Environmental Science & Technology Letters

Letter

pubs.acs.org/journal/estlcu

Thiol-Facilitated Cell Export and Desorption of Methylmercury by Anaerobic Bacteria

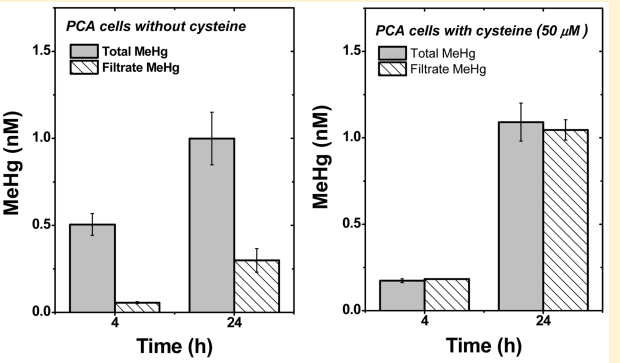
Hui Lin,[†] Xia Lu,[†,‡] Liyuan Liang,[†] and Baohua Gu*[,†]

[†]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830, United States

[‡]School of Nuclear Science and Technology, Lanzhou University, Lanzhou, China

Supporting Information

ABSTRACT: Methylmercury (MeHg) toxin, formed by anaerobic bacteria, is rapidly excreted from cells, but the mechanism of this process is unclear. We studied the factors affecting MeHg export and its distribution in cells, on cell surfaces, and in solution by two known mercury methylators, *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132. Thiols, such as cysteine, were found to greatly facilitate desorption and export of MeHg, particularly by PCA cells. In cysteine-free assays (4 h), less than 10% of the synthesized MeHg was found in solution and greater than 90% was associated with PCA, of which about 73% was sorbed on the cell surface and 19% remained inside the cells. In comparison, 77% of MeHg was in solution, leaving about 13% of MeHg sorbed and about 10% inside the ND132 cells. Our results demonstrate that MeHg export is bacteria specific, time dependent, and influenced by thiols, implicating important roles of ligands, such as natural organic matter, in MeHg production and mobilization in the environment.



INTRODUCTION

Microbial conversion of inorganic mercury species [Hg(II)] to organometallic methylmercury (MeHg, i.e., CH_3Hg^+) has been a global concern because of the high toxicity of MeHg and its biomagnification in food chains.[1−3] Reducing emission of mercury has been called for in a recent treaty signed by 147 nations.[4] Our understanding of the mechanisms responsible for Hg(II) methylation by anaerobic bacteria has improved greatly over the past few years, especially the identification of a gene pair, *hgcA* and *hgcB*, that is essential for Hg methylation.[1] Furthermore, recent studies have established that microbial methylation is tightly coupled with mercury redox transformation, adsorption on cell surface, cellular uptake of Hg(II), and MeHg export.[5−9] Intracellular Hg(II) uptake has been proposed to involve active transport and is sensitive to Hg(II) chemical speciation in the external medium, particularly influenced by specific thiol compounds such as cysteine.[5,9,10] However, despite these advances, it remains unclear how Hg(II) is taken up, how MeHg is exported by methylating bacteria, and what environmental factors may affect these processes. Studies have shown that once inside the cell Hg(II) is methylated and rapidly exported from the cell.[5,6,10,11] For example, in assays with either *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132, no significant delay was observed for the excretion of the synthesized MeHg to the external medium.[5,6,11] It is thus postulated that bacteria may employ Hg(II) methylation and export as a means of mercury detoxification, i.e., maintaining low intracellular Hg(II) concentrations.[5,12−14] However, these previous studies involved the use of relatively high concentrations of thiols, such as cysteine, glutathione, and thioglycolate.[5,6,11] Whether these thiols may have facilitated the export of MeHg or prevented it from sorption onto cell surfaces of the methylating bacteria is unknown because thiols can compete for MeHg binding in solution.[15,16] MeHg sorption on cell surfaces is expected because of the presence of abundant cellular thiol functional groups[17,18] and the thiophilic nature of Hg.[19]

We hypothesized that thiol compounds in solution compete with the cell surface receptors and sorption sites for MeHg and thus facilitate the export and desorption of MeHg to the external media. Using both *G. sulfurreducens* PCA and *D. desulfuricans* ND132, the two well-studied Hg methylators,[1,5−9] we determined time-dependent MeHg export and distribution in Hg(II) methylation assays as influenced by thiol compounds. Additionally, we examined the effect of thiols on MeHg sorption and desorption by cells, in which bacterial synthesis of MeHg was minimized by adding no inorganic Hg(II).

