Standard Project

Experimental Report template

Proposal title: X-ray Absorption Spectroscopy to progress in the understanding of Hg methylation in sulfate-reducing bacteria		**Proposal number:** 20141413
Beamline: FAME (BM30) ESRF	**Date(s) of experiment:** from: 24/06/2015 to: 30/06/2015	**Date of report:** 22/10/2015
Shifts: 15	**Local contact(s): Isabelle Kieffer**	*Date of submission:* *27/10/2015*

Objective and expected results:

Methylmercury (MeHg), mainly produced by sulfate-reducing bacteria (SRB), is biomagnified in aquatic food chains and poses a risk to human health. However, very little is known regarding the mechanism of inorganic Hg (Hg(II)) uptake and methylation by these organisms as well as the demethylation process. Our objective was to determine the Hg speciation in two SRB strains with contrasted methylation capacities to progress in the involved metabolic pathways. For that, we applied Hg L_{III}-edge High Resolution X-ray Absorption Near Edge Structure (HR-XANES) and Extended X-ray Absorption Fine Structure (EXAFS) using the new set up with Si analyzer crystals available on FAME beamline.

Experimental:

Two anaerobic strains were studied: strain *BerOc1* (*Desulfovibrio dechloroacetivorans*) able to both methylate Hg(II) and demethylate MeHg and strain *G200* (*Desulfovibrio desulfuricans*) only able to demethylate MeHg. Bacteria were grown in a synthetic medium in anaerobic conditions. They were spiked with 0, 0.1, 1 and 10 ppm Hg(II)Cl_2 (IHg) and MeHgCl (MeHg), and incubated for 24 h (growth parameters were followed in parallel to adjust the time of Hg exposure). Then, the culture was centrifuged, rapidly washed and prepared as frozen pressed pellets. Hg-references were also synthesized and analyzed as solid pellets or aqueous solutions. Samples were positioned in the He cryostat operating at 12K. Hg L_3-edge XANES and EXAFS spectra were collected in fluorescence mode. To limit diffusion from background and increase Hg sensitivity, Hg $L_{\alpha 1}$ fluorescence (L_3-M_5, 9988.8 eV) line was selected using 5 Si(111) crystals while the diffracted intensity was collected with a one Si detector.

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

Results confirmed the higher sensitivity of HR-XANES in comparison to standard fluorescence measurements. For instance, αHgS and HgO spectra have a pronounced near-edge peak at 12.286 keV (arrow in Fig. 1 left) which is not present in standard fluorescence measurements and is characteristic of Hg(II) linearly coordinated (Manceau et al., 2015). This peak is absent in Hg_2Cl_2 where Hg has a +I oxidation state and a 5 coordination, and when Hg(II) is tetracoordinated, for intance in βHgS or HgSe (not shown). Furthermore, the position of the near –edge peak depends on the nature of the ligands: it is shifted to a higher value when Hg is digonally bound to one methyl plus one other group (S, Cl, OH, Fig.1 right). HR-XANES spectra recorded on *BerOc1* exposed to 0.1, 1 and 10 ppm IHg have different features, indicating that Hg speciation differs for the three conditions. Moreover, the near-edge peak at 12.286 keV is absent in the three *BerOc1* cultures, and thus Hg is not linearly coordinated: the initial $HgCl_2$ species have been modified by the bacteria cultures and the assimilation and/or sequestration depends on the Hg level. Comparison of these spectra with literature data suggests that tetracoordinated Hg such as βHgS (or at least

a non negligible part of this species) could play a role in Hg associated to the bacteria, particularly for the 0.1 and 1 ppm IHg. Additional measurements are necessary to validate this hypothesis. EXAFS spectra (Fig. 2) confirm that Hg speciation is different in the three cultures, particularly for *BerOc1* exposed to 10 ppm IHg, and radial structure functions also suggest that sulfur atoms can be present in the first Hg coordination sphere. Data treatment by linear combination fitting and determination of the structural parameters by shell simulations are in progress, and these data must be completed by measurements of other Hg references.

Justification and comments about the use of beam time:

The alignment of the five analyzer crystals is time consuming and 2 shifts were used for this step. The use of the cryostat also takes time since it is equipped with a single sample position and it is necessary to warm it up between two samples. Beamtime was dedicated to the measurement of different Hg standards and to *BerOc1* exposed to three IHg concentrations. HR-XANES proved very powerful to discriminate Hg speciation. We have initially planed to investigate the non-methylating strain *G200* as well as the exposure to methylmercury but 15 shifts were not sufficient for that. We thus applied for additional beamtime to carry on these encouraging results.

