Journal of Nuclear and Radiochemical Sciences, Vol. 6, No.1, pp. 81-84, 2005

Separation of Rare Earth Elements by Microorganisms

Takehiko Tsuruta*

Department of Applied Chemistry, Tohwa University, 1-1-1 Chikushigaoka, Fukuoka 815-8510, Japan

Received: November 15, 2004; In Final Form: April 21, 2005

The selective accumulation of rare earth elements in Gram-positive bacteria and actinomycetes was examined. The resting cells of 18 strains having high capacities to accumulate rare earth elements were screened for selectivity using a solution containing 5 elements: Y, La, Sm, Er, and Lu. Among the strains tested, *Bacillus megaterium* accumulated Sm, *Streptomyces albus* accumulated Lu, and *Arthrobacter nicotianae* accumulated both Sm and Lu in higher quantities than the other metals. Similar results were also obtained from a solution containing Y and 14 rare earth elements (La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu). The amount of Sm accumulated in *B. megaterium* and that of Lu accumulated in *S. albus* increased as the initial metal concentration increased. *S. albus* and *B. megaterium* cells show the highest capacity to accumulate Lu and Sm, respectively, from a solution containing 5 elements, Y, La, Sm, Er, and Lu when each metal concentration ranged from 20 to 100 μM. These results suggest that the separation of these two rare earth elements using microorganisms should be possible.

1. Introduction

The separation of rare earth elements from solutions containing many kinds of rare earth elements is difficult because of their very similar chemical properties.[1] Solvent extraction methods performed for the separation of rare earth elements pose environmental problems because a large amount of organic solvent is inevitably used. As a consequence, many researchers have been studying the removal of uranium using microorganisms such as actinomycetes,[2-5] bacteria,[3, 6–10] fungi,[3, 11–15] and yeasts.[3, 7, 16]

The adsorption of several actinides (thorium and uranium) and lanthanides (lanthanum, europium, and ytterbium) by *Mycobacterium smegmatis* has been investigated; however, the amount of adsorbed lanthanide on cells was low and there was almost no difference in the amounts of different lanthanides extracted.[10] Recently, it was reported that Gram-negative bacteria, such as *Variovorax paradoxus,* reduced the amount of light rare earth elements such as Y, La, Ce, and Nd, more than that of the other rare earth elements from solutions containing all of the lanthanides.[17] It was also reported that *Streptomyces* sp. decreased the amount of Yb.[18]

We have investigated the accumulation of rare earth elements using various microorganisms from solutions containing only one kind of rare earth element (not published). The amounts of rare earth elements accumulated by Gram-positive bacteria were much higher than those by Gram-negative bacteria, fungi, and yeasts. Most species of the Gram-positive bacteria have higher rare-earth-element accumulating abilities than actinomycetes. However, actinomycetes and other Gram-positive bacteria showed different accumulating features in the case of the uranium.[3, 19] The amounts of uranium accumulated at pH 5.8 using Gram-positive bacteria such as *Arthrobacter nicotianae* IAM12342, *Bacillus subtilis* IAM1024, and *Micrococcus luteus* IAM1056, were higher than those using all the actinomycetes strains tested (20 strains).[3] On the other hand, the amounts of uranium accumulated at pH 3.5 using half strains of actinomycetes tested were higher than those using all the Gram-positive bacteria except *A. nicotianae*.[19] Therefore, actinomycetes and other Gram-positive bacteria are expected to have different features for accumulating rare earth elements from solutions containing many kinds of rare earth elements. Accordingly, in order to clarify the possibility of separating rare earth elements, actinomycetes and other kinds of gram-positive bacteria that have a high accumulating ability for rare earth elements were examined in this study. First, the screening of microorganisms for the selective accumulation of Y, La, Sm, Er, and Lu from a solution containing these 5 kinds of metals was examined using 18 kinds of strains. After that, the accumulation pattern of Y and all lanthanides elements and the effect of the concentration of rare earth elements on the accumulation of these elements were studied using strains that showed both high accumulating ability and selectivity.

2. Experimental

Materials. The strains used in this study 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); and the Faculty of Engineering, Hiroshima University (HUT). The chemicals (guaranteed reagents) were obtained from Nacalai Tesque, Inc., Kyoto.

Culture conditions of microorganisms. The medium for growing bacteria contained 3 g/L meat extract, 5 g/L peptone, and 5 g/L NaCl in deionized water. The medium for growing actinomycetes contained 4 g/L yeast extract, 10 g/L malt extract, and 4 g/L glucose in deionized water, pH 7.1. 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) or by filtration through filter paper (for actionomycetes) and washed thoroughly with deionized water for use in the accumulation experiments.

