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Studies on the Accumulation of Heavy Metal Elements in Biological Systems

XIX. Accumulation of Uranium by Microorganisms

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Summary. Ten species of bacteria, 13 actinomycetes, 11 yeasts and 18 fungi were screened to determine which have the greatest ability to accumulate uranium. The abilities of the microorganisms to accumulate uranium were in the following order: actinomycetes > bacteria, yeasts > fungi. Two species of actinomycetes, *Actinomyces levoris* and *Streptomyces viridochromogenes* have extremely high accumulation abilities.

The uptake of uranium by these two species of actinomycetes, *Actinomyces levoris* and *Streptomyces viridochromogenes* is very rapid and is affected by environmental factors such as the pH and the concentration of carbonate ion but is not affected by temperature of metabolic inhibitors. Most of the absorbed uranium was released by washing the organisms with an EDTA solution. These results suggest that the accumulation of uranium by actinomycetes depends on physico-chemical adsorption at the cell surface rather than on a biological activity and that uranium is coupled to the cells by ligands which were easily substituted with EDTA. The uptake of uranium followed Freundlich isotherm which confirms this suggestion.

Introduction

The recovery of uranium from aqueous systems, especially sea water, merits attention because a severe shortage of uranium is expected by the end of the century. Previous investigations on the recovery of uranium have concentrated almost exclusively on inorganic adsorbents such as hydrous titanium oxide (Kanno 1980). There is very little information on the recovery by microorganisms of uranium from aqueous systems. Some microorganisms are suitable for recovering uranium from aqueous systems; they bind uranium selectively, they produce biomass easily and inexpensively and pose no disposal problems.

We have recently studied the recovery of uranium from aqueous systems by using microalgae. We have shown (Sakaguchi et al. 1978; Horikoshi et al. 1979a, b; Nakajima et al. 1979a) that some microalgae accumulate large amounts of uranium from solution and that uranium uptake by algae is markedly inhibited by carbonate ions. It would therefore be useful to find further microorganisms having an enhanced ability to accumulate uranium.

In this paper some bacteria, actinomycetes, yeasts and fungi have been screened for their ability to accumulate large amounts of uranium. The basic features of uranium uptake by two species of actinomycetes, *Actinomyces levoris* and *Streptomyces viridochromogenes* which accumulated large amounts of uranium were investigated.

Materials and Methods

Microorganisms

Strains used in this study were generously donated by the Institute of Applied Microbiology, University of Tokyo (IAM), the Faculty of Agriculture, Hokkaido University (AHU) and the Faculty of Engineering, Hiroshima University (HUT).

Culture

The medium for growing the bacteria contained 3 g/l meat extract; 5 g/l peptone; and 5 g/l NaCl in deionized water, pH 6.5. The medium for growing actinomycetes, yeasts and fungi 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 yeasts and fungi). The mircoorganisms were maintained on agar slants and grown in 100 ml of medium in a 500 ml culture flask (60 rpm). Cells for uranium uptake experiments were obtained by culturing at 30 °C for 48–72 h. Bacteria and yeast cells were collected by centrifu-

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Table 1. Screening of microorganisms for their ability to accumulate large amounts of uranium

