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Articles

Accumulation of Thorium and Uranium by Microbes – the Effect of pH, Concentration of Metals, and Time Course on the Accumulation of Both Elements Using *Streptomyces levoris*

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The accumulation of thorium and uranium by various microorganisms from a solution containing both metals at pH 3.5 was examined. Among the tested species, a high accumulation ability for thorium was exhibited by strains of gram-positive bacteria, such as *Arthrobacter nicotianae, Bacillus megaterium, B. subtilis, Micrococcus luteus*, *Rhodococcus erythropolis*, and *Streptomyces levoris*. Though uranium was accumulated in small amounts by most of microorganisms, *A. nicotianae*, *S. flavoviridis,* and *S. levoris* had relatively high uranium accumulation abilities. In these high performance thorium- and uranium-accumulating microorganisms, *S. levoris*, which accumulated the largest amount of uranium from the solution containing only uranium at pH 3.5, accumulated about 300 μmol thorium and 133 μmol uranium per gram dry weight of microbial cells from a solution containing both thorium and uranium at pH 3.5. The amount and time course of the thorium accumulation were almost unaffected by the co-existing uranium, while those of uranium were strongly affected by the co-existing thorium. The effects of pH, the thorium and uranium concentrations, and time course on both metal accumulations were also evaluated by numerical formulas.

1. Introduction

The removal of radionuclides, such as uranium (U) and thorium (Th) from aqueous solutions, especially from contaminated sources, seems to be a significantly useful subject for exploiting unused or abandoned energy resources. In this regard, efforts have been especially concentrated on studying the accumulation of U using microorganisms, such as the actinomycetes,[1–3] bacteria,[4–8] fungi,[9–13] and yeasts.[7, 14]

Thorium accumulation has also been investigated by some researchers. Tzezos and Volesky[12, 15] reported the biosorption of U and Th by some microorganisms and the mechanism of Th biosorption by *Rhizopus arrhizus*. White and Gadds[13] reported the biosorption of Th by fungal biomass. Andres et al[4] reported the adsorption of Th and U by *Mycobacterium smegmatis*.

Recently, various species and strains of actinomycetes, bacteria, fungi, and yeasts were screened for their abilities to accumulate U or Th from a solution containing only each metal at pH 3.5.[16, 17] Among the microorganisms, a high Th accumulating ability was exhibited by the gram-positive bacterial strains, especially *Arthrobacter nicotianae* IAM12342, *Bacilles subtilis* IAM1026, and *Micrococcus luteus* IAM1056. The amount of Th accumulated by the gram-positive bacteria was greater than those by actinomycetes, gram-negative bacteria, fungi, and yeasts. On the other hand, high ability to accumulate U was found in some actinomycetes strains, such as *S. albus* HUT6047 and *S. levoris* HUT6156. The amount of U accumulated by actinomycetes was higher than those by most of the other gram-positive bacteria, gram-negative bacteria, fungi, and yeasts. The effect of Th (or U) addition on the U (or Th) accumulation rate and the amount of accumulated U (or Th) using *S. levoris* cells was also investigated.[16, 17] Thorium strongly affected the U accumulation, whereas U had only a very slight affect on the Th accumulation. Additionally, the U-Th exchange reaction occurred in the solution containing an excess amount of U.

In this study, various species and strains of actinomycetes, bacteria, fungi, and yeasts were screened for their ability to accumulate Th and U from a solution containing the same amount of both elements at pH 3.5. The ability to accumulate Th and U, the effect of pH on the accumulation, and the time course of the Th and U accumulation were also investigated by *S levoris* cells, which accumulated the largest amount of U from an aqueous solution containing only U at pH 3.5.[16] Furthermore, an attempt was made to evaluate these effects by numerical formulas.

2. Experimental

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

2.2. Culture of microorganisms. The medium for growing the bacteria (except actinomycetes) consisted of 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 at 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. Uranium and Th accumulation experiments. The U and Th ions were supplied as $Th(NO_3)_4$ and $UO_2(NO_3)_2$. The pH of the solution was adjusted to the desired values with 0.1 M HNO_3 or 0.1 M NaOH. Unless otherwise stated, the accumulation experiments were conducted as follows. The resting microorganisms (15 mg dry weight (wt.) basis) were suspended in 100 mL solution at pH 3.5 containing each 50 μM Th and U,

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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 U and Th accumulated by the microorganisms was determined by measuring their contents in the filtrate using an inductively coupled plasma quantometer (ICPS8000, Shimadzu Corporation, Kyoto). Uranium and Th were not adsorbed on the filter and the wall of the vessel under this experimental condition.

