Supporting Information

Microbial methylation of mercury sulfide nanoparticles

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SI Materials and Methods

Methylmercury Degradation Bioassay. The cultures of *D. propionicus* 1pr3 were pre-grown in a fermentative medium and incubated until exponential growth phase prior to dosing with methylmercury chloride (MeHgCl). All methylmercury degradation bioassays were conducted under the same conditions as in the mercury methylation bioassays. Abiotic control consisted of uninoculated media amended with MeHgCl.

Characterization of HgS Particles. The average hydrodynamic diameter of HgS nanoparticles and microparticles were analyzed by light-intensity weighted dynamic light scattering (DLS) (Malvern Zetasizer NS). The diameters of the monomers within the aggregates were analyzed by transmission electron microscopy (TEM). Samples for DLS were directly quantified in their respective stock suspensions. Samples for TEM were prepared by depositing droplets of the particle stocks on a carbon-coated copper grid (200 mesh), wiping the excess liquid with a lint-free tissue, and allowing the grid to air dry under protective cover. Micro- and nano-HgS particles (aged for 16 h or 1 week) were examined by a FEI Technai TEM at 200 keV and characterized with selected area electron diffraction (SAED) to assess crystal structure of the particles (Figure S4a-f). Images of HgS nanoparticles (aged for 3 days) were captured on a Hitachi HF2000 TEM operating at 200 keV and analyzed for elemental composition by energy dispersive X-ray (EDX) spectroscopy (Oxford Inca EDX system) (Figure S4g and h).

For X-ray diffraction (XRD) analysis, the HgS nanoparticle stocks were filtered through 0.025 μm pore size mixed cellulose ester membranes (Millipore). The material that deposited on the filter membrane was analyzed for crystallographic structure by synchrotron XRD performed at the Stanford Synchrotron Radiation Laboratory (SSRL) BL 11-3. Samples were placed in the path of a 12,700 eV beam measuring 0.150 mm^2 and the diffraction pattern was captured using a MAR 345 detector set at a distance of 149.9 mm away from the sample, as determined through calibration with a LaB_6 standard. The diffraction image was integrated and peak matched in a manner similar to Reinsch *et al.* (2010) (1). The resulting spectra indicated that the commercial HgS microparticles consisted of a mixture of metacinnabar and cinnabar (approximately 50-50

proportion based on Rietveld analysis of XRD data in Figure S5c). The spectra for the humic-HgS nanoparticles indicated metacinnabar-like structure, but peaks were broader and less defined than the micro-HgS spectrum, signifying that nano-HgS consisted of smaller crystallite size and less crystallinity (Figure S5c).

The HgS particles that deposited on the 0.025 μm mixed cellulose ester filters (Millipore) were also analyzed for elemental composition by X-ray photoelectron spectrometry (XPS) (Figure S5a and b). A PHI VersaProbe Scanning XPS Microprobe was used for these measurements. XPS data were calibrated using the binding energy of C(1s) (285.00 eV) as the internal standard. In the XPS spectra, binding energy and peak width of the Hg(4f) and S(2p) transitions were comparable for nano-HgS and micro-HgS, indicating similar local structure.

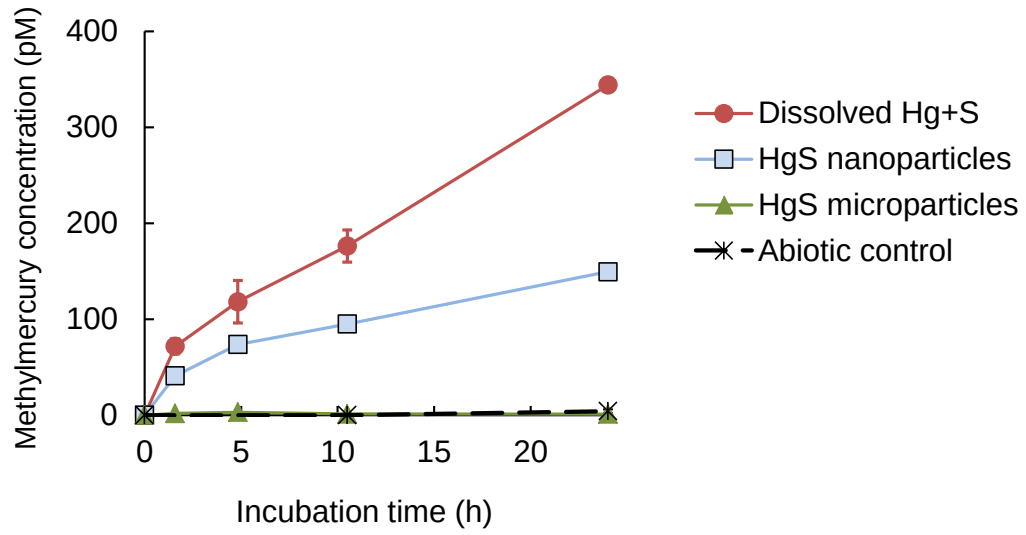
Centrifugation and Ultracentrifugation of HgS-Amended Cultures. In mercury methylation bioassays, total mercury in *D. propionicus* 1pr3 cultures was also fractionated using centrifugation and ultracentrifugation (Figure S9). HgS-treated bacteria were first separated from the bulk medium by centrifugation at 6,700 *g* for 5 min. The pellets (containing mercury associated with bacterial cells and/or large particle aggregates) were washed with 10 mM PBS (pH 7.4) by centrifugation and resuspended in nanopure-filtered water (>18 MΩ-cm). The cells within the washed pellets were then lysed through four freeze-thaw cycles plus 15-min sonication (90 W). This procedure was adequate to fully compromise the cell membrane integrity as determined from the LIVE/DEAD® viability assay (Invitrogen). The mercury released from the lysed cells was collected from the supernatant after centrifugation at 10,800 *g* for 30 min and the cell debris- and/or large particle-associated mercury was collected from the pellets. Total mercury in the supernatant of the centrifuged (6,700 *g*, 5 min) sample was further fractionated by ultracentrifugation at 370,000 *g* for 2 h. After ultracentrifugation, we considered the mercury remaining in the supernatant to be nominally dissolved, while mercury in the pellet after ultracentrifugation consisted of nanoparticulate/colloidal mercury. The applicability of this procedure for separating dissolved, nanoparticulate and microparticulate mercury was demonstrated by (ultra)centrifugation of bacteria-free media that were treated by these different

