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Bioaccumulation of uranium and thorium from the solution containing both elements using various microorganisms

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Abstract

The effects of proton, thorium and uranium on the bioaccumulation of thorium and uranium from the solution (pH 3.5) containing uranium and thorium using *Streptomyces levoris* cells were examined. The amount of thorium accumulated using the cells decreased by the pre-contact between the cells and the solution (pH 3.5) containing no metals, whereas that of uranium was almost unaffected by the treatment. The amount of thorium was almost unaffected by the existence of uranium. On the other hand, the amount of uranium accumulated was strongly affected by the thorium, especially thorium addition after uranium accumulation. The decrease of uranium accumulated by the addition of thorium after the accumulation of uranium was higher than that from the solution containing both elements. Therefore, the contribution of uranium–thorium exchange reaction was higher than that of competition reaction. Accordingly, proton-uranium–thorium exchange reaction was occurred in the accumulation of thorium from the solution containing thorium and uranium. The gram-positive bacteria, such as *Micrococcus luteus*, *Arthrobacter nicotianae*, *Bacillus subtilis* and *B. megaterium*, has a much higher separation factor as thorium/uranium than that of actinomycetes. These gram-positive bacterial strains can be used for the accumulation of thorium from the solution containing uranium and thorium.

Keywords: Thorium bioaccumulation; Uranium bioaccumulation; Microorganism; Proton-uranium–thorium exchange reaction; Separation factor

1. Introduction

The removal of radionuclide and toxic heavy metals such as uranium and thorium from aqueous solutions, especially from contaminated sources, seems to be a significantly useful subject for environmental control. Many researchers have been studying the removal of uranium using microorganisms, such as actinomycetes [1–4], bacteria [2,5–9], fungi [2,10–14], and yeasts [2,6,15].

Thorium removal has also investigated by some researchers. Tsezos and Volesky [10,16] reported biosorption of uranium and thorium by some microorganisms and the mechanism of thorium biosorption by *Rhizopus arrhizus*. White and Gadds [14] reported biosorption of thorium by fungal biomass. Andres et al. [9] reported adsorption of thorium and uranium by *Mycobacterium smegmatis*. However, little detailed knowledge is available regarding which types of microorganisms can adsorb large amounts of thorium.

Recently, various species and strains of actinomycetes, bacteria, fungi, and yeasts were screened for their ability to accumulate uranium at pH 5.8 [2], and 3.5 [17]. Among the microorganisms, a high uranium accumulating ability was exhibited by the gram-positive bacterial strains, especially *Arthrobacter nicotianae* IAM12342, *Bacillus subtilis* IAM1026 and *Micrococcus luteus* IAM1056 at pH 5.8. The amount of uranium accumulated by gram-positive bacteria was larger than those by actinomycetes, gram-negative bacteria, fungi and yeasts. However, the amount of uranium accumulated using gram-positive bacteria was strongly affected with the pH of the solution. The amount of uranium accumulated using gram-positive bacteria was decreased sharply with increasing acidity below pH 5 [2]. On the other hand, a

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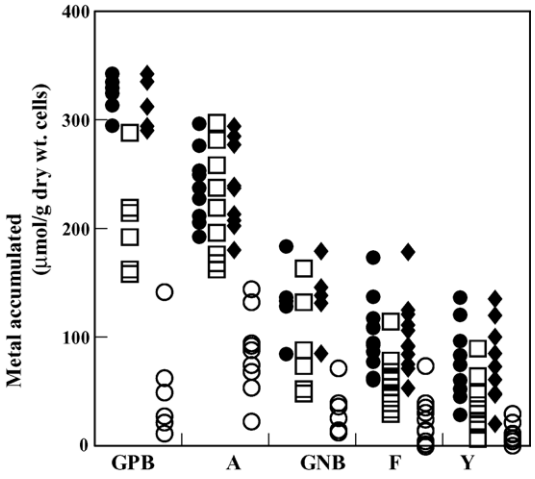

Fig. 1. Bioaccumulation of uranium and/or thorium using various microorganisms. Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.5) containing 50 μM uranium and/or thorium for 1 h at room temperature. GPB: gram-positive bacteria, A: actinomycetes, GNB: gram-negative bacteria, F: fungi, Y: yeasts. Symbols: closed circles, thorium accumulated (μmol/g dry wt. cells) from the solution containing thorium only; squares, uranium accumulated from the solution containing uranium only; diamonds, thorium accumulated from the solution containing uranium and thorium; opened circles, uranium accumulated from the solution containing uranium and thorium.

high uranium accumulating ability at pH 3.5 was exhibited by the actinomycetes strains, such as *Streptomyces levoris* HUT6156 and *S. albus* HUT6047 and gram-positive bacterial strain, such as *A. nicotianae*. The amounts of uranium accumulated using half strains of actinomycetes were higher than those using all gram-positive bacterial strains except *A. nicotianae*, all fungi and yeasts [17].

