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Biosorption of Actinides from Dilute Waste Actinide Solution by Egg-Shell Membrane

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Abstract

Removal of radioactive elements from the effluent and waste aqueous solutions is an important problem. In previous laboratory batch experiments, hen egg-shell membrane (ESM) was stable as an insoluble protein and was very capable of binding heavy metal ions from aqueous solution. Batch laboratory pH profile, time dependency, and capacity experiments were performed to determine the binding of uranium (U) and thorium (Th) to ESM. Batch pH profile experiments indicated that the optimum pH for binding these actinides was approx 6.0 (U) or 3.0 (Th). The adsorption isotherms were developed at pH 5.0 (U) or 3.0 (Th) at 25°C, and the adsorption equilibrium data fitted both Langmuir and Freundlich models. The maximum uptakes by the Langmuir model were about 240 mg U/g and 60 mg Th/g dry weight ESM. In addition, their adsorption capacities increased as salt concentration increased. ESM could also accumulate uranium from dilute aqueous solution by adjusting to the optimum pH. These results showed that ESM was effective for removing actinides from solution and would be useful in filtration technology to remove actinides from aqueous solution.

Index Entries: Biosorption; egg-shell membrane; actinide; uranium; thorium.

Introduction

Recent world concern has focused attention on the disposal of toxic metals and radionuclides and supported research on the effects of such elements on the environment, since they ultimately reach and accumulate

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in animal and human tissues. The existence of toxic metals and radionuclides in the environment has represented a significant environmental hazard. Metal mining and refining industries produce aqueous waste streams containing low metal concentration. In addition, radionuclides continue to seep through soil and groundwater at the sites where uranium was mined. Removal of radionuclides and heavy metals from dilute aqueous streams currently depends on physical or chemical means and includes such treatment methods as chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment, evaporative recovery, and solvent extraction. However, these procedures have significant disadvantages, such as incomplete metal removal, nonselective removal, high reagent or energy requirements, generation of toxic sludge, or high cost at low metallic concentrations.

Fungi and yeast biomass have a high affinity for actinide elements. Results using batch techniques have shown that *Rhizopus arrhizus*, *Mucor miehei*, and *Saccharomyces cerevisiae* can accumulate significant amounts of uranium *(1–5)*. In previous studies, it was found that biological substances, such as phosphorylated polysaccharides, immobilized phytic acid and immobilized Chinese gallotannin were very capable of adsorbing uranium from aqueous systems *(6–9)*. Recently, we showed that various heavy metals were accumulated in high yield by hen egg-shell membrane (ESM) *(10)*, and the ESM removed gold from dilute aqueous solutions *(11)*.

The purpose of this article is to test ESM for its ability to adsorb uranium and thorium ions. Furthermore, the performance of ESM to remove uranium from a dilute hot-spring water solution is studied.

Materials and Methods

Sample Preparation

ESMs were mechanically stripped from the shells after immersion of the hen (White leghorn) egg shells, which were collected from a local confectionery, in 0.5 *M* HCl overnight and then further in 0.5 *M* NaOH for 1 h followed by rinsing with distilled, deionized water 10 times. All ESMs were desiccated over phosphorus pentaoxide under reduced pressure at room temperature for over 10 h.

Chemicals and Metal Solutions

Solutions of uranium and thorium were prepared by dissolving exact quantities of uranyl nitrate and thorium nitrate (Wako, Inc., Tokyo, Japan) in distilled, deionized water. Diluting was done by addition of deionized, distilled water daily as required. All pH adjustments were carried out with 1 *M* HNO_3 and 1 *M* NaOH. The hot-spring water sample was obtained from Miasa Hot-Spring Resort (Tottori, Japan), and was prefiltered using a 0.45-μm membrane filter.

Adsorption Experiments

In the batch procedure, the amount of ESMs (around 45–55 mg) was precisely weighed, then placed directly into the aqueous solution containing actinide ions in polypropylene tubes. To prevent metal contamination during the experiments, the following cleaning procedure was used. The test tubes were soaked with 10% (w/v) HCl overnight. Then, they were washed and rinsed with distilled, deionized water. Unless otherwise stated, the volume of uranium or thorium solution was always 100 (U) or 25 mL (Th). The pH values of reaction mixtures were adjusted with 1 *M* HNO_3 or 1 *M* NaOH at the start of the experiment and maintained when possible. The solutions containing samples were shaken in a thermostated water bath at 25°C. After an appropriate time, aliquots of the solution of reaction mixture were analyzed for the remaining metal ions.

