

***Microbial impacts on the migration of actinides  
–Effects of exudates on adsorption–***

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# Microbial impacts on the migration of actinides -Effects of exudates on adsorption-

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## Abstract

The interaction of actinides with microorganisms has been extensively studied to elucidate migration behavior of actinides in the environments. However, the mechanisms of interaction of microorganisms and actinides are poorly understood. We have been conducting basic science on microbial accumulation of actinides in order to elucidate the environmental behavior of actinides under relevant conditions. The effect of exudates from bacteria cells on the sorption of Eu(III) and Cm(III) by *Chlorella vulgaris* was studied by a batch method. The pH dependence of  $\log K_d$  of Eu(III) and Cm(III) for cellulose, major component of *C. vulgaris* cell, differed from that for *C. vulgaris*. On the contrary,  $\log K_d$  of Eu(III) and Cm(III) for cellulose in the solution containing exudates from *C. vulgaris* cells in a 0.5% NaCl solution showed a similar pH dependence to that by *C. vulgaris*. These results strongly suggested that exudates affect on the sorption of Eu(III) and Cm(III) on *C. vulgaris*. Effect of desferrioxamine B (DFO), one of exudates to chelate with insoluble Fe(III), on the sorption of Pu(IV), Th(IV) and Eu(III) by *Pseudomonas fluorescens* was studied. In the presence of DFO the sorption of Pu(IV), Th(IV) and Eu(III) on the cells increased with a decrease in pH from 7 to 4. In contrast, without DFO most of Pu(IV), Th(IV) and Eu(III) were precipitated from solution. Adsorption of DFO on the cells was negligible in the solution with and without metals. Adsorption of Pu(IV), Th(IV) and Eu(III) on *P. fluorescens* cells decreased in the order Eu(III) > Th(IV) > Pu(IV), which corresponds to increasing stability constant of the DFO complexes. These results indicate that Th(IV), Pu(IV) and Eu(III) dissociate when in contact with cells, after which the metals are adsorbed.

## 1. Introduction

The presence of actinides in nuclear reactors and radioactive wastes is a major environmental concern due to their long radioactive half-lives, their high energy radiation emissions, and their chemical toxicity. In order to estimate the potential impact of actinides on human beings, the mobility of actinides has been examined in terms of its interactions with soils and subsoils composed of abiotic and biotic components, principally minerals and bacteria (Ticknor, 1994; Waite et al., 1994; Sylwester et al. 2000; Haas et al., 2001; Dent et al., 2004; Suzuki et al., 2005). Among the biotic components, some microorganisms have cells whose surfaces sorb actinides (Haas et al., 2001; Brantley et al., 2001; Panak & Nitsche,

2001; John et al., 2001; Francis et al., 2004; Suzuki et al., 2005). The high capacity of microbial surfaces to bind actinides may affect the migration of actinides in the environment. However, we have only limited knowledge of the role of microorganisms in the migration of actinides in the environment.

The microbiology research group of JAEA is conducting basic scientific research on microbial interactions with actinides. Fundamental research on microbial transformations of actinides involves elucidating the mechanisms of dissolution and precipitation of various chemical forms such as ions, oxides, and organic and inorganic complexes of actinides by aerobic or anaerobic microorganisms under relevant microbial process conditions. In the present report, recent findings from the heavy elements biogeochemistry group of JAEA are summarized.

## 2. Effects of exudates on $K_d$ of Eu(III) and Cm(III)

At neutral pH, the mobility of An(III) is low because of their low solubility and high affinity with inorganic particles (Seki & Suzuki, 1998; Rollemberg et al., 2000). On the other hand, in the presence of chelating substances, An(III) show high solubility and low affinity with these particles because they form soluble complexes. Exudates released with cells are known to interact with actinides, i.e. lichen exudates reduce Pu(VI) to Pu(V) and Pu(IV) (Ohnuki, et al., 2004). However, the effects of exudates on the sorption of An(III) is not fully understand. Ozaki et al. (2003) have carried out the sorption experiments of Eu(III) and Cm(III) on *Chlorella vulgaris* and cellulose which is major components of cell of *C. vulgaris*.

