Coupled mercury-cell sorption, reduction, and oxidation on methylmercury production by *Geobacter sulfurreducens* PCA

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ABSTRACT

G. sulfurreducens PCA cells have been shown to reduce, sorb, and methylate Hg(II) species, but it is unclear whether this organism could oxidize and methylate dissolved elemental Hg(0) as shown for *Desulfovibrio desulfuricans* ND132. Using Hg(II) and Hg(0) separately as Hg sources in washed cell assays in phosphate buffered saline (pH 7.4), we study and report how cell-mediated Hg reduction and oxidation compete or synergize with sorption, thus affecting the production of toxic methylmercury by PCA cells. Methylation is found to be positively correlated to Hg sorption (r = 0.73) but negatively correlated to Hg reduction (r = –0.62). These reactions depend on the Hg and cell concentrations or the ratio of Hg to cellular thiols (–SH). Oxidation and methylation of Hg(0) are favored at relatively low Hg to cell–SH molar ratios (e.g., <1). Increasing Hg to cell ratios from 0.25×10^{-19} to 25×10^{-19} moles-Hg/cell (equivalent to Hg/cell–SH of 0.71 to 71) shifts the major reaction from oxidation to reduction. In the absence of five outer membrane *c*-type cytochromes, mutant *ΔomcBESTZ* also shows decreases in Hg reduction and increases in methylation. However, the presence of competing thiol-binding ions such as Zn^{2+} leads to increased Hg reduction and decreased methylation. These results suggest that the coupled cell-Hg sorption and redox transformations are important in controlling the rates of Hg uptake and methylation by *G. sulfurreducens* PCA in anoxic environments.

INTRODUCTION

Neurotoxic monomethylmercury (MeHg) is produced predominantly by some anaerobic microorganisms, such as sulfate-reducing bacteria, dissimilatory iron reducing bacteria, and methanogens in anoxic environments.[1-7] While the recent identification of two genes (*hgcA* and *hgcB*) in methylating bacteria provided the genetic basis for mercury methylation,[7] the fundamental mechanisms and the key biogeochemical reactions involved in Hg uptake and methylation remain poorly understood.[8-11] One important factor affecting Hg bioavailability and uptake is the redox transformation resulting in changes of the chemical speciation of Hg.[2,12-14] Both chemical and biological processes can mediate these reactions in natural water and sediments. For example, naturally dissolved organic matter (DOM) not only forms complexes with Hg[15-17] but also affects Hg reduction and oxidation.[12-14] Microorganisms, such as dissimilatory metal-reducing bacteria *Shewanella oneidensis* MR-1, *Geobacter metallireducens* GS-15, and *Geobacter sulfurreducens* PCA, are known to reduce Hg(II).[18-20] PCA can also methylate Hg(II)[5-7] and is found in various environments including the contaminated East Fork Poplar Creek watershed in Oak Ridge, Tennessee, our study site.[21,22] Recent studies indicate that *G. sulfurreducens* PCA uses a mer-independent pathway for Hg(II) reduction at low concentrations (nM) under anaerobic conditions.[18,19] This mer-independent reduction of Hg(II) is of great environmental significance because microbial reduction can occur concurrently with methylation leading to decreased Hg bioavailability in dark anoxic environments.[19,23]

Hg reduction and sorption by *G. sulfurreducens* PCA are closely linked due to the presence of both Hg-binding and reduction sites on cell envelops and the redox-reactive, thiophilic properties of Hg.[19] As two competing processes, Hg(II) reduction and sorption proceed concurrently on PCA cells and are both influenced by the cellular thiol content.[19,24] Additionally,

Hg-binding ligands can affect these reactions: the presence of thiol ligands, such as cysteine and glutathione has been shown to eliminate Hg(II) reduction by PCA cells and cause thiol-induced oxidation of dissolved Hg(0).[14,19] However, these reactions are microbial species specific: bacterium *Desulfovibrio desulfuricans* ND132 does not reduce Hg(II), and its methylation is not affected by cysteine and glutathione.[8,9] ND132 can also directly oxidize and methylate Hg(0) under anaerobic conditions,[14,25] and the reaction has been attributed to thiol functional groups on the cell envelope. Despite notable quantities of thiols also being present on *G. sulfurreducens* PCA,[19,26] oxidation of Hg(0) was not observed on this organism until recently.[11] It is unclear whether the lack of Hg(0) oxidation by PCA[14] is caused by its low thiol content or by its high reduction potential, thereby inhibiting Hg(0) oxidation. Furthermore, although *G. sulfurreducens* PCA has been studied extensively in laboratory for Hg uptake and methylation,[5,7,8] little is known regarding the effects of coupled cell reduction, oxidation, and sorption on Hg methylation by this organism.

In this study we investigate how cell-induced Hg redox transformations may compete or synergize with sorption, thus affecting the production of MeHg, and under what conditions *G. sulfurreducens* PCA can oxidize and methylate dissolved elemental Hg(0). Using washed cell assays under dark anaerobic conditions, we determined (1) the kinetics of Hg reduction/oxidation and sorption reactions, and their correlations to Hg methylation, (2) Hg-cell interactions and methylation under varying Hg to cell (or Hg/cell-SH) ratios, and (3) the effect of either deletion of five outer-membrane (OM) c-type cytochromes (*ΔomcBESTZ*)[27] or addition of Zn^{2+} (as a Hg^{2+} competing ion) on Hg-cell interactions and methylation.

MATERIALS AND METHODS

Bacterial strains and growth conditions

G. sulfurreducens PCA (ATCC 51573) wild type (WT) strain was cultured anaerobically in the nutrient broth Basal salts containing 40 mM fumarate and 20 mM acetate at 30°C.[19,28] Cells were harvested at the exponential growth phase (optical density of ~0.5) and subsequently washed three times by repeated centrifugation (10 min at 1200×*g*, 25°C) and resuspension in de-oxygenated 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 and was boiled and purged with ultra-high purity N_2 gas for more than 2 h. After purging, the PBS was kept in an anaerobic glove chamber (Coy) (98% N_2 and 2% H_2) and equilibrated for at least 24 h before use. Deoxygenated PBS was used throughout the experiment to minimize abiotic redox transformation of Hg. The cell density was obtained by measuring optical density at 600 nm and validated by direct cell count under microscope.[14,19]

A mutant strain of PCA, Δ*omcBESTZ*, was used in comparative studies of the Hg-cell interactions. The strain is deficient in genes for five outer-membrane *c*-type cytochromes OmcB-OmcE-OmcS-OmcT-OmcZ (donated by Dr. Derek Lovley and colleagues at the University of Massachusetts, Amherst).[27] The five heme-based cytochromes are known to be involved in the reduction of humic acids[27] and metal ions and/or oxides such as Hg(II),[19,20] Fe(III)/Mn(IV),[29] and U(VI).[30] The mutant was cultured and prepared following the same procedures as used for the PCA-WT strain.

