ESRF	**Experiment title:** Dynamics of selenite reduction and local structure of elemental selenium in Selenium nano-particles produced by bacterial cultures of Stenotrophomonas maltophilia SeITE02	**Experiment number**: 0801-1030
Beamline:	**Date of experiment**: from: 30/11/2016 to: 6/12/2016	**Date of report**: 5/3/2017
Shifts: 18	**Local contact(s)**: Giovanni Orazio Lepore	*Received at ESRF:*

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Report:

This experiment is a continuation of experiment 0801-1015. Our aim was to apply X-ray Absorption Spectroscopy (XAS) at the K-edge of selenium 1) to characterize the evolution of selenium speciation and putative metabolic intermediates during the transformation of selenite (SeO_3^{2-}, oxidation state 4^+) into elemental selenium by the bacterial strain *Stenotrophomonas maltophilia* SeITE02, by collecting samples at increasing incubation times after exposure to Na_2SeO_3 at 0.5 mM concentration; and 2) to characterize the short range order of Se^0 in the biogenic nano-particles (SeNPs) produced during this transformation.

Experimental Details:

Unlike our previous 0801-1015 run, most of the measured samples were grown, incubated and extracted at ESRF thanks to the EMBL Laboratory support.

XANES measurements were performed at room temperature at the Se K-edge in fluorescence mode:

a) on dry bacterial cells deposited on millipore filters, after addition of selenite Na_2SeO_3 at 0.5 mM concentration after 0, 0.5, 1, 2, and 3 hrs incubation time;
b) on bacterial cells as liquid suspensions, after addition of selenite at 0.5 mM concentration after 6, 12, 16, 20, 24 hrs incubation time;
c) on the cytoplasm proteic fractions - extracted from *Stenotrophomonas maltophilia* SeITe02 grown for 24 hrs in nutrient broth - as liquid suspension, after addition of selenite at 0.5 mM after 0, 6, 12, 24 and 48 hrs reaction time;
d) on the cytoplasm - purified from the bacterial cells grown with selenite at 0.5 mM after 0, 3, 6 and 12 hrs incubation time - as liquid suspension;
e) on selected Selenium standards: home-prepared elemental red Selenium, elemental grey Selenium, sodium-selenite Na_2SeO_3, and seleno-L-cysteine (oxidation state 2+), either on millipore filters or as liquid samples.

EXAFS measurements were performed at room temperature (and, for a subset, also at 77 K) either in transmission or in fluorescence mode:

a) on biogenic SeNPs extracted through sonication from the bacterial cells after addition of selenite at 0.5 mM concentration at 6, 24 and 48 hrs incubation time (deposited on millipore filters);
b) as for the previous set, on biogenic Se-nanoparticles extracted without sonication after 24 and 48 hrs;
c) on chemical SeNPs, synthesized through cysteine reaction with Na_2SeO_3;
d) on selected selenium standards: home-prepared red elemental Selenium, grey elemental Selenium, sodium-selenite Na_2SeO_3 and seleno-L-Cysteine, either on millipore filters or as liquid samples.

In most cases two or more measurements were performed for each sample, in particular for fluorescence detection. The S/N ratio for XANES was good on both type of samples (filters and liquid cells). EXAFS measurements were characterized by some noise and irreproducibilities due to the instability of the old BM08-Lisa monochromator. Absolute energy scale of the spectra was set by comparison with the Se edge which was simultaneously measured at each time on a metallic selenium foil placed after the samples.

Main Results:

Preliminary data processing and analysis were performed using the ATHENA Data Processing software (https://bruceravel.github.io/demeter/) after alignment of the XAS spectra.

We found that inside the bacterial cells (millipore filters samples) intermediate oxidation states between selenite (4+) and elemental selenium (0) are present in the very early stages of incubation and that already after 0.5 hour elemental selenium neatly prevails (Fig.1, left side); while in the suspension at least up to 12/16 hours of incubation the main component is selenite (Fig.1, right side), thus indicating that the original selenite content has not been fully reduced.