No	Species	Concentration of uranium in the cells (mg/g dry wt.)	Distribution coefficients[a]
Bacteria			
1.	*Bacillus badius* IAM 11059	31.12	11060
2.	*Bacillus cereus* AHU 1030	30.70	29580
3.	*Bacillus cereus* AHU 1355	30.28	24902
4.	*Bacillus cereus* AHU 1356	27.92	22140
5.	*Bacillus stearothermophilus* IAM 11062	32.72	11560
6.	*Bacillus subtilis* AHU 1219	36.33	12500
7.	*Bacillus subtilis* AHU 1390	52.81	20560
8.	*Bacillus subtilis* IAM 11062	48.94	234200
9.	*Bacillus thuringiensis* IAM 11064	37.04	6907
10.	*Escherichia coli* AHU 1520	22.59	42710
Yeasts			
11.	*Candida albicans* IAM 4966	15.72	3011
12.	*Candida mycoderma* IAM 12190	15.82	2794
13.	*Candida robusta* AHU 3402	27.92	12960
14.	*Candida robusta* AHU 3405	26.63	25980
15.	*Hansenula anomala* AHU 3743	40.40	18750
16.	*Hansenula misumaiensis* AHU 3753	30.52	26380
17.	*Hansenula misumaiensis* AHU 3754	36.48	19460
18.	*Rhodotorula glutinis* IAM 4988	36.52	19570
19.	*Rhodotorula glutinis* IAM 12228	34.83	6021
20.	*Rhodotorula glutinis* IAM 12263	21.11	274200
21.	*Saccharomyces cerevisiae* AHU 3818	33.79	24120
Actinomycetes			
22.	*Actinomyces levoris* HUT 6156	45.47	115100
23.	*Streptomyces albidoflavus* HUT 6126	48.38	47950
24.	*Streptomyces albidus* HUT 6129	40.03	34640
25.	*Streptomyces albosporeus* HUT 6130	38.84	19280
26.	*Streptomyces albus* HUT 6132	27.49	35790
27.	*Streptomyces antibioticus* HUT 6137	55.69	36300
28.	*Streptomyces chartreusis* HUT 6140	38.18	49320
29.	*Streptomyces cinereoruber* HUT 6142	58.03	70250
30.	*Streptomyces griseoflavus* HUT 6153	143.52	99320
31.	*Streptomyces novaecaesareae* HUT 6158	45.55	16110
32.	*Streptomyces violaceus* HUT 6164	28.27	14310
33.	*Streptomyces viridochromogenes* HUT 6166	23.35	65770
34.	*Streptomyces viridochromogenes* HUT 6167	20.19	354200
Fungi			
35.	*Aspergillus cervinus* IAM 2752	46.16	8414
36.	*Aspergillus niger* AHU 7120	80.73	12010
37.	*Aspergillus niger* AHU 7296	46.53	6347
38.	*Aspergillus oryzae* AHU 7216	63.42	9398
39.	*Aspergillus oryzae* AHU 7411	57.64	7919
40.	*Chaetomium globosum* AHU 9270	54.21	6896
41.	*Chaetomium globosum* AHU 9272	53.38	7458
42.	*Chaetomium globosum* AHU 9427	48.22	6191
43.	*Gibberella fujikuroi* AHU 9078	31.04	5811
44.	*Neurospora sitophila* IAM 5503	35.56	12520
45.	*Neurospora sitophila* IAM 5504	62.83	13600
46.	*Penicillium janthinellum* AHU 8312	52.70	7029
47.	*Penicillium janthinellum* AHU 8313	47.21	5851
48.	*Penicillium lilacinum* AHU 8357	25.17	3250
49.	*Rhizopus oryzae* AHU 6590	60.49	19960
50.	*Rhizopus oryzae* AHU 6591	57.61	20850
51.	*Trichoderma viride* AHU 9485	27.63	5772
52.	*Trichoderma viride* AHU 9503	42.50	9006

Accumulation experiments were carried out by suspending the cells in 100 ml uranium solution (10 ppm, pH 5) for 1 h

[a] Uranium (μg) accumulated in the cells per g dry weight/residual uranium (μg) in the solution per ml