MATERIALS AND METHODS

G. sulfurreducens PCA was cultured in the nutrient broth Basal salts containing 40 mM fumarate and 20 mM acetate at 30 °C, and *D. desulfuricans* ND132 was cultured in a modified MOY

Received: August 7, 2015
Revised: August 31, 2015
Accepted: September 1, 2015

DOI: 10.1021/acs.estlett.5b00209
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

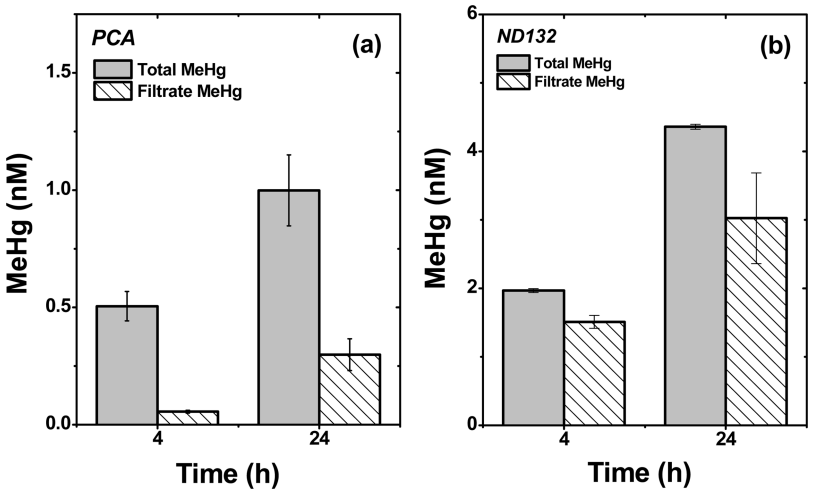


Figure 1. Determination of total methylmercury (MeHg) and soluble MeHg ($MeHg_{sol}$) in filtrate solution during Hg(II) methylation assays with washed cells of (a) *G. sulfurreducens* PCA and (b) *D. desulfuricans* ND132 in a phosphate buffered saline (pH 7.4). The initial Hg(II) concentration was 25 nM, and cell concentration was 10^8 cells/mL. Error bars represent one standard deviation of replicate samples (n = 4−6) from two independent batch experiments.