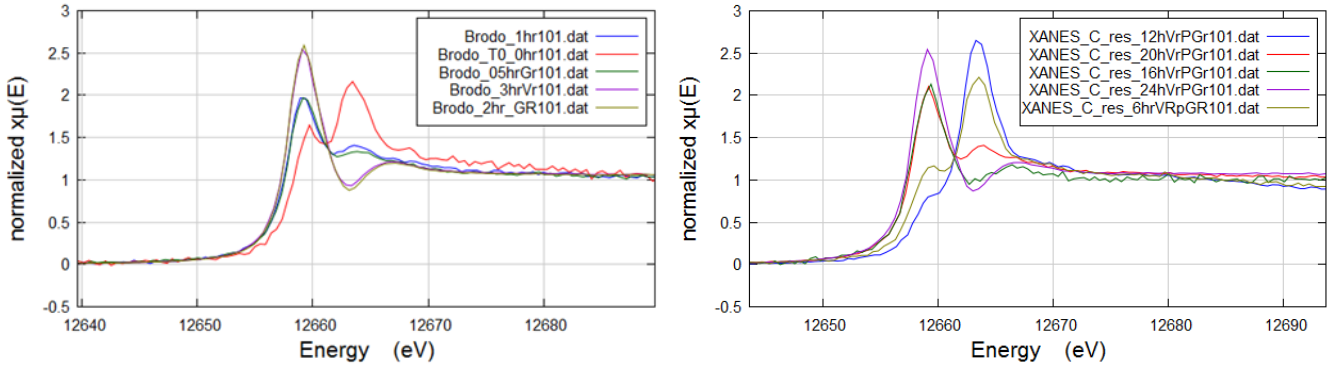

Fig. 1. On the left: XANES on dry bacterial cells of Stenotrophomonas maltophilia SeITE02 grown in nutrient broth after addition of 0.5 mM Na_2SeO_3 at different incubation times: from 0 to 3 hrs. On the right: XANES on bacterial cells as suspension, grown in nutrient broth after incubation from 6 to 24 hrs.

The expected role of the cytoplasm in selenite reduction was confirmed. Moreover, we found that the process is slower in the extracted proteic fraction than in the cytoplasm inside the cell. The reduction of selenite by the proteic fraction occurs between 12 and 24 hours (Fig.2, right side), while it appears earlier, between 6 and 12 hours, and is completed after 12 hours, in the cytoplasm purified from the bacterial cells grown with selenite (Fig. 2, left side), suggesting that different mechanisms of selenite reduction could be involved inside the cells.

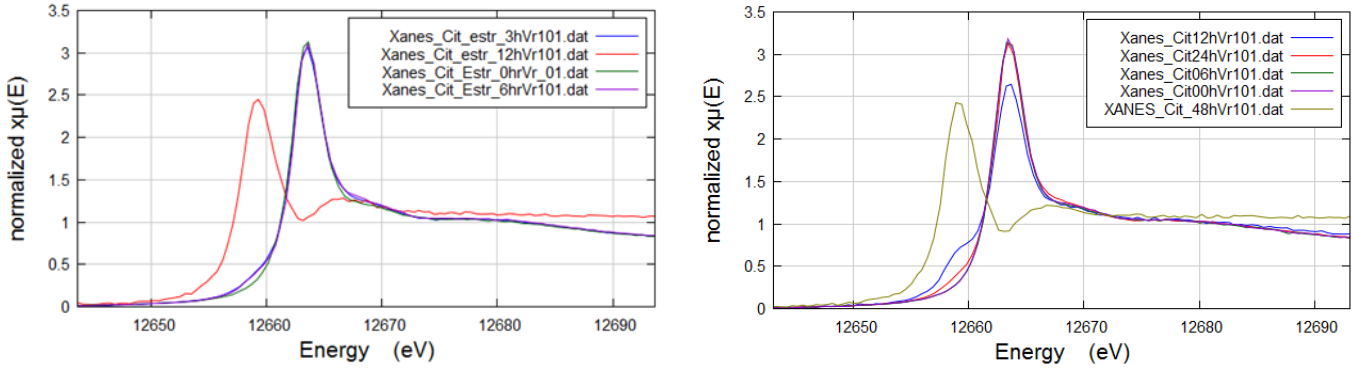

Fig. 2. On the left: XANES on the cytoplasm purified from Stenotrophomonas maltophilia SeITE02 grown in nutrient broth with 0.5 mM Na_2SeO_3 after 0, 3, 6, 12 hrs of incubation time. On the right: XANES on the cytoplasm protein fractions, obtained from Stenotrophomonas maltophilia SeITE02 grown in nutrient broth for 24 hrs, exposed to 0.5 mM Na_2SeO_3 for 0, 6, 12, 24, 48 hrs.

As regards SeNPs, the absorption edge resulted to be always that of red selenium, independently from the sonication process, indicating that the transition to the grey form seen in the previous experiment is due to the spontaneous transition of Se to the more stable grey form. Moreover, EXAFS measurements at liquid nitrogen temperature on the home-prepared red selenium and on the SeNPS show that there is no long range order in red selenium in any sample, hence suggesting that red selenium is always in the amorphous form.