forms of mercury (Figure S9d).

SI References

1. Reinsch BC, Forsberg B, Penn RL, Kim CS, & Lowry GV (2010) Chemical Transformations during Aging of Zerovalent Iron Nanoparticles in the Presence of Common Groundwater Dissolved Constituents. *Environmental Science & Technology* 44(9):3455-3461.

a.



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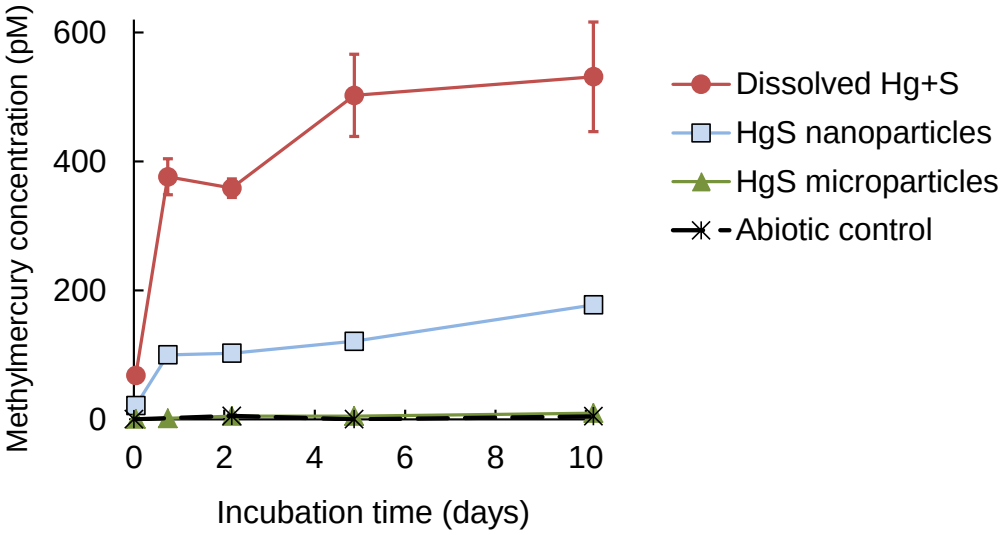


Figure S1. Net MeHg production in *D. propionicus* 1pr3 cultures exposed to different forms of mercuric sulfides. Test cultures were exposed to 5 nM dissolved $Hg(NO_3)_2$ and Na_2S, 5 nM humic-HgS nanoparticles (aged for 16 h), and 20 nM HgS microparticles during **(a)** 1-day time course experiments and **(b)** 10-day time course experiments. Abiotic controls were uninoculated medium solutions amended with 5 nM dissolved $Hg(NO_3)_2$ and Na_2S. The error bars represent ±1 s.d. for duplicate samples.