2.3.1. Effect of Th and U concentrations on both metal accumulation. The effect of external Th and U concentrations on both metal accumulation was examined as follows. The resting cells of *S. levoris* (15 mg dry wt. basis) were suspended in 100 mL of solution at pH 3.5 containing the desired amounts of Th and U (10–200 μM), and their suspensions were shaken for 1 h at room temperature.

2.3.2. Time course of Th and U accumulations on both metal accumulation. The resting cells of *S. levoris* (15 mg dry wt. basis) were suspended in 100 mL of solution at pH 3.5 containing each 50 μM of Th and U, and their suspensions were shaken for the desired time at room temperature. Separation of the cells from the solution and the analysis of the metals content are the same as those mentioned above.

3. Results and discussion

3.1. Accumulation of Th and U by microorganisms from a solution containing both metals. The amounts of accumulated Th and U by 41 strains of 37 species (9 actinomycetes, 12 bacteria, 11 fungi, and 9 yeasts) are summarized in Tables 1–4. A preliminary experiment showed that the amount of accumulated Th and U using these microorganisms increased with increasing pH of the solution; however, thorium hydroxide was precipitated at pH 4.0.[12] Therefore, the pH of the solution was adjusted to 3.5 throughout the screening. Among the tested microorganisms, a high Th accumulation ability was exhibited by certain gram-positive bacterial strains, notably *A. nicotianae* IAM12342, *Bacillus megaterium* IAM1166, *B. subtilis* IAM1026, *Micrococcus luteus* IAM1056, *Rhodococcus erythlopolis* IAM1399, and *Streptomyces levoris* HUT6156. On the other hand, though U was accumulated in small amounts by all these microorganisms, *A. nicotianae* IAM12342, *S. flavoviridis* HUT6147, and *S. levoris* HUT6156 had relatively high U accumulation abilities.

The relative degree of Th accumulation by the microorganisms was observed in the following order: gram-positive bacteria (except actinomycetes) > actinomycetes > gram-negative bactera, fungi, and yeasts, indicating that gram-positive bacteria can accumulate Th more readily than the other types of microorganisms. As shown in Table 5, there were significant differences. On the other hand, half of the actinomycetes species accumulated U much more than most of the gram-positive bacteria species (except *A. nicotianae*). There were not significant differences between gram-positive and gram-negative bacteria in the amount of accumulated U from the solution containing both elements. Additionally, all of the microorga-

TABLE 1: Accumulation of Th and U from the Solution Containing Th and U by Actinomycete

Species and strains	Metals accumulated (μmol/g dry wt. cells) Th	U
Streptomyces albogriseolus HUT6045	241±3	23±2
S. albus HUT6047	281±3	93±1
S. flavoviridis HUT6147	290±1	145±4
S. fradiae HUT6054	202±4	54±8
S. griseoflavus HUT6153	183±1	69±4
S. levoris HUT6156	297±7	133±0
S. olivaceus HUT6061	216±1	89±3
S. scabies HUT6027	215±4	95±3
S. viridochromogenes HUT6030	241±2	75±2

Resting cells (15 mg dry wt. basis) were suspended in 100 mL of solution (pH 3.5) containing 50 μM of Th and U for 1 h at room temperature.

TABLE 2: Accumulation of Th and U from the Solution Containing Th and U by Bacteria

Species and strains	Metals accumulated (μmol/g dry wt. cells) Th	U	Gram type
Arthrobacter nicotianae IAM12342	339±1	142±5	+
Bacillus licheniformis IAM11054	294±1	22±2	+
B. megaterium IAM1166	314±2	28±3	+
B. subtilis IAM1026	295±4	12±5	+
Escherichia coli IAM1268	135±0	15±3	–
Micrococcus luteus IAM1056	346±1	50±1	+
Rhodococcus erythropolis IAM1399	316±3	63±2	+
Pseudomonas aeruginosa IAM1054	135±4	40±2	–
P. fluorescens IAM12022	153±3	26±3	+
P. saccharophilia IAM1504	143±1	13±4	+
P. stutzeri IAM12097	183±1	72±4	+
Starkeya novela IAM 12100	89±2	37±1	+

See footnote of Table 1.