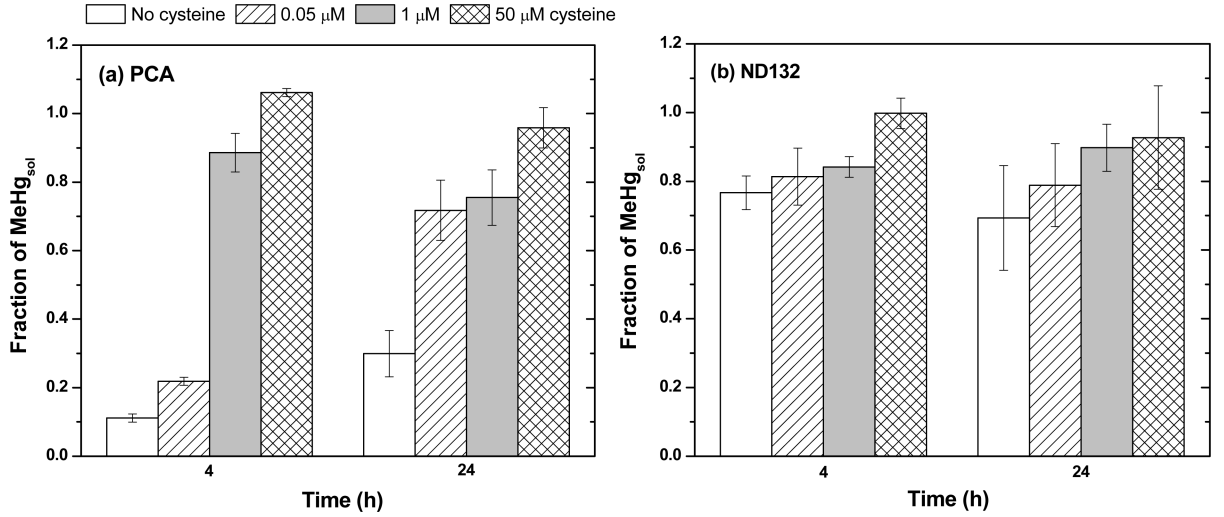


Figure 2. Effects of cysteine concentrations on methylmercury (MeHg) excretion by washed cells of (a) *G. sulfurreducens* PCA and (b) *D. desulfuricans* ND132 at 10^8 cells/mL in PBS. The excreted MeHg was expressed as the fraction of the soluble MeHg ($MeHg_{sol}$) to the total synthesized MeHg at 4 and 24 h. The initial added Hg(II) concentration was 25 nM, and cysteine concentrations were 0, 0.05, 1, and 50 μM. Error bars represent one standard deviation of replicate samples (n = 4−6) from two independent batch experiments.

medium containing 60 mM fumarate and 60 mM pyruvate at 33 °C.[7−9,20] Cells were harvested during mid-exponential phase with an optical density (OD) of 0.5 to 0.6 and then washed three times by repeated centrifugation (at 1200*g*, 10 min, 25 °C) and resuspension in the assay buffer, a deoxygenated phosphate buffer saline (PBS) at pH 7.4. The PBS consisted of 0.14 M NaCl, 3 mM KCl, 10 mM Na_2HPO_4, and 2 mM KH_2PO_4. The buffer was first autoclaved and then deoxygenated by boiling and purging with ultrahigh purity N_2 gas for at least 2 h and subsequently kept in an anaerobic glove chamber (Coy) (98% N_2 and 2% H_2) for at least 24 h before use. All the washing steps and subsequent Hg assays were conducted in the glove chamber, as previously described.[7,9,20,21]

The Hg(II) methylation assay was conducted in 4 mL amber glass vials (National Scientific) in the anaerobic glove chamber. Each vial contained washed cells of PCA or ND132 (at 10^8 cell/mL) and Hg(II) at 25 nM (as $HgCl_2$) in 1 mL PBS or otherwise specified. Acetate (or pyruvate for ND132) and fumarate (1 mM each) were added initially as the respective electron donor and acceptor. The Hg(II) working solution was freshly prepared from the stock solution of 50 μM $HgCl_2$ in 1% HCl. Cysteine (0.05−50 μM) was used to study the effect of thiols on the export of MeHg. Glutathione, 2-mercaptoacetic acid (2-MPA), and penicillamine (50 μM each) were also used in some assays. All vials were immediately sealed with a PTFE/silicone cap screw and kept in the dark on an orbital shaker. At each selected time point, replicate sample vials (2−3) were taken out of the glove chamber and sacrificed for the determination of both total MeHg ($MeHg_{Total}$) (without filtration) and soluble MeHg in filtrate ($MeHg_{sol}$, filtered through 0.2-μm filters). The cell-associated MeHg, including both adsorbed and intracellular MeHg, was obtained by subtracting $MeHg_{sol}$ from $MeHg_{Total}$. All samples for MeHg analysis were preserved with trace metal-grade H_2SO_4 (0.2%, v/v) and kept at −20 °C until analysis.

To determine MeHg distribution either on the cell surface or inside the cell (including periplasmic, membrane, and cytosol

DOI: 10.1021/acs.estlett.5b00209
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

spaces), a modified washing procedure[5,21] was adopted based on MeHg sorption−desorption assays (described below). After cells were incubated with Hg(II) for either 4 or 24 h, a set of sample vials (4−6) was removed from the glove chamber, and half of them were used for the determination of $MeHg_{sol}$ and $MeHg_{Total}$, as described above. The remaining vials were used to determine the washable MeHg ($MeHg_{wash}$) after equilibrating samples with glutathione (100 μM final concentration) for about 10 min. Samples were subsequently filtered, and $MeHg_{wash}$ in the filtrate was determined (equals to the sum of the cell-adsorbed MeHg ($MeHg_{ad}$) and $MeHg_{sol}$). The intracellular MeHg ($MeHg_{cell}$) was then calculated by subtracting $MeHg_{wash}$ from $MeHg_{Total}$.

