteria and in wastewater effluent.[135−138] (The term "polynuclear clusters" refers to aqueous molecules with multiple metal centers[139] that are the precursors to more crystalline phases during nanoparticle synthesis.) Because of the high affinity between Hg(II) and inorganic sulfide, a portion of mercury in anaerobic settings could be expected to associate with metal sulfide clusters or nanoparticles, possibly through sorption of Hg^{2+} ions or coprecipitation of HgS on sulfide nanoparticle surfaces.[140] Discrete nanoparticles of HgS have been detected directly in soil, sediment, and biofilms on plant

roots.[119,141,142] However, these examples were highly contaminated settings, such as mining and industrial sites where mercury-enriched materials were actively processed. Methods to directly detect nanoparticles (e.g., electron microscopy) generally require high concentrations of the target element in the sample (e.g., greater than one part-per-million). Most natural settings have much more dilute mercury concentrations. Therefore, we expect that nanoscale mercuric sulfides will likely comprise of a mixture of metal sulfides, such as Hg sorbed to or coprecipitated with FeS.[140]

Nanoscale particles are expected to behave differently than the compositionally identical, larger materials due to the high specific surface areas and unique reactivity of materials at the nanoscale.[132,134] Indeed, the defining characteristics of nanoparticles are not only the small size (i.e., at least one dimension smaller than 100 nm) but also size-specific reactivity exhibited by the nanomaterials.[143] Nanoscale-specific reactivity is generally observed in monomer particles smaller than 30 nm and stems from the relatively large specific surface area and crystal lattice imperfections in a material with a large proportion of atoms on the surface. Nanoscale-specific reactivity may include increased sorption capacity (normalized to surface area), enhanced transport, and faster rates of dissolution and renucleation.[132,143]

The reactivity of nanoparticles can lend them to unique pathways for uptake into organisms, and at the very least, will influence the microbial bioavailability of the metal constituents of the nanoparticle. Clues toward understanding the importance of nano-HgS for microbial uptake and methylation could be gained from more established research on biouptake of iron originating from nanostructured iron oxides. For example, in microbial iron reduction, nanosized iron oxide colloids exhibited up to 100 times greater iron transformation rates than their respective bulk minerals.[144] This observation was attributed to the enhanced solubility[145] and larger mineral particle−bacteria contact for nanoparticulate Fe(III).[146,147] Moreover, microscopic analysis revealed that iron oxide nanoparticles could penetrate the outer membrane of iron reducing bacteria, *Shewanella putrefaciens*, without collapsing the cells,[146] and this bacterium tended to dissolve Fe(III) at the bacteria−mineral interface.[148]

Recent work has suggested that mercury derived from HgS nanoparticles is more available to methylating bacteria than bulk minerals (e.g., metacinnabar), even when normalized to surface area.[18] Greater dissolution rates of small, more amorphous particles may account for the observed relationship between MeHg production and the "age" of the Hg−sulfide species. However in this study, attempts to quantify the dissolved fraction of Hg in the growth media could not fully account for the enhanced MeHg production in cultures exposed to HgS nanoparticles.

In natural aquatic systems, nanoparticles commonly exist as aggregates.[132] Therefore, while nanostructured materials may appear as larger particles in conventional fractionation methods, they would differ in their reactivity toward transformations such as dissolution and bioreduction.[149−152] The aggregation of metal sulfide nanoparticles (e.g., HgS, ZnS, CdS) are controlled by various environmental factors, including ionic strength, pH, the concentration and type of natural organic matter, and metal:sulfide ratio.[131,153−157] Humic substances, in particular, are known to interfere with precipitation reactions of minerals. Geochemists have long recognized that polynuclear clusters and nanoparticles are formed as intermediates of heterogeneous precipitation reactions,[158] yet the role of NOM for controlling cluster formation, particle nucleation, growth, and aggregation rates remains unclear.[80] Most of the work in this area has involved metal oxides and hydroxides, rather than metal sulfides. For example, the reaction mechanisms of metal hydroxide flocculation (e.g., aluminum and iron hydroxides) in the context of organic matter-containing water has been studied using spectroscopic tools.[159,160] This body of work has led to evidence for the formation of polynuclear clusters and nanoparticle compounds during the initial stages of precipitation. Furthermore, the formation of nanoscale iron hydroxides may be part of a reaction mechanism in which the dissolved metal, Fe^{3+} in this case, can proceed through two pathways: either direct coordination with Fe-binding ligands on the NOM or hydrolysis to form polynuclear Fe-hydroxide clusters that are coated with organic matter or infused in the NOM matrix.[159]

For metal sulfides such as metacinnabar, the nanoscale materials produced during the initial stages of precipitation are expected to be structurally different from metal hydroxide minerals. During the early stages of Hg−S polymerization, Hg takes on a 2-coordinate linear structure (−S−Hg−S−Hg−S−) that evolves into four-coordinate cubic HgS structure.[161,162] This transformation is likely to involve polynuclear Hg−S cluster species as intermediates,[162] particularly if precipitation is occurring in the presence of dissolved organic matter[15,16] that caps the nanoclusters and prevents them from growing further. HgS nanoclusters also differ from metal hydroxides in the specific interactions with natural organic matter.[14] Because metals such as Hg persist at low levels in surface water and sediment porewater (picomolar to nanomolar), complexation with high affinity, low abundance ligands such as thiols must be considered. Thiol-containing organic compounds are capable of altering the growth kinetics of metal sulfide nanoparticles.[14,163] This phenomena could explain why low molecular weight thiols enhanced the uptake of Hg(II) for sulfate-reducing bacterial cultures:[55,64] the thiols slowed the precipitation of HgS particles as the microorganisms were producing inorganic sulfide at trace levels. The simultaneous interactions between Hg(II), organic matter, and sulfide ultimately determine the collection of species that make up this continuum of dissolved, polynuclear, nanoparticulate, and particulate mercury in anoxic settings. These species could be expected to exhibit differences in Hg^{2+} release rates that may be a limiting step toward biouptake in methylating bacteria.