TABLE 3: Accumulation of Th and U from the Solution Containing Th and U by Fungi

Species and strains	Metals accumulated (μmol/g dry wt. cells) Th	U
Aspergillus niger IAM2086	81±6	0±0
Giberella fujikuroi AHU9078	95±5	15±3
Mucor hiemalis IAM6088	91±5	74±2
Neurospora sitophira AHU9213	182±5	40±1
N. sitophira IAM5502	110±3	25±2
Penicillium chrysogenum IAM7114	88±2	2±1
P. lilacinum AHU8357	57±3	25±2
P. notatum IAM7168	128±2	5±4
Rhizopus arrhizus AHU6573	115±3	36±0
R. oryzae IAM6006	125±0	31±2
Trichoderma viride AHU9503	76±3	14±4

See footnote of Table 1.

TABLE 4: Accumulation of Th and U from the Solution Containing Th and U by Yeasts

Species and strains	Metals accumulated (μmol/g dry wt. cells) Th	U
Candida utilis AHU3210	53±1	12±2
C. utilis IAM4220	50±1	5±2
Cryptococcus laurentii AHU3671	104±4	22±4
C. laurentii IAM12264	137±2	8±6
Debaryomyces hansenii AHU3759	124±1	30±2
Endomycopsis fibligera AHU4113	77±4	1±1
Hansenula anomala AHU3702	65±1	10±3
Saccharomyces serevisiae AHU3818	87±3	11±1
S. serevisiae IAM4512	24±2	7±3

See footnote of Table 1.

TABLE 5: T-authorization of the Difference in the Average Amount of Each Metal Accumulated by Various Microorganisms from the Solution Containing Both Metals

Group of microorganisms	Amounts of thorium versus				Group of microorganisms	Amounts of uranium versus			
	GPB	A	GNB	F		A	GPB	GNB	F
A	****				GPB	–			
GNB	****	****			GNB	****	–		
F	****	****	**		F	****	–	–	
Y	****	****	****	–	Y	****	****	****	*

See footnote of Table 1.

Symbols: **** There are significant differences at a dangerous rate of 1%.
*** Dangerous rates 2%, ** 5%, * 10%.
– There are no significant differences (at dangerous rates of 10%).
GPB: gram-positive bacteria, A: actinomycetes, GNB: gram-negative bacteria, F: fungi, Y: yeasts.

nisms accumulated greater amounts of Th than U under these experimental conditions.

Generally, the teichoic acid polymers in gram-positive bacteria definitely confer a strong negative charge on the surface of the cell wall because of their high content of ionized phosphate groups, although little if any teichoic acid is found in the gram-negative bacteria.[18] It is tentatively considered that the chelate formation between the cell surface of gram-positive bacteria and Th ions becomes stronger than that of the gram-negative bacteria and Th ion. Consequently, it is reasonable that the amount of Th accumulated by the gram-positive bacteria is greater than that of the gram-negative bacteria. It is tentatively considered that the chelate formation between the cell surface of gram-positive bacteria and Th ion becomes stronger than that of the gram-negative bacteria and Th ion. Consequently, it is reasonable that the amount of Th accumulated by the gram-positive bacteria is greater than that of the gram-negative bacteria.

In the case of the amount of accumulated U from the solution containing both metals, the effect of the kind of species on U accumulation was not clear, because the effect of Th was far larger.

3.2. Effect of pH on accumulation of Th and U by *S. levoris* cells. Recently, the effect of pH on the U accumulation from a solution containing only U using *S. levoris* was reported.[16] Though the amount of U accumulated gradually increased with increasing pH of the solution under pH 2.00 and above pH 2.50, it rapidly increased with increasing pH of the solution between pH 2.00–2.50. On the other hand, the amount of Th accumulated from the solution containing only Th rapidly increased with increasing pH of the solution under pH 2.00, whereas it gradually increased with increasing pH of the solution between pH 2.00–3.25.[18]

The amounts of Th (squares) and U (circles) accumulated by the *S. levoris* cells from the mixed solution containing the same molar amount of each metal linearly increased with increasing pH of the solution (Figure 1). The amount of accumulated Th (Q_{Th}) (μmol/g dry wt. cells) was well described by $Q_{Th}=84.0 \times pH + 31.9$, and that of accumulated uranium (Q_U) (μmol/g dry wt. cells) was well described by $Q_U=44.5 \times pH - 3.88$. The amount of accumulated Th was far greater than that of U at all pHs from 1.00 to 3.75. As a result, the amounts of Th and U accumulated from the solution containing each metal[16, 17] and the amount of Th accumulated from the mixed solution containing both metals were very similar. However, the amount of U from the mixed solution containing both metals was far lower than the amounts of Th and U accumulated from the solution containing each metal only[16, 17] and the amount of Th accumulated from the mixed solution containing both metals.