MeHg sorption−desorption experiments were also performed, in which stock MeHg standard (5 μM, Brooks Rand Laboratories) was added to washed cells in PBS (10^8 cells/mL) to a final concentration of 5 nM. After equilibration for 4 and 24 h (or otherwise specified), samples were filtered or washed and determined for MeHg distributions, as described. To determine MeHg sorption on PCA cells, cells were exposed to different concentrations of MeHg, ranging from 0.05 to 1 nM, and samples were equilibrated for 4 h before analysis of $MeHg_{sol}$ and $MeHg_{Total}$.[9,20,21]

A modified EPA Method 1630 was used for MeHg analysis via GC-ICP-MS with enriched $CH_3{}^{200}Hg^+$ as an internal standard, as described previously.[1,20,21] The recovery of spiked MeHg standards was 100 ± 10%, and the detection limit was ∼6 pg MeHg. Control assays (with or without cells) were performed in the same manner, and Hg_{Total} and soluble Hg_{sol} were also analyzed after samples were oxidized by BrCl (5%, v/v) to ensure a good mass balance.[20,21]

RESULTS AND DISCUSSION

G. sulfurreducens PCA and *D. desulfuricans* ND132 were used first to determine whether MeHg can be effectively exported during Hg(II) methylation. In the absence of cysteine or complexing organic ligands, *G. sulfurreducens* PCA cells produced a total of 0.5 and 1.0 nM MeHg in PBS at 4 and 24 h, respectively, but only about 0.06 and 0.3 nM MeHg were found in the filtrate solutions, designated as $MeHg_{sol}$ (Figure 1a). A large percentage of MeHg (>70%) remained associated with PCA cells after 24 h of incubation; only about 11% and 30% of MeHg were in solution after 4 and 24 h, respectively (Figure 2a). The ND132 strain is a more efficient Hg methylator than PCA[1,5,11,20] and produced a total of 2.0 and 4.4 nM MeHg in 4 and 24 h, respectively (Figure 1b). Compared to PCA, ND132 cells appeared to be more efficient in exporting MeHg because 76% and 69% of MeHg were found in the filtrate solutions of the 4 and 24 h assays, respectively (Figures 1b and 2b). In control experiments, we found no significant loss of MeHg during filtration or sample preservation (Figure S1 of the Supporting Information).

These results clearly indicate that MeHg was not exported instantaneously, particularly by *G. sulfurreducens* PCA cells. Previous studies reported no significant delays in the export of the synthesized MeHg[5,6,10,11] and thus postulated that the excreted MeHg had little affinity or low selectivity for the cell surface receptors and transporters.[6] This is in contrast with the extremely high affinity of inorganic Hg(II) sorption onto cell surfaces.[5,8]

To determine whether the presence of thiols may compete with the cell surface receptors for MeHg binding, we investigated the effect of cysteine in facilitating MeHg export and desorption during Hg(II) methylation by both PCA and ND132 cells. Results (Figure 2) show that soluble MeHg in the external media increased dramatically with increasing cysteine concentrations (from 0.05 to 50 μM). With the addition of only 0.05 μM cysteine, i.e., Hg(II):Cysteine = 1:2, about 20% of the synthesized MeHg was excreted at 4 h and about 72% at 24 h by PCA cells (Figure 2a). Note that the excreted $MeHg_{sol}$ was normalized to the total MeHg produced at a given cysteine concentration or time because cell production of MeHg varies with the cysteine concentration and incubation time (Figure S2 of the Supporting Information).[9] With increasing cysteine concentrations, more MeHg was exported to the external media by PCA, and at 50 μM cysteine, greater than 96% of MeHg was found in the filtrate solution at either 4 or 24 h (Figure 2a). Similarly, the presence of cysteine increased MeHg export by ND132 cells (Figure 2b), although the increased export was not as much as those by PCA cells because ND132 itself is much more efficient at exporting MeHg (Figure 1b). At the cysteine concentration of 50 μM, greater than 93% of MeHg was in the external solution of ND132 (Figure 2b). Together these results demonstrate the critical influence of cysteine on the export and desorption of MeHg. We also evaluated the effects of other thiol ligands, including glutathione, penicillamine, or 2-mercaptoacetic acid (50 μM each) on MeHg export. Results similar to the effects of cysteine were obtained (Figure S3 of the Supporting Information); no notable differences between the filtered and unfiltered samples were observed, suggesting that all these thiols either facilitated the export of MeHg or prevented it from sorption onto the cell surface.

