

The implementation of Pollutant Release and Transfer Register (PRTR) obliged us to report the total amount of chemical substances, such as toxic metals, released into the atmosphere, hydrosphere and soil. The total amount of these substances that have been released into the environment due to human activity is now becoming clear. However, the chemical state changes of toxic metals have not been clarified. We should clarify the behavior of toxic metals in the environment to ensure safety against damage due to toxic metal contamination, because the toxicity of a metal depends on its valence or combined states.

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In many cases, microorganisms have assumed a vital role in the chemical state changes of metals. The use of microorganisms is expected to lead to the development of environmental restoration technologies by revealing the biological reaction pathway of metals.

Recently, a selenate reduction bacterium, Sulfurospirillum barnesii [1], was isolated from a freshwater sediment. S. barnesii has the ability to reduce selenic acid [Se(VI)] under anaerobic conditions, using organic acid as an electron donor.

We present here the chemical state changes observed for selenium by X-ray absorption fine structure (XAFS), focusing our attention on the reduction ability of *S. barnesii* to reveal the behavior of selenium in a biological reactive process.

We used a DSM 10660 strain [2] (= ATCC 700032 strain) for *S. barnesii*, cultivated in a 500 ml sealed bottle with *Sulfurospirillum* II medium containing 83 mg/l Se(VI). Cultivation was carried out at constant temperature (30° C) under anaerobic conditions with the injection of inert gases (N₂/CO₂).

XAFS measurement was carried out at beamline **BL01B1** with a two-crystal Si(111) monochromator. XAFS spectra of *S. barnesii* culture were collected in the fluorescence mode using a Ge 19-element solid-state detector.

Figure 1 shows Se *K*-edge XANES spectra of *S. barnesii* culture and reference materials. A peak due to metallic selenium [Se(0)] was clearly observed in the spectra after 28, 37, 43, 53 and 66 hours. Also, a peak due to tetravalent selenium [Se(IV)] was observed in the same spectra. This result shows that the reduction pathway from Se(VI) to Se(0) by *S. barnesii* goes through Se(IV).

To estimate the chemical composition of selenium in each moment, we calculated the ratio of each chemical states by the curve-fitting method using the spectra of the reference materials. The result is shown in Fig. 2. The change of the chemical composition of selenium indicates that Se(VI) constantly decreases with increasing *S. barnesii* growth rate. In this case, although a rate-limiting step of the reduction pathway from Se(IV) to Se(0) occurred for 20 hours after seeding, the reduction of Se(VI) to Se(IV) proceeded constantly. Thus, from the above finding, it is assumed that the reduction of Se(VI) to Se(IV) by *S. barnesii* is faster than that of Se(IV) to Se(0) under such a high selenium concentration (83 mg/l selenium).



Fig. 1. Se *K*-edge spectra of culture after 0, 12, 21, 28, 37, 43, 53 and 66 hours from seeding *S. barnesii*, along with Se, SeO₂ and CaSeO₄ for reference.



Environmental Science

The results also indicate that selenium released in to the hydrosphere is immobilized as metallic selenium by anaerobic bacteria, such as *S. barnesii*, which have the ability to reduce selenium. Figure 3 shows pattern diagrams of dynamics of selenium in aquatic sediments. If we can accelerate the growth of selenium reduction bacteria, and keep them in high density, we will be able to effectively render selenium harmless.





Fig. 2. Change in chemical composition of selenium with time. (Result of calculation by the curve-fitting method using the spectra of reference materials).

Fig. 3. Pattern diagrams of dynamics of selenium in aquatic sediments.

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98

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