As shown in Fig. 1, the amounts of thorium accumulated from the solution containing thorium only [18] or thorium and uranium (unpublished data) using most of gram-positive bacterial strains are higher than those using most of actinomycetes, all of fungi and yeasts strains. The amounts of uranium accumulated from the solution containing thorium and uranium (unpublished data) using all microorganisms became lower than those from the solution containing uranium only [17]. However, the amounts of uranium accumulated from the solution containing both elements using half strains of actinomycetes were also higher than those using all gram-positive bacteria except *A. nicotianae*, all fungi and yeasts.

In this paper, the effects of uranium, thorium, and pre-contact between microorganisms and acidic solution at pH 3.5 on uranium and/or thorium accumulation using *Streptomyces levoris* cells, which accumulated the largest amount of uranium from the aqueous acidic solution containing uranium only at pH 3.5, were investigated. Furthermore, the separation factor as thorium/uranium from the solution containing same amounts of uranium and thorium using some microorganisms, which could accumulate large amount of thorium, was also calculated.

2. Experimental

2.1. Materials

The strains used in this research were generously donated by the IAM Culture Collection, Center for Cellular and Molecular Research, Institute of Molecular and Cellular Biosciences, The University of Tokyo (IAM); the Faculty of Engineering, Hiroshima University (HUT); and the Faculty of Agriculture, Hokkaido University (AHU). The chemicals (guaranteed reagents) used were obtained from Nacalai Tesque, Inc., Kyoto.

2.2. Culture of microorganisms

The medium for growing bacteria (except actinomycetes) contained 3 g/l meat extract, 5 g/l peptone, and 5 g/l NaCl in deionized water. The medium for growing the actinomycetes, fungi, and yeasts contained 4 g/l yeast extract, 10 g/l malt extract, and 4 g/l glucose in deionized water, pH 7.1 (for actinomycetes) and pH 5.7 (for fungi and yeasts). The microorganisms were maintained on agar slants and grown in 300 ml of the medium in a 500 ml flask with continuous shaking (120 rpm) for 72 h at 30 °C. Cells were collected by centrifugation (for bacteria except actinomycetes and for yeasts) or by filtration through filter paper (for actionomycetes and fungi), washed thoroughly with deionized water, and then used in the following accumulation experiments.

2.3. Metal accumulation experiments

Metal was supplied as $Th(NO_3)_4$ and/or $UO_2(NO_3)_2$. The pH of the solution was adjusted to 5.0 with 0.1 M NaOH. Unless otherwise stated, the accumulation experiments were conducted as follows. Resting microorganisms (15 mg dry wt. basis) was suspended in 100 ml solution (pH 3.5) containing 50 μM thorium and/or uranium and the suspension was shaken for 1 h at room temperature. The microorganisms were then collected by filtration through a membrane filter (pore size 0.2 μm). The amount of metal accumulated by the cells was determined by measuring the metal content in the filtrate using an inductively coupled plasma quantometer (ICPS8000, Shimadzu Corporation, Kyoto).

2.4. Effects of thorium or uranium addition, and pre-contact between microbial cells and acidic solution (pH 3.5) containing no metals on uranium and/or thorium accumulation using Streptomycin levers cells

Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.5) containing (1) 50 μM uranium and/or thorium for desired time (1 or 2 h) at room temperature, (2) 50 μM uranium or thorium for 1 h at room temperature. At 1 h, the same molar amount of thorium or uranium was added to the solution and suspended at room temperature for 1 h, (3) no metals for 1 h at room temperature. At 1 h, 50 μM

thorium, uranium or the mixture of thorium and uranium was added to the solution and suspended at room temperature for 1 h.

3. Results and discussion

3.1. The effects of thorium, uranium and pre-contacting between microbial cells and the acidic solution on the accumulation of uranium and/or thorium

In order to determine, the effect of thorium, uranium and pre-contacting of microbial cells and the acidic solution containing no metal on the accumulation of uranium and/or thorium were investigated in detail.