To examine whether the cell-associated MeHg remained inside the cell or was sorbed on the cell surface, we used a modified washing procedure to distinguish the sorbed versus the intracellular $MeHg_{cell}$.[5,21] $MeHg_{cell}$ in the Hg(II) methylation assay was estimated by the difference between the total cell-associated MeHg and $MeHg_{ad}$ (Figure 3a,b). This washing procedure is effective as evidenced in MeHg sorption and desorption studies, where only MeHg was added to both PCA and ND132 cells (Figure 3c,d and Figure S3 of the Supporting Information). Results (Figure 3a) indicate that a significant fraction of MeHg was retained inside the PCA cell, and $MeHg_{cell}$ accumulated initially but decreased with time. At 4 and 24 h, about 19.1% and 8.6% of MeHg were intracellular. A majority of MeHg was sorbed on PCA cell surfaces ($MeHg_{ad}$, ∼72.6% and 66.4% at 4 and 24 h, respectively) (Figure 3a), leaving only a small fraction (8.3% and 25%) in solution. In comparison, ND132 cells retained about 13% and 8% of MeHg inside the cells after 4 and 24 h, respectively (Figure 3b), and a much lower amount of MeHg (10% and 22% at 4 and 24 h, respectively) was sorbed on the cell surface. A majority of MeHg (70−77%) was thus found in solution (Figure 3b). This comparison again shows that ND132 cells are more efficient in exporting MeHg or possess a lower affinity to sorb MeHg than PCA. These results also suggest that thiols not only caused desorption of MeHg from the cell surface but facilitated MeHg export because in the presence of cysteine (50 μM) greater than 96% and greater than 93% of MeHg were found in the external media of the PCA and ND132 cells, respectively.

To further verify the interactions between MeHg and cell surfaces, additional experiments were performed by adding stock MeHg to the cell suspensions, which were then filtered and washed to determine the sorption or uptake of MeHg. In control experiments, we found no demethylation or notable loss of MeHg even after 5 days in the presence or absence of cysteine (50 μM) (Figure S1b of the Supporting Information). However, substantial amounts of MeHg were found to be associated with

DOI: 10.1021/acs.estlett.5b00209
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