92 **Cell-Hg interaction assays**

93 All cell-Hg interaction assays were conducted in sealed amber glass vials (4-mL) in an
94 anaerobic chamber.[8,19] In brief, a series of sample solutions was prepared in PBS, to which
95 acetate and fumarate were added as the respective electron donor and acceptor (final
96 concentration = 1 mM each, added once at time zero). For selected experiments, 20 μM Zn^{2+} (as
97 $ZnCl_2$) was added as a competing ion for Hg^{2+}. Washed cells were then added to each vial to
98 reach a final concentration of 10^8 cells/mL, unless otherwise specified. The effects of Hg to cell
99 ratios were studied either at a fixed Hg concentration (25 nM) with varying cell concentrations
100 (10^7–10^9 cells/mL) or at a fixed cell concentration (10^8 cells/mL) with varying Hg concentrations
101 (5–250 nM). Hg stock solutions of either Hg(II) (as 50 μM $HgCl_2$ in 1% HCl) or dissolved
102 gaseous Hg(0) (150–200 nM)[12,31] were used, and the final reaction volume was 1 mL in each
103 vial. All vials were sealed immediately and placed on an orbital shaker and kept in the dark.
104 Blank and control experiments were performed in the same manner with (1) PBS only (no cells),
105 (2) spent medium (cells removed by filtration), and (3) heat-killed cells (3 h at 55°C).[8,11,19] Cell
106 viability and metabolic activity during Hg(II) assays were evaluated and showed a decreased cell
107 density (~ 30%) after 144 h (Supporting Information, Figure S1). However, cells remained alive
108 (>80%) (Figures S1a and S1b) and metabolically active, as evidenced by the consumption of
109 fumarate or the production of succinate (Figure S1d).[32]

110 At selected time points, a set of sample vials (4–5) were removed from the glove chamber
111 and immediately determined for purgeable gaseous Hg(0). The purged samples were then used
112 for Hg speciation analysis, in which 2–3 samples were analyzed for total non-purgeable Hg
113 (Hg_{NP}), and other samples were filtered through a 0.2-μm, 13-mm syringe filter (to remove cells)
114 and analyzed for total non-purgeable soluble Hg (Hg_{sol}). The loss of Hg via sorption on vials

and/or filters was about 2–7%, as observed previously.[19] For samples containing relatively high concentrations of Hg(0), ~10% loss of Hg occurred in the later period of assays (120-144 h). For MeHg analysis, a separate set of sample vials (without purging and filtration) was removed from the glove chamber and immediately preserved with trace-metal grade H_2SO_4 (0.2%, v/v) and kept at –20°C until analysis. Cell-associated or sorbed Hg (Hg_{cell}) was calculated by the difference between Hg_{NP} and Hg_{sol} (here no distinction was made between Hg adsorbed and/or taken up by the cell). Selected samples were analyzed for the total Hg (Hg_{Total}), and a mass balance was determined by the ratio between Hg_{Total} and the sum of Hg(0) and Hg_{NP} and usually within the range of 96-105%. The measured Hg_{Total} at each time point varied slightly between 22-27 nM (Figure S2), indicating good reproducibility and negligible loss of Hg during the experiment. For comparisons, Hg speciation data were presented in percentages by normalizing the Hg(0), Hg_{cell}, Hg_{sol}, and MeHg concentrations (Figure S2) to the measured Hg_{Total} at each time point.

Analytical methods

The purgeable Hg(0) was analyzed directly by purging dissolved gaseous Hg(0) from cell suspension with ultrapure N_2 for 2 min into a Hg(0) analyzer (detection limit ~0.05 ng) (Lumex 915+, Ohio Lumex).[12,19] Hg_{Total}, Hg_{NP} and Hg_{sol} were determined first by oxidizing samples in BrCl (5%, v/v) overnight at 4°C and then analyzing Hg concentrations via $SnCl_2$ reduction, gold-trap amalgamation, and detection using a cold-vapor atomic fluorescence spectrometer (CVAFS) (Tekran 2600, Tekran Instruments) (detection limit ~0.02 ng). The initial rate constants (k_{obs}) for Hg(II) reduction were calculated based on the first order rate law: $d[Hg(0)]/dt = -k_{obs}\,[Hg_{NP}]$, where k_{obs} is determined by the slope of the linear regression between natural logarithm of the Hg_{NP} concentration and time.[13,33]

A modified EPA Method 1630 was used for MeHg analysis with enriched $^{200}HgCH_3^+$ as an internal standard.[7,14] Briefly, MeHg was extracted from the sample matrix via distillation, ethylation, and trapping on a Tenax column via N_2-purging. Thermal desorption and separation by gas chromatography were used prior to the detection of Hg by an inductively coupled plasma mass spectrometer (ICP-MS) (Elan-DRCe, Perkin-Elmer, Inc.). The recovery of spiked MeHg standards was 100±10%, and detection limit of MeHg was ~6 pg.

The reduction capacity of bacterial cells was quantified by titration with 2,6-anthraquinone disulphonate (AQDS) and subsequent measurement of the production of reduced 2,6-anthrahydroquinone disulphonate (AHQDS) after 24-h reaction.[27,34] AHQDS standards were prepared via reduction of AQDS by H_2/Pd and quantified by the specific absorbance at 450 nm wavelength.[34] Cell thiol contents were determined using a maleimide ThioGlo-1 (TG-1) based fluorescent probe, as described previously.[11,26] Reference thiols and standard additions to bacterial cells were used to ensure measurement accuracy between signal intensity and thiols on cells.

RESULT AND DISCUSSION

Hg reduction, oxidation, and sorption by *G. sulfurreducens* PCA

Hg reduction, sorption, and methylation have been shown to occur concurrently on *G. sulfurreducens* PCA cells.[11,19] Exposing PCA cells (10^8 cells/mL) to 25 nM Hg(II), we also found that approximately 65% of the Hg(II) was reduced to gaseous Hg(0) in 4 h, but Hg(0) production was stabilized at this level up to about 48 h and then decreased to 34% after 6 days (Figure 1). The initial reduction rate constant (k_{obs}) was 0.41±0.05 h^{-1}. Coincided with this observation, cell-sorbed Hg (Hg_{cell}) increased rapidly to a maximum of 34% within 30 min and

then decreased to 25% after 2 h, and finally reached 63% after 6 days. Control experiments with PBS only, heat-killed cells, or cell filtrates all showed < 12% of Hg(II) reduction in 144 h (Figure S3), indicating that Hg(II) reduction was biologically mediated and required contacts between live PCA cells and Hg(II). The dissolved Hg_{sol} (0.2-μm filter-passing) decreased concomitantly as Hg(II) was reduced or sorbed, and only a small amount of Hg_{sol} (including MeHg) remained in solution after 24 h (< 5% of the Hg_{Total}) (Figure 1).

The time-dependent change of Hg reduction and sorption, particularly at the later stage of the incubation (after 48 h), was not observed previously.[11,19] To interpret this result, we considered reactions including Hg reduction, oxidation, cell sorption and methylation and the competition or synergy of these reactions in the system. Cell sorption of Hg was faster than reduction, resulting in about 34% Hg sorbed in 30 min. However, the sorbed Hg decreased to 25%, whereas Hg(II) reduction continued up to ~60% after 2 h, suggesting that some Hg(II) weakly-sorbed onto such functional groups as carboxyl, amine, and phosphoryl were reduced over time.[19,35] The AQDS-reducing capacity of PCA-WT was $1.7(\pm0.4)\times10^{9}$ electrons per cell (or ~280 μM at 10^{8} cells/mL) (Table S1), much greater than that of the Hg(II). The fact that 25% of the Hg(II) remained sorbed indicates that this portion of Hg(II) is not reducible by PCA cells. Binding between Hg(II) and cell–SH functional groups has been suggested to inhibit Hg(II) reduction.[19,35] We thus quantitatively determined –SH content on PCA-WT, which was $3.46(\pm0.55)\times10^{-20}$ moles –SH/cell (Table S1),[26] equivalent to a Hg to cell–SH ratio of 7.1 (Hg in excess) at 10^{8} cells/mL. Both reducing capacity and cellular thiol content data suggest that reduction was favored in the system, resulting in a majority of the Hg(II) reduced within 2 h (Figure 1). Although 34% of the Hg(II) was sorbed in 30 min, only about 14% may be attributed to thiol binding (based on the cell–SH content), leading to reduction of some sorbed Hg(II) and

thus decreased Hg_{cell} between 30 min to 2 h. These concurrent reactions of Hg reduction, sorption, and possible rearrangements of Hg on cell surfaces thus led to the dynamic changes of the Hg_{cell} concentration over time (up to ~ 48 h) (Figure 1).