Actinide Analysis

Determinations of uranium and thorium concentrations were carried out using Arsenazo III methods described previously *(13)*. Arsenazo III was used as the color-developing agent, and a spectrophotometer (U-1000, Hitachi Ltd., Tokyo, Japan) was used for the quantitative determinations. Hot-spring water analyses were performed by an inductively coupled plasma mass spectrometer (ELAN 6000 ICP-MS, Perkin Elmer, Norwalk, CT).

Sorption capacity was calculated by $q = (C_0 - C_e)V/m$; where q is the sorption capacity (mg/g sorbent), C_0 the initial metal concentration, C_e the residual metal concentration in solution (mg/L), V the volume of solution (L), and m the sorbent mass (g). During the adsorption, equilibrium was established between the adsorbed metal ions on the sample (q) and unadsorbed metal ions in solution (C_e), and was represented by either the Freundlich or the Langmuir adsorption isotherms, which are widely used to present data for water and waste water treatment applications *(1–4,14,15)*.

The Freundlich isotherm, used to describe the adsorption of solutes from dilute solutions, can be given by:

$$q = K_F C_e^{1/n} \tag{1}$$

$$\log q = \log K_F + (1/n)\log C_e \tag{2}$$

where K_F (adsorption capacity) and $1/n$ (adsorption intensity) are Freundlich constants. This equilibrium equation should be linearized by plotting $\log C_e$ vs $\log q$ to determine the Freundlich constants from the slope ($1/n$) and intercept ($\log K_F$).

Unlike the empirical Freundlich isotherm, the Langmuir isotherm has a theoretical basis, which relies on a postulated chemical or physical interaction between solute and vacant sites on the adsorbent surface. The Langmuir isotherm model has the form:

$$q = Kq_{max}C_e/(1 + KC_e) \tag{3}$$

$$C_e/\mathrm{q} = (1/\mathrm{K}q_{max}) + (1/q_{max})C_e \tag{4}$$

where q_{max} is the maximum amount of adsorbate per weight of adsorbent and K is a constant related to the energy of adsorption. This equilibrium equation also can be linearized by plotting C_e vs C_e/q to determine the Langmuir constants from the slope ($1/q_{max}$) and the intercept ($1/Kq_{max}$).

Results and Discussion

Effect of pH on Uranium and Thorium Adsorption

To find the optimum conditions, adsorption of uranium and thorium by ESM was studied at solutions of differing pH levels. As shown in Fig. 1, the pH of the solution had a significant effect on the actinides' adsorption. The uranium uptake by ESM increased as the pH increased from 2.0 to 6.0, and the maximum uranium uptake was obtained at pH 6.0. On the other hand, the maximum thorium uptake was obtained at pH 3.0, below and above which the uptake declined greatly. Similar results have been observed in fungi *(4,16)*.

In the case of actinide uptake by fungi, it has been reported that the pH acts on the cell wall chemistry and metal chemistry in solution *(4)*. ESM, like the cell wall, consists mainly of protein, which contains amines, amides, and carboxylic surface functional groups, which are protonated or deprotonated depending on the pH of the solution.

Uranyl UO_2^{2+} chemistry is complicated by a hydrolysis phenomenon: When pH increases from an acidic value to a neutral value, various hydrolyzed species exist, the repartition of which is conditioned by the pH and total uranium concentration *(1,4,17)*. Ion-species repartition is determined according to following reactions and equilibrium constants:

$$UO_2^{2+} + 2H_2O \rightleftharpoons UO_2(OH)^+ + H_3O^+ \quad pK = 5.8 \tag{5}$$

$$2UO_2^{2+} + 4H_2O \rightleftharpoons (UO_2)_2(OH)_2^{2+} + 2H_3O^+ \quad pK = 5.62 \tag{6}$$

$$3UO_2^{2+} + 10H_2O \rightleftharpoons (UO_2)_3(OH)_5^+ + 5H_3O^+ \quad pK = 15.63 \tag{7}$$

This fact plays an important role in the uranium adsorption mechanisms: With changes in pH, uranyl hydrolyzed species repartition changes and influences the nature, charge, and size of ions. For a total uranium concentration of 100 ppm, at pH 2.0 about 100% of uranium exists in free-form UO_2^{2+}, whereas at pH 6.0 about 100% of uranium exists in the hydrolyzed form $(UO_2)_3(OH)_5^+$ *(4)*.