5. RESEARCH NEEDS

Key questions remain to be answered concerning the mechanisms by which microorganisms methylate mercury. An understanding of these mechanisms is critical to the development of models that predict methylation potential in contaminated settings, and particularly in settings altered by remediation or change in mercury deposition. The greatest gaps in knowledge are related to the molecular processes that control the speciation of mercury, the route of Hg(II) uptake into methylating bacteria, and the enzymatic pathways toward methylation. In this respect, recent developments in molecular biology and nanogeochemistry can lend clues to address these questions. The breakthrough discovery of the *hgcAB* system[52] will undoubtedly pave the way for a much greater understanding of mercury methylation mechanisms, the distribution of methylators in the environment, and the factors that govern the rate of mercury methylation.

In terms of the geochemical speciation of mercury at biological interfaces, recent developments in the nano-geosciences provide much promise for enabling the understanding of mercury reactivity in complex mixtures such as sediments and sulfidic bottom waters. These developments include not only the realization of unique reactivity associated with nanoparticles but also new tools and novel applications of older approaches (such as spectroscopy, photon scattering, and microscopy) to help us answer these questions. These methods could be particularly powerful if combined with more "conventional" tools such as size separation and complexation. For example, synchrotron-based X-ray spectroscopic methods have been widely used for analyzing metal speciation in environmental samples due to their element specificity, minimal sample manipulation and nondestructive nature.[164] However, these techniques generally require samples with at least part-per-million amounts of mercury, a concentration applicable only to highly contaminated settings.[165] This limitation can be alleviated by the application of a preconcentration step such as a C18 resin,[16] a technique that was modified from mercury−ligand competitive exchange experiments. DGT techniques also hold much promise in enabling measurements of the reactive or bioavailable Hg(II), particularly if the devices can mimic the environment immediately surrounding a methylating bacterium. If a kinetics-based approach is developed to assess mercury bioavailability in anaerobic settings, then the DGT probes could be used as proxies for Hg(II) flux at biological interfaces. The utilization of Hg stable isotopes can also be valuable in tracking simultaneous transformation reactions in microcosm studies.[43,121,166]

The mechanistic understanding of microbial mercury methylation will be greatly improved if the biogeochemical reactions occurring at the microorganism−mineral interface (e.g., adsorption, complexation, dissolution, precipitation, aggregation) can be directly investigated, rather than indirectly implied by the measurements of bulk samples. The investigation of these interfacial processes requires powerful analytical tools with both high spatial resolution and chemical sensitivity. High-resolution transmission electron microscopy, synchrotron-based X-ray microscopy, and microprobe mapping have been utilized to examine the distribution of mercury and other trace elements and to identify the hot spots of these elements in biological samples.[141,167,168] These techniques can also be coupled with metal speciation analysis, including X-ray absorption spectroscopy, X-ray diffraction, selected-area electron diffraction, and energy dispersive X-ray spectroscopy, and have shown great promise in elucidating the mechanisms of nanoparticle−microorganism transformation processes.[18,169]

In addition to the molecular-level speciation of Hg in anoxic settings, we also lack a good understanding of the process by which methylating microorganisms take up inorganic mercury before converting it to methylmercury. Perhaps the characteristics of the microorganisms themselves can provide clues toward the mechanism of uptake. For example, Hg uptake by sulfate-reducers could occur via metal transporters,[55] and these organism would need mechanisms for acquiring metabolically necessary soft-sphere metals such as Cu or Zn from their sulfidic surroundings.

Additional questions on the biochemical mechanism of mercury methylation remain to be fully answered. New and faster capabilities in the "-omics" of molecular biology (e.g., genomics, proteomics, metabolomics) could provide assistance to this problem.[66] For example, scientists are now realizing the diversity of yet uncultured microorganisms that are capable of methylating mercury in the environment.[42,44,47] With the ongoing improvements in tools utilized for microbial ecology, this list of organisms will continue to grow. Advances in pyrosequencing are improving the efficiency of large scale DNA sequencing, allowing for a metagenomics approach to characterizing genetic material recovered from environmental samples.[170,171] The use of comparative metagenomics[172] can potentially provide additional insights into the differences among microbial populations that perform the same biological function (e.g., mercury methylation) but have evolved in different environments. Researchers are also successfully utilizing proteomics tools to characterize the proteins involved in metabolic pathways and to determine the proteome of microorganisms exposed to contaminants and environmental stressors.[173,174] Even with these new advances, however, an unresolved challenge is how to perform these experiments at Hg exposure levels representative of environmental concentrations (e.g., parts-per-billion or less).

While much progress has been made in the past decades towards understanding the multifaceted aspects of mercury methylation, many fundamental questions remain. Hopefully, recent advances in the geochemical and biological sciences will help provide insights to those elusive questions. Ultimately, an improved comprehension of the factors that control MeHg production in the environment should enable the development of effective mercury remediation strategies, support the implementation of sound mercury emissions policies, and decrease human exposure to methylmercury.

AUTHOR INFORMATION

Corresponding Author

*E-mail: hsukim@duke.edu. Phone: (919) 660-5109. Fax (919) 660-5219.

Notes

The authors declare no competing financial interest.

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