

## Research Group for Bioactinides Chemistry

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The research objectives of the group are to elucidate a chemical state change of actinides and lanthanides including nano-particles formation in the biological reaction environments, and to develop biological remediation system for radiocesium contaminated land. In the year of 2014, we have studied the effects of 1) nanoparticle of Ce oxide on microbial activity, 2) Ce phosphate nanoparticle grown up at the surface of microbial cells on the sorption by Mn oxide, and 3) minerals on accumulation of Cs by fungus *Saccharomyces cerevisiae*.

### Metal nanoparticles affect microbial activity

Microbial activity can alter the mobility of radionuclides through biosorption, biomineralization, or the production of chelating compounds in the environment [1]. In previous studies, we demonstrated that microorganisms precipitate nanoparticles comprising heavy metals such as rare earth elements and uranyl ions outside the cell and release phosphate from inside the cell. Although the mechanism of nanoparticle formation by microorganisms has been explored in many previous studies, the role of nanoparticles still remain unknown. Specifically, the influence of nanoparticles on microbial activity is poorly understood, even though microbial activity potentially affects local and global ecosystems.

To understand the effects of nanoparticles on microorganisms, we examined the effects of CeO<sub>2</sub> nanoparticles (CeNPs) on yeast focusing on microbial metabolites and intracellular proteins. The yeast was harvested from a yeast extract-peptone-dextrose (YPD) medium containing 0, 10, 100 and 250 ppm of CeNPs. It was incubated for 120 h in 1mM NaCl solution at three different pH values: 3, 5 and 7. The yeast released organic matter, P, K and Mg into the NaCl solution at all pH values, even without CeNPs. Distinct differences were detected in the released organic species and intracellular proteins after exposure to CeNPs. High-performance liquid chromatography revealed that the yeast expressed or suppressed various organic species after exposure to CeNPs. Although CeNPs did not have cytotoxicity for the yeast, the results of the peptide mass fingerprint analysis of the intracellular protein revealed that Eno2p, a glycolysis enzyme, was expressed after exposure to CeNPs. These results suggest that nanoparticles have the potential to alter microbial metabolism, leading to change of the composition of the substances released in the surrounding environment.

### Ce phosphate nano-particles are participate in Ce oxidation

Sorption of Ce by mixtures of synthetic Mn oxides and microbial cells of *Pseudomonas fluorescens* was examined to elucidate the role of microorganisms on Ce(III) oxidation in the environment [2]. The mixtures sorbing Ce by exposure to solutions containing  $1.0 \times 10^{-4}$  or  $1.0 \times 10^{-5}$  mol L<sup>-1</sup> Ce(III) were analyzed by scanning electron microscopy energy dispersive X-ray spectroscopy (SEM-EDS) and micro-X-ray fluorescence (micro-XRF) at synchrotron facilities. A Ce LIII-edge micro XANES spectra analysis was also performed to determine the oxidation states of Ce adsorbed to the Mn oxides and microbial cells in the mixtures. The distribution ratios (K<sub>d</sub>) of Ce between the individual solids and solution rose with increasing in pH of

the solution. The K<sub>d</sub> was nearly the same in the mixtures containing varying amounts of microbial cells. SEM-EDS and micro-XRF analyses showed that Ce was sorbed by both MnO<sub>2</sub> and microbial cells ( $1.7 \times 10^{-1}$  or  $3.3 \times 10^{-1}$  g·L<sup>-1</sup>). In addition, nano-particles containing Ce and P grew up on the surface of the microbial cells. XANES analysis showed that lower fractions of Ce(III) were oxidized to Ce(IV) in the mixtures containing greater amounts of microbial cells. Micro-XANES analysis revealed that Ce was present as Ce(III) on the microbial cells and as Ce(IV) on Mn oxides. These results strongly suggest that the association of Ce(III) with the microbial cell surface and the formation of Ce phosphate nano-particles are responsible for suppressing the oxidation of Ce(III) to Ce(IV) in the mixtures.

### Yeast accumulates radioactive Cs competitively with minerals

In concerned with Fukushima Daiichi Nuclear Power Plant Accident, it is important to elucidate migration behavior of radioactive Cs deposited on the ground for determination of the dose rate and the fate of the radioactive Cs in the terrestrial environment. The migration of radioactive Cs in the environment is affected by minerals and fungal microorganisms. However, the accumulation of radioactive Cs by fungal microorganisms in the presence of minerals and role of microorganisms in this migration are not completely understood.

The accumulation of Cs by unicellular fungus of *Saccharomyces cerevisiae* in the presence of minerals has been studied to elucidate the role of microorganisms against the migration of radioactive Cs in the environment. Two types of experiments were employed. The one is the experiment using stable Cs to examine the effect of a carbon source on the accumulation of Cs. The other one is accumulation experiment of radioactive Cs from agar medium containing <sup>137</sup>Cs and mineral supplements such as zeolite, vermiculite and so on. In the former experiments, the Cs-accumulated cells were analyzed by scanning electron microscopy equipped with energy dispersive X-ray analysis (SEM-EDS). In the latter one, the radioactivity in the yeast cells was measured by an autoradiography technique.

When a carbon source was present, higher amounts of Cs accumulated in the cells than in the resting condition without a carbon source. Analysis by SEM-EDS showed that no mineral formed on the cell surface. These results indicate that the yeast cells accumulate Cs by adsorption on the cell surface and intracellular accumulation. In the case of the presence of minerals in the agar medium, the radioactivity in the yeast cells was in the order of mica > smectite, illite >> vermiculite, phlogopite, zeolite. This order is inversely correlated to the ratio of the concentration of radioactive Cs between the minerals and the medium solution. These results strongly suggest that the yeast accumulates radioactive Cs competitively with minerals.

### References

- [1] K. Pedersen, In: Fredrickson, J.K., Fletcher, M. (Eds.), *Subsurface Microbiology and Biogeochemistry*. Wiley Liss Inc., New York (2001).
- [2] T. Ohnuki, *Geochim. Cosmochim. Acta* **163**, 1 (2015).