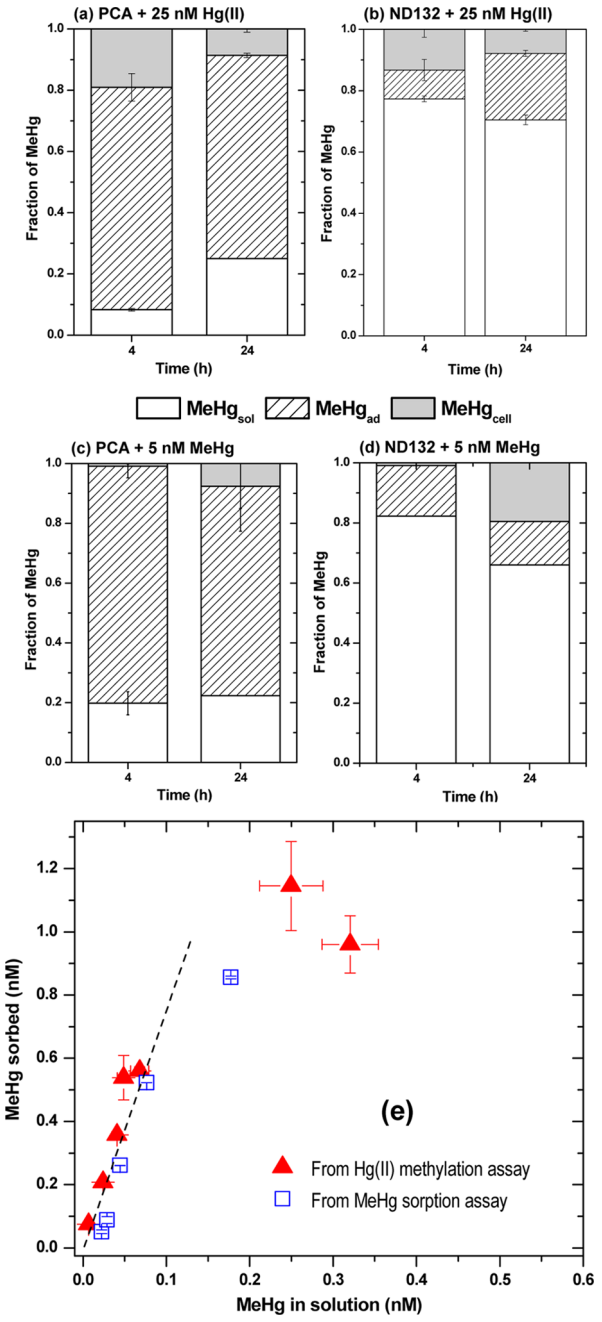


Figure 3. (a, b) Determination of methylmercury (MeHg) distribution inside cells, on cell surfaces, and in solutions during Hg(II) methylation assays with washed cells of (a) *G. sulfurreducens* PCA and (b) *D. desulfuricans* ND132 at 10^8 cells/mL in PBS. The initial added Hg(II) concentration was 25 nM. The soluble MeHg ($MeHg_{sol}$) was determined in filtrate solution. The adsorbed MeHg ($MeHg_{ad}$) was determined by washing cells with 100 μM glutathione followed by filtration. The intracellular MeHg ($MeHg_{cell}$) was estimated by subtracting $MeHg_{sol}$ and $MeHg_{ad}$ from the total MeHg. (c, d) Determination of MeHg distribution in MeHg sorption and desorption assays. The initial added MeHg concentration was 5 nM, and no Hg(II) was added. Other experimental conditions were the same as those used in the Hg(II) methylation assay. (e) MeHg sorption and partitioning on washed cells of PCA (10^8 cells/mL) in PBS. Solid symbols represent data obtained from Hg(II) methylation assays, and open symbols were from MeHg sorption assays. Error bars represent one standard deviation of replicate samples (n = 2–6).

PCA cells ($MeHg_{ad}$ + $MeHg_{cell}$, ~ 80%) (Figure 3c), whereas only about 18% and 34% of MeHg associated with ND132 cells at 4 and 24 h, respectively (Figure 3d). PCA cells exhibited a high affinity in sorbing MeHg as shown by the linear partitioning of MeHg in either the Hg(II) methylation assay or MeHg sorption–desorption assay studies (Figure 3e). Importantly we found that after 24 h a fraction of MeHg could be internalized to the PCA and ND132 cells since a significant amount of MeHg (8% on PCA or 20% on ND132) could not be desorbed by 100 μM glutathione. In contrast, in the 4-h assay, nearly 100% of the sorbed MeHg on the cell surface could be desorbed, or little internalization of MeHg occurred (Figure 3c,d). These results indicate that cells were capable of taking up MeHg with time when MeHg was added to the external media. Slightly more MeHg was taken up by ND132 than PCA cells in 24 h (Figure 3c,d). This observation appears in line with that ND132 cells are more efficient than PCA in exporting or transporting MeHg in and out of the cell. Taken together, these results demonstrate that MeHg can be either exported (while MeHg is being synthesized by cells) or internalized across the cell outer surface (when biosynthesis is absent and MeHg is added, Figure 3c,d), and these processes are time dependent.

Hg(II) methylation followed by efficient export of MeHg has been suggested as one of the potential detoxification mechanisms of cells to get rid of Hg.[5,12−14] We show that biosynthesized MeHg is distributed inside the cells, on the cell surface, and in solution, and the proportion of the distribution is time and ligand dependent. In the absence of thiols, most MeHg was found on surface of cells; more MeHg is associated with the surface of *G. sulfurreducens* PCA than *D. desulfuricans* ND132 cells (Figure 3). PCA cells showed a high sorption affinity for MeHg (Figure 3e), which may partially explain why its MeHg production usually levels off in 24 h in cysteine-free assays[8,9,20] but increases continuously to a high level (up to 5 times) in the presence of cysteine.[9] Sorption and accumulation of MeHg on PCA cells in the absence of thiols may lead to toxic effects on PCA, resulting in suppressed MeHg production. Cysteine could allow desorption and continuous export of MeHg, although different thiols (e.g., cysteine or glutathione) also impact the overall MeHg production due to competitive interactions between cells and thiols for Hg(II) in solution.[5,9,16] In contrast, ND132 is much more efficient in producing and excreting MeHg, likely due to its ability to produce thiolate compounds[11,22] which facilitate the export and desorption of MeHg. Therefore, addition of thiols showed less influence on MeHg production by ND132 cells. Interestingly, ND132 exhibits a lower sorption affinity for MeHg than for inorganic Hg(II), whereas the PCA cells sorb Hg(II) more weakly than MeHg, the opposite.[5,8] These observations suggest that different binding domains or mechanisms may be involved in Hg(II) and MeHg sorption, uptake, and export in different strains of bacteria and thus highlight the need for future studies to better understand the mechanisms. Although free thiols are generally low in freshwater,[23] orders of magnitude higher levels of thiol exist in biofilms,[24] where bacterial consortia thrive. Abundant thiol functional groups are also known to exist in natural organic matter.[17,18,25] These environmental thiols may thus exert an important control on MeHg export from cells, in addition to their influences on Hg(II) uptake and methylation in the environment.[26−28]

DOI: 10.1021/acs.estlett.5b00209
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.5b00209.

