Standard Project

Experimental Report template

Proposal title: Kinetic of Hg incorporation and methylation in the sulfate-reducing bacteria *Desulfovibrio dechloroacetivorans*		**Proposal number:** 20160609
Beamline: FAME	**Date(s) of experiment:** from: November 9 2016 to: November 15 2016	**Date of report:** 10 February 2017
Shifts: 18	**Local contact(s): Isabelle Kieffer**	*Date of submission:*

Objective & expected results (less than 10 lines):

The highly toxic methylmercury (MMHg) is mainly produced by sulfate-reducing bacteria (SRB) in aquatic environments. It is biomagnified in the food chain, thus constituting a risk to human health. The mechanisms of Hg methylation are still poorly understood, and we have started the investigation of the anaerobic strain *Desulfovibrio dechloroacetivorans BerOc1*, isolated from Etang de Berre and able to methylate inorganic mercury (IHg) and demethylate methylmercury (MMHg). We had preliminary evidence that βHgS was the dominant Hg form in *BerOc1* exposed to IHg. The objective of this proposal was to charaterize the kinetics of the biotransformation of IHg using Hg L_{III}-edge High Resolution X-ray Absorption Near Edge Structure (HR-XANES) and Extended X-ray Absorption Fine Structure (EXAFS) spectroscopy with the Crystal Analyser Spectrometer (CAS) operating on FAME beamline.

Results and the conclusions of the study (main part):

The SRB *BerOc1* strain was grown in anaerobic conditions and in fumarate medium to avoid HgS precipitation. It was spiked with 1 ppm Hg(II)Cl_2, and incubated for 14 minutes, 1h, 4h and 24 h. Times of the kinetic were chosen according to the literature (Graham et al. 2012, *Appl. Environ. Microbiol.*). The culture was centrifuged, rapidly washed and prepared as frozen pressed pellets for measurements. Hg-references were synthesized and analyzed as solid pellets or aqueous solutions. Hg L_{III}-edge HR-XANES and EXAFS spectra were collected using the five Si(111) crystals analyzer spectrometer selecting Hg Lα1 fluorescence (L3-M5, 9988.8 eV) line for better sensitivity. The diffracted intensity was measured with a one Si detector.

Additional Hg references measured in this experiment confirmed that methyl group induced a slight shift to higher energy values on the HR-XANES spectra (Fig. 1). Spectra recorded after various times of exposure had a similar pattern, with spectral features comparable to βHgS (Fig. 1). This was confirmed by EXAFS spectra (Fig. 2). Data treatment is still in progress but preliminary results with linear combination fitting indicated that the proportion of βHgS accounted for more than 70% in all samples. These results suggest that (1) mercury transformations are rapid and (2) that maybe some potentially produced Hg molecules are exported from the cell. Finally, βHgS, possibly formed by nucleation from thiol-containing compounds (see report 20151061) would be the remaining non mobile Hg form in the cell.
BerOc1 exposed to a low amount of IHg (0.01 ppm) was also measured to approach environmental concentrations often encountered in the natural medium. A good signal/noise HR-XANES spectrum was obtained and was comparable to the 0.1 ppm exposure, with again βHgS as the predominant phase.