3.3. Dependence of Th and U concentrations on accumulation by *S. levoris* cells. As shown in Figure 2-a), the amount of Th accumulated from the mixed solution containing the same molar amounts of both Th and U by the *S. levoris* cells (μmol/g dry wt. cells) increased with increasing Th concentration. When the Th and U concentration was 150 μM (residual concentration of Th was 93.5 μM and that of U was 141 μM), a high Th accumulation of about 365 μmol of Th/g dry wt. cells was observed.

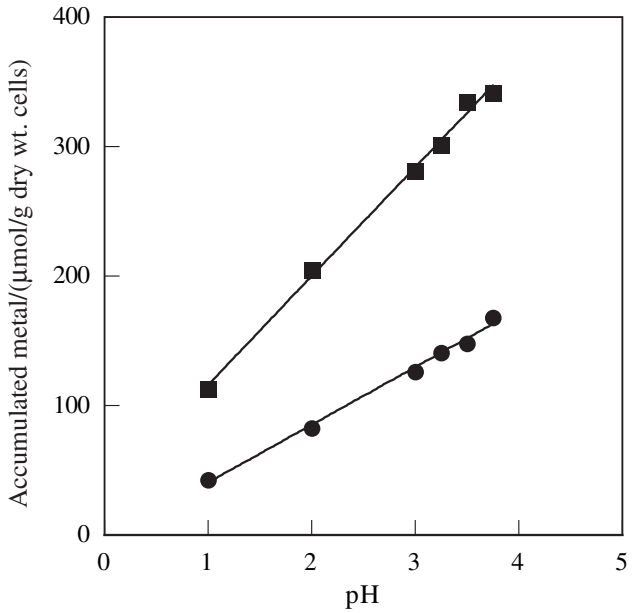

Figure 1. Effect of pH on Th and U accumulation by *S. levoris* cells. Resting cells (15 mg dry wt. basis) were suspended in a 100 mL of solution containing 50 μM Th (squares) and U (circles) for 1 h at room temperature.

Figures 2-a) and b) indicate that the accumulation of Th from the mixed solution containing Th and U by the *S. levoris* cells does not obey the Langmuir isotherm over the entire concentration region (dotted line). However, the relationship between the amount of accumulated Th (μmol/g dry wt. cells) and the residual Th concentration from 0 to 10.0 μM (as the initial thorium concentration from 0 to 51.3 μM), and from 10.0 to 93.5 μM (as the initial thorium ion concentration at 51.3 to 150 μM) obeys different Langmuir isotherms (solid line) respectively, which depend only on the residual Th concentration. The curve in Figure 2-a) indicates the values calculated from each Langmuir isotherm, $C_e(Th)/Q_{Th}= m_{Th}C_e(Th)+n_{Th}$, where Q_{Th} indicates that the amount of accumulated Th (μmol Th/g dry wt. cells), $C_e(Th)$ is the residual Th concentration in the solution (μM) and m_{Th} and n_{Th} are the Langmuir constants. Accordingly, the result obtained in this experiment has dual patterns. When the initial Th concentration is varied from 0 to 51.3 μM (as 0 to 10.0 μM of residual Th concentration), the amount of accumulated Th is shown on the left side in Figure 2-a). When the initial Th concentration increased from 51.3 to