Method controls, effect of cysteine concentrations on MeHg production and export, and MeHg sorption and desorption with different thiols. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: gub1@ornl.gov. Phone: (865)-574-7286. Fax: (865)-576-8543.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Dwayne Elias and Alex Johs for reviewing the manuscript and Xiangping Yin for assistance in mercury and methylmercury analyses. This research was supported by the Office of Biological and Environmental Research, Office of Science, U.S. Department of Energy (DOE) as part of the Mercury Science Focus Area at Oak Ridge National Laboratory, which is managed by UT-Battelle LLC for the DOE under contract DE-AC05-00OR22725.

■ REFERENCES

(1) Parks, J. M.; Johs, A.; Podar, M.; Bridou, R.; Hurt, R. A.; Smith, S. D.; Tomanicek, S. J.; Qian, Y.; Brown, S. D.; Brandt, C. C.; Palumbo, A. V.; Smith, J. C.; Wall, J. D.; Elias, D. A.; Liang, L. The genetic basis for bacterial mercury methylation. *Science* **2013**, *339*, 1332−1335.

(2) Krabbenhoft, D. P.; Sunderland, E. M. Global change and mercury. *Science* **2013**, *341*, 1457−1458.

(3) Lamborg, C. H.; Hammerschmidt, C. R.; Bowman, K. L.; Swarr, G. J.; Munson, K. M.; Ohnemus, D. C.; Lam, P. J.; Heimburger, L. E.; Rijkenberg, M. J. A.; Saito, M. A. A global ocean inventory of anthropogenic mercury based on water column measurements. *Nature* **2014**, *512*, 65−68.

(4) Minamata Convention on Mercury, Text and Annexes. U.N. Environment Programme, 2013. www.mercuryconvention.org (accessed September 2015).

(5) Schaefer, J. K.; Rocks, S. S.; Zheng, W.; Liang, L.; Gu, B.; Morel, F. M. M. Active transport, substrate specificity, and methylation of Hg(II) in anaerobic bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 8714−8719.

(6) Graham, A. M.; Bullock, A. L.; Maizel, A. C.; Elias, D. A.; Gilmour, C. C. Detailed assessment of the kinetics of hg-cell association, Hg methylation, and methylmercury degradation in several *Desulfovibrio* species. *Appl. Environ. Microbiol.* **2012**, *78*, 7337−7346.

(7) Hu, H.; Lin, H.; Zheng, W.; Rao, B.; Feng, X. B.; Liang, L.; Elias, D. A.; Gu, B. Mercury reduction and cell-surface adsorption by *Geobacter sulfurreducens* PCA. *Environ. Sci. Technol.* **2013**, *47*, 10922−10930.

(8) Lin, H.; Morrell-Falvey, J. L.; Rao, B.; Liang, L.; Gu, B. Coupled mercury-cell sorption, reduction, and oxidation affecting methylmercury production by *Geobacter sulfurreducens* PCA. *Environ. Sci. Technol.* **2014**, *48*, 11969−11976.

(9) Lin, H.; Lu, X.; Liang, L.; Gu, B. Cysteine inhibits mercury methylation by *Geobacter Sulfurreducens* PCA mutant Δ*omcBESTZ*. *Environ. Sci. Technol. Lett.* **2015**, *2*, 144−148.

(10) Schaefer, J. K.; Morel, F. M. M. High methylation rates of mercury bound to cysteine by Geobacter sulfurreducens. *Nat. Geosci.* **2009**, *2*, 123−126.

(11) Gilmour, C. C.; Elias, D. A.; Kucken, A. M.; Brown, S. D.; Palumbo, A. V.; Schadt, C. W.; Wall, J. D. Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. *Appl. Environ. Microbiol.* **2011**, *77*, 3938−3951.

(12) Hamdy, M. K.; Noyes, O. R. Formation of methyl mercury by bacteria. *Appl. Microbiol.* **1975**, *30*, 424−432.

(13) Robinson, J. B.; Tuovinen, O. H. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds - Physiological, biochemical, and genetic analyses. *Microbiol. Rev.* **1984**, *48*, 95−124.