Metal accumulation experiments

Screening of microorganisms for selective accumulation of Y, La, Sm, Er, and Lu. In order to determine which types of microorganisms have greater separation ability, 18 strains of 15 species (9 actinomycetes, 9 other kinds of Gram-positive bacteria) were examined. The metal was supplied as $Ln(NO_3)_3$. The accumulation experiments were conducted as follows. Resting microorganisms (15 mg dry wt. basis) were

*Corresponding author. E-mail: ttsuruta@tohwa-u.ac.jp. FAX: +81-92-552-2707.

Published on Web 07/31/2005

suspended in a 100 mL solution (pH 5.0) containing 66.5 μM of Y, La, Sm, Er, and Lu, and the suspension was shaken for 3 h at room temperature. The pH of the solution was adjusted to 5.0 with 0.1 M NaOH because the precipitation of hydroxide from NaOH occurred at pH 6.0, while, in a solution containing only Sm, the amount of accumulated Sm increased with increasing pH (data not shown). The microorganisms were then collected by filtration through a membrane filter (pore size 0.2 mm). 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).

Selective accumulation of Y and rare earth elements in microorganisms. In order to determine the selectivity of Sm and/or Lu accumulation, the selective accumulation of Sm and/or Lu was tested in *S. albus, B. megaterium*, and *A. nicotianae*. The resting cells (15.0 mg dry wt. basis) were suspended in 100 mL of the solution (pH 5.0) containing 66.5 each μM of Y and all the lanthanides elements for 3h at room temperature.

Rare-earth-element accumulation capacity of microorganisms. In order to determine the Lu accumulation capacity of *S. albus* and the Sm accumulation capacity of *B. megaterium* cells, the cell-specific accumulation effect of each equilibrium metal concentration in solutions containing only that metal was examined. Resting cells (15.0 mg dry wt. basis) were suspended in 100 mL of the solution (pH 5.0) containing the desired amounts of Sm or Lu for 3h at room temperature.

Effect of rare-earth-element concentrations on selective accumulation in microorganisms. In order to determine the effect of the initial concentration of the rare earth elements on their accumulation in *S. albus* and *B. megaterium* cells, the accumulation from a solution containing equivalent molar amounts of Y, La, Sm, Er, and Lu were examined. Resting cells (15.0 mg dry wt. basis) were suspended in 100 mL of the solution (pH 5.0) containing the desired amounts of each metal for 3h at room temperature.

3. Results and Discussion

Screening of microorganisms for selective accumulation of Y, La, Sm, Er, and Lu using various microorganisms. The amounts of total accumulated metals in some Gram-positive bacteria and actinomycetes, such as *A. nicotianae*, *B. subtilis* IAM11060, *S. albus*, and *S. levers*, were higher than those in others (Table 1). Among these, the strains *S. albus* and *S. levers* had higher accumulating abilities for Lu than for Y, La, Sm, and Er. These strains can accumulate Lu at about 150 μmol/g dry wt. cells. On the other hand, in *A. nicotianae*, the amounts of accumulated Sm and Lu were higher than the amounts of Y, La, and Er. This strain can accumulate Sm and Lu about 150 μmol/g dry wt. cells. The amount of Sm accumulated in *B. megaterium* was higher than the amounts of Y, La, Er, and Lu, though the total amount of the rare earth elements accumulated in this cell was lower than that in *A. nicotianae, B. subtilis, S. albus,* and *S. levoris*.

The results of these experiments are quite different from those of Reference 17. Gram-negative bacteria, such as *Variovorax paradoxus* and *Comamonas acidovorans*, show a higher capacity to accumulate light rare earth elements, such as Y, La, and Nd than to accumulate heavy rare earth elements.[17]

Selective accumulation of Y and rare earth elements in microorganisms. As shown in Figure 1-(A), in *S. albus*, Lu was accumulated at higher amounts than other elements while in *B. megaterium,* Sm had the highest accumulation (Figure 1-(B)). Furthermore, as shown in Figure 1-(C), the amounts of Sm and Lu accumulated in *A. nicotianae* were higher than that of Y and other rare earth elements. Based on the results of these experiments, Sm and/or Lu can be selectively accumu-

TABLE 1: Accumulation of Rare Earth Elements Using Various Microorganisms from the Solution Containing Five Kinds of Rare Earth Elements

