ESRF	**Experiment title:** Biotransformation of CeO_2 nanomaterials by bacteria: mechanisms and impact of NMs design	**Experiment number**: EV-202
Beamline: BM16	**Date of experiment**: from: 01-02-2017 to: 07-02-2017	**Date of report**: 10-03-2017
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Report:

Nanomaterials (NMs) possess unique intrinsic properties that have led industrialists to use them in many new everyday products. Alteration of those products, their accidental release, and their dumping, lead to NMs being discharged into the different compartments of the environment, including soil. Bacteria in soil provide critical ecosystem services and contribute to plant growth and health. Understanding of mechanisms involved in NMs/bacteria interactions is decisive to assess their ecotoxicological impact.

We measure Ce speciation in *Pseudomonas brassicacearum* exposed to CeO_2 NMs. As response to an environmental stress, this soil bacterium can undergo a phenotypic variation to adapt. The mutant, D*gacA*, impaired in the regulator GacA mimics the behavior of the phenotypic variant. The tested conditions are summarized in this table :

Bacterium	CeO_2 NMs size	Concentration Ce NMs
P. brassicacearum wild type (WT)	• 4 nm (Rhodia) • 30 nm (Umicore)	• 5 mg/L and 0.1 mg/L • 5 mg/L
P. brassicacearum DgacA	• 4 nm • 30 nm	• 5 mg/L • 5 mg/L

We also tested the effects on Ce speciation of selected bacterial metabolites likely to act as reducers. These metabolites had been identified by analysis of the metabolome of cells in interaction with CeO_2 NMs. CeO_2 NMs (4 nm-Rhodia) were incubated during 4h with metabolites: vitamin B6, 2-ketogulonate, citric acid, ascorbic acid. The suspension was then frozen and kept in liquid nitrogen until the analysis.

A set of references have been analyzed: several CeO_2 NMs design (Rhodia, Umicore, Envirox) in liquid and in pellets, micro-CeO_2, Ce(IV) sulfate, Ce(III) compounds (Ce-sulfate, Ce-phosphate, Ce-acetate, Ce-cysteine) (Figure 1A).

First results in Figure 1 indicated that:

- **CeO_2 NMS size controls the reduction kinetics.** While Umicore CeO_2 NMs (30 nm) are not reduced after contact with bacteria, Rhodia CeO_2 NMs (4 nm) undergo a total reduction in the two tested bacteria (Figure 1B). Differences between the wild type and the mutant were not measured; possibly because of the total dissolution of the 4 nm NMs or the non-detected reduction of the 30 nm.
- **CeO_2 NMS/bacteria ratio controls the reduction.** The 4 nm CeO_2 NMs undergo reduction at 5 mg/L but not at a lower concentration (0.1 mg/L) (Figure 1B). One hypothesis to explain this result is a dose-dependent response of the bacteria to deal with the NMs contamination. This hypothesis will have to be validated. This result is unexpected and illustrates the need to work at low and environmentally relevant NMs concentration as well as the new potential this beamline offers.
- **Metabolites do not induce a strong Ce reduction.** Among the tested metabolites, only ascorbic acid reduces CeO_2 NMs (Figure 1C). Linear combination fitting indicates a Ce(III) contribution of 20 %.