In the experiments, adsorption kinetics and the distribution coefficients ( $K_d$ ) of Eu(III) and Cm(III) were determined as function of solution pH. The concentrations of Eu and Cm in the suspension containing the algae were approximately  $1 \times 10^{-6}$  M and  $1 \times 10^{-8}$  M, respectively. Radionuclides of  $^{152}\text{Eu}$  and  $^{244}\text{Cm}$  were added for the measurement of the concentrations. The distribution coefficient,  $K_d$  (ml/g), was calculated by Eq. 1

$$K_d = (C_0 - C)V/CW \quad (1)$$

where  $C_0$  is initial concentration of metal ion in the solution;  $C$  the equilibrium concentration of the metal in the solution;  $V$  the volume of solution (ml); and  $W$  is the weight of the *C. vulgaris* on a dry weight. Adsorption of Eu(III) and Cm(III) on cellulose was measured using cellulose powder obtained from Kanto Chemical, Tokyo, Japan. The cellulose was used without purification.

The amounts of organic carbon exudated from *C. vulgaris* cells during incubation, possibly including the constituents of dead and dying cells, were determined as dissolved organic carbon (DOC). Approximately 0.025 mg of the algal cells was added to 5 ml of distilled water. The concentration of DOC in the filtrate was determined by total organic carbon analyzer, TOC-5000 (Shimadzu, Kyoto, Japan). The distribution coefficients of Eu and Cm for cellulose were obtained in the exudates solution that was collected from *C. vulgaris*.

Fractions of Eu and Cm sorbed on *C. vulgaris* as a function of time at pH 4, 5, and 6 (Fig. 1) showed that the sorbed fractions increased immediately after contact the elements with *C. vulgaris* cells. The maximum sorbed fractions were attained within 3 min, when all values exceeded 75%. Both elements showed higher sorbed fractions in the lower pH solution. Beyond 3 min the sorbed fractions decreased with increasing exposure time. No marked difference in sorption was observed between Eu and Cm.

Figure 2 shows the  $\log K_d$  of Eu and Cm for *C. vulgaris* and cellulose in a 0.5% NaCl solution of pH between 2 and 9. For *C. vulgaris*  $\log K_d$  of Eu and Cm rose with an increase in pH from 2 to 3 by a unit of 1; beyond pH 3, the  $\log K_d$  declined with increasing pH. No significant difference was observed between  $\log K_d$  of Eu and Cm within the pH range examined. The highest  $\log K_d$  was approximately 4.0, observed at pH 3; the lowest one was 2.1 at pH 8. Adsorption of Eu and Cm on cellulose was almost identical. The  $\log K_d$  of Eu and

Cm increased with increasing pH from 2 to 6. Log  $K_d$  at pH 2 and pH 6 were approximately 0.7 and 5.1, respectively. Beyond pH 6, log  $K_d$  decreased with increasing pH, and it was 4.0 at pH 9.

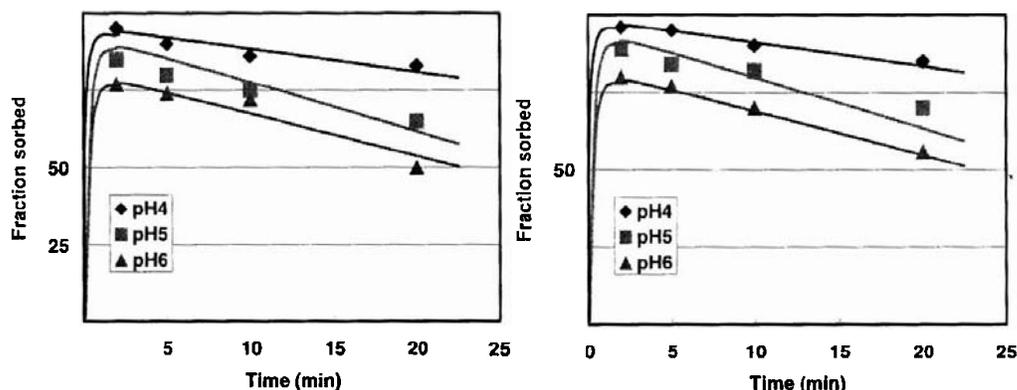


Fig. 1 Time course of adsorption of Eu(III) and Cm(III) by *Chlorella vulgaris*.