Strain	Metals accumulated (μmol/g dry wt. cells)					
	Y	La	Sm	Er	Lu	Total
Arthrobacter nicotianae IAM12342	82	72	142	90	139	524
Bacillus megaterium IAM1166	46	50	106	65	73	342
B. subtilis IAM1026	51	40	91	60	94	335
B. subtilis IAM1633	38	23	71	53	89	275
B. subtilis IAM11060	68	65	104	78	107	422
B. licheniformis IAM11054	45	40	81	59	83	308
Brevibacterium helovolum IAM1637	35	29	78	53	83	279
*Corynebacterium equi*IAM1038	39	29	74	44	73	259
Nocardia erythropolis IAM1399	48	38	77	59	105	328
Streptomyces albus HUT6047	53	37	92	65	153	401
S. aurantiacogriceus HUT6201	27	28	56	35	35	182
S. flavoviridis HUT6147	39	33	67	53	75	267
S. griseoflavus HUT6153	30	27	58	39	44	198
S. levoris HUT6156	63	42	95	77	151	427
S. olivaceus HUT6061	43	50	74	60	52	278
S. phaeochromogenus HUT6013	68	64	100	73	87	392
S. viridochromogenes HUT6030	48	36	78	53	68	282
S. viridochromogenes HUT6031	49	39	83	56	66	294

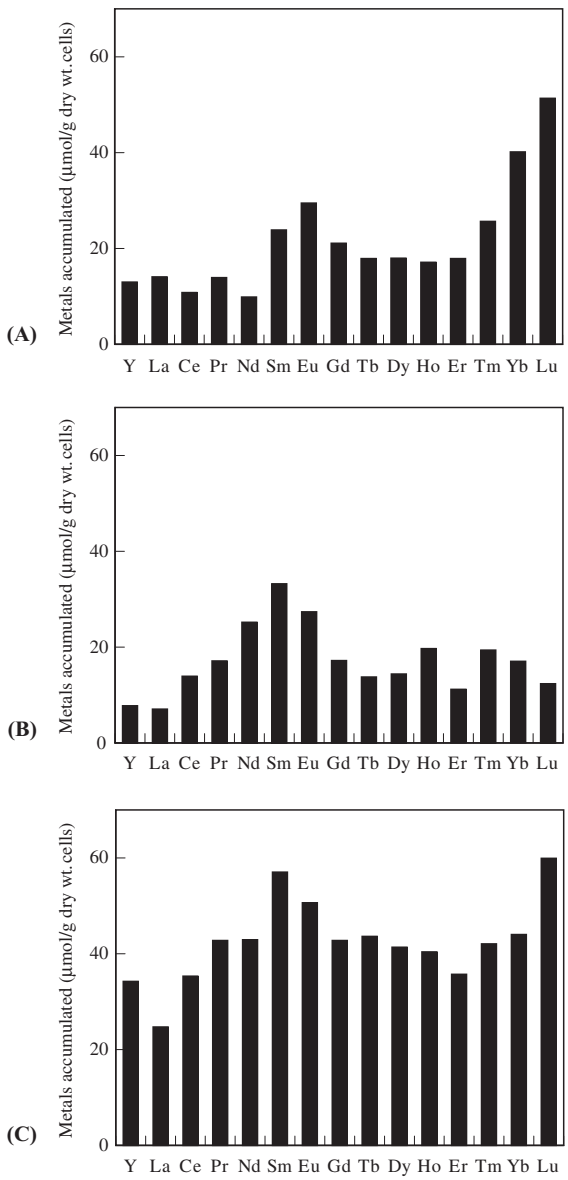

Figure 1. Accumulation of rare earth elements using (A) *S. albus* (B) *B. megaterium* and (C) *A. nicotianae* from the solution containing yttrium and all the lanthanoid elements.

