Using mercury stable isotopes to investigate bacterial Hg(II) uptake

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Introduction: Methylmercury (MeHg) is neurotoxic and its production by anaerobic microorganisms is a dominant process influencing mercury bioaccumulation and human exposures. The fractionation of mercury stable isotopes provides a new tool to examine the cellular mechanisms by which anaerobic bacteria take up and convert inorganic Hg (Hg(II)) to MeHg. Recently, it was observed that the δ^{202}Hg value of cumulative MeHg produced by iron- and sulfate-reducing bacteria exceeded that of the Hg(II) to which they were exposed, possibly due to fractionation associated with Hg(II) transport or intracellular compartmentalization [1]. In this study, we traced changes in Hg isotope ratios of cellular Hg(II) in *Geobacter sulfurreducens* to investigate the transport of Hg(II) into the cells and its associated isotope fractionation prior to methylation.

Materials and Methods: Hg uptake experiments were performed with wildtype *G. sulfurreducens* PCA and a mutant (generated at Rutgers) lacking the mercury methylation genes *hgcAB* exposed to Hg(II)-cysteine. Whole cell, intracellular, and extracellular Hg was collected and analyzed for Hg concentration and δ^{202}Hg values. The potential influence of Hg(II) adsorption/desorption on Hg isotope fractionation in cells was examined in an abiotic experiment with a thiol resin.

Results and Discussion: Both strains accumulated Hg(II) intracellularly. In 24 h, little Hg(II) (<10%) was reduced to Hg(0) by wild-type *G. sulfurreducens*, while about 40% of Hg(II) was reduced by the mutant. In both strains, δ^{202}Hg of intracellular Hg(II) was higher than that outside the cells, perhaps reflecting isotope fractionation during Hg(II) transport. No equilibrium fractionation was observed between aqueous cysteine-complexed Hg(II) and thiol resin sorbed Hg(II), indicating that fractionation between dissolved and cell surface adsorbed Hg during uptake was unlikely. These results indicate that intracellular Hg(II) in *G. sulfurreducens* is subject to isotopic fractionation, becoming enriched in δ^{202}Hg relative to Hg(II) outside the cell, prior to its methylation.

Reference: [1] Janssen *et al*. (2016). *ES&T*, **50**(15), 8077-8083.