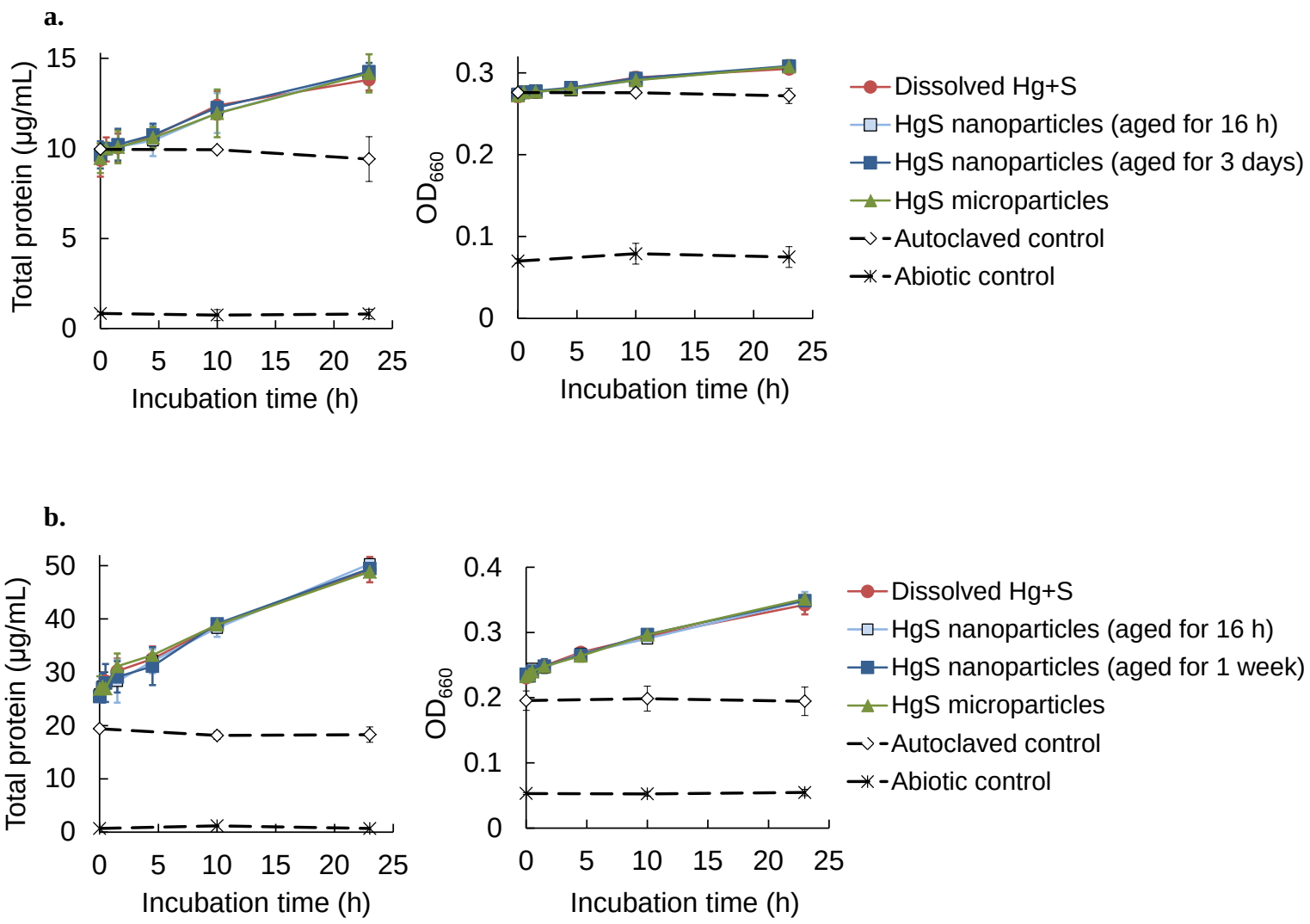
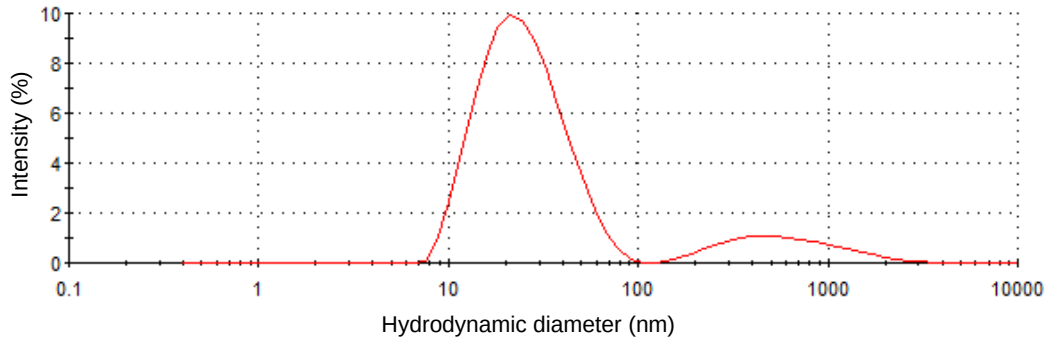


Figure S2. Bacterial growth in SRB cultures exposed to different forms of mercuric sulfides. Total protein content and optical density (OD_{660}) of **(a)** *D. propionicus* 1pr3 cultures and **(b)** *D. desulfuricans* ND132 cultures.

a.



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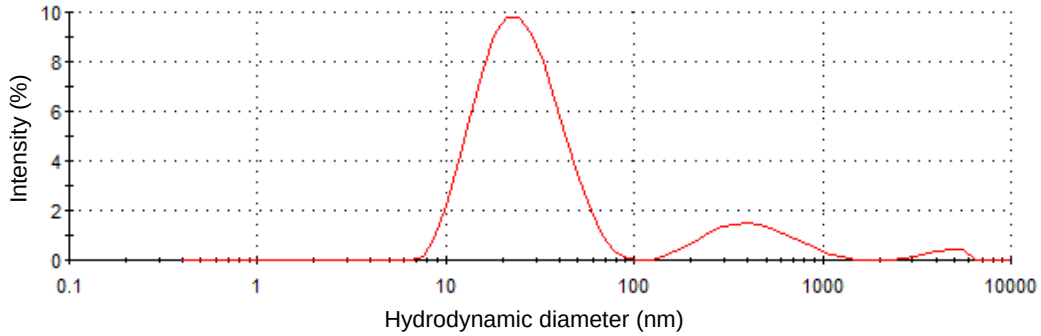