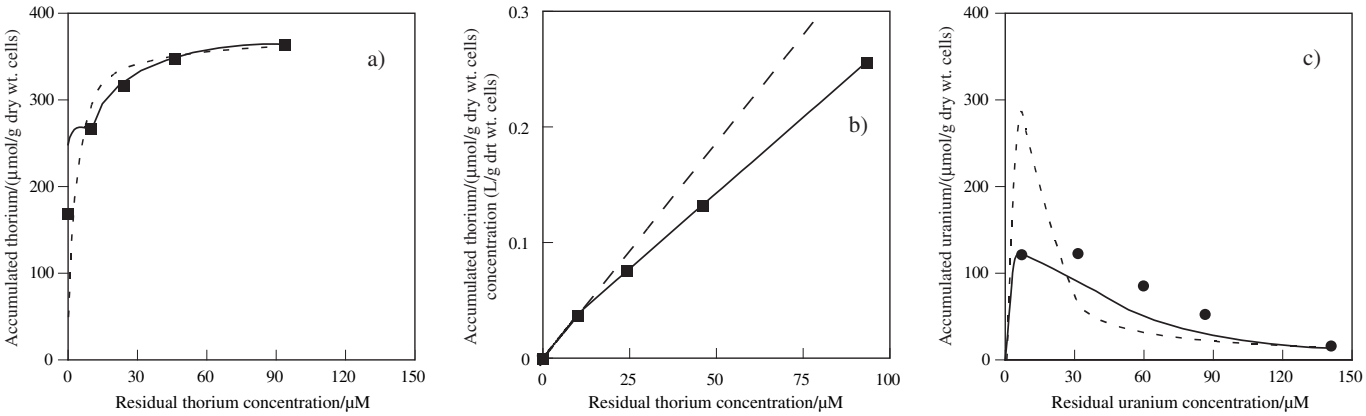

Figure 2. Effect of metal concentrations on Th (closed squares) and U (closed circles) accumulation by *S. levoris* cells. Resting cells (15 mg dry wt. basis) were suspended in 100 mL of solution (pH 3.5) containing the desired same molar amounts of Th and U for 1 h at room temperature. Symbols indicate the amount of accumulated Th (squares) and U (circles) calculated by the Langmuir equations using entire concentration data (dotted line) or using data from two separate concentrations (solid line).

150 μM (as 10.0 to 93.5 μM of residual Th concentration), the amount of accumulated Th is shown on the right side of Figure 2-a). A similar result was obtained[19] from the absorption of potassium using barley roots. This fact seems to indicate that Th accumulation using *S. levoris* cells is not a monolayer accumulation but a dual-layer accumulation. The constants m_{Th}, n_{Th}, and the maximum amount of accumulated Th Q_{max}(Th) that was used to estimate $1/m_{Th}$ are summarized in Table 6. As a result, the amount of accumulated Th was almost unaffected by the accumulation of U by the *S. levoris* cells.

TABLE 6: Estimated Constants and Q_{max}(Th) from the Langmuir Isotherm

Initial Th concentration (μM)	m_{Th}	n_{Th}	Q_{max}(Th) (μmol/g dry wt. cells)
0–50	3.74×10^{-3}	5.69×10^{-5}	267
50–150	2.61×10^{-3}	1.19×10^{-2}	382
0–150	2.69×10^{-3}	7.01×10^{-3}	372

See footnote of Figure 2.

It was reported that the accumulation of U from the solution containing only U by the *S. levoris* cells obeys the Langmuir isotherm, depending on the U concentration.[16] The amount of U (μmol/g dry wt. cells) accumulated from the mixed solution containing the same molar amount of Th and U is shown in Figure 2-c). As shown in this figure, the amount of U accumulated was far lower than that from the solution containing only U[16] due to the presence of Th. The amount of U increased with increasing initial U and Th concentrations from 0 to 50 μM (the residual concentration of U was from 0 to 31.3 and that of Th was 0 to 10 μM). On the other hand, the amount of U decreased with increasing initial U and Th concentrations from 50 to 150 μM (residual concentration of U was from 31.3 to 141 μM and that of Th was 10 to 93.5 μM).

It was reported that the accumulated U was easily desorbed by the co-existing Th.[16] Therefore, the amount of accumulated U from the mixed solution containing the same molar amounts of U and Th was assumed to be the value, (the amount of accumulated U from the solution containing only U) – (the amount of accumulated Th from the mixed solution containing U and Th). Accordingly, $Q_U = C_e(U)/(m_U \cdot C_e(U) + n_U) - C_e(Th)/(m_{Th} \cdot C_e(Th) + n_{Th})$, where Q_U indicates the amount of U accumulated (μmol U/g dry wt. cells) from the mixed solution containing U and Th, $C_e(U)$ is the residual U concentration in the solution containing both metals (μM), $C_e(Th)$ is the residual Th concentration in the solution containing both metals (μM), and m_U and n_U are the Langmuir constants calculated from the solution containing only U[16]; m_{Th} and n_{Th} are the Langmuir constants mentioned in the previous section. The calculated amount of accumulated U based on the entire concentration data (dotted line) was much higher from the experimental data especially for low concentration regions. On the other hand, though the value of U accumulated based on this equation using two separated concentration regions (solid line) was slightly lower than the experimental data, both values are relatively similar. Therefore, it seems almost reasonable that the amount of U accumulated from the mixed solution containing the same molar amounts of U and Th can be calculated from the difference in the amount of U accumulated from the solution containing only U and that of Th accumulated from the mixed solution containing both elements. Based on the result in the literature[16] and the evaluated and experimental data, it was tentatively assumed that the 1:1 U–Th ion exchange reaction occurred from the solution containing both elements using *S. levoris* cells.