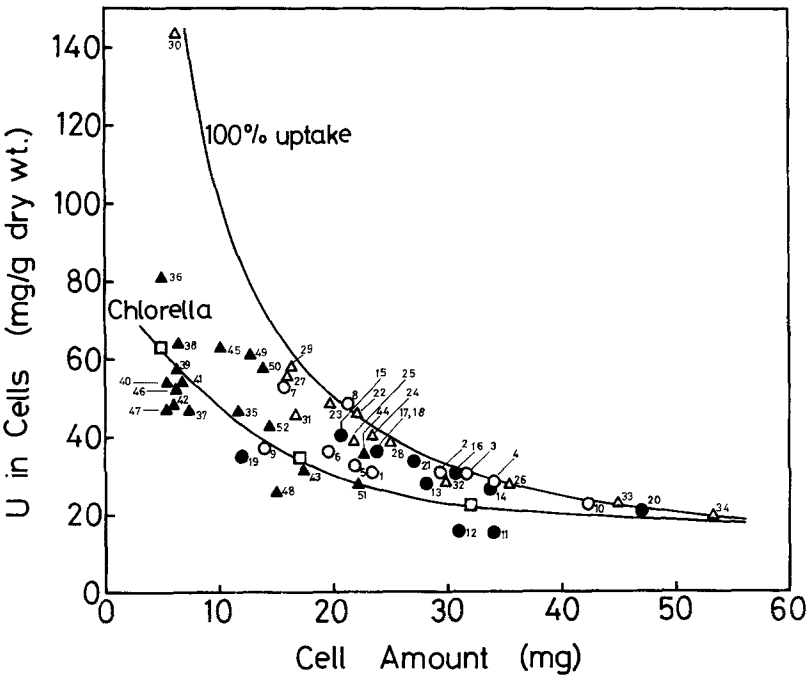


Fig. 1. Screening of microorganism for their ability to accumulate uranium. The conditions for uptake experiments by: ○, bacteria; ●, yeasts; △, actinomycetes; ▲, fungi and □, *Chlorella regularis* were as described in Table 1. The 100% uptake line was drawn by postulating that all the uranium supplied is absorbed by the microorganisms. The closer a line is to the theoretical 100% line, the greater is the uptake of the microorganism. Results for *Chlorella regularis* are indicated for comparison. Numbers on the lines correspond to the strain numbers shown in Table 1

gation (6,000 x g for 20 min) and the actinomycete and fungal cultures were collected by filtration through filter paper. The cells were washed thoroughly with deionized water.

Uptake Experiments

The precultured cells were suspended in a pure solution of uranium which was added as $UO_2(NO_3)_2$. The solution was adjusted to the desired pH value with 0.1 N NaOH solution. The uptake experiments were carried out with continuously stirring cultures at room temperature. Following their exposure to uranium, the bacteria and yeasts were collected by centrifugation and the actinomycetes and fungi by filtration on membrane filters (pore size, 1.0 μm). Uranium taken up by cells was determined by measuring the residual uranium in the supernatant or filtrate spectrophotometrically using Arsenazo III (Motojima et al. 1969).

Results

Screening of Microorganisms for Their Ability to Accumulate Uranium

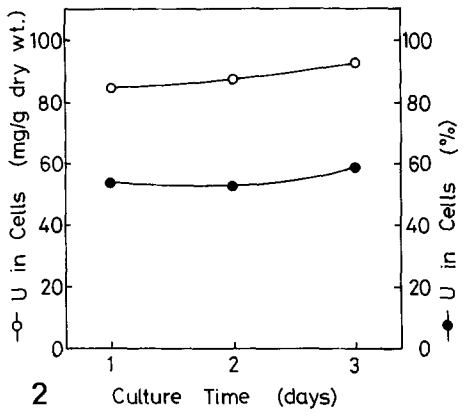
To determine the ability of various microorganisms to accumulate uranium, 10 species of bacteria, 13 actinomycetes, 11 yeasts and 18 fungi were screened (Table 1 and Fig. 1). Figure 1 shows the relationship between the amount of uranium absorbed and the cell concentration. The 100% uptake line, indicated in Fig. 1, is obtained by postulating that all the uranium supplied is absorbed by the microorganisms. The closer the line for a given microorganism is to 100%, the greater is the ability of that microorganism to accumulate uranium. The amount of uranium absorbed by *Chlorella regularis* is also shown in the figure. The abilities of microorganisms to accumulate uranium are in the following order; actinomycetes > bacteria, yeasts > fungi, *Chlorella regularis*. In the following experiments, the basic features of uranium uptake by *Actinomyces levoris* (HUT 6156) and *Streptomyces viridochromogenes* (HUT 6167), which accumulated the most uranium were investigated.

Effect of Culture Time on the Uptake of Uranium

The effect of the age of the culture on the uptake of uranium was studied using *Streptomyces viridochromogenes* (Fig. 2). The amounts of uranium absorbed did not change much with the age of the culture. In the following uranium accumulation experiments cells from 2-day old culture were used.

Time Course of the Uptake of Uranium

The time course of the uptake