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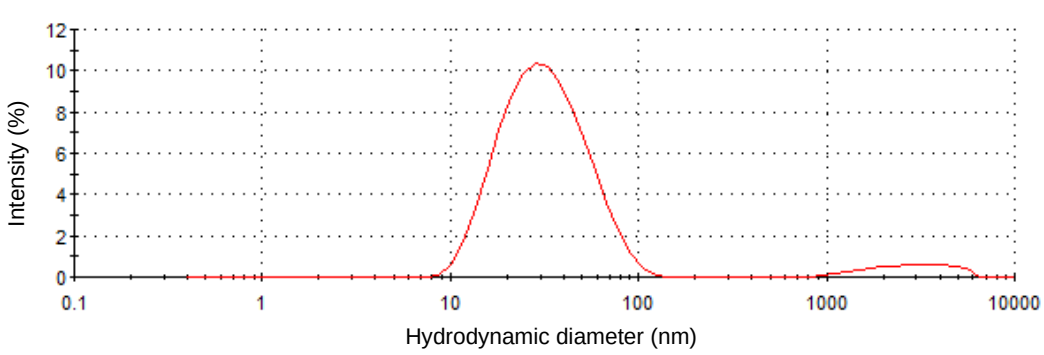

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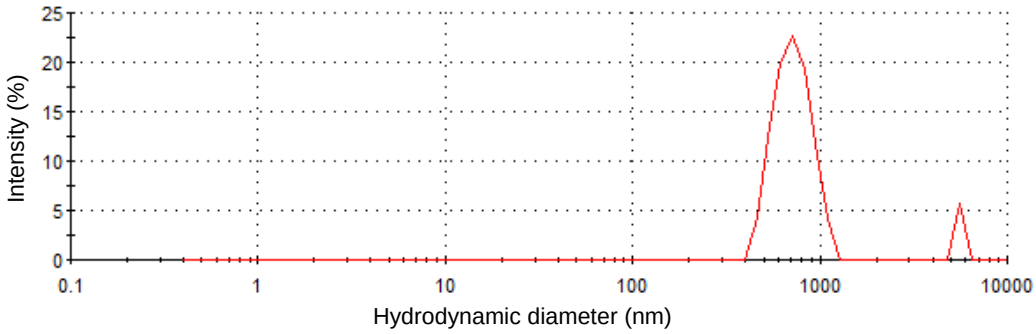


Figure S3. Intensity-weighted size distribution of HgS particles measured by dynamic light scattering. **(a)** HgS nanoparticles (aged for 16 h); **(b)** HgS nanoparticles (aged for 3 days); **(c)** HgS nanoparticles (aged for 1 week); **(d)** Commercial HgS microparticles.