The sorbed Hg_{cell} increased up to 63% at the later stage of the incubation, which coincided with decreased Hg(0) from 66% at 48 h down to 34% at 144 h (Figure 1). Since Hg_{NP} (including both Hg_{cell} and Hg_{sol}) is primarily composed of the oxidized form of Hg(II),[14,19] this result indicates that about 50% of the Hg(0), produced in the first 48 h, was re-oxidized at the later stage. Bacterial Hg(0) oxidation has been reported with *D. desulfuricans* ND132 and *D. alaskensis* G20 strains,[14] but not with *G. sulfurreducens* PCA cells under anaerobic conditions.[19] However, comparative analyses showed that a twice higher Hg concentration (or a Hg to cell-SH ratio of 14) was used in the previous study[19] than in the present study, which may offers an explanation for the observed difference. Studies using DOM and small thiol compounds also showed that decreasing Hg to thiol ratios can lead to increased Hg(0) oxidation under anoxic conditions.[12,31]

To further illustrate the ability of PCA cells to oxidize Hg(0) and the effect of Hg to cell ratios on Hg redox transformation, washed cells at different concentrations (10^8 and 10^9 cells/mL) were exposed to dissolved Hg(0) (~25 nM) as the sole source of Hg under the same conditions (Figure 2 and S4). At 10^9 cells/mL (equivalent to 0.25×10^{-19} moles Hg/cell or 0.71 Hg/cell–SH), about 85% of the Hg(0) was oxidized and recovered as non-purgeable Hg_{NP} after 144 h (Figure 2a and S4), compared to about 17% of Hg(0) oxidized in heat-killed cells (Figure 2b) and 5% in the abiotic control (Figure 2c). Most of the Hg_{NP} (81%) was sorbed onto cells (Figure 2a). Coincided with Hg(0) oxidation, MeHg was produced [up to 1% of the added Hg(0)] (Figure 2a) but at a rate slower than that in the Hg(II) assay (Figure 1).

At the low cell concentration of 10^8 cells/mL (Hg/cell–SH=7.1), however, <10% of Hg(0) was oxidized and negligible amounts of MeHg was produced (Figure 2d), consistent with those observed by Hu et al.[14] This amount of Hg(0) oxidation was similar to that in the abiotic control (Figure 2c) but lower than that with heat-killed cells (17% at 10^8 cells/mL) (Figure 2e). This result is explained by the high reduction potential that was maintained with the live cells, leading to a net Hg(0) oxidation lower (Figure 2d) than that with heat-killed cells (with reduction terminated) (Figures 2e and S3). Similarly, we observed higher Hg(0) oxidation rate in the later period of the Hg(II) assay (Figure 1) than in the Hg(0) assay (Figure 2d), although the same cell concentration (10^8 cells/mL) or the same Hg/cell–SH ratio was used. In the Hg(0) assay we expected negligible or no Hg(II) reduction because no Hg(II) was added (Figure 2). Thus, cells maintained a relatively high reduction potential, or Hg(II) reduction outcompeted Hg(0) oxidation throughout the Hg(0) assay. In contrast, in the Hg(II) assay, the added Hg(II) was reduced rapidly (Figure 1); the reduction then slowed down or terminated so that Hg(0) oxidation became favored after 48 h of the reaction.

Hg/cell ratios and correlations between Hg methylation and reduction or sorption

To further examine whether the coupled Hg redox transformation and sorption events influence methylation, and also how changes of the Hg to cell ratio affect the net balance between Hg(II) reduction and Hg(0) oxidation, we performed experiments at a fixed Hg(II) concentration (25 nM) and tested three Hg to cell ratios at 25, 2.5, and 0.25 ($\times 10^{-19}$ moles Hg/cell), equivalent to Hg/cell-SH ratios of 71, 7.1 and 0.71 (Figure 3a,b,c,d). At the low cell density (10^7 cells/mL) (or Hg to cell ratio of 25×10^{-19} moles Hg/cell), Hg(II) reduction outcompeted cell sorption (Figure 3a and b). Since Hg was present in a large excess over cell thiols (Hg/cell–SH = 71), about 75% of the Hg(II) was reduced after 144 h. However, the

reduction appeared relatively slow; only about 6% of the Hg(II) was reduced in 4 h (Figure 3a), and >80% of the Hg(II) remained in solution (Figure 3c). After 24 h reaction, about 40% of the Hg(II) was reduced, compared to 65% reduced at 2.5×10^{-19} moles Hg/cell (or Hg/cell–SH = 7.1) (Figure 3a). Slow reduction at the low cell density is attributed to limited cell numbers and the fact that the reduction required contacts or interactions between Hg(II) and cell surfaces.[19] A decrease of the Hg to cell ratio to 2.5×10^{-19} moles Hg/cell (or Hg/cell–SH=7.1 at 10^8 cells/mL) resulted in a much higher Hg(II) reduction (66%) in 4 h (Figure 3a), but reduction decreased with time to 34% at 144 h due to re-oxidation of Hg(0), as described earlier. Further decreasing Hg to cell ratio to 0.25×10^{-19} moles Hg/cell (or Hg/cell–SH=0.71 at 10^9 cells/mL) led to an even lower amount of Hg(II) reduced (25% at 4 h) than that observed at 2.5×10^{-19} moles Hg/cell, and Hg(0) decreased to non-detectable levels at 144 h (Figure 3a). The inhibited Hg(II) reduction at the low Hg to cell ratio (0.25×10^{-19} moles Hg/cell) (Figure 3a) agrees well with increased Hg(0) oxidation at the same ratio (Figure 2a), indicating a shift toward Hg(0) oxidation as a dominant process. Coincided with inhibited Hg(II) reduction, cell sorbed Hg_{cell} was the highest (Figure 3b) and showed a clear inverse correlation with the reduction. More than 60% of the Hg(II) was sorbed on cells at 4 h, and Hg_{cell} increased to 85% at 144 h, as compared to < 20% of the Hg(II) sorbed at the Hg/cell–SH ratio of 71 (Figure 3b). Cell sorption of Hg outcompeted Hg(II) reduction at decreasing Hg to cell ratios or increasing cell densities. These two competing processes thus resulted in net amounts of the Hg(II) either reduced or sorbed by the cell or remained in solution at any given time points (Figure 3a,b,c). MeHg concentrations increased continuously over time (Figure 3d) with increasing Hg_{cell}, but Hg_{sol} concentrations decreased to background levels after 4 h (Figure 3c). The highest MeHg (16%) was produced in the high cell density assay (10^9 cells/mL) (Figure 3d).