3.4. Time course of Th and U accumulations from the solution containing both metals. In order to solve the time course of the Th and U accumulations, the relationships of the amounts of accumulated Th and U and the retention time were analyzed.

The accumulation rate of Th using *S. levoris* is considered to be the difference in the real adsorption rate and the desorption rate.[20] The time course of the Th accumulation was almost unaffected by the co-existing U.[16] Therefore, the real accumulation rate of Th is considered to be proportional to the difference in the maximal amounts of the total metal accumulated and the amount of Th accumulated at a certain time. The amount of accumulated U is ignored in this consideration. On the other hand, the real desorption rate is considered to be proportional to the amount of Th accumulated at the same time. Accordingly,

$$dQ_{Th}/dt = k_{1Th}(Q_{maxTotal} - Q_{Th}) - k_{2Th} Q_{Th}.$$

At equilibrium, $dQ_{Th}/dt = 0$ and $Q_{Th} = Q_e(Th)$,

$$Q_{maxTotal} = (k_{1Th} + k_{2Th}) Q_e(Th)/ k_{1Th}.$$

$$dQ_{Th}/dt = (k_{1Th} + k_{2Th}) Q_e(Th) - k_{1Th} Q_{Th} - k_{2Th} Q_{Th} = (k_{1Th} + k_{2Th}) \{Q_e(Th) - Q_{Th}\}.$$

$$Q_{Th} = Q_e(Th)[1 - \exp\{-(k_{1Th} + k_{2Th}) t\}].$$

As $Q_{Th}/Q_e(Th) = \theta_{Th}$, $1 - \theta_{Th} = \exp\{-(k_{1Th} + k_{2Th})t\}$,

$$\ln\{1/(1 - \theta_{Th})\} = (k_{1Th} + k_{2Th})t.$$

Q_{Th} : amount of Th adsorbed (μmol/g dry wt. cells)
k_{1Th} : real adsorption rate constant of Th
$Q_{maxTotal}$: the maximum total amounts of Th and U adsorbed (μmol/g dry wt. cells)
k_{2Th} : real desorption rate constant of Th
Q_{eTh} : amount of Th adsorbed (μmol/g dry wt. cells) at equilibrium

From the equation, one can see that $\ln\{1/(1-\theta_{Th})\}$ is proportional to the adsorbing time.

As shown in Figure 3, the relationship of $\ln\{1/(1-\theta_{Th})\}$ and t showed a good linearity; however, the line did not fit the original point but was fitted to another line under and over 60 min. The accumulation was very fast after contact between the metals and the microbes. Furthermore, most of the metals were accumulated within 1 h; therefore, the accumulation rate after 1h became slower. Accordingly, in the case of within 60 min, $\ln\{1/(1-\theta_{Th})\}$ was estimated to be $1.88 \times 10^{-2} t + 0.444$. On the other hand, in the case of the time from 60 to 180 min, that was estimated to be $7.95 \times 10^{-3} t + 1.14$.

As shown in Figure 4, the accumulated Th was in good agreement with the calculated curve, and the amount of accumulated Th at equilibrium was estimated to be 353 μmol/g dry wt. cells. Therefore, the accumulation rate of Th from the solution containing Th and U using the *S. levoris* cells was almost described by the amount of accumulated Th only. Accordingly, in the case of within 60 min, the amount of accumulated Th, Q_{Th}, was estimated to be $353\{1-\exp(-1.88 \times 10^{-2} t - 0.444)\}$. On the other hand, in the case of the time from 60 to 180 min, that was estimated to be $353\{1-\exp(-7.95 \times 10^{-3} t - 1.14)\}$.