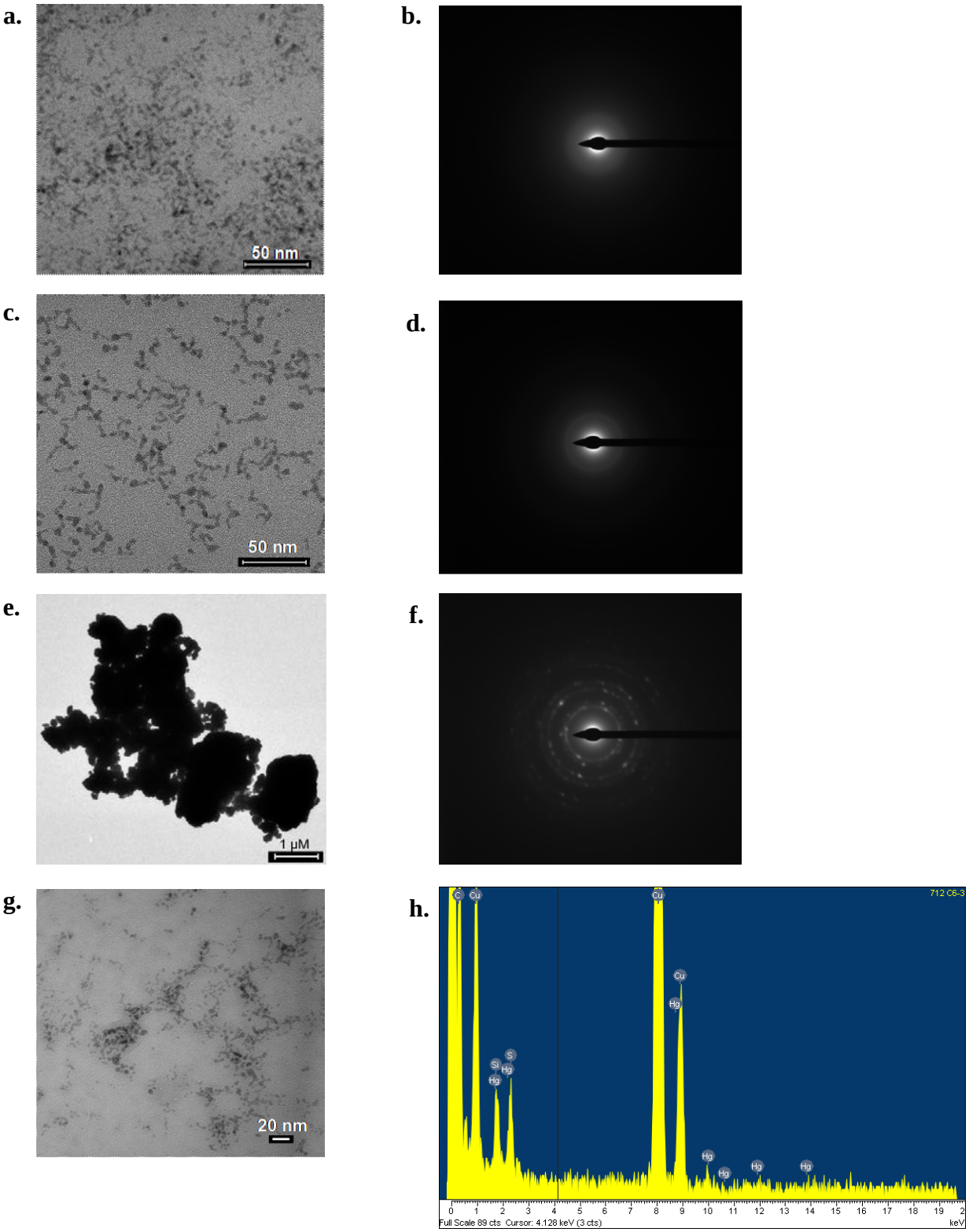


Figure S4. TEM and SAED patterns of HgS nanoparticles aged for 16 h **(a,b)**, HgS nanoparticles aged for 1 week **(c,d)**, and commercial HgS microparticles **(e,f)**. The EDX spectra for HgS nanoparticles aged for 3 days **(g)** is shown in **(h)**. EDX spectrum indicated the presence of Hg and S in the nano-HgS sample. In SAED patterns, defined rings indicate long range crystal structure.

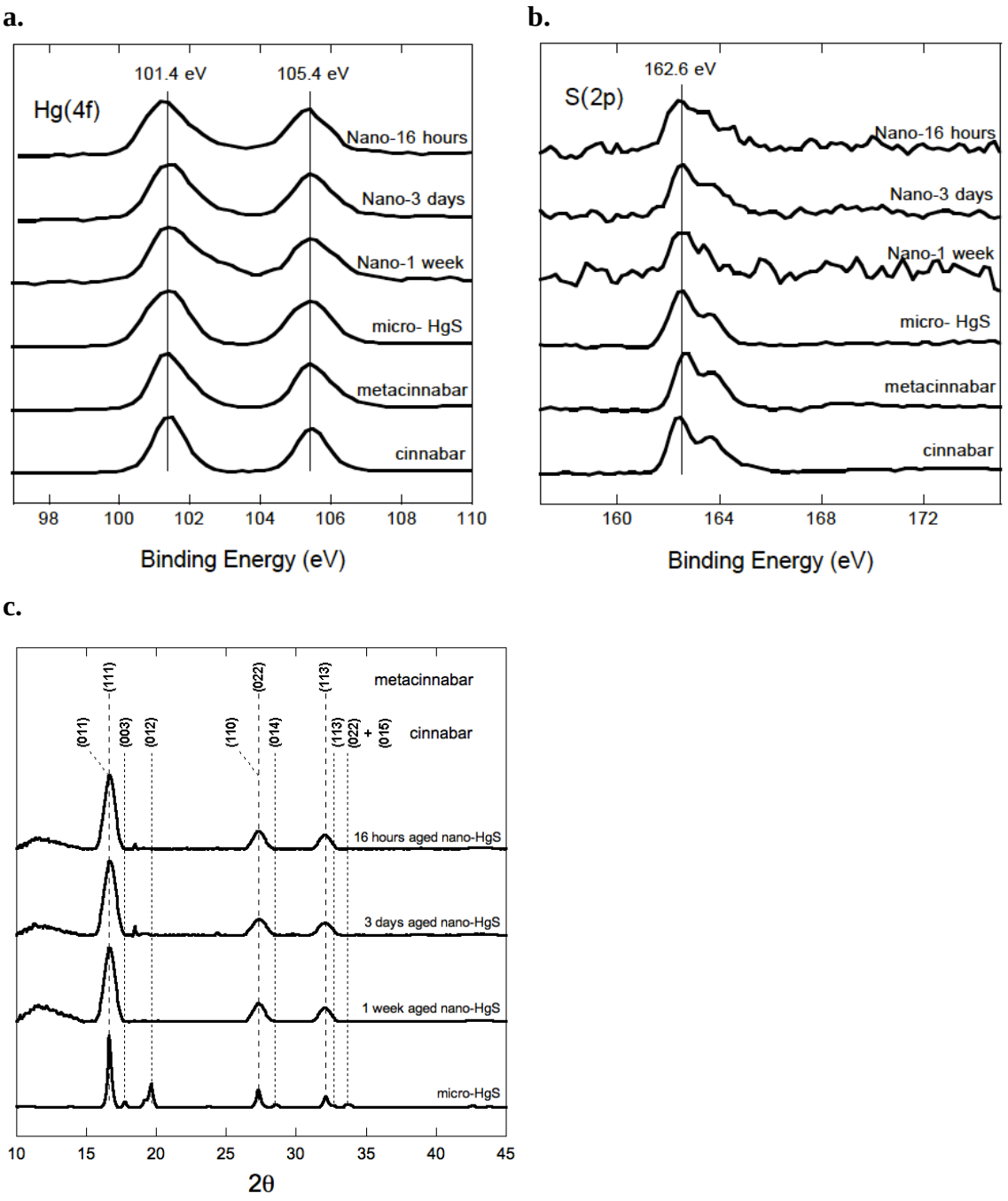


Figure S5. X-ray photoelectron spectroscopy (XPS) and X-ray diffraction (XRD) results of HgS particles. XPS data corresponding to **(a)** Hg(4f) and **(b)** S(2p) electrons for the HgS nanoparticles (aged for 16 h, 3 days or 1 week) and HgS microparticles used in methylation experiments. XPS spectra were also collected for pure minerals of metacinnabar and cinnabar. **(c)** XRD spectra of the same samples. Dotted lines correspond to expected peak positions for pure cinnabar and pure metacinnabar. The spectra for humic-HgS nanoparticles indicated metacinnabar-like structure. HgS microparticles consisted of a mixture of metacinnabar and cinnabar.