We also examined the cell-Hg interactions at a fixed cell density (10^8 cells/mL) but varying Hg(II) concentrations (5, 25, 50 and 250 nM) to yield Hg to cell ratios of 0.5, 2.5, 5, and 25 ($\times 10^{-19}$ moles Hg/cell) or Hg/cell–SH ratios of 1.4, 7.1, 14, and 71 (Figure 3e–h). In general, similar trends were observed: Hg(II) reduction was the highest (~90%) at the highest Hg to cell ratio (25×10^{-19} moles Hg/cell) and the lowest (< 25%) at the lowest Hg to cell ratio (0.5×10^{-19} moles Hg/cell) (Figure 3e). Re-oxidation of Hg(0) occurred in the later period of the incubation (after 24 h) at relatively low Hg to cell ratios (or Hg/cell–SH=1.4 and 7.1) but was insignificant at high Hg/cell–SH ratios (14 and 71). Note that data sets at Hg/cell–SH=7.1 (or Hg(II) = 25 nM) were not plotted as they were already shown in Figure 3a–d. The sorbed Hg_{cell} showed an inverse trend and was the highest at the lowest Hg to cell ratio (Figure 3f). Comparing the two sets of experiments (Figures 3a-d and 3e-h), we found that, at the same Hg to cell ratio of 25×10^{-19} moles Hg/cell (or Hg/cell–SH = 71), higher Hg(II) reduction occurred in the presence of higher Hg and higher cell concentrations (250 nM and 10^8 cells/mL) (Figure 3f) than that at lower Hg and lower cell concentrations (25 nM and 10^7 cells/mL) (Figure 3a). About 50% and 90% of the Hg(II) were reduced in 4 and 24 h, respectively, at the higher Hg and cell concentrations (Figure 3f) as compared to only about 6% and 40% reduced at the lower Hg and cell concentrations within the same period (Figure 3a). As a result, a lower percentage of Hg_{sol} was observed at high Hg(II) and high cell concentrations (10^8 cells/mL) (Figure 3g) than that at low Hg(II) and low cell concentrations (Figure 3c). Higher Hg(II) and cell concentrations thus resulted in higher Hg(II) reduction, or vice versa, since both reduction and sorption required cell surface contact with Hg(II). We also found a lower production of MeHg (4%) at the lower Hg(II) (5 nM) and cell (10^8 cells/mL) concentrations (Figure 3h) than that (16%) at higher Hg(II) (25 nM) and cell (10^9 cells/mL) concentrations (Figure 3d). The low Hg and cell concentrations limit Hg uptake

and methylation; increasing Hg(II) concentration alone (at the same cell density) could result in even lower percentages of Hg becoming methylated (Figure 3h).

Together by plotting MeHg against Hg(0) or Hg_{cell} from Figure 3, we observe a significant negative correlation between MeHg production and Hg reduction ($r = –0.62$ at $p < 0.02$) (Figure 4a) because these two processes compete for the same Hg(II) source. However, MeHg was positively correlated with cell-sorbed Hg_{cell} ($r = 0.73$ at $p < 0.02$) (Figure 4b), consistent with previous findings for other methylating bacteria.[9] No significant correlation ($p < 0.02$) was observed between MeHg and soluble Hg_{sol} (Figure S5), partly due to rapid reduction and sorption of Hg(II) by cells, leaving negligible amounts of Hg in the solution phase. These results suggest that MeHg production is likely coupled to Hg_{cell} rather than Hg_{sol}, as evidenced by continued MeHg production when Hg in solution approached zero (Figures 1, 3, and 5). Similarly Lin et al.[11] reported that an increased intracellular Hg uptake coincided with a decreased Hg_{cell} when Hg_{sol} approached zero.

These findings can be further elaborated by examining the Hg sorption capacity (Q_m) on cells (at 10^8 cells/mL) at different levels of Hg(II) exposure. By re-plotting data from Figure 3 (at 4 and 24 h) based on the Langmuir equation,[36] we show a near linear correlation between $\frac{[Hg_{sol}]}{[Hg_{cell}]}$ and $[Hg_{sol}]$ (Figure S6). Despite the fact that the Hg_{sol} concentrations decreased about 2 orders of magnitude from 4 h to 24 h, the estimated Q_m values were similar at $2.4(\pm0.5)\times10^{-19}$ and $2.2(\pm0.5)\times10^{-19}$ moles Hg/cell, respectively (Figure S6). Note that data at 144 h were not used because of the re-oxidation of Hg(0) at the later period of the incubation. The average Q_m value was $2.3(\pm0.7)\times10^{-19}$ moles Hg/cell for all sorbed Hg. The measured thiol content on PCA cells, $3.46(\pm0.55)\times10^{-20}$ moles SH/cell, thus accounts for about 15% of the sorption capacity. Therefore,

Hg-cell interactions and their effects on Hg methylation may be interpreted by comparing the cellular Hg load (or Hg to cell ratio) and the sorption capacity (Q_m). When the Hg to cell ratio << Q_m, Hg sorption dominates over Hg(II) reduction and thus promotes Hg(0) oxidation and methylation. When the ratio >> Q_m, Hg(II) reduction dominates over cell sorption and thus outcompetes Hg(0) oxidation and methylation.

Deletion of OM cytochromes or addition of competing ions (Zn^{2+}) on Hg-cell interactions

The correlations between Hg redox transformation, cell sorption and methylation were subsequently studied using a five-cytochrome-deficient PCA mutant strain *ΔomcBESTZ*, which has twice higher amounts of thiols (6.81×10^{-20} moles/cell)[26] but ~50% lower reducing capacity than the WT (Table S1). Deletion of the five OM *c*-type cytochromes in this organism resulted in a substantially decreased initial reduction rate (k = 0.11±0.01 h^{-1}) but an increased methylation rate in comparison with the WT at 10^8 cells/mL (Figure 5a and Table S1). About 10% of the Hg was methylated by *ΔomcBESTZ*, as compared to < 5% methylated by the WT in 24 h. Hg(II) reduction by *ΔomcBESTZ* was not completely absent because of its overall reduction capacity (0.9×10^9 AQDS/cell) and a relatively high Hg to thiol ratio (3.7) used in the experiment. The relatively high –SH binding capacity of the mutant also resulted in a higher Hg-cell sorption; about 39% of the Hg was sorbed on *ΔomcBESTZ* cells as compared with 27% on the WT at 4 h. These results again indicate an inverse correlation between Hg(II) reduction and cell sorption and/or methylation. The Hg_{sol} in the presence of Δ*omcBESTZ* cells decreased slower than that of the WT due to slower reduction of Hg(II). However, despite its higher thiol content,[26] the initial sorbed Hg_{cell} by *ΔomcBESTZ* (Figure 5a) was lower than that by PCA-WT (within the first 1 h) (Figure 1 and Table S1). This result suggests that OM cytochromes may be involved in the initial sorption of Hg(II) as well.

The decrease in Hg(II) sorption and increase in Hg(II) reduction can also be illustrated by comparing Hg(II) reduction in the presence of Zn^{2+} as a competing ion with Hg(II) by WT cells (Figure 5b). Zn is in the same group 12 elements as Hg and thus has a relatively high binding affinity with thiols (log β = 17 for Zn-cysteine bis-complexes), albeit much lower than that of Hg(II) (log β = 33-43 for Hg-cysteine bis-complexes).[37,38] The addition of 20 μM Zn^{2+} increased both the rate and the extent of Hg(II) reduction by WT cells (Figure 5b), but no significant effect of Zn^{2+} was observed in the abiotic control (PBS only) (Figure S3). The initial reduction rate constant is 0.66±0.08 h^{-1}, much higher than that in the absence of Zn^{2+} (0.41±0.05 h^{-1}) (Table S1). Most notably the production of MeHg decreased by ~50% in the presence of Zn^{2+} (Figure 5b and Table S1). Hg_{sol} and Hg_{cell} were also lower than those in the absence of Zn^{2+} because of increased Hg(II) reduction (Figure 5b). The increased Hg(II) reduction and decreased Hg methylation cannot be attributed to the toxic effect of Zn^{2+} to PCA cells because, even at mM-concentration levels, Zn^{2+} has shown little effect on the activity of PCA-WT in Fe(III) respiration.[39] Instead, the competition between Zn^{2+} and Hg(II) for cell thiol-binding or for cellular uptake likely caused the decrease of Hg(II) sorption, which in turn increased the available Hg(II) for cell reduction. These results are consistent with findings that increasing Hg to cell or Hg to cell–SH ratios promotes Hg(II) reduction, as discussed earlier. The increased Hg(II) reduction and decreased MeHg production in the presence of Zn^{2+} further support a negative correlation between Hg(II) reduction and methylation (Figure 4). The presence of competing ions, especially those with high binding affinities to thiols, results in decreased Hg sorption and MeHg production.