We considered the effect of pH on the uranium adsorption mechanisms as follows. If the pH in solutions is too acidic (pH ≈ 2.0), protons compete with uranyl ions, and protonation of the ESM inhibits uranium adsorption by an electrostatic repulsive force between protonated ESM and positively charged ions. Conversely, at the optimum pH (pH ≈ 6.0), uranium exists mainly as uranyl hydroxide in solution, the solubility of uranium decreases, and the effect of electrostatic repulsive force stays minor; therefore, the maximum uranium uptake is observed.

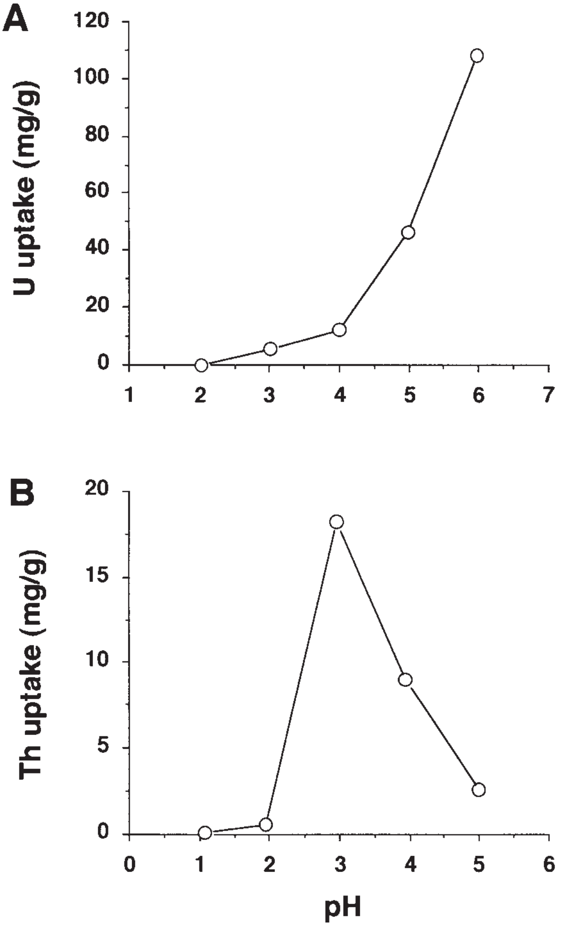


Fig. 1. Effect of pH on the uranium **(A)** and thorium **(B)** adsorption by ESM. The ESMs (50 mg dry weight basis) were soaked in 100 (U) or 25 mL (Th) of a solution containing 100 ppm uranium or thorium for 24 h at 25°C.

Thorium hydrolysis, however, is more complicated than uranium hydrolysis *(18)*. Indeed, thorium solubility changes dramatically with pH. At pH 2.0, thorium solubility is high, with Th^{4+} as the soluble species. When the solubility decreases with pH increase, various hydrolyzed species, such as $Th(OH)^{3+}$, $Th(OH)_2^{2+}$, $Th_2(OH)_2^{6+}$, $Th(OH)_3^{+}$, and $Th_6(OH)_{15}^{9+}$, appear in solution, with Th^{4+} decreasing. The existence of negatively charged hydrolyzed thorium is suggested by Tsezos et al. *(1)*. It seems that this phenomenon is significantly related to the thorium adsorption by ESM.