The time course of U accumulation using *S. levoris* was strongly affected by the co-existing Th. Therefore, the real accumulation rate of uranium is considered to be proportional to the difference in the maximal amounts of the total accumulated metal and the amounts of Th and U accumulated at a certain time. The real desorption rate of U is considered to be proportional to the amount of Th and U accumulated at the same time.

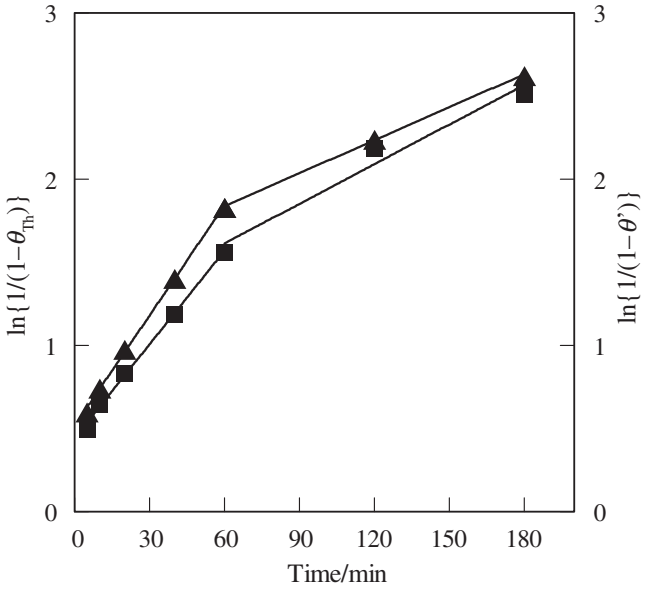

Figure 3. Parameter determination for the time course of Th and U accumulation by *S. levoris*. Resting cells (15 mg dry wt. basis) were suspended in 100 mL of solution (pH 3.5) containing 50 μM of Th and U at room temperature. Square and triangle symbols indicate the $\ln\{1/(1-\theta_{Th})\}$ and $\ln\{\{1/(1-\theta')\}$, respectively. θ_{Th} expresses the ratio of accumulated Th at a time and that at equilibrium, Q_{Th}/Q_{eTh}, θ' expresses the ratio of the amount of accumulated Th and U at a time and that at equilibrium, $(Q_{Th}+Q_U)/(Q_{eTh}+Q_{eTh})$.

Accordingly,

$$dQ_U/dt = k_{1U}(Q_{maxTotal} - Q_U - Q_{Th}) - k_{2U}(Q_U + Q_{Th}\}.$$

At equilibrium, $dQ_U/dt = 0$, $Q_U = Q_e(U)$, and $Q_{Th} = Q_e(Th)$,

$$Q_{maxTotal} = (k_{1U} + k_{2U})\{Q_e(U) + Q_e(Th)\}/k_{1U}.$$

$$\therefore dQ_U/dt = (k_{1U} + k_{2U})\{Q_e(U) + Q_e(Th)\} - (k_{1U} + k_{2U})(Q_U + Q_{Th})$$
$$= (k_{1U} + k_{2U})\{Q_e(U) + Q_e(Th) - Q_U - Q_{Th}\}.$$

$$\int[1/\{Q_e(U) + Q_e(Th) - Q_U - Q_{Th}\}]dQ_U = \int(k_{1U} + k_{2U})dt.$$

Because the amount of Th accumulated was unaffected by the amount of co-existing U,

$$-\ln\{Q_e(U) + Q_e(Th) - Q_U - Q_{Th}\} = (k_{1U} + k_{2U})t + \text{Const.}$$

When $t = 0$, as $Q_U = Q_{Th} = 0$ and $\text{Const} = -\ln\{Q_e(U) + Q_e(Th)\}$,

therefore,

$$-\ln\{Q_e(U) + Q_e(Th) - Q_U - Q_{Th}\} = (k_{1U} + k_{2U})t - \ln\{Q_e(U) + Q_e(Th)\}.$$

$$\ln[\{Q_e(U) + Q_e(Th)\}/\{Q_e(U) + Q_e(Th) - Q_U - Q_{Th}\}] = (k_{1U} + k_{2U})t.$$

$$\ln(1/[1-(Q_U + Q_{Th})/\{Q_e(U) + Q_e(Th)\}]) = (k_{1U} + k_{2U})t.$$

As $(Q_U+Q_{Th})/\{Q_e(U) + Q_e(Th)\} = \theta'$,

$$\ln\{1/(1-\theta')\} = (k_{1U}+k_{2U})t.$$

Q_U : amount of U adsorbed (μmol/g dry wt. cells)
k_{1U} : real adsorption rate constant of U
k_{2U} : real desorption rate constant of U
$Q_e(U)$: amount of U adsorbed (μmol/g dry wt. cells) at equilibrium

From the equation, one can see that $\ln\{1/(1-\theta')\}$ is proportional to the adsorbing time.