Taken together, the present study demonstrates strongly coupled Hg redox transformation, sorption, and methylation by *G. sulfurreducens* PCA and its mutant

343 Δ*omcBESTZ*. These findings have important implications to Hg speciation, uptake, and
344 methylation in the environment and to the design of laboratory incubation studies, in which the
345 Hg to cell ratio can potentially influence the outcome and/or interpretation of the experimental
346 results. Our results indicate that, even under exactly the same experimental conditions,[19] a small
347 change in Hg to cell ratios can tip the net balance between Hg reduction and oxidation reactions
348 and thus the rates of Hg methylation (Figures 1–3). Increasing the Hg to cell ratio (e.g., >
349 2.3×10^{-19} moles Hg/cell sorption capacity) increases Hg(II) reduction and decreases Hg-cell
350 sorption, methylation, and Hg(0) oxidation, or *vice versa*. *Geobacter* strains are found in various
351 natural environments including our contaminated East Fork Poplar Creek watershed in Oak
352 Ridge, Tennessee.[21] Previous studies reported that high Hg concentrations in contaminated water
353 do not necessarily lead to higher percentages of Hg methylation,[40,41] whereas low Hg
354 concentrations in water often show a high percentage of Hg methylation (up to 30–50%) such as
355 those observed in pristine environments.[42,43] Concentrations of Hg in natural or contaminated
356 water and sediments typically vary between 1 pM to 1 nM,[40,42] equivalent to a Hg to cell ratio of
357 about 10^{-20} to 10^{-17} moles Hg/cell by assuming a microbial population of 10^8 cells/mL in pore
358 waters, of which *Geobacter* accounts for ~ 10^5 cells/mL.[21] A high Hg to cell ratio (> 10^{-17} moles
359 Hg/cell) (or Hg/cell-SH ratio ~ 300) found in contaminated environments results in increased Hg
360 reduction but decreased Hg sorption and methylation. In the pristine environment, however, Hg
361 sorption and methylation could be favored because of a relatively low Hg to cell ratio (10^{-20}
362 moles Hg/cell) or a low Hg/cell-SH ratio.

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SUPPORTING INFORMATION AVAILABLE

Rate constants of Hg reduction and methylation, cell surface thiol contents, AQDS-reducing capacity; measurements of cell viability, and metabolic activity; absolute concentrations of Hg_{Total}, Hg(0), Hg_{sol}, Hg_{cell}, and MeHg during Hg(II) assays and in control samples; purgeable Hg(0) in Hg(0) assays; determination of the Hg sorption capacity via Langmuir isotherm. This information is available free of charge via the Internet at http://pubs.acs.org/.

REFERENCES

(1) Compeau, G. C.; Bartha, R. Sulfate-reducing bacteria - principal methylators of mercury in anoxic estuarine sediment. *Appl. Environ. Microbiol.* **1985**, *50* (2), 498-502.

(2) Barkay, T.; Wagner-Dobler, I. Microbial transformations of mercury: Potentials, challenges, and achievements in controlling mercury toxicity in the environment. In *Advances in Applied Microbiology, Vol 57*; Elsevier Academic Press Inc: San Diego, 2005; Vol. 57, pp 1-52.

(3) Jay, J. A.; Murray, K. J.; Gilmour, C. C.; Mason, R. P.; Morel, F. M. M.; Roberts, A. L.; Hemond, H. F. Mercury methylation by *Desulfovibrio desulfuricans* ND132 in the presence of polysulfides. *Appl. Environ. Microbiol.* **2002**, *68* (11), 5741-5745.

(4) Hamelin, S.; Amyot, M.; Barkay, T.; Wang, Y. P.; Planas, D. Methanogens: Principal Methylators of Mercury in Lake Periphyton. *Environ. Sci. Technol.* **2011**, *45* (18), 7693-7700.

(5) Kerin, E. J.; Gilmour, C. C.; Roden, E.; Suzuki, M. T.; Coates, J. D.; Mason, R. P. Mercury methylation by dissimilatory iron-reducing bacteria. *Appl. Environ. Microbiol.* **2006**, *72* (12), 7919-7921.

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

(7) 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. Y. The genetic basis for bacterial mercury methylation. *Science* **2013**, *339* (6125), 1332-1335.

(8) Schaefer, J. K.; Rocks, S. S.; Zheng, W.; Liang, L. Y.; Gu, B. H.; 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* (21), 8714-8719.

(9) 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* (20), 7337-7346.

(10) Poulain, A. J.; Barkay, T. Cracking the Mercury Methylation Code. *Science* **2013**, *339* (6125), 1280-1281.

(11) Lin, H.; Hurt, R. A.; Johs, A.; Parks, J. M.; Morrell-Falvey, J.; Liang, L.; Elias, D. A.; Gu, B. Unexpected effects of gene deletion on interactions of mercury with the methylation-deficient mutant Δ*hgcAB*. *Environ. Sci. Technol. Lett.* **2014**, *1* (5), 271-276.

(12) Gu, B. H.; Bian, Y. R.; Miller, C. L.; Dong, W. M.; Jiang, X.; Liang, L. Y. Mercury reduction and complexation by natural organic matter in anoxic environments. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (4), 1479-1483.

(13) Zheng, W.; Liang, L. Y.; Gu, B. H. Mercury reduction and oxidation by reduced natural organic matter in anoxic environments. *Environ. Sci. Technol.* **2011**, *46* (1), 292-299.

(14) Hu, H. Y.; Lin, H.; Zheng, W.; Tomanicek, S. J.; Johs, A.; Feng, X. B.; Elias, D. A.; Liang, L.; Gu, B. H. Oxidation and methylation of dissolved elemental mercury by anaerobic bacteria. *Nat. Geosci.* **2013**, *6* (9), 751-754.

(15) Dong, W. M.; Bian, Y. R.; Liang, L. Y.; Gu, B. H. Binding Constants of Mercury and Dissolved Organic Matter Determined by a Modified Ion Exchange Technique. *Environ. Sci. Technol.* **2011**, *45* (8), 3576-3583.

(16) Skyllberg, U.; Bloom, P. R.; Qian, J.; Lin, C. M.; Bleam, W. F. Complexation of mercury(II) in soil organic matter: EXAFS evidence for linear two-coordination with reduced sulfur groups. *Environ. Sci. Technol.* **2006**, *40* (13), 4174-4180.

(17) Aiken, G. R.; Hsu-Kim, H.; Ryan, J. N. Influence of dissolved organic matter on the environmental fate of metals, nanoparticles, and colloids. *Environ. Sci. Technol.* **2011**, *45* (8), 3196-3201.

(18) Wiatrowski, H. A.; Ward, P. M.; Barkay, T. Novel reduction of mercury(II) by mercury-sensitive dissimilatory metal reducing bacteria. *Environ. Sci. Technol.* **2006**, *40* (21), 6690-6696.

(19) Hu, H. Y.; Lin, H.; Zheng, W.; Rao, B.; Feng, X. B.; Liang, L. Y.; Elias, D. A.; Gu, B. H. Mercury reduction and cell-surface adsorption by *Geobacter sulfurreducens* PCA. *Environ. Sci. Technol.* **2013**, *47* (19), 10922-10930.

(20) Lovley, D. R.; Giovannoni, S. J.; White, D. C.; Champine, J. E.; Phillips, E. J. P.; Gorby, Y. A.; Goodwin, S. *Geobacter-metallireducens* gen-nov sp-nov, a microorganism capable of coupling the complet oxidation of organic-compounds to the reduction of iron and other metals. *Arch. Microbiol.* **1993**, *159* (4), 336-344.