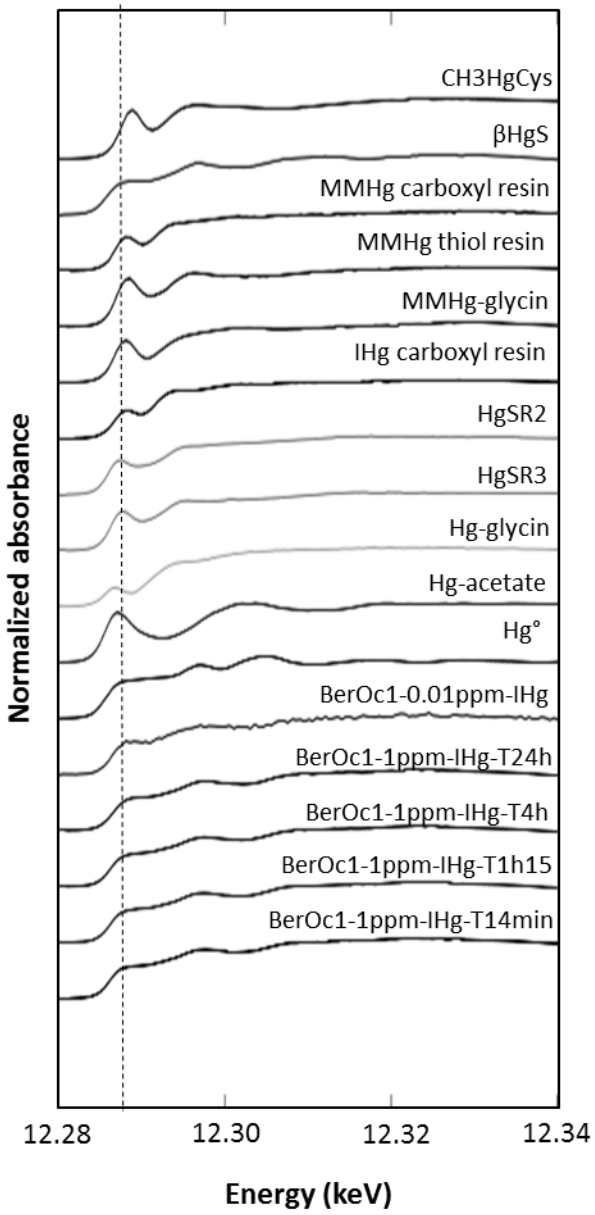

Fig. 1 : Hg L_{III}-edge HR-XANES spectra of *BerOc1* exposed to 1 ppm IHg during 14 min, 1h, 4h and 24h, compared to Hg references. *BerOc1* exposed to 0.01 ppm IHg during 24h was also reported.

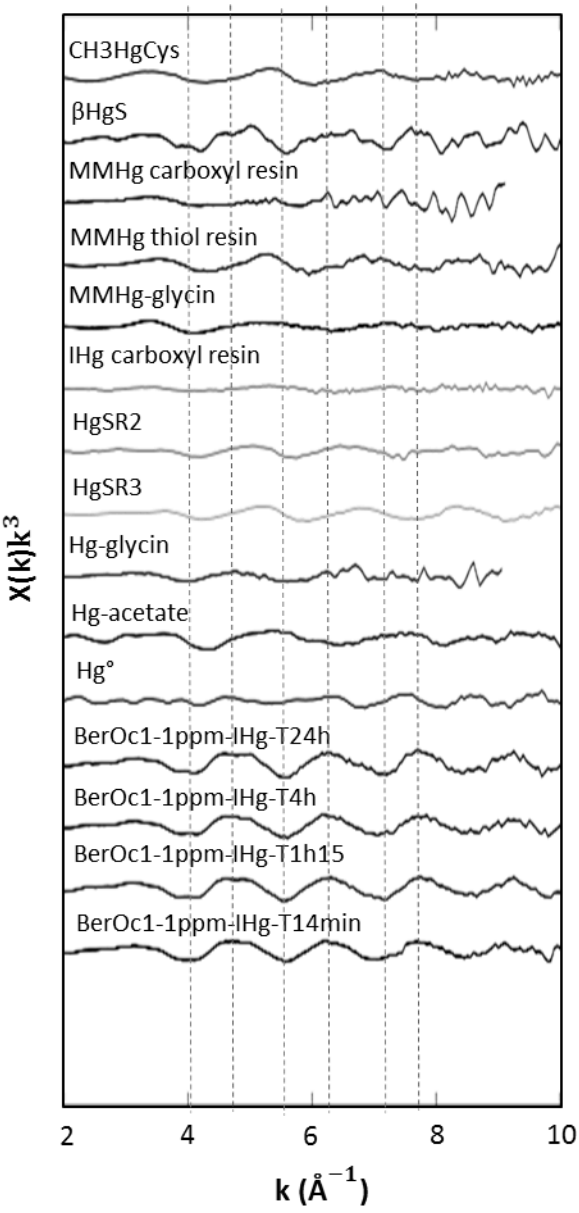

Fig. 2 : EXAFS spectra of *BerOc1* exposed to 1 ppm IHg during 14 min, 1h, 4h and 24h, compared to Hg references.

Justification and comments about the use of beam time (5 lines max.):

Sixteen shifts were dedicated to the XANES and EXAFS measurements of Hg references and bacteria samples. One shift was used for the alignment of the CAS, and one other for the use of the cryostat and change of samples (holder with only one sample position available).

Publication(s):

- Albertelli M, Isaure MP, Kieffer I, Tucoulou R, Tessier E, Monperrus M, Goñi-Urriza M. Understanding the mercury transformation in sulfate-reducing bacteria cells. Submitted to the *13th International Conference on Mercury as a Global Pollutant (ICMGP)*, July 16-21 2017, Providence, Rhode Island, USA.

- This work is part of Marine Albertelli PhD work. Publications are in progress.