Role of Microorganisms on the Transformation of Lanthanides during the Biooxidation of Divalent Manganese

We found for the first time that the biomolecule released from *Acremonium strictum* KR21-2 during the formation of MnO_2 specifically binds tetravalent Ce. This Ce-binding biomolecule was not found to associate with any other trivalent lanthanides tested or with Fe. The oxidation states of Mn and Ce in the biogenic MnO_2 were determined to be IV by XANES measurements. Most of the desorbed Ce was in the colloidal phase and associated with a biomolecule produced by the active fungus. The biomolecule was characterized as ca. 4700 Da in size, and contained saccharides that differed from those non-nuclide-specific organic substances released from resting cells, as reported previously.

Ce oxidation and reduction reactions were found to play an important role in Ce mobility on the mineral surface. Mn(II)-oxidizing microorganisms strongly affect the migration of Ce because they can produce biogenic Mn oxide in the subsurface [1, 2]. We assessed how the presence of lanthanides affects the oxidation rate of Mn(II) as well as the production of cellular secretions by Mn-oxidizing microorganisms, and determined the influence on lanthanide mobility at the solid/liquid interface. To accomplish this, active fungus *A. strictum* KR21-2 was incubated under various conditions to compare the oxidation rate of Mn(II), the sorption rate of lanthanides, and the production of cellular secretions.

The HAY medium (50 mL), which initially contained 0–1 mmol/L Mn(II), 0–100 μg/L of each lanthanide element, and 0–100 μL spore suspension, was contacted with *A. strictum* KR21-2 at pH 7.0. To determine the oxidation state of Ce in the solid phase, Ce K-edge XANES (X-ray-absorption near-edge structure) analysis was carried out at AR-NW10A. The spectra of $CeCl_3$ and $Ce(SO_4)_2{\cdot}nH_2O$ were collected as references. The chemical shift of the absorption edge for Ce(III) (40,442 eV) and Ce(IV) (40,453 eV) is 11 eV, which can be used to quantify the Ce oxidation state in solid phases [3]. We used LCF fitting for quantifying the Ce oxidation state. The analysis procedure was similar to that in our previous work [4]. It was confirmed that the samples were not damaged by repeated X-ray irradiation.

When fungal spores were added, more than 99% of the lanthanides were removed from the liquid phase after 24 h (**Fig. 1a**). The sorption of all the light lanthanides (La, Pr - Gd) except Ce was almost constant up to 72 h. The sorbed Ce started to be desorbed after 32 h and desorption increased with increasing time. The desorption behavior of Ce was markedly different from that of its neighboring elements, and the large desorption of Ce resulted in the formation of a negative Ce anomaly.

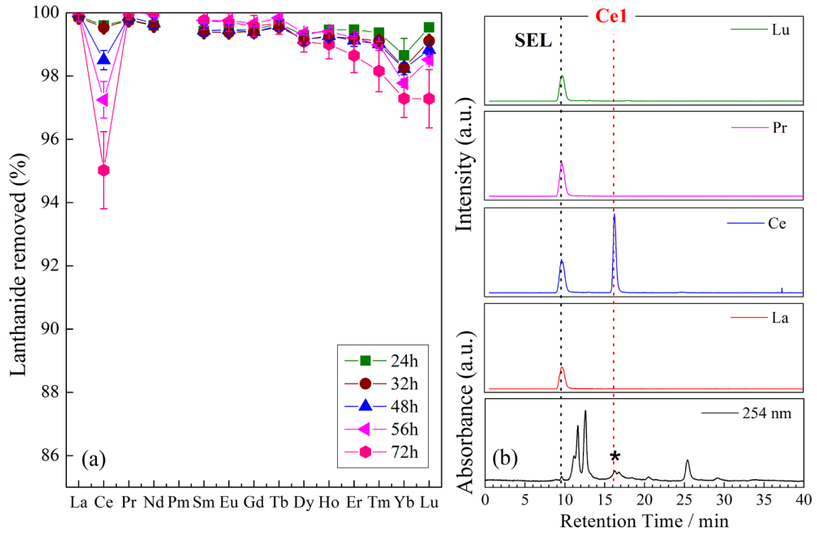

Figure 1: Change in lanthanide removal fraction with time **(a)**, and SEC-UV-ICP-MS chromatograms in the liquid phase **(b)**.

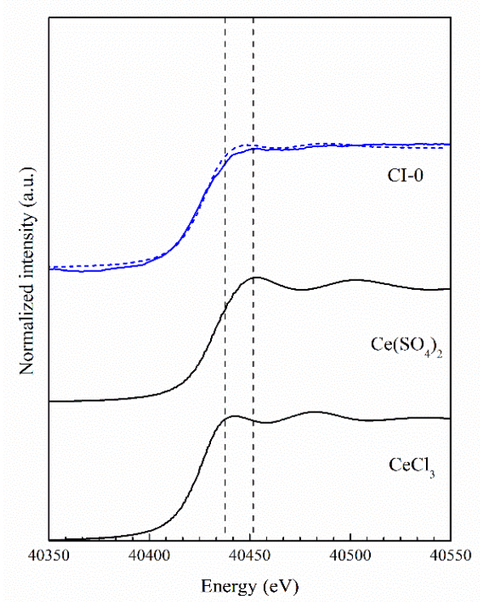

Figure 2: Ce K-edge XANES spectra of the solid phase after 100 h incubation (CI-0). The spectra of $CeCl_3$ and $Ce(SO_4)_2$ are also plotted as references. Blue dashed line is linear combination fitting using $Ce(SO_4)_2$ and $CeCl_3$ standards.

The SEC-UV-ICP-MS chromatograms of the liquid phase are shown in **Fig. 1b**. A new intense SEC-ICP-MS peak for Ce was observed at a retention time of 15.9 min. This intense Ce peak corresponded to Ce desorbed from the solid phase containing the fungal cells and Mn oxides. The SEC-UV peak (marked with an asterisk in **Fig. 1b**) accompanying the Ce1 peak was more intense than in the control test without the fungus. These results suggest that the fungal cell may have released a biomolecule with a retention time of 15.9 min that formed a complex with Ce in solution. The estimated molecular size of this biomolecule was 4700 Da; therefore, we refer to this Ce-binding biomolecule as “CB4700”. Quantitative determination of total carbohydrates by a colorimetric method showed that the carbohydrate content for CB4700 and whole medium was 53 and 103 mg/g, whereas the uronic acid content was 3 and 27 mg/g, respectively. The direct observation of saccharide-containing compounds produced by Mn-oxidizing fungi and their potential effects on the scavenging of Ce(IV) has never been reported before. Protein was not detected from CB4700, although *A. strictum* releases a variety of proteins into solution [1].

The XANES spectrum of Ce in the solid phase without Mn(II) (HY-Ln) showed an absorption edge similar to $CeCl_3$ (**Fig. 2**), indicating that the fungus did not oxidize Ce(III) to Ce(IV) in the absence of Mn, even under metabolically active conditions. The absorption edge of Ce in the solid phase was shifted to a higher energy compared with that of HY-Ln, indicating that a fraction of Ce was present in the tetravalent state. The estimated Ce(IV) content obtained by linear combination fitting was 36%.

To our knowledge, this is the first report of the release of a biomolecule with specific affinity for Ce(IV) as an adaptive response of fungi to lanthanides. The findings of this study will be helpful for understanding lanthanide cycling in the environment, and for long-term management of nuclear waste sites, where microbial activity is involved in Mn oxide formation and radionuclide migration.

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