(21) Mosher, J. J.; Vishnivetskaya, T. A.; Elias, D. A.; Podar, M.; Brooks, S. C.; Brown, S. D.; Brandt, C. C.; Palumbo, A. V. Characterization of the Deltaproteobacteria in contaminated and uncontaminated stream sediments and identification of potential mercury methylators. *Aquat. Microb. Ecol.* **2012**, *66* (3), 271-282.

(22) Vishnivetskaya, T. A.; Mosher, J. J.; Palumbo, A. V.; Yang, Z. K.; Podar, M.; Brown, S. D.; Brooks, S. C.; Gu, B. H.; Southworth, G. R.; Drake, M. M.; Brandt, C. C.; Elias, D. A. Mercury and other heavy metals influence bacterial community structure in contaminated Tennessee streams. *Appl. Environ. Microbiol.* **2011**, *77* (1), 302-311.

(23) Barkay, T.; Miller, S. M.; Summers, A. O. Bacterial mercury resistance from atoms to ecosystems. *Fems Microbiology Reviews* **2003**, *27* (2-3), 355-384.

(24) Bae, W.; Wu, C. H.; Kostal, J.; Mulchandani, A.; Chen, W. Enhanced mercury biosorption by bacterial cells with surface-displayed MerR. *Appl. Environ. Microbiol.* **2003**, *69* (6), 3176-3180.

(25) Colombo, M. J.; Haa, J. Y.; Reinfelder, J. R.; Barkay, T.; Yee, N. Anaerobic oxidation of Hg(0) and methylmercury formation by *Desulfovibrio desulfuricans* ND132. *Geochim. Cosmochim. Acta* **2013**, *112*, 166-177.

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

(27) Voordeckers, J. W.; Kim, B. C.; Izallalen, M.; Lovley, D. R. Role of *Geobacter sulfurreducens* outer surface *c*-type cytochromes in reduction of soil humic acid and anthraquinone-2,6-disulfonate. *Appl. Environ. Microbiol.* **2010**, *76* (7), 2371-2375.

(28) Caccavo, F.; Lonergan, D. J.; Lovley, D. R.; Davis, M.; Stolz, J. F.; McInerney, M. J. *Geobacter sulfurreducens* Sp-Nov, a hydrogen-oxidizing and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl. Environ. Microbiol.* **1994**, *60* (10), 3752-3759.

(29) Mehta, T.; Coppi, M. V.; Childers, S. E.; Lovley, D. R. Outer membrane c-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Appl. Environ. Microbiol.* **2005**, *71* (12), 8634-8641.

(30) Shelobolina, E. S.; Coppi, M. V.; Korenevsky, A. A.; DiDonato, L. N.; Sullivan, S. A.; Konishi, H.; Xu, H. F.; Leang, C.; Butler, J. E.; Kim, B. C.; Lovley, D. R. Importance of *c*-type cytochromes for U(VI) reduction by *Geobacter sulfurreducens*. *BMC Microbiol.* **2007**, *7, 16*.

(31) Zheng, W.; Lin, H.; Mann, B. F.; Liang, L. Y.; Gu, B. H. Oxidation of Dissolved Elemental Mercury by Thiol Compounds under Anoxic Conditions. *Environ. Sci. Technol.* **2013**, *47* (22), 12827-12834.

(32) Galushko, A. S.; Schink, B. Oxidation of acetate through reactions of the citric acid cycle by *Geobacter sulfurreducens* in pure culture and in syntrophic coculture. *Arch. Microbiol.* **2000**, *174* (5), 314-321.

(33) Lin, H.; Szeinbaum, N. H.; DiChristina, T. J.; Taillefert, M. Microbial Mn(IV) reduction requires an initial one-electron reductive solubilization step. *Geochim. Cosmochim. Acta* **2012**, *99*, 179-192.

(34) Lovley, D. R.; Coates, J. D.; BluntHarris, E. L.; Phillips, E. J. P.; Woodward, J. C. Humic substances as electron acceptors for microbial respiration. *Nature* **1996**, *382* (6590), 445-448.

(35) Mishra, B.; O'Loughlin, E. J.; Boyanov, M. I.; Kemner, K. M. Binding of Hg^{II} to high-affinity sites on bacteria inhibits reduction to Hg^{0} by mixed $Fe^{II/III}$ phases. *Environ. Sci. Technol.* **2011**, *45* (22), 9597-9603.

(36) Ruzic, I. Theoretical Aspects of the Direct Titration of Natural-Waters and Its Information Yield for Trace-Metal Speciation. *Anal. Chim. Acta* **1982**, *140* (1), 99-113.

(37) Van Der Linden, W. E.; Beers, C. Determination of composition and stability-constants of complexes of mercury(II) with amino-acids. *Anal. Chim. Acta* **1974**, *68* (1), 143-154.

(38) Basinger, M. A.; Casas, J. S.; Jones, M. M.; Weaver, A. D. Structural requirements for Hg(II) antidotes. *Polyhedron* **1981**, *43* (6), 1419-1425.

(39) Burkhardt, E. M.; Bischoff, S.; Akob, D. M.; Buchel, G.; Kusel, K. Heavy metal tolerance of Fe(III)-reducing microbial communities in contaminated creek bank soils. *Appl. Environ. Microbiol.* **2011**, *77* (9), 3132-3136.

(40) Brooks, S. C.; Southworth, G. R. History of mercury use and environmental contamination at the Oak Ridge Y-12 Plant. *Environ. Pollut.* **2011**, *159* (1), 219-228.

492 (41) Burns, D. A.; Woodruff, L. G.; Bradley, P. M.; Cannon, W. F. Mercury in the Soil of
493 Two Contrasting Watersheds in the Eastern United States. *PLoS One* **2014**, *9* (2).
494 (42) Balogh, S. J.; Nollet, Y. H.; Swain, E. B. Redox chemistry in Minnesota streams during
495 episodes of increased methylmercury discharge. *Environ. Sci. Technol.* **2004**, *38* (19), 4921-
496 4927.
497 (43) Hollweg, T. A.; Gilmour, C. C.; Mason, R. P. Mercury and methylmercury cycling in
498 sediments of the mid-Atlantic continental shelf and slope. *Limnol. Oceanogr.* **2010**, *55* (6), 2703-
499 2722.

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Figure Captions

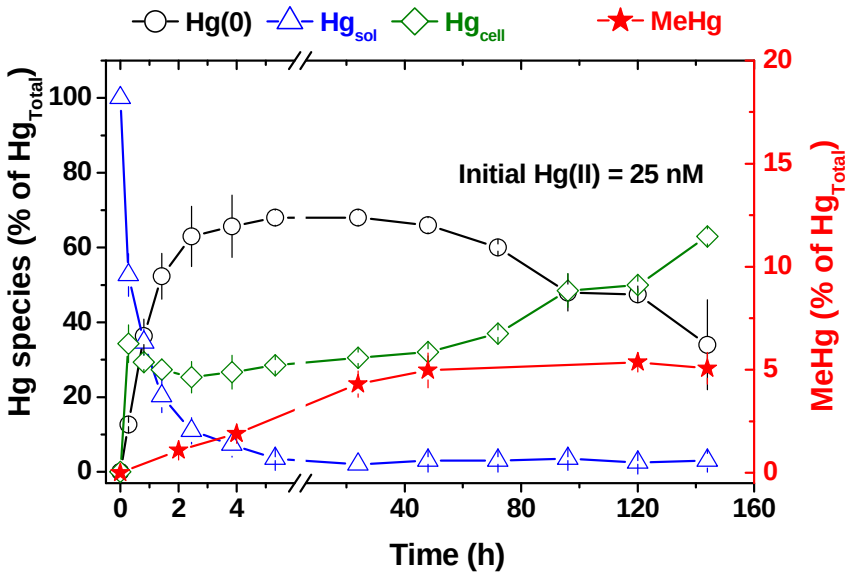
Figure 1. Mercury (Hg) species distribution, expressed as the percentage of the measured total Hg (Hg_{Total}), as a function of time during Hg(II) assays with washed cells of *G. sulfurreducens* PCA (WT) (10^8 cells/mL) in PBS (pH 7.4). Hg(0) = purgeable Hg, Hg_{sol} = soluble Hg, Hg_{cell} = cell-sorbed Hg, and MeHg = methylmercury. The initial Hg(II) concentration added was about 25 nM, and error bars represent one standard deviation of replicate samples (n=2–3) from different batch experiments (n=4–6).