Publication(s):

Data treatment is still in progress (linear combination fitting and determination of structural parameters). We need additional data on bacteria exposed to MeHg and on the non methylating strain to have a global view for a high impact publication.

References :

Manceau A et al. 2015. Formation of Mercury Sulfide from Hg(II)-Thiolate Complexes in Natural Organic Matter. *Environ Sci Technol* 49: 9787-9796.

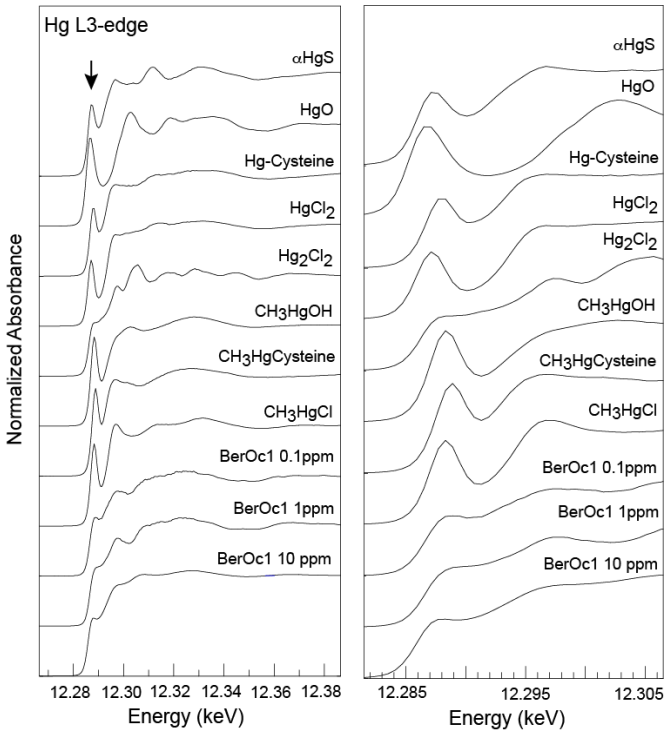


Fig. 1: Hg L_3-edge HR XANES spectra of *BerOc1* exposed to 0.1, 1 and 10 ppm IHg compared to Hg-reference compounds. A detailed observation of the edge (right) shows that the position of the near-edge peak changes with the nature of the ligands.

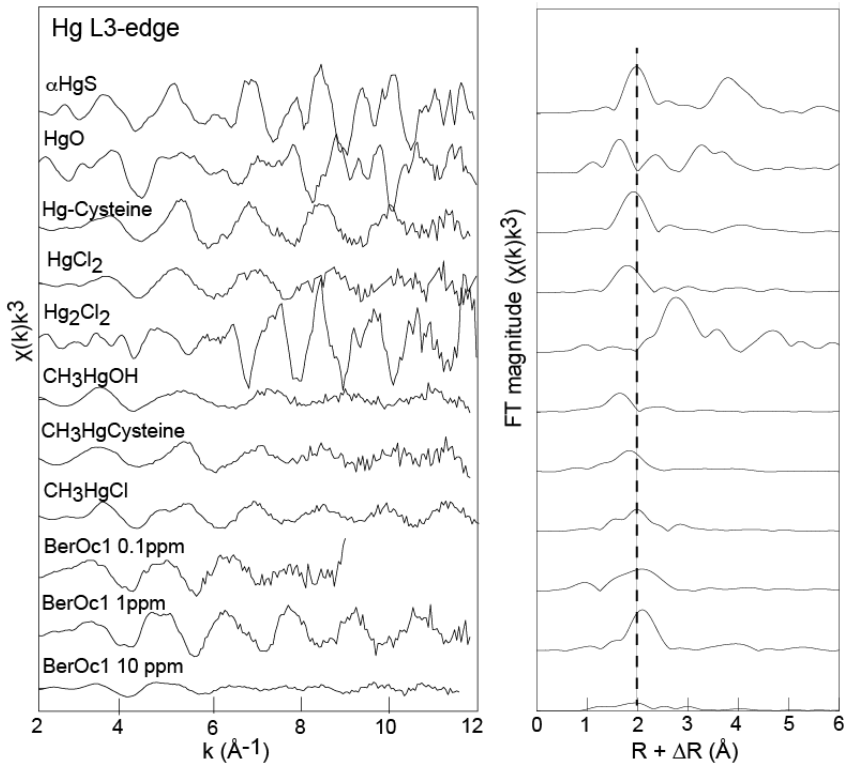
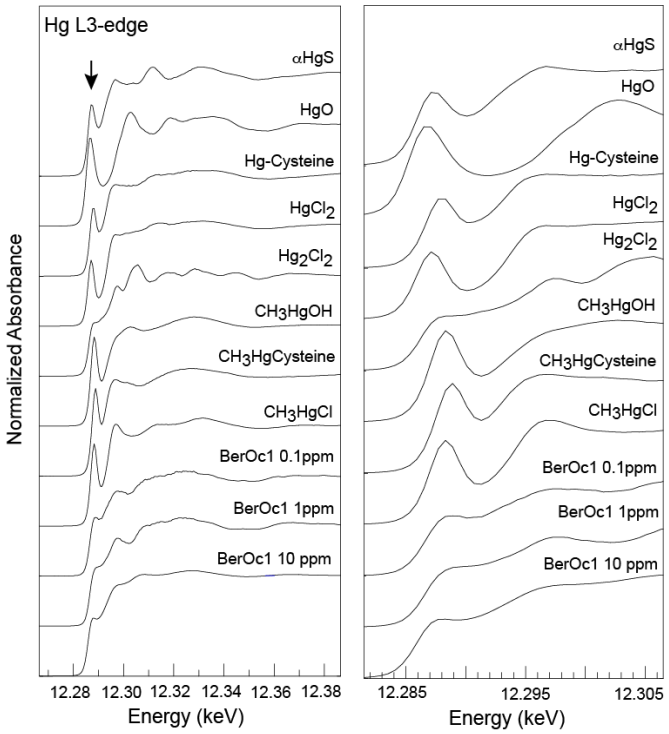