As shown in Figure 3, the relationship of $\ln\{1/(1-\theta')\}$ and t also showed a good linearity; however, the line also did not fit the original point but was fitted to another line under and over 60 min. The accumulation was very fast after contact between the metals and the microbes. Furthermore, most of the metals were also accumulated within 1 h; therefore, the accumulation rate after 1h became slower. Accordingly, in the case of within 60 min, $\ln\{\{1/(1-\theta')\}$ was estimated to be $2.21 \times 10^{-2} t + 0.517$. On the other hand, in the case of the time from 60 to 180 min, that was estimated to be $6.62 \times 10^{-3} t + 1.14$.

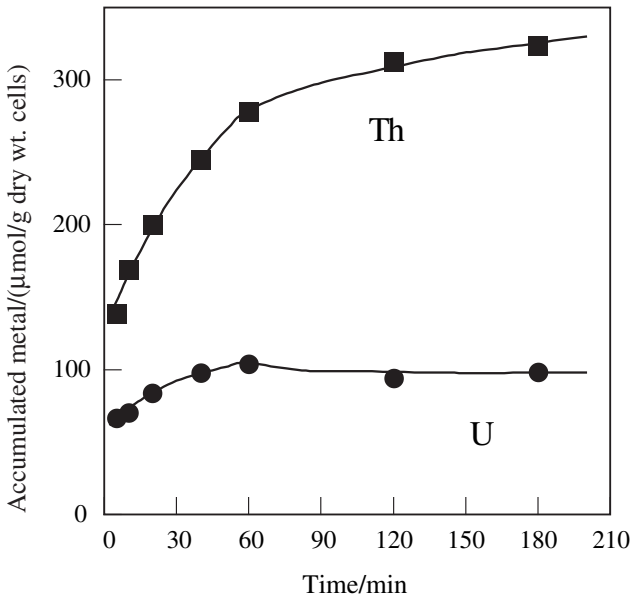

Figure 4. Time course of Th and U accumulation from the solution containing both metals by *S. levoris* cells. The experimental conditions were the same as in Figure 3. Symbols are the same as in Figure 1.

As shown in Figure 4, the accumulated U was in good agreement with the calculated curve, and the amount of accumulated U at equilibrium was estimated to be 104 μmol/g dry wt. cells. Therefore, the accumulation rate of U from the solution containing Th and U using the *S. levoris* cells was described by the amount of total accumulated Th and U. Accordingly, in the case of within 60 min, the amount of accumulated U, Q_U, was estimated to be $104-457\exp(-2.21\times10^{-2}\,t-0.517)+353\exp(-1.88\times10^{-2}\,t-0.444)$. On the other hand, in the case of the time from 60 to 180 min, that was estimated to be $104-457\exp(-6.62\times10^{-3}\,t-1.44)+353\exp(-7.95\times10^{-3}\,t-1.14)$.

4. Conclusions

Among the species tested, a high Th accumulation ability was exhibited by strains of gram-positive bacteria, such as *Arthrobacter nicotianae, Bacillus megaterium, B. subtilis, Micrococcus luteus*, *Rhodococcus erythropolis*, and *Streptomyces levoris*. Though U was accumulated in small amounts by all these microorganisms, *A. nicotianae* IAM12342, *S. flavoviridis* HUT6147, and *S. levoris* HUT6156 had relatively high uranium accumulation abilities. In these high performance metal-accumulating microorganisms, *S. levoris* could accumulate about 300 μmol Th and 133 μmol U per gram dry wt. of microbial cells from a solution containing both Th and U at pH 3.5. The accumulated amounts of both elements linearly increased with the increasing pH of the solution. The accumulated amount of Th was increased with increasing Th concentration; however, it was almost unaffected by the co-existing U. On the other hand, the accumulated amount of U is strongly affected by the co-exsisting Th, and it can be evaluated by the U–Th ion exchange mechanism. The time course of accumulated Th was also unaffected by co-exsisting U and that of U was strongly affected by co-existing Th.

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