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Reduction of selenite by biofilm of *Shewanella putrefaciens*

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1 Introduction

Selenium-79 is a long-lived randionuclide contained in high-level radioactive waste. It is important to understand migration behavior of selenium at deep underground. Selenium solubility is largely controlled by selenium oxidation state. Microbial reduction of highly soluble selenate ($Se^{VI}O_4^{2-}$) and selenite ($Se^{IV}O_3^{2-}$) to insoluble elemental selenium is one of important phenomena affecting the mobility of selenium. The microbial reduction of selenate and selenite have been widely studied using planktonic bacteria [1,2]. On the other hand, it has been known that in natural settings bacteria are predominantly found within surface-associated cell assemblages, or biofilms. However, there are little information on the interaction between selenium and biofilms. In this study, we examined the reduction of selenite by biofilm of *Shewanella putrefaciens* to reveal the selenium immobilization ability of the biofilms.

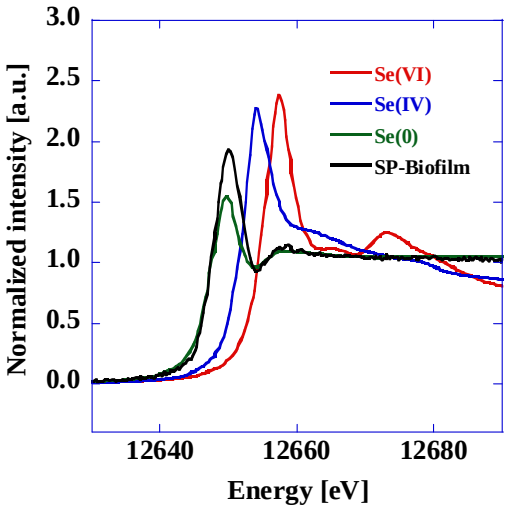
2 Experiment

Biofilms of *S. putrefaciens* were made on poly-L-lysine coated circular cover glasses in a sterile 24-well microplate containing a 1 mL of nutrient broth in each well at 30°C. After a week incubation, biofilm like membranes were formed on the cover glasses. The membranes were washed with a deionized water, stained with safranin solution, and observed by an optical microscope. To investigate the reduction of selenite by the biofilms, a 1 mL of aqueous solution containing 500 μM sodium selenite as an electron acceptor, 20 mM sodium lactate as an electron donor, and 20 mM HEPES buffer (pH 7.0) was added to the biofilms formed on the cover glasses in the microplate. The microplate was placed in an anerobic plastic sack, where an oxygen absorbent was placed to attain an anaerobic condition. After 3 days, the biofilms were washed with a deionized water, and observed by the optical microscope without staining. The selenium concentration in the solution was measured by ICP-AES. Se K-edge X-ray absorption near edge structure (XANES) spectra of the precipitates appeared on the biofilms were collected in fluorescens mode at beamline 12C. XANES spectra of sodium selenate, sodium selenite, and elemental gray selenium were collected as reference materials in transmission mode.

3 Results and Discussion

The microscopic observation of the cover glasses after the incubation with *S. putrefaciens* revealed that the cells were heterogeneously distributed on the cover glass indicating the formation of biofilms. After 3 days incubation of the biofilms with selenite, red precipitates were observed at the place where the biofilms were formed. The precipitates were not dissociated from the biofilms by washing with a deionized water indicating that they associated tightly with the biofilms. The selenium concentration in the solution decreased from 500 to 16 μM. Se K-edge XANES spectra of the reference materials and the red precipitates formed at the biofilms are shown in Fig. 1. The edge peak of the red precipitates appeared at the almost same energy of that of gray Se, however, the peak height was higher than that of gray Se. These characteristics of XANES spectrum indicate that the red precipitates are nanoparticulate elemental selenium. These results suggest that the biofilms with iron-reducing bacteria in the environment can immobilize the selenium at the biofilms through selenite reduction to nanoparticulate elemental selenium.

Fig. 1: Se K-edge XANES spectra of reference materials and precipitates at biofilm.



References

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