	Experiment title: Interactions between ceria nanoparticles and bacteria: relation between Ce speciation and biologial effects	**Experiment number**: EC-183
Beamline: BM 30b FAME	**Date of experiment**: from: 11/04/2007 to: 17/04/2007	**Date of report**: 30/07/07
Shifts: 18	**Local contact(s)**: Olivier Proux	*Received at ESRF:*

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REPORT

Introduction

The very fast development of new synthetic nanomaterials and their widespread commercial diffusion raises questions about their impact on the environment and the human health [1-3]. The unknown toxicity of NPs is a key issue since unforeseen exposure to humans through air pathway or the food web are possible.

Cerium is used in the form of nanoparticles for catalysis. It is used, for example, in car exhaust system to burn diesel particles. One of the specificity of cerium di-oxide is to be a strong oxidant. Therefore redox reactions may occur in aquatic media. In the case of E. coli, it has been observed previously that CeO2 NPs are toxic in some cases and that the contact between cerium nanoparticles and E. coli bacteria could induce a cerium reduction.
Synechocistis is an environmentally relevant micro algae that displays a very different response with respect to ceria NPs bacteria survival compared to E. coli. The cerium modifications after contact with Synechocystis were unknown before the experiments. The main goal of the experiments were to both to determine the kinetic of Cerium reduction in contact with E.coli and to follow the redox state of cerium nanoparticles in contact with Synechocistis.

The modifications of the cerium speciation after contact with two different living cells (E. Coli, Synechocystis) was studied during this XANES/EXAFS experiment on BM30b.

Material and methods

The general frame of the experiment was as follows. Both bare NPs and NPs covered with PAA polymers (to change the charge of NP surface) were mixed with i)the cells ii)cells equilibrated medium iii)water. The bacteria solution were grown at the ESRF laboratory to obtain 5.10^9 cells/ml for the mixture with CeO2 NPs. Concentration ranging from 0 to 500 mg/l of CeO2 and PAA/CeO2 will be used. In the case of Escherichia

coli, the NPs strongly adsorb on the cell up to a concentration of 16 mg/m2 (50 ppm) of CeO2. The reduction kinetic was expected to depend on the surface states of the NPs and the bacteria. The kinetic of Ce reduction was followed by quick-XANES at Ce L3 edge. Some EXAFS spectra were also recorded during the kinetic experiments.

Experiments

The kinetic evolution of the cerium redox state was studied in-situ (in solution) using a specially build liquid cell adapted for measurements in the fluorescent mode. The XANES spectra were scanned in the Quick-XANES mode. The 30 elements fluorescent detector was used. The quality of the data (signal/noise ratio) was good enough to determine the Ce redox state using linear combination of spectra of reference compounds. An other set-up was used to determine the redox state (XANES) as well as the atomic environment of cerium (EXAFS) at the end of the experiments. The samples were placed in a liquid nitrogen cryostat for longer acquisition time and avoid any beam damage.

Main results

Surprisingly, no modification of the cerium oxidation state was observed after contact between Nano-CeO2 and E. coli in water during one hour. However, in these conditions, it is known from biological tests that the NPs induce a strong cytotoxicity [3]. This conclusion is still very qualitative and deserves quantitative verifications, but a strong oxidative stress initiated by cerium reduction may not be the main cause of the observed cytotoxic effect of the cerium NPs as it was previously expected.

An other Quick-XANES series of experiments concerned the contact between cerium NPs and bacteria in the growth medium instead of water. In this case, it is known that the NPs induce no cytotoxic effects[3]. We observed however in this case a slow reduction of the cerium NPs. The degree of reduction has still to be quantified. The bacteria in this case seemed to be able to produce specific enzymes that reduce the NPs. The reduction is not observed in pure growth medium or in a medium were bacteria have grown. We clearly demonstrated that the reduction in this case is a biological reaction of the bacteria toward cerium NPs.

For Synechocistis, no toxic effects have been observed contrary to E. coli. The question therefore was to determine whether a reduction of Cerium occurred as we expected or not. One of our hypothesis was that the cerium reduction exists at the surface of the Np (since Ce4+ is a strong oxidant), but that a biological mechanism could induce a protection against CeO2 adverse effect.

Surprisingly, no modification of the cerium speciation was observed even after several hours of contact with the bacteria whatever the medium was (water or growth medium).

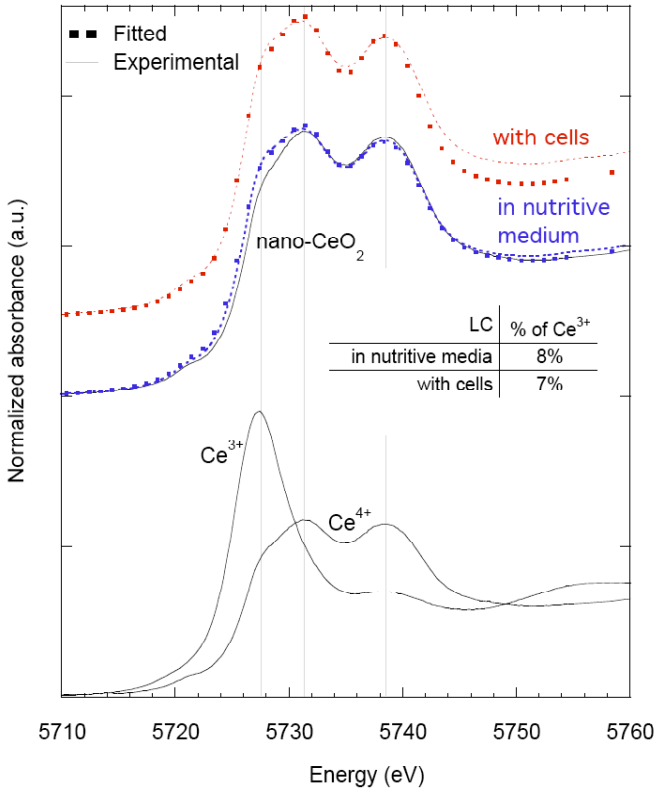

Figure 1: quantification of the redox state of cerium for NPs in nutritive medium with and without cells.

The protective mechanism against cerium oxidative stress for Synechocystis is not based on a biologically passivation of the nanoparticles like in the case of E. Coli. The data are still to be quantified to validate this first qualitative interpretation of the Quick-XANES results.

Beam effect on the Cerium redox state

Several solid samples were also measured in fluorescent mode using the nitrogen cryostat. Some of these samples were already measured using a micro focused x-ray beam at the Swiss Light Source installed on an Insertion Device. Surprisingly, different results were obtained. In the case of the micro-focus beam line installed on a ID, a reduction of the cerium was observed. We tried to determined whether or not a beam effect existed. XANES were recorded in less than 3 minutes. We never observed an evolution between the first and other scans. This meant that if a redox effect occurred it occurred during the first 3 minutes. The samples were scanned at room temperature.
On the Fame beamline (installed on a bending magnet (BM)) we scanned XANES of samples already scanned at the SLS on the ID beam line. It appeared that the redox state was higher that the one measured at the SLS. But a slow reduction of cerium was observed. Therefore the evolution of the redox state of cerium can be modified by the beam (very rapidly on ID (less than 3 minutes), after 20 to 30 minutes of BM…) and particular care is required (using cryostat, moving the sample between scans…).

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