Figure 2. Production of non-purgeable Hg (Hg_{NP} = Hg_{cell} + Hg_{sol}, left y-axis) and MeHg (right y-axis), expressed as the percentage of the total Hg (Hg_{Total}), at 4, 24, and 144 h during Hg(0) (~25 nM) assays with (a) 10^9 cells/mL of live *G. sulfurreducens* PCA (WT) cells, (b) 10^9 cells/mL of heat-killed (dead) cells, (c) abiotic control (PBS only), (d) 10^8 cells/mL of live cells, and (e) 10^8 cells/mL of heat-killed (dead) cells. Error bars represent one standard deviation of replicate samples (n=2–3) from two independent batch experiments (n=2).

Figure 3. Hg species distribution, expressed as the percentage of the total Hg (Hg_{Total}), during Hg(II) assays with washed cells of *G. sulfurreducens* PCA (WT) at a fixed Hg(II) concentration (25 nM) but varying cell concentrations (a – d), or at a fixed cell concentration (10^8 cells/mL) but varying Hg(II) concentrations (e – h). Hg(0) = purgeable Hg, Hg_{sol} = soluble Hg, Hg_{cell} = cell-sorbed Hg, and MeHg = methylmercury. Error bars represent one standard deviation of replicate samples (n=2–3) from two independent batch experiments (n=2).

Figure 4. Correlations between MeHg production and (a) Hg reduction [Hg(0)] or (b) cell-sorbed Hg_{cell} during Hg(II) assays with washed cells of *G. sulfurreducens* PCA (WT) at varying Hg(II) and cell concentrations (data from Figure 3). Error bars represent one standard deviation of replicate samples (n=2–3) from two independent batch experiments (n=2). The Pearson correlation coefficient (r) was calculated using software OriginPro 8.1 at the significance level p = 0.02.

Figure 5. Mercury (Hg) species distribution, expressed as the percentage of the measured total Hg (Hg_{Total}), as a function of time during Hg(II) (25 nM) assays with washed cells of (a) PCA mutant, Δ*omcBESTZ* and (b) PCA-WT the presence of 20 μM Zn^{2+}. The cell concentration was fixed at 10^8 cells/mL. Hg(0) = purgeable Hg, Hg_{sol} = soluble Hg, Hg_{cell} = cell-sorbed Hg, and MeHg = methylmercury. Error bars represent one standard deviation of replicate samples (n=2–3) from three independent batch experiments.



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535 Figure 1.

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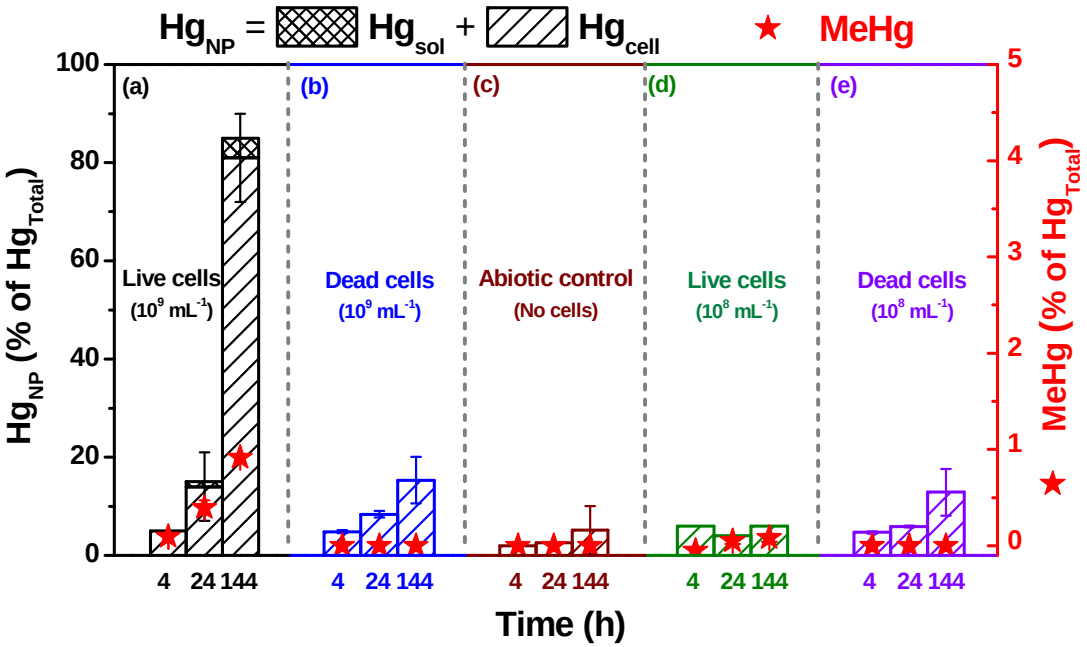

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541 Figure 2.