As shown in Fig. 2, the amounts of thorium accumulated were also almost unaffected by co-existed uranium and addition of uranium after thorium accumulation. On the other hand, the amounts of uranium were decreased with co-existing thorium. Furthermore, the amounts of uranium were strongly affected by the addition of thorium after uranium accumulation. As a result from these experiments, the effect of uranium–thorium exchange reaction were stronger than competitive reaction from the solution containing both metals. Therefore, uranium accumulated on the surface of microorganisms can be exchanged thorium easily.

On the other hand, the amount of thorium accumulated from the solution containing thorium only was decreased, when the thorium accumulation was carried out after contacting the microorganisms and the acidic solution (pH 3.5) containing no metals, though that of uranium accumulated were almost unaffected. The amount of thorium accumulated from the solution containing both metals was also decreased when the both metals accumulation was carried out after contacting the microorganisms and the acidic solution (pH 3.5) containing no metals. The amount of uranium accumulated in this case was affected by co-existed thorium, however almost unaffected by the pre-contacting of microorganisms and the acidic solution. As the results from these experiments, proton-uranium exchange is easier than proton-thorium exchange reaction. Therefore, proton-uranium–thorium exchange reaction can be contributed in the accumulation of both elements from the solution (pH 3.5) containing both elements.

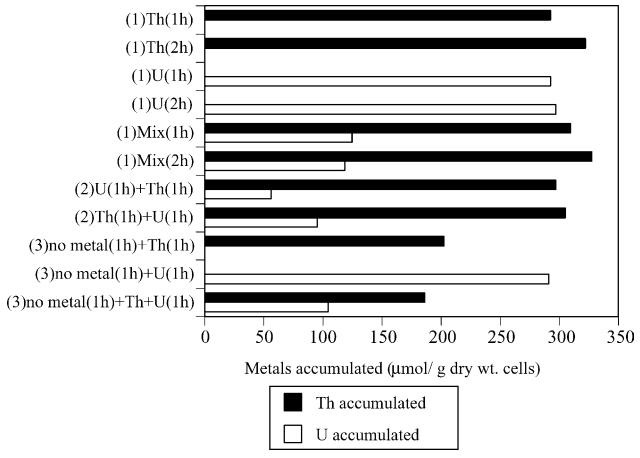

Fig. 2. Effect of thorium or uranium addition, and pre-contact between microbial cells and acidic solution (pH 3.5) containing no metals on uranium and/or thorium accumulation using *Streptomycin levers* cells. Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.5) containing (1) 50 μM uranium and/or thorium for desired time (1 or 2 h) at room temperature, (2) 50 μM uranium or thorium for 1 h at room temperature. At 1 h, the same molar amount of thorium or uranium was added to the solution and suspended at room temperature for 1 h, (3) no metals for 1 h at room temperature. At 1 h, 50 μM thorium, uranium or the mixture of thorium and uranium was added to the solution and suspended at room temperature for 1 h.

3.2. Screening of the separation factor of thorium accumulation/uranium accumulation

In order to determine the selectivity of thorium and uranium accumulation, separation factors [S.F.] were calculated by next equation from the results of the thorium and uranium accumulation from the solution containing same molar amount of both element, S.F. = $([Th]_{cells}/[U]_{cells})/([Th]_{solution}/[U]_{solution})$, where $[Th]_{cells}$ indicates the amount of thorium accumulated in the cells, $[U]_{cells}$ is the amount of uranium accumulated in the cells, $[Th]_{solution}$ is the amount of thorium remained in the solution, and $[U]_{solution}$ is the amount of uranium remained in the solution.