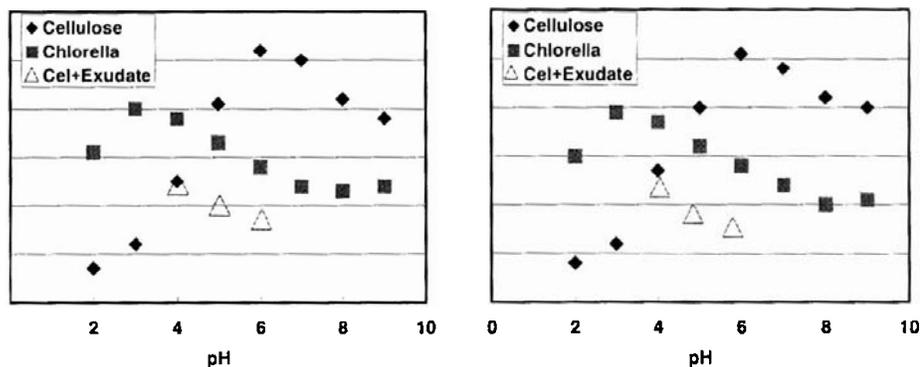


Fig. 2 Measured  $\log K_d$  of Eu(III) and Cm(III) for *C. vulgaris* and cellulose in the solution of different pH. The  $\log K_d$  of Eu(III) and Cm(III) for cellulose in the solution containing exudates from *C. vulgaris*.

The amount of DOC excreted from *C. vulgaris* cells showed a slight increase with an increase in pH (Fig. 3). This result indicated that exudates were released from the cells during the sorption experiments. The  $\log K_d$  of Eu and Cm for cellulose in the exudates solution were plotted in Fig. 2. The  $\log K_d$  of Eu and Cm were almost identical and both showed a tendency to decrease with an increase in pH with values of approximately 2.3, 1.9, and 1.7 at pH 4, 5, and 6, respectively.

The pH dependence of Eu and Cm sorption on cellulose differed from the one observed for *C. vulgaris*. The  $\log K_d$  versus pH plots from pH 2 to 6 showed a positive slope. The slope of  $\log K_d$  for *C. vulgaris* showed a negative slope at pH between 3 and 8. On the contrary, the  $\log K_d$  for cellulose in the exudates solution showed a negative slope at pH between 4 and 6. These results indicated that the exudates changed sorption behavior of Eu and Cm on cellulose, and resembled to that on *C. vulgaris*.

Our results strongly suggested that exudates released from bacterial cells affect sorption behavior of actinides on bacterial cells.

### 3. Effects of metabolites on the sorption of actinides

As mentioned above, exudates from microorganisms reduce the sorption of Eu(III) by bacteria. Naturally occurring chelating substances also have the potential to reduce the sorption of actinides and lanthanides by forming complexes in the environment. Siderophores, produced by microorganisms, access insoluble cations and form complexes not only with Fe but also with actinides, causing their solubility to increase (Brainard, 2000). Yoshida et al. (2004a, 2004b, 2005). have studied the effects of desferrioxamine (DFO) B on the sorption of trivalent and tetravalent lanthanides and actinides by soil bacteria of *P. fluorescens* and *B. subtilis*.