Kinetics of Uranium and Thorium Adsorption

Figure 2 illustrates the effect of pH on biosorption kinetics. This figure shows that pH plays an important role in adsorption capacities and biosorption kinetics. The adsorption rate of these actinides is very low, and equilibrium needs over 24 h with pH at the maximum uptakes for uranium

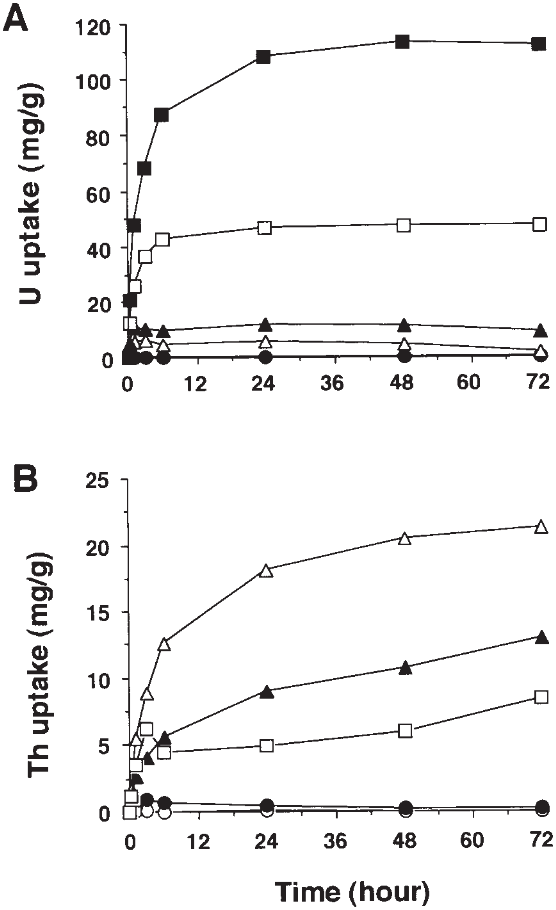


Fig. 2. Kinetics of adsorption of uranium **(A)** and thorium **(B)** by ESM. The ESMs (50 mg dry weight basis) were soaked in 100 (U) or 25 mL (Th) of a solution (pH 1.0 [○], 2.0 [●], 3.0 [△], 4.0 [▲], 5.0 [□], and 6.0 [■]) containing 100 ppm uranium or thorium at 25°C.

and thorium, respectively. Although various hydrolyzed species appear as pH changes, we do not think that it takes over 24 h for their diffusion into ESM. Therefore, we postulate that the rate-determining step for the adsorption of these actinides is a hydrolysis reaction.

Uranium and Thorium Isotherms

Representative biosorption isotherms for uranium at pH 5.0 and thorium at pH 3.0, plotted in Fig. 3, show the evolution of metal concentration in ESM vs residual metallic concentration in solution. To avoid precipitation of uranium hydroxide at high concentration, the pH of the uranium solution was adjusted to 5.0. Figures 4 and 5 show the isotherms fitting both Langmuir (Figs. 4A, 5A) and Freundlich (Figs. 4B, 5B) models and their correlation coefficients (r^2). These results indicate that in uranium adsorp-

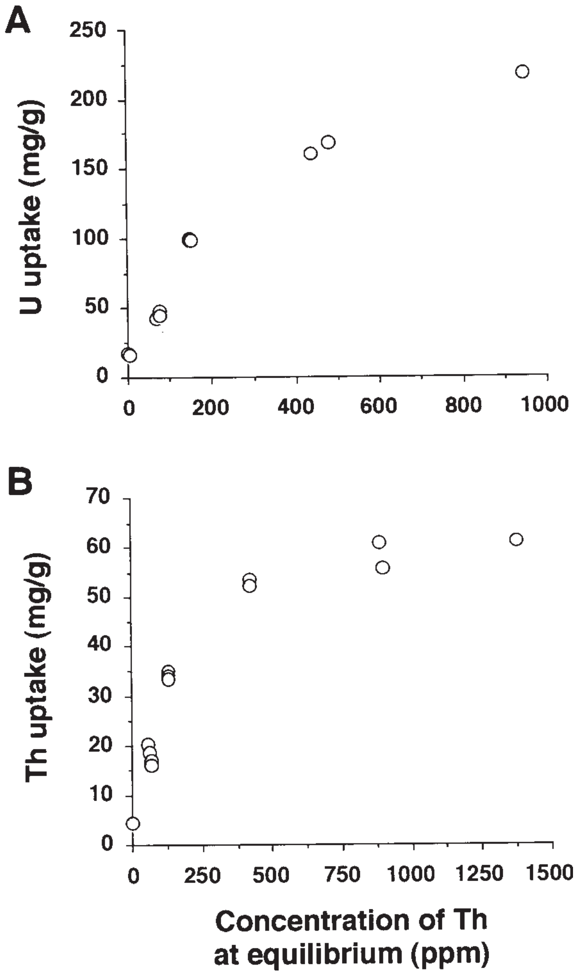
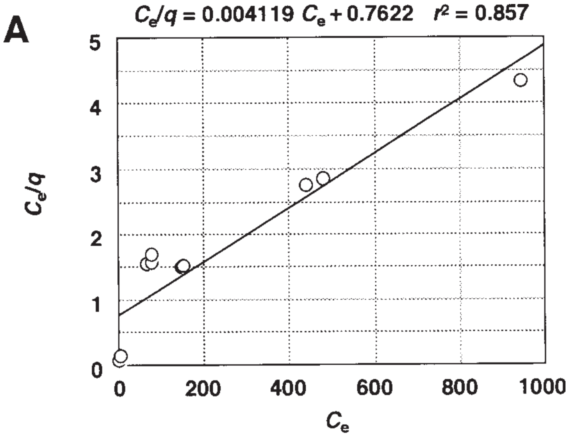