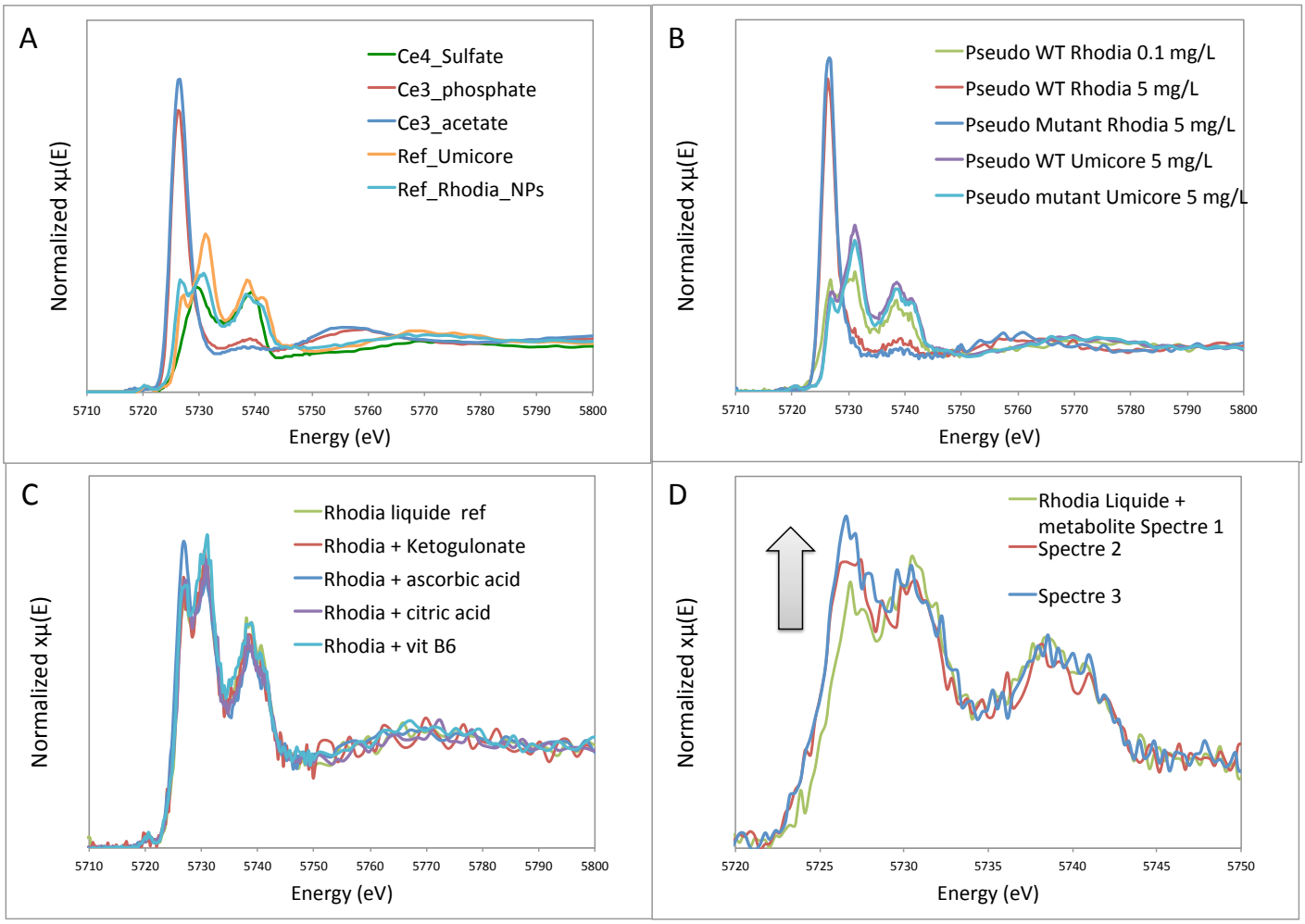

Figure 1 - Ce L_3-edge XANES spectra of A - references compounds, B – bacteria exposed to CeO_2 NMs, C - incubated CeO_2 NMs with metabolites. D – Rhodia incubated with ketogulonate showing the spectra evolution under the beam

This first experiment on BM16 illustrated the **great sensitivity** of this beamline, with the possibility to measure Ce speciation at the ppm range (concentration of bacteria exposed to 0.1 mg/kg).

Special attention should be paid to the **beam-induced reduction**, even with the use of a helium cryostat. We observed a reduction of Ce in all frozen liquid samples after the first spectra acquisition (Figure 1D) and for the Ce(IV) sulfate standard (dry pellet). An option to minimize this phenomenon will be to change the position on the sample between each spectrum, which is difficult (and time consuming) due to the set up of the crystal analyzers spectrometers. This may require the development of a dedicated procedure to optimize the sample adjustment between each position.