The sorption density of Pu(IV) and Th(IV) on bacterial cells, and Fe(III) and Eu(III) on *P. fluorescens* in the presence of DFO (Fig. 3) indicated that sorption of Pu(IV) on *P. fluorescens* increased from 3 to 19  $\mu\text{M g}^{-1}$  with a decrease of pH from 7.3 to 3.0, while sorption of Pu(IV) on *B. subtilis* and Fe(III) on *P. fluorescens* was smaller than 3  $\mu\text{M g}^{-1}$  at about pH 3-8. Sorption of Th(IV) on *P. fluorescens* increased from 21 to 42  $\mu\text{M g}^{-1}$  with a decrease of pH from 7.5 to 4.0, and sorption of Th(IV) on *B. subtilis* increased from 3 to 31  $\mu\text{M g}^{-1}$  with a decrease of pH from 7.8 to 3.3. On the contrary, adsorption of DFO on both species was negligible at 3 hours after contact of the 1:1 Th(IV)-DFO complex with *P. fluorescens* or *B. subtilis* cells at pH 5.5. No DFO was sorbed on *P. fluorescens* cells from Eu(III)-DFO complexes. These results indicate that Th(IV)-, Pu(IV)- and Eu(III)- dissociate by contact with cells, after which the metals are sorbed.

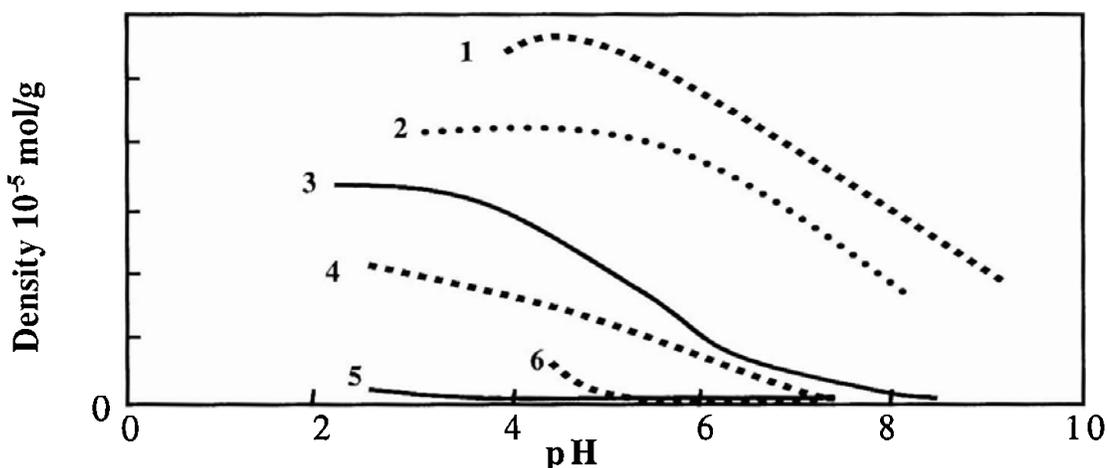


Fig. 3 Sorption density of Th(IV) and Pu(IV) on *P. fluorescens* and *B. subtilis* in the presence of DFO. Initial concentrations of Fe(III), Eu(III), Th(IV), Pu(IV), and DFO were 20  $\mu\text{M}$ . 1: Eu on *P. fluorescens*, 2: Th on *P. fluorescens*, 3: Th on *B. subtilis*, 4: Pu on *P. fluorescens*, 5: Pu on *B. subtilis*, 6: Fe on *P. fluorescens*. Data are plotted based on the refs (Yoshida et al., 2004a, 2005).

Stability constants of the metal-DFO complexes decrease in the order of Pu(IV) ( $\log K = 30.8$ ) > Fe(III) ( $\log K = 30.6$ ) > Th(IV) ( $\log K = 26.6$ ) > Eu(III) ( $\log K = 15$ ) (see references in Yoshida 2005). Adsorption density of Eu(III), Th(IV), and Pu(IV) on *P. fluorescens* cells decreased in the order Eu(III) > Th(IV) > Pu(IV), which corresponds to the increasing order of the stability constant of the DFO complexes. Adsorption of hydrated Eu(III) on *P. fluorescens* cells does not change significantly at pH 3 – 8 (Suzuki et al. 2005, Yoshida et al., 2003), indicating that the affinity of *P. fluorescens* cell surfaces with metal ions is not changed significantly at these pHs. These facts indicate that pH dependence of adsorption density of metal ions on cells is dominated by the stability of the metal-DFO complexes.