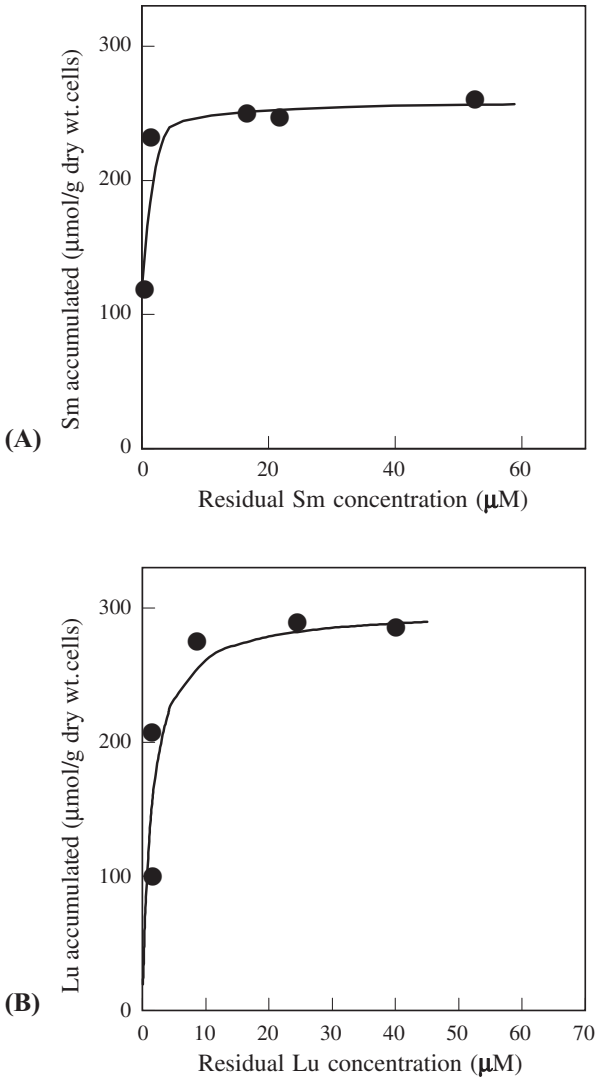

Figure 2. Effect of the external (A) lutetium or (B) samarium concentration on each accumulation from the solution containing each metal only using (A) *S. albus* and (B) *B. megaterium*. The lines were estimated by the Langmuir isotherms mentioned in Table 2.

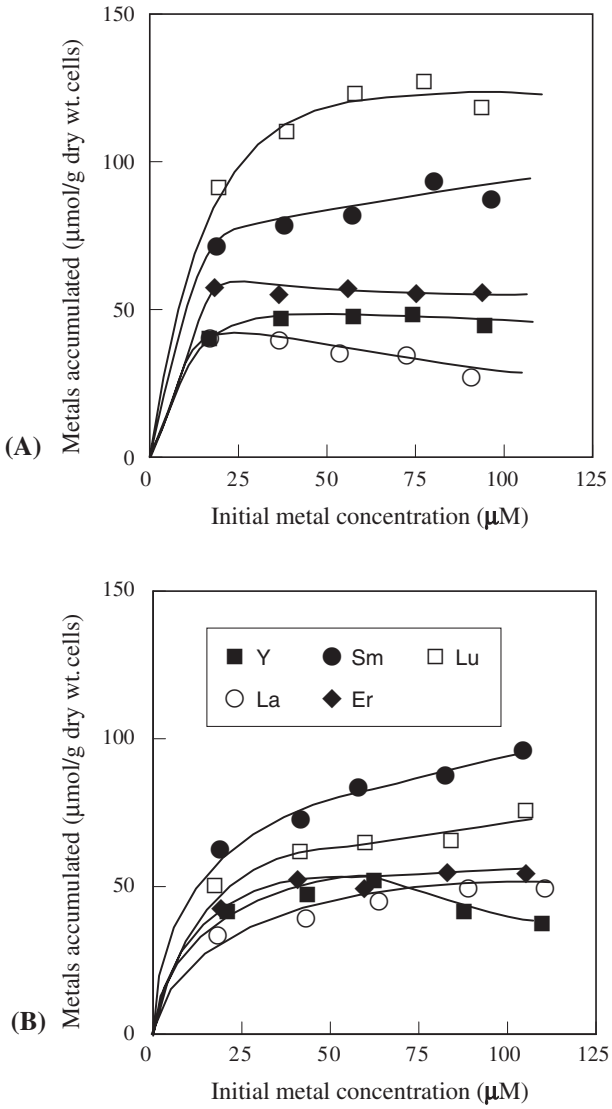

Figure 3. Effect of the concentrations of external rare earth elements on the accumulation of yttrium, lanthanum, samarium, erbium, and lutetium using (A) *S. albus* and (B) *B. megaterium*.

lated in microorganisms, such as *S. albus*, *A. nicotianae*, and *B. megaterium*.

Rare earth elements accumulation capacity of microorganisms. The amount of Lu accumulated in *S. albus* and Sm accumulated in *B. megaterium* cells increased with an increase of each metal concentration (Figure 2). The amount of each metal accumulated obeys the Langmuir isotherm, $Q_M = C_e(M)/(m_M\, C_e(M) + n_M)$, where Q_M indicates the amount of each accumulated metal (μmol each metal/g dry cells), $C_e(M)$ is the concentration of each residual metal concentration (μM), and m_M and n_M are Langmuir constants. On the basis of this finding, it seems reasonable to postulate that the accumulations of Lu and Sm by these microorganisms are mostly dependent on physico-chemical binding to the cell components. The Langmuir constants and the maximum amounts of each metal are shown in Table 2.