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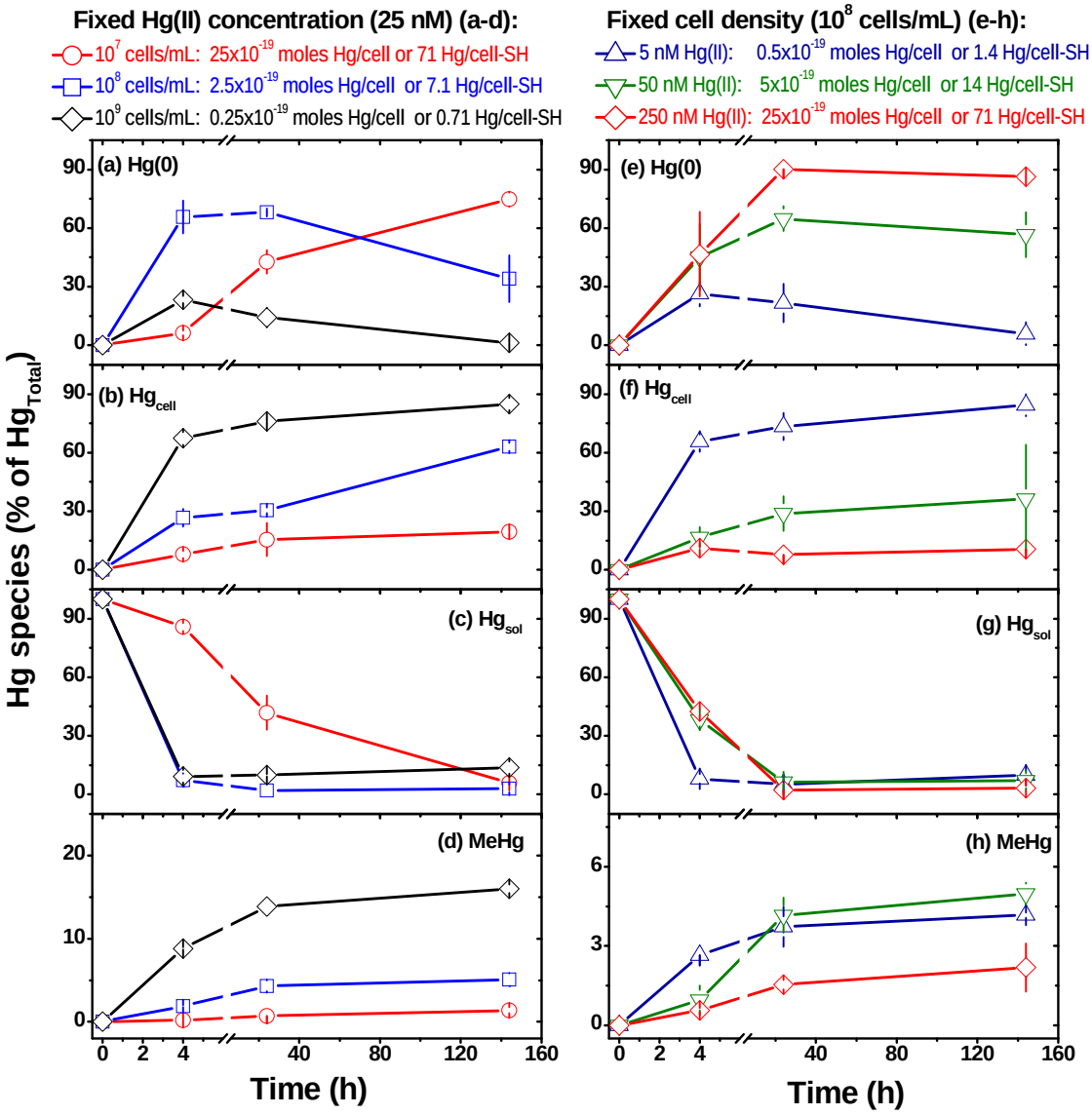
Fixed Hg(II) concentration (25 nM) (a-d):
10^7 cells/mL: 25x10^-19 moles Hg/cell or 71 Hg/cell-SH
10^8 cells/mL: 2.5x10^-19 moles Hg/cell or 7.1 Hg/cell-SH
10^9 cells/mL: 0.25x10^-19 moles Hg/cell or 0.71 Hg/cell-SH
Fixed cell density (10^8 cells/mL) (e-h):
5 nM Hg(II): 0.5x10^-19 moles Hg/cell or 1.4 Hg/cell-SH
50 nM Hg(II): 5x10^-19 moles Hg/cell or 14 Hg/cell-SH
250 nM Hg(II): 25x10^-19 moles Hg/cell or 71 Hg/cell-SH
(a) Hg(0)
(b) Hg_cell
(c) Hg_sol
(d) MeHg
(e) Hg(0)
(f) Hg_cell
(g) Hg_sol
(h) MeHg
Hg species (% of Hg_Total)
Time (h)
Time (h)

Figure 3.

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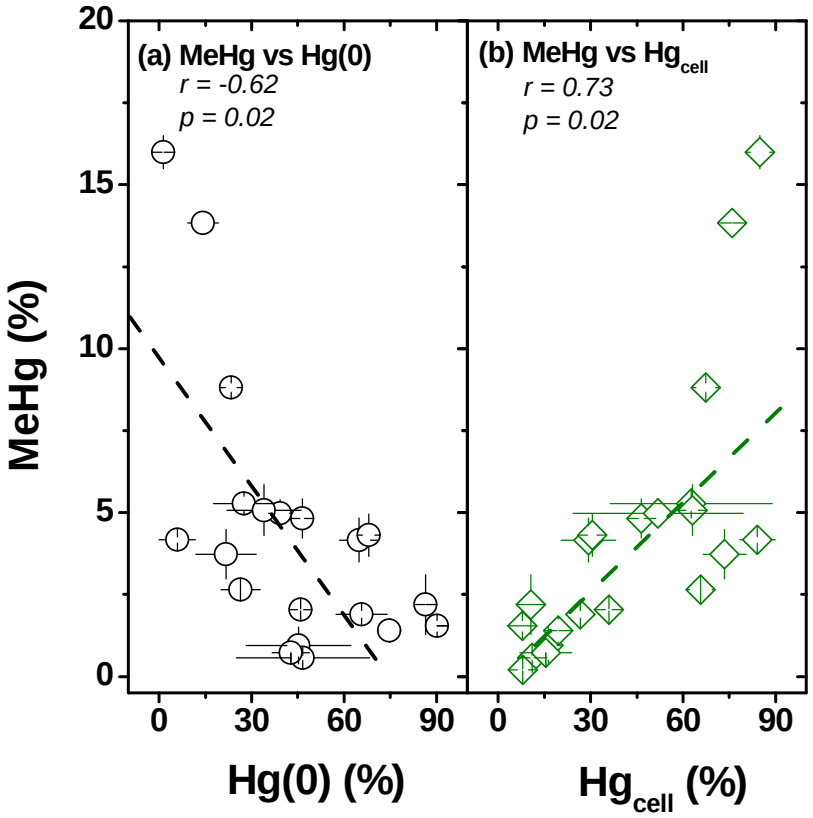

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555 Figure 4.

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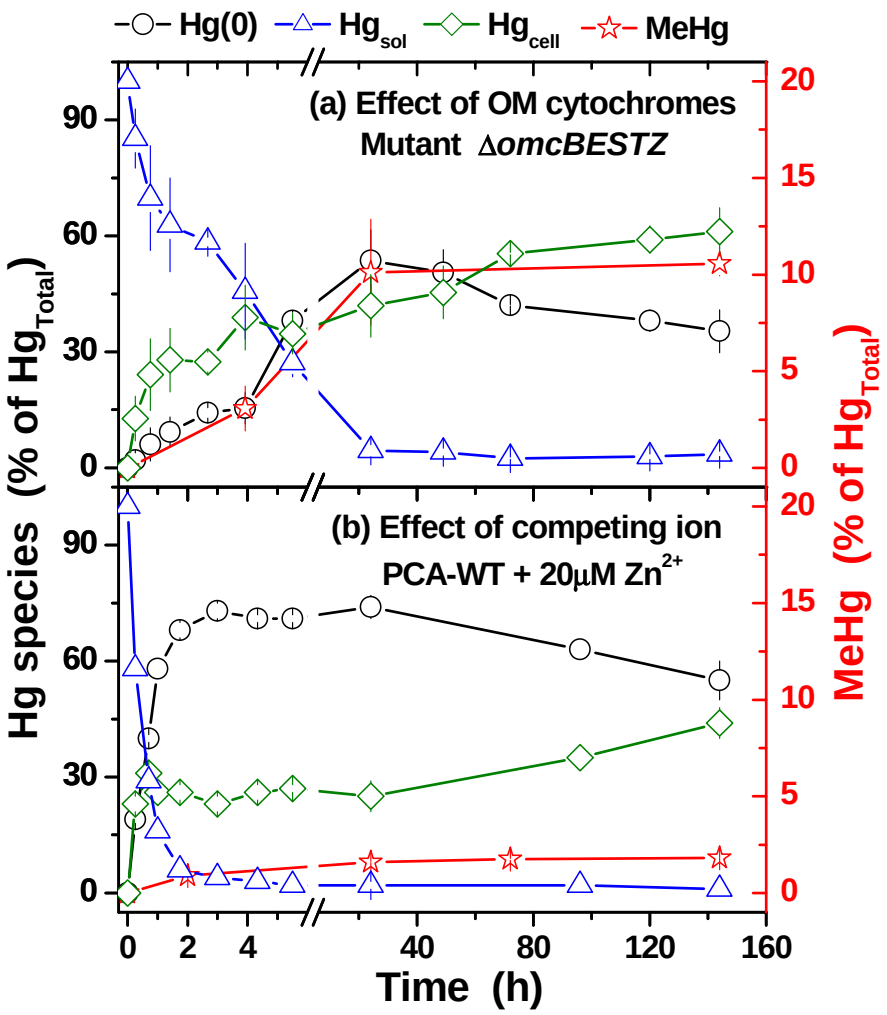

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560 Figure 5.

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