Fig. 2: Hg L_3-edge EXAFS spectra of *BerOc1* exposed to 0.1, 1 and 10 ppm IHg compared to Hg-reference compounds (left) and Fourier transforms of the corresponding spectra (right). The dashed line at 2 Å (R+ ΔR) indicates the first S coordination sphere for αHgS and Hg-cysteine.



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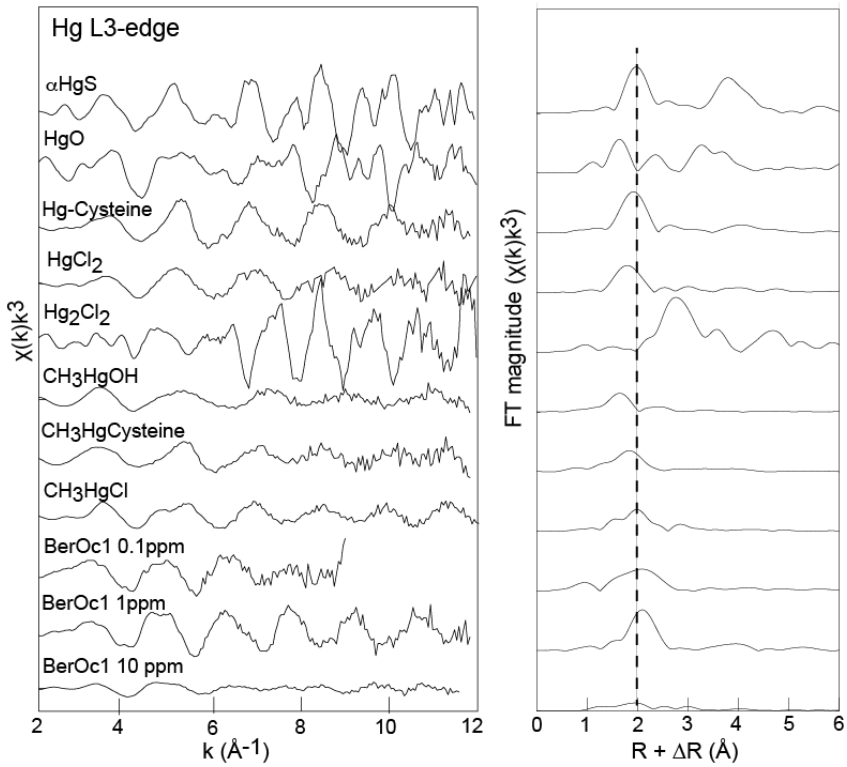

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