Effect of initial rare-earth-element concentration on selective accumulation in microorganisms. As shown in Figure 3, the amount of Lu accumulated in *S. albus* cells increased from 91 to 127 μmol/g dry wt cells with an increase of initial Lu concentration from 20 to 94 μM. The amount of Sm accumulated in *B. megaterium* cells also increased from 61 to 96 μmol/g dry wt cells with an increase of initial Sm concentration from 20 to 100 μM. The amount of Lu accumulated in *S. albus* cells and that of Sm in *B. megaterium* cells were higher than the accumulated amounts of the other 4 kinds of metal combined in each cell within whole metals concentration. As a result, the selective accumulation ability of Lu in *S. albus* and that of Sm in *B. megaterium* cells were kept within the whole concentration.

Accordingly, from the results of these experiments, it is shown that the cells of these microorganisms can be used to separate rare earth elements.

TABLE 2: The Estimated Langmuir Constants and Q_{max} from the Langmuir Isotherm

Metal	Initial metal concentration (μM)	Residual metal concentration (μM)	m	n	Q_{max} [μmol/g dry cells]
Lutetium	0–84.6	0–39.9	3.35×10^{-3}	4.86×10^{-3}	299
Samarium	0–91.1	0–52.3	3.86×10^{-3}	1.92×10^{-3}	259

Acknowledgements. This work was supported by the Metal Mining Agency of Japan (MMAJ). We thank grateful to MMAJ.

References

(1) R. Miyawaki and I. Nakai, *Handbook of the physics and chemistry of rare earths*, Elsevier, **16**, 249 (1993).
(2) Z. Golab, B. Orlowwska, and R. W. Smith, Water Air Soil Pollut. **60**, 99 (1991).
(3) T. Tsuruta, J. Biosci. Bioeng. **94**, 23 (2002).

(4) N. Friiss and P. Myers-Keith, Biotechnol. Bioeng. **28**, 21 (1986).
(5) J. J. Byerley, J. M. Scharer, and A. M. Charles, Chem. Eng. J. **36**, B49 (1987).
(6) T. Sakaguchi, T. Tsuruta, and A. Nakajima, Proc. the technical solutions for pollution prevention in the mining and mineral processing industries. Engineering Foundation Conference, eds. B. S. Richardson and B. J. Schneiner (Palm Coast, FL, USA, 1996), p. 183.
(7) G. W. Strandberg, S. E. Shumate II, and J. R. Parrott Jr., Appl. Env. Microbiol. **41**, 237 (1981).
(8) M. Z.-C. Hu, J. M. Norman, B. D. Faison, and M. E. Reeves, Biotechnol. Bioeng. **51**, 237 (1996).
(9) A. M. Marques, X. Roca, M. D. Simon-Pujol, M. C. Fusto, and F. Congregado, Appl. Microbiol. Biotechnol. **35**, 406 (1991).
(10) Y. Andres, H. J. Maccordick, and J.-C. Hubert, Appl. Microbiol. Biotechnol. **39**, 413 (1993).
(11) M. Tsezos and B. Volesky, Biotechnol. Bioeng. **23**, 583 (1981).
(12) M. E. Treen-Sears, B. Volesky, and R. J. Neufeld, Biotechnol. Bioeng. **26**, 1323 (1984).
(13) M. Galun, P. Keller, D. Malki, H. Fedelstein, E. Galun, S. Siegel, and B. Siegel, Water Air Soil Pollut. **20**, 221 (1983).
(14) M. Galun, P. Keller, D. Malki, H. Feldstein, E. Galun, S. M. Siegel, and B. Z. Siegel, Science **219**, 285 (1983).
(15) C. White and G. M. Gadds, J. Chem. Technol. Biotechnol. **49**, 331 (1990).
(16) S. E. Shumate II, G. W. Strandberg, and J. R. Parrott Jr., Biotechnol. Bioeng. Symp. **8**, 13 (1978).
(17) M. Kamijo, T. Suzuki, K. Kawai, and H. Murase, J. Ferment. Bioeng. **86**, 564 (1998).
(18) M. Kamijo, T. Suzuki, K. Kawai, T. Fujii, and H. Murase, J. Biosci. Bioeng. **87**, 340 (1999).
(19) T. Tsuruta, J. Biosci. Bioeng. **97**, 275 (2004).