Fig. 3. Uranium **(A)** and thorium **(B)** biosorption isotherms for ESM. The ESMs (50 mg dry weight basis) were soaked in 100 (U) or 25 mL (Th) of a solution (U: pH 5.0, Th: pH 3.0) containing 10–1500 ppm of uranium or thorium for 24 h at 25°C.

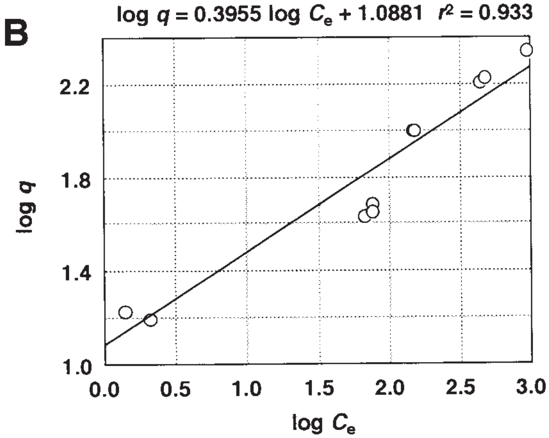
tion the Freundlich isotherm model provides a better representation of the data than the Langmuir model, but in thorium adsorption the Langmuir model provides the better fit. It is unclear whether this has any physical relevance for adsorption. Perhaps the mechanisms are different for uranium and thorium adsorption by ESM. Also, according to the Langmuir model constant, the slope $1/q_{max}$, it was found that the maximum uptake was about 240 mg uranium/g dry weight ESM, or about 60 mg thorium/g.

Effect of ESM Pretreatment on Uranium and Thorium Adsorption

To examine the effect of ESM pretreatment on biosorption of uranium and thorium, the adsorption of uranium and thorium by ESM treated without acid and alkaline was compared with the adsorption ability of



Langmuir isotherm



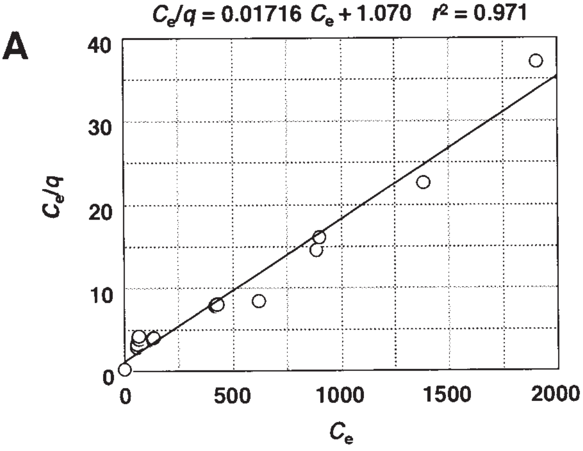
Freundlich isotherm

Fig. 4. Langmuir **(A)** and Freundlich **(B)** isotherms of the uranium adsorption by ESM. r^2 values are given to show the best fit for both Langmuir and Freundlich models (1.0 = perfect fit).

