ESRF	**Experiment title:** XANES at the Se K-edge to study the process of reduction of selenite and formation of Se nano-particles in bacterial cultures of Stenotrophomonas maltophilia	**Experiment number**: 0801-1015
Beamline:	**Date of experiment**: from: 30/3/2016 to: 3/4/2016	**Date of report**: 8/5/2016
Shifts: 12	**Local contact(s)**: Giovanni Orazio Lepore	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

*** Francesca Monti**

*** Emanuele Zonaro**

*** Francesco Rocca**

Report:

XANES measurements were performed at the Se K-edge in fluorescence mode on samples obtained through deposition on **millipore filters** (*Samples*: bacterial cells after addition of selenite Na_2SeO_3 at 0.5 mM concentration after 3, 6, 9, 12, 24, 48 h incubation time. *Standard*: chemically home-prepared elemental red selenium, either amorphous or crystalline monoclinic) and on **liquid samples** (*Samples*: bacterial cells in suspension after addition of selenite at 0.5 mM concentration after 10 min, 6, 12, 24 h incubation time; liquid surnatant after extraction of the bacterial cells after 12 h incubation time. *Standards*: selenite (oxidation state 4+), seleno-glutathione and seleno – L-Cystein (oxidation state 2+)). XANES and EXAFS measurements were performed either in transmission or in fluorescence mode on Boron Nitride **pellets** (*Standards*: purchased elemental trigonal crystalline (grey) selenium; selenite and selenium dioxide (oxidation state 4+); seleno-glutathione and seleno–L-Cystein (oxidation state 2+); *Samples*: biogenic Selenium-nanoparticles extracted from the bacterial cells after addition of selenite at 0.5 mM concentration at 24h and 48h incubation time; Chemical Se-nanoparticles). In most cases two or more measurements were performed for each sample, in particular for fluorescence detection. About 45 absorption spectra have been acquired. The S/N ratio was good on all the three types of samples (pellets, filters, liquid cells). 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.

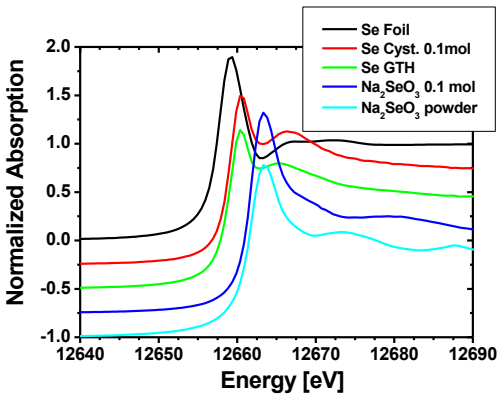

Fig.1

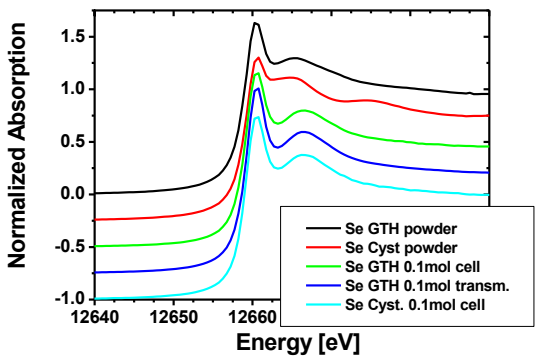

Fig. 2

The shift of the absorption edge towards higher energy going from elemental selenium to the oxidation state 2+ (in selenium glutathione and in selenium cystein) to the oxidation state 4+ (in selenite

Na_2SeO_3 and in selenium dioxide) has been clearly seen (Fig. 1). Moreover, the shape of the XANES spectra measured on selenite, seleno-glutathione (GTH) and seleno-cysteine in their solid form measured in pellets resulted to be quite different from those measured on the same standards in solution (Fig. 2). Red (either amorphous or crystalline in the monoclinc form) and grey (crystalline in the trigonal form) elemental selenium are clearly distinguished through a second feature appearing only in grey selenium at around 12672 eV after the one at 12666 eV (Fig. 3). In the biogenic (Bio-NPs) and chemically produced (Chem-NPs) selenium nanoparticles (Fig. 3) the absorption edge corresponds to elemental selenium as expected.