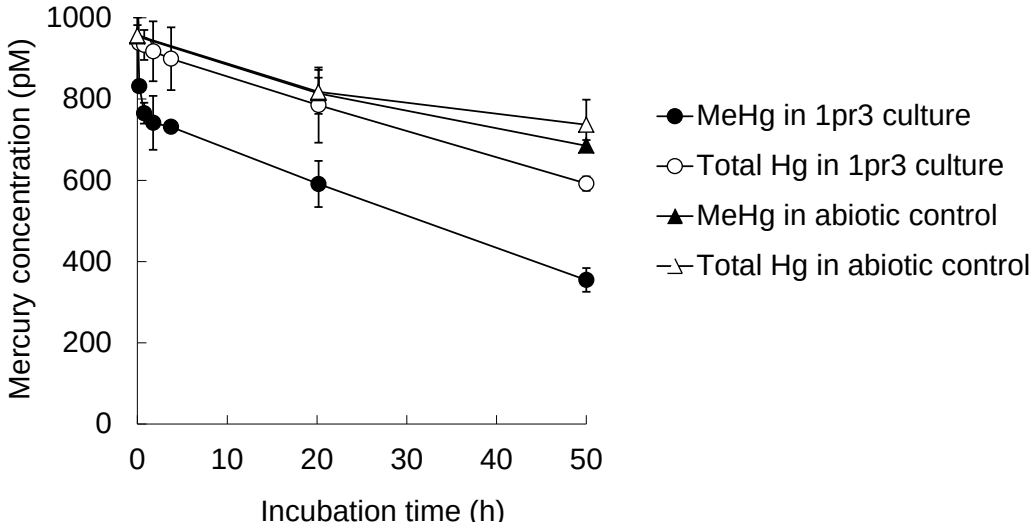
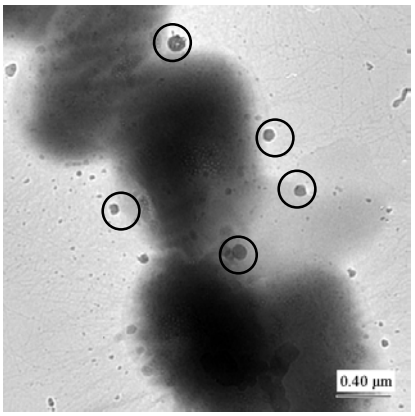
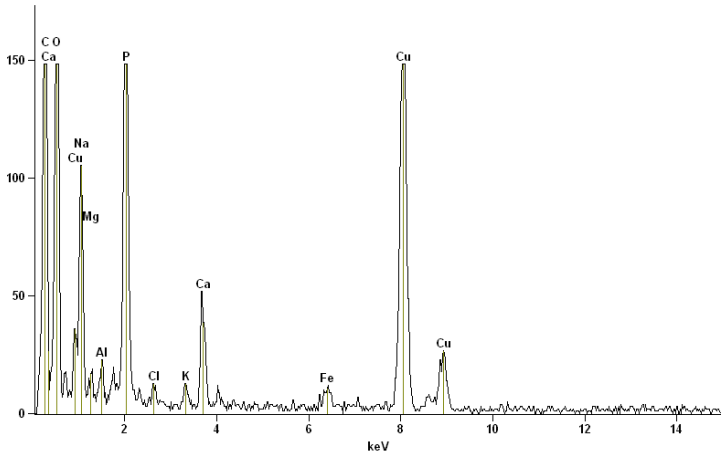


Figure S6. MeHg degradation in *D. propionicus* 1pr3 cultures. Test cultures were exposed to 1 nM methylmercury chloride (MeHgCl) during 2-day time course experiments. Abiotic controls were uninoculated medium solutions amended with 1 nM MeHgCl. The error bars represent ±1 s.d. for duplicate samples.

a.