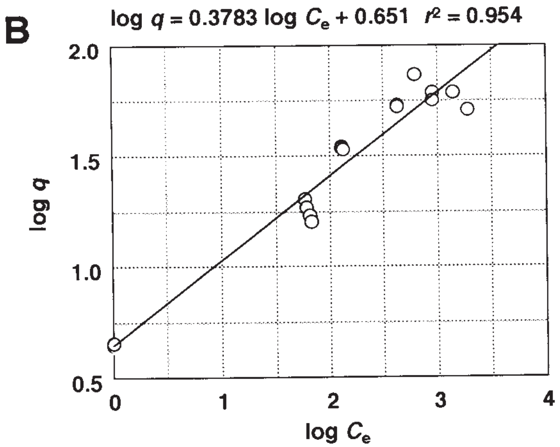
ESM prepared by exposure to 0.5 *M* HCl overnight and then a further 0.5 *M* NaOH for 1 h. All samples were washed with distilled, deionized water 10 times.

The results obtained following analysis of treated ESMs for uranium and thorium uptake are shown in Table 1, and demonstrate that with HCl treatment the observed adsorption capacity reduced to about 20–40% compared to without treatment sample, whereas with NaOH treatment it increased to about 30%.

It has previously been reported that pretreatment with KOH contributed to an increase in the availability of latent metal-binding sites in biomass derived from the fungus *Penicillium digitatum (19)*. Pretreatment of



Langmuir isotherm



Freundlich isotherm

Fig. 5. Langmuir **(A)** and Freundlich **(B)** isotherms of the thorium adsorption by ESM.

Table 1
Effect of ESM Pretreatment on Uranium and Thorium Adsorption[a]

Pretreatment	Uptake, mg/g U	Uptake, mg/g Th
Without treatment	78.8 ± 3.1	18.0 ± 0.4
With HCl treatment	44.9 ± 2.4	13.5 ± 0.5
With HCl and NaOH treatment	105.2 ± 4.9	22.9 ± 3.4

[a] The ESMs (50 mg dry weight basis) were soaked in 100 (U) or 25 mL (Th) of a solution (U: pH 6.0, Th: pH 3.0) containing 100 ppm uranium or thorium for 24 h at 25°C.

Table 2
Effect of Salt Concentration in Solution
on Uranium and Thorium Adsorption by ESM[a]

	Uptake, mg/g	
Salt concentration	U	Th
Control	44.5 ± 2.2	9.2 ± 1.4
0.01 M $NaNO_3$	72.6 ± 5.2	11.6 ± 0.2
0.05 M $NaNO_3$	89.2 ± 2.7	14.3 ± 0.5
0.10 M $NaNO_3$	90.3 ± 3.3	15.5 ± 1.2

[a] The ESMs (50 mg dry weight basis) were soaked in 100 (U) or 25 mL (Th) of a solution (U: pH 5.0, Th: pH 3.0) containing 100 ppm uranium or thorium for 24 h at 25°C.

R. arrhizus biomass with NaOH resulted in higher uptake capacities for zinc *(20)*. Conversely, it has been reported that polysaccharides contained in marine algae, such as *Ascophyllum nodosum*, are extremely sensitive to acid or alkaline pH *(21)*, and that cell-wall components, such as proteins and protein–carbohydrate complexes from the fungus *Talaromyces emersonii*, are removed with acid and alkaline treatment *(22)*. It is recognized that ESMs are intricate networks of stable and water-insoluble fibers with high surface area, and that they are relatively resistant to treatments with acid and alkaline. The results obtained with the ESM may suggest that binding-surface functional groups are protonated or deprotonated following pretreatment with acid or alkaline pH.

Effect of Salt Concentration in Solution on Uranium and Thorium Adsorption

To determine whether salt concentration in solution is affected by the uranium and thorium adsorption by ESM, adsorption experiments were conducted using 100 ppm uranium or thorium solution containing 0.01, 0.05, or 0.10 *M* sodium nitrate. As shown in Table 2, the results indicate that the uptake of uranium and thorium increases as the concentrations of sodium nitrate in the solutions increase.