As shown in Table 1, the separation factors for Th/U of some gram-positive bacteria, such as *M. luteus*, *A. nicotianae*, *B. subtilis* and *B. megaterium*, were much higher than those of actinomycetes, such as *S. levoris* and *S. albus*. Therefore,

Table 1
Separation factor as thorium/uranium

Species	Separation factor
Arthrobacter nicotianae IAM12342	211
Bacillus licheniformis IAM11054	14
B. megaterium IAM1166	91
B. subtilis IAM1026	153
Corynebacterium equi IAM1038	14
Micrococcus luteus IAM1056	572
Rhodococcus erythropolis IAM1399	21
Streptomyces albogriseolus HUT6045	31
S. flavoviridis HUT6147	2
S. albus HUT6047	27
S. fradiae HUT6054	7
S. griseoflavus HUT6153	5
S. levoris HUT6156	28
S. olivaceus HUT6061	3
S. scabies HUT6027	3
S. viridochromogenes HUT6030	14

Resting cells (15 mg dry weight basis) were suspended in 100 ml solution (pH 3.5) containing 50 μM of thorium and uranium for 1 h at room temperature. Separation factors [S.F.] were calculated by next equation. S.F. = $([Th]_{cells}/[U]_{cells})/([Th]_{solution}/[U]_{solution})$, where $[Th]_{cells}$ is the amount of thorium accumulated in the cells, $[U]_{cells}$ the amount of uranium accumulated in the cells, $[Th]_{solution}$ the amount of thorium remained in the solution, and $[U]_{solution}$ the amount of uranium remained in the solution.

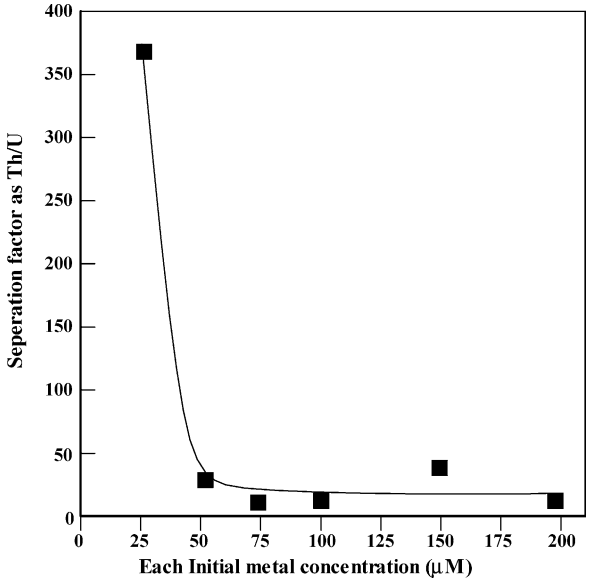

Fig. 3. Effect of external metal concentration on separation factor as thorium/uranium of the accumulation of both metals from the solution containing same molar amount of uranium and thorium using *S. levoris* cells. Resting cells (15 mg dry wt. basis) were suspended in a 100 ml solution (pH 3.5) containing a desired amount of thorium and uranium (each 25–200 μM) for 1 h at room temperature. The calculation method is shown in Table 1.

these gram-positive bacterial strains are useful for the bioseparation of thorium and uranium from the solution containing both metals. On the other hand, actinomycetes strains are useful for the bioaccumulation of uranium after removed thorium.

3.3. Effect of external concentrations of thorium and uranium on separation factor as thorium/uranium using S. levoris cells

In order to determine the effect of external concentration of thorium and uranium on separation factor as thorium/uranium, the separation factors were calculated from the results of the accumulation of thorium and uranium from the solution containing same molar amount of uranium and thorium using *S. levoris* cells.

As shown in Fig. 3, the separation factor was increased with decreasing the external metal concentration. Therefore selectivity of thorium becomes higher from the solution containing small amount of uranium and thorium.

4. Conclusions

The effect of thorium addition after uranium accumulation using *S. levoris* cells was stronger than that of co-existed thorium. Therefore, the thorium accumulation mechanism from the solution containing uranium and thorium was explained by uranium–thorium exchange rather than competitive mechanism. On the other hand, uranium was almost unaffected on thorium accumulation. Pre-contact between *S. levoris* cells and acidic solution (pH 3.5) was unaffected on uranium accumulation, however that was strongly affected thorium accumulation. Therefore, proton-uranium exchange is easier than proton-thorium exchange reaction. Accordingly, proton-uranium–thorium exchange reaction is occurred in the bioaccumulation from the acidic solution containing uranium and thorium.

The separation factors as Th/U using gram-positive bacteria, which have high thorium accumulate ability, such as, *M. luteus*, *A. nicotianae*, *B. subtilis* and *B. megaterium* are far higher than those using actinomycetes, such as *S. levoris* and *S. albus*. Therefore, these gram-positive bacterial strains can be used for the selective bioaccumulation of thorium. On the other hand, these actinomycetes strains can be used for the accumulation of uranium after removed thorium.

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