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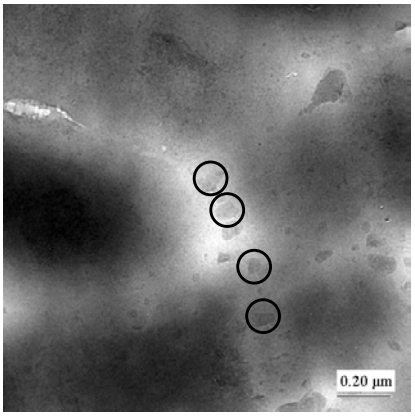

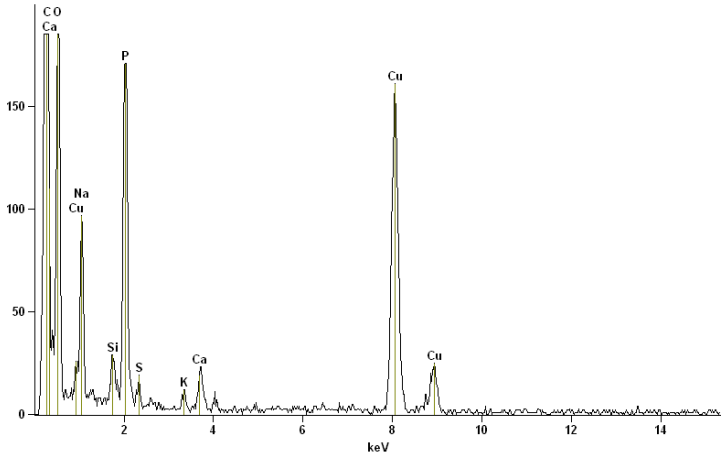

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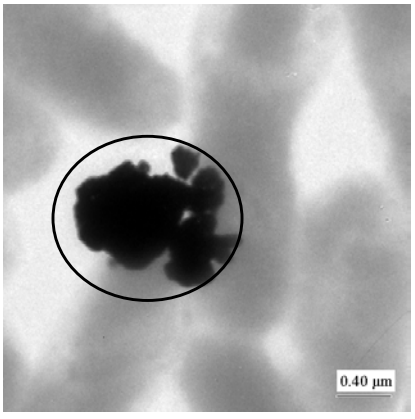

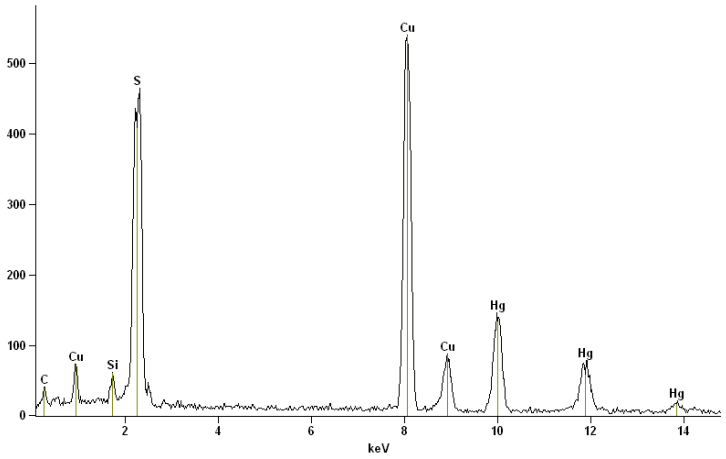

Figure S7. TEM images and EDX spectra of *D. propionicus* 1pr3 cultures. Cultures were exposed to **(a)** 1 nM dissolved $Hg(NO_3)_2$ and Na_2S, **(b)** 1 nM humic-HgS nanoparticles (aged for 16 h), and **(c)** 6 nM HgS microparticles. Test cultures for TEM imaging were collected 14 h after exposure to HgS. Elemental composition of the particles around and/or on the bacterial cells (in black circles) was determined by EDX. The Cu, C, O and Si peaks are from the sample grid. Hg-containing particles were not observed in the cultures exposed to dissolved and nanoparticulate mercury; however, other nanoparticles comprising of elements in the culture media were present, highlighting the heterogeneous nature of the bacteria cultures.

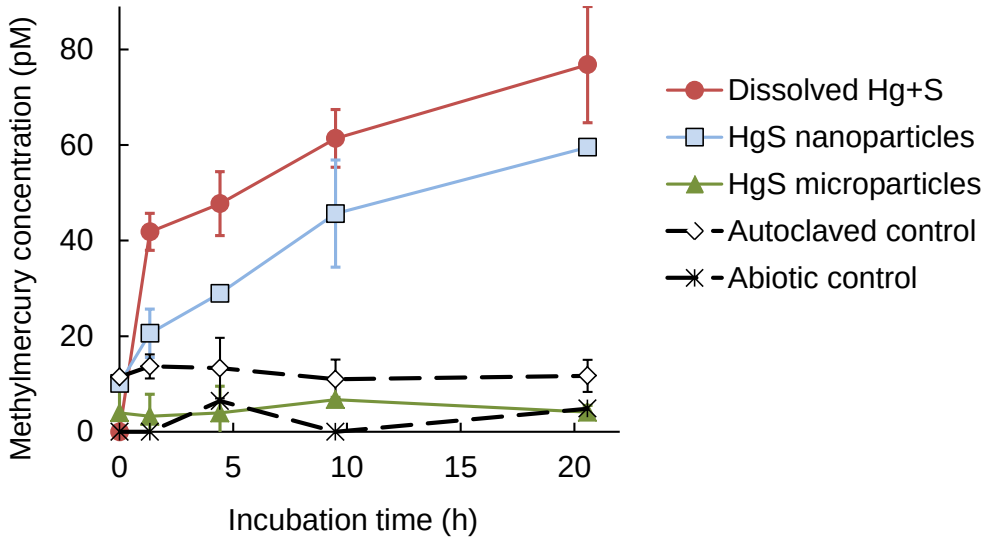

Figure S8. Net MeHg production in *D. propionicus* 1pr3 cultures exposed to different forms of mercuric sulfides. Test cultures were exposed to 1 nM dissolved $Hg(NO_3)_2$ and Na_2S, 1 nM humic-HgS nanoparticles (aged for 16 h), and 6 nM HgS microparticles. The error bars represent ±1 s.d. for duplicate samples. Results of Hg fractionation by filtration and (ultra)centrifugation of these samples are shown in Figure 2 and Figure S9, respectively.