Recovery of Uranium from Dilute Solution

In the batch procedure, to determine the recovery of uranium from dilute aqueous solutions by ESM, adsorption experiments were conducted. Uranyl ions at concentrations of 71 ppm were adsorbed from dilute aqueous solutions (20 mL) at an initial pH 6.0 by ESM (200 mg dry weight basis). As shown in Fig. 6, ESM was capable of accumulating only about 25% of total uranium concentration during the initial 6 h. However, when the pH in the solution was measured after 6 h, it increased from 6.0 to 9.5. It is thought that this effect of pH results in the buffer capacity of ESM in solution. Furthermore, when a small amount of HNO_3 is added to the solution

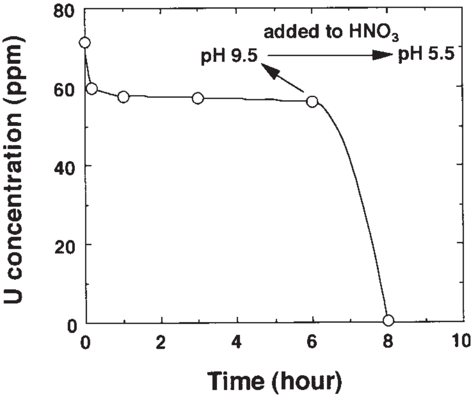


Fig. 6. Removal of uranium from a dilute solution by ESM.

Table 3
Effect of pH on Removal of Uranium from Hot-Spring Water with ESM[a]

Initial pH	U removal, %
1.3	13
6.0	97
8.6	6
11.4	17

[a] The ESMs (30 mg dry weight basis) were soaked in 10 mL of a solution for 6 h at room temperature, expressed as a removal percentage from initial uranium concentration (119 ppb).

containing ESM and the pH lowers to pH 5.5, ESM was able to remove uranyl ions from the dilute solution completely. This result shows that pH plays an important role in the removal of uranium from dilute solution.

Biosorption of Uranium from Hot-Spring Water

To determine whether ESM was capable of uranium uptake from a natural medium containing various ions, the ability of the biosorbent to take up uranium from hot-spring water was examined. Solutions were prepared by adding very small amounts of uranium to hot-spring water and filtering through a 0.45-μm membrane filter. The solution was equilibrated for 3 d before filtering. Recovery of uranium from hot-spring water containing various metal and metalloid ions appears to be suitable for the modeling applications.

Previously, to determine the effect of pH on the removal of uranium from those solutions, adsorption of uranium by ESM was examined using various pH solutions: acid (pH 1.3), alkaline (11.4), optimum condition (6.0), and nontreated condition (8.6). As shown in Table 3, the recovery of

Table 4
Removal of Metal and Metalloid Ions from Hot-Spring Water with ESM[a]

	Metal and metalloid ions								
	Mn	Fe	Cu	As	Sr	Mo	Cs	Ba	U
Removal, %	32	5	86	4	6	7	0.8	5	97

[a] The ESMs (30 mg dry weight basis) were soaked in 10 mL of a solution (pH 6.0) for 6 h at room temperature, expressed as a removal percentage from initial metal concentration (Mn: 110, Fe: 132, Cu: 35, As: 201, Sr: 486, Mo: 23, Cs: 51, Ba: 21, U: 119 [ppb]).

uranium was markedly affected by the pH of the hot-spring water solution; it was the highest at optimum pH (6.0) by ESM. ESM accumulated uranium from hot-spring water solution with high efficiency at pH 6.0, and the removal rates were more than 90%.

Furthermore, the recoveries of heavy metal and metalloid ions from the hot-spring water solution (pH 6.0) containing different concentrations of manganese (110 ppb), iron (132), copper (35), arsenic (201), strontium (486), molybdenum (23), cesium (51), barium (21), and uranium (119) were analyzed. As shown in Table 4, ESM sorbed uranium far more readily than other metals, but the removal percentage from initial metal concentration of iron arsenic, strontium, molybdenum, cesium, and barium by ESM was <10%. These results show that ESM can accumulate uranium highly selectively from a solution containing various species of heavy metals; that is, this protein can remove uranium from used nuclear fuel containing various nuclear fission products.

As described above, ESM has a good capacity to adsorb uranium and thorium and has industrial applications for recovery of uranium and thorium. Furthermore, in this study it was found that ESM has the ability to remove selectively low concentrations of uranium. Further studies will be undertaken to more surely characterize the mechanisms of uranium and thorium adsorption and to devise more practical approaches to the recovery of uranium and thorium from aqueous systems using ESM.

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