(14) Barkay, T.; Miller, S. M.; Summers, A. O. Bacterial mercury resistance from atoms to ecosystems. *FEMS Microbiol. Rev.* **2003**, *27*, 355−384.

(15) Ndu, U.; Mason, R. P.; Zhang, H.; Lin, S. J.; Visscher, P. T. Effect of inorganic and organic ligands on the bioavailability of methylmercury as determined by using a mer-lux bioreporter. *Appl. Environ. Microbiol.* **2012**, *78*, 7276−7282.

(16) Thomas, S. A.; Tong, T. Z.; Gaillard, J. F. Hg(II) bacterial biouptake: the role of anthropogenic and biogenic ligands present in solution and spectroscopic evidence of ligand exchange reactions at the cell surface. *Metallomics* **2014**, *6*, 2213−2222.

(17) Joe-Wong, C.; Shoenfelt, E.; Hauser, E. J.; Crompton, N.; Myneni, S. C. B. Estimation of reactive thiol concentrations in dissolved organic matter and bacterial cell membranes in aquatic systems. *Environ. Sci. Technol.* **2012**, *46*, 9854−9861.

(18) Rao, B.; Simpson, C.; Lin, H.; Liang, L.; Gu, B. Determination of thiol functional groups on bacteria and natural organic matter in environmental systems. *Talanta* **2014**, *119*, 240−247.

(19) Riccardi, D.; Guo, H. B.; Parks, J. M.; Gu, B. H.; Summers, A. O.; Miller, S. M.; Liang, L. Y.; Smith, J. C. Why mercury prefers soft ligands. *J. Phys. Chem. Lett.* **2013**, *4*, 2317−2322.

(20) Hu, H.; Lin, H.; Zheng, W.; Tomanicek, S. J.; Johs, A.; Feng, X. B.; Elias, D. A.; Liang, L.; Gu, B. Oxidation and methylation of dissolved elemental mercury by anaerobic bacteria. *Nat. Geosci.* **2013**, *6*, 751−754.

(21) Lin, H.; Hurt, R. A., Jr; Johs, A.; Parks, J. M.; Morrell-Falvey, J. L.; Liang, L.; Elias, D. A.; Gu, B. Unexpected effects of gene deletion on mercury interactions with the methylation-deficient mutant Δ*hgcAB*. *Environ. Sci. Technol. Lett.* **2014**, *1*, 271−276.

(22) Fahey, R. C.; Brown, W. C.; Adams, W. B.; Worsham, M. B. Occurrence of glutathione in bacteria. *J. Bacteriol.* **1978**, *133*, 1126−1129.

(23) Liem-Nguyen, V.; Bouchet, S.; Bjorn, E. Determination of subnanomolar levels of low molecular mass thiols in natural waters by liquid chromatography tandem mass spectrometry after derivatization with p-(hydroxymercuri)benzoate and online preconcentration. *Anal. Chem.* **2015**, *87*, 1089−1096.

(24) Leclerc, M.; Planas, D.; Amyot, M. Relationship between extracellular low-molecular-weight thiols and mercury species in natural lake periphytic biofilms. *Environ. Sci. Technol.* **2015**, *49*, 7709−7716.

(25) Dong, W.; Liang, L.; Brooks, S. C.; Southworth, G.; Gu, B. Roles of dissolved organic matter in the speciation of mercury and methylmercury in a contaminated ecosystem in Oak Ridge, Tennessee. *Environ. Chem.* **2010**, *7*, 94−102.

(26) Chiasson-Gould, S. A.; Blais, J. M.; Poulain, A. J. Dissolved organic matter kinetically controls mercury bioavailability to bacteria. *Environ. Sci. Technol.* **2014**, *48*, 3153−3161.

(27) Graham, A. M.; Aiken, G. R.; Gilmour, C. C. Dissolved organic matter enhances microbial mercury methylation under sulfidic conditions. *Environ. Sci. Technol.* **2012**, *46*, 2715−2723.

(28) Skyllberg, U. Competition among thiols and inorganic sulfides and polysulfides for Hg and MeHg in wetland soils and sediments under suboxic conditions: Illumination of controversies and implications for MeHg net production. *J. Geophys. Res.* **2008**, *113*, G00C03.

DOI: 10.1021/acs.estlett.5b00209
Environ. Sci. Technol. Lett. XXXX, XXX, XXX−XXX