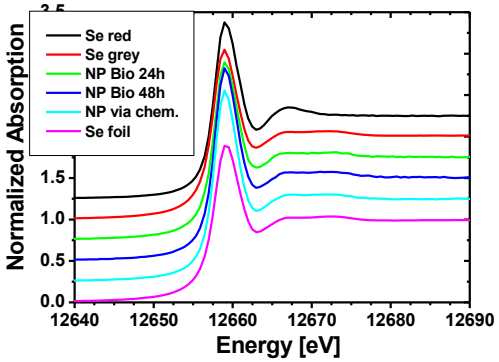

Fig. 3

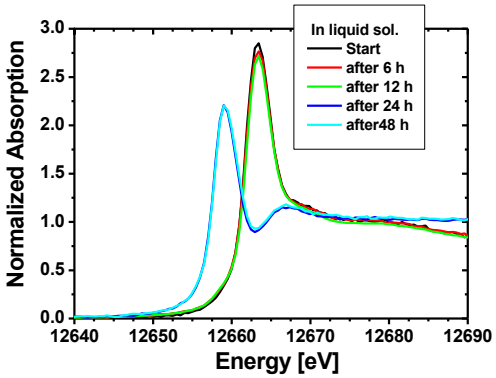

Fig. 4

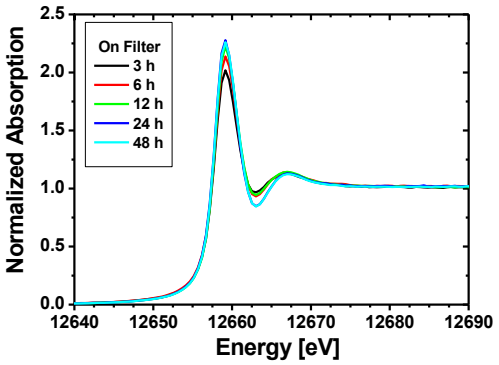

Fig. 5

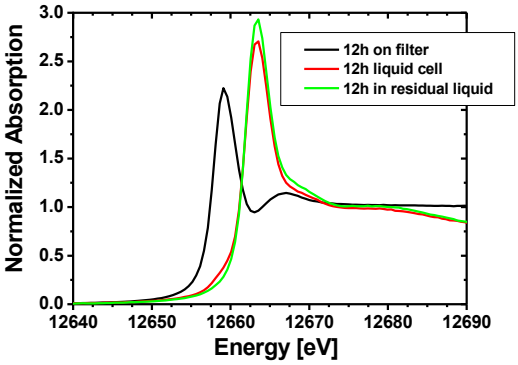

Fig. 6

Values of the edge jump as related to sample weight (data not shown) confirm that selenium concentration in Bio-NPs and Chem-NPs is very low with respect to the organic fraction (around 10% and 30% respectively). Spectral features indicate a prevailing structural contribution from trigonal selenium although the color of these nanoparticles is still red. This new result deserves further investigation, since, on the other side, the local structure of elemental selenium inside the cell samples corresponds red selenium (Figg. 4 and 5). As regards bacterial cells in suspension, the Se oxidation state 4+ prevails up to 12 h of incubation, while the elemental form (“red") prevails after 24 and 48 h (absorption edges, Fig. 4). Further analysis and investigation are required to understand what happens in the liquid samples between 12 and 24 hours (and for higher selenite concencentration in the incubation solution). As regards bacterial cells after deposition on filters, at a first sight a kind of evolution is seen from the shortest measured incubation times to the last two ones (24 and 48 hours), although the contribution from elemental selenium (“red” local structure) is well identifyed in all cases (Fig. 5). A little contribution from an intermediate oxidation state 2+ at the first incubation times cannot be excluded: further analysis and investigation are needed. In the case of bacterial cells after 12 hours of incubation, measurements were also performed on the liquid surnatant after bacterial cell extraction: the elemental state (“red”) prevails in the deposited cells, while in the liquid surnatant only selenite (4+) is present. Note that in the original liquid sample (cells measured as suspension in their incubation liquid) the oxidation state 4+ prevails but some neat difference with respect to the surnatant can be seen in the height of the white line and in the shape of the XANES (Fig. 6). These results indicate that already in the samples at first incubation times and up to 12 hours a small amount of Selenium in the elemental form is clearly detectable inside the cells (or on the cell walls), although the most part of it is still in the liquid solution, mainly maintaining the original 4+ oxidation state.