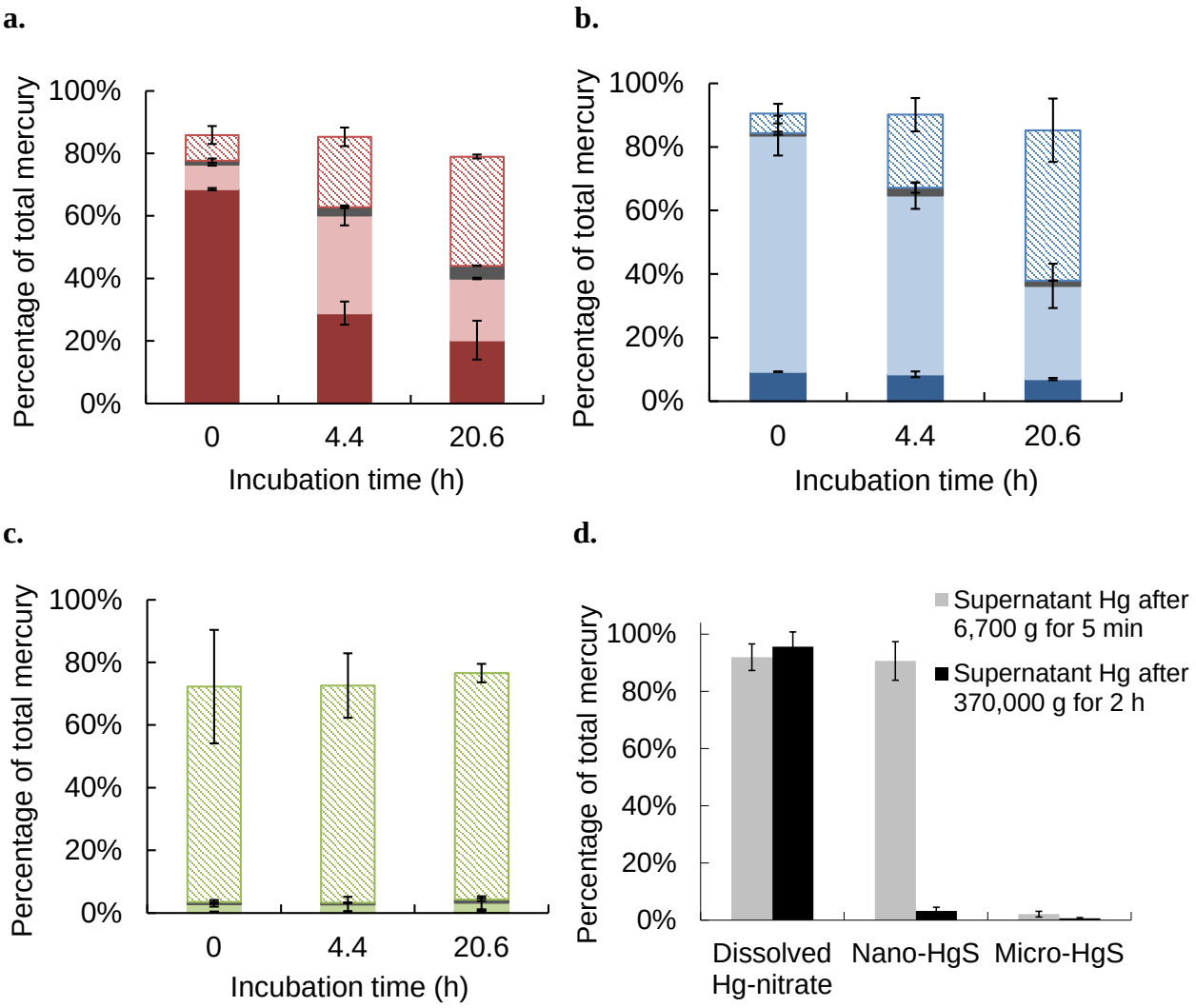

Figure S9. Centrifugation and ultracentrifugation of mercury-amended *D. propionicus* 1pr3 cultures. Cultures were treated with different Hg-sulfide species, including **(a)** 1 nM dissolved $Hg(NO_3)_2$ and Na_2S, **(b)** 1 nM humic-HgS nanoparticles (aged for 16 h), and **(c)** 6 nM HgS microparticles. Cultures were incubated at room temperature for up to 1 day after mercury addition. At each time point, cultures were first centrifuged at 6,700 *g* for 5 min. The pellets were lysed through freeze-thaw cycles plus sonication and then centrifuged at 10,800 *g* for 30 min. The supernatant (after 6,700 *g* for 5 min) was ultracentrifuged at 370,000 *g* for 2 h. **(d)** Bacteria-free media were amended with 1 nM $Hg(NO_3)_2$, 1 nM humic-HgS nanoparticles (aged for 16 h) or 6 nM HgS microparticles, and (ultra)centrifuged immediately (less than 10 min) after mercury addition. The error bars represent ±1 s.d. for duplicate samples.