Yoshida et al. (2004a) investigated the influence of DFO on the sorption behavior of 11 rare-earth elements (REEs), La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, and Er on *P. fluorescens* cells at neutral pH. *Pseudomonas fluorescens* cell suspensions (1.3 g (dry wt.) L<sup>-1</sup>) in the suspensions were incubated for 30 minutes in 10 ml of 0.1 M Tris-HCl solutions containing 1.0 mg L<sup>-1</sup> of each REE (La, 7.20 μM; Ce, 7.14 μM; Pr, 7.10 μM; Nd, 6.93 μM; Sm, 6.65 μM; Eu, 6.58 μM; Gd, 6.36 μM; Tb, 6.29 μM; Dy, 6.15 μM; Ho, 6.06 μM; Er, 5.98 μM) and 0.5 mM DFO in air at room temperature. The oxidation state of Ce was determined by X-ray absorption near edge structure (XANES) spectroscopy in fluorescence mode at the BL27B line at High Energy Accelerator Research Organization (Tsukuba, Japan). In the presence of DFO, the percent fraction of REEs in the solution after exposure to *P. fluorescens* cells (Fig. 4) showed a tendency to increase with an increase of their atomic number, except for Ce. The adsorption of Ce was significantly lower than those of the neighboring REEs, La and Pr. On the contrary, no Ce anomaly in the sorption was distinguished in the solution with hydroxylammonium. XANES analysis of Ce in the Ce-DFO complex showed that Ce was in the tetravalent state. Adding hydroxylammonium reduced the tetravalent Ce in the complex to its trivalent form and erased the Ce anomaly (Fig. 4). These results show that DFO can oxidize Ce(III) to Ce(IV). Cyclic voltammetry revealed that the redox potential of the Ce(IV)/Ce(III) couple in the DFO complex was much lower than the standard redox potential, and that the stability of Ce(IV)-DFO is much higher than that of Ce(III)-DFO. These findings suggest that the observed Ce anomaly is due to the oxidation of the Ce(III)-DFO complex to the more stable Ce(IV)-DFO complex, and that naturally occurring organic ligands can contribute to this Ce anomaly in the natural environment.

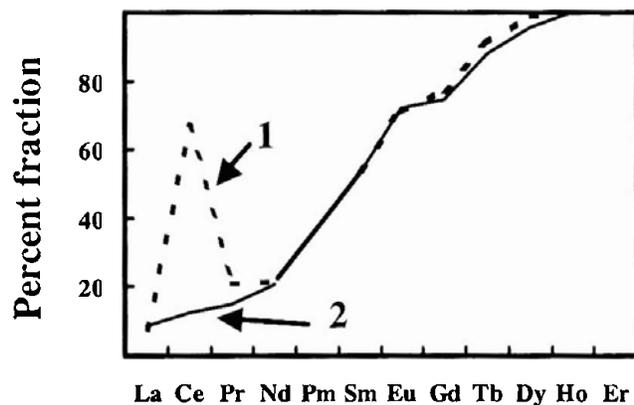


Fig. 4 Percent fraction of REEs complexed with DFO in the solutions after exposure to *P. fluorescens* cells. 1: without hydroxylammonium and 2: with hydroxylammonium.

#### 4. Conclusion

We have studied the effects of exudates and siderophore on the sorption of trivalent and tetravalent actinides by a batch experiments. We obtained the following conclusions.

1. The sorption of Eu(III) and Cm(III) by *Chlorella vulgaris* was well expressed by the sorption by cellulose in the solution containing exudates from *C. vulgaris* cells in a 0.5% NaCl solution.
2. The sorption of Pu(IV), Th(IV) and Eu(III) by *Pseudomonas fluorescens* reduced by the presence of DFO. pH dependence of the sorption indicates that Th(IV), Pu(IV) and Eu(III) dissociate when in contact with cells, after which the metals are adsorbed.

These results strongly indicated that the effects of exudates should involve the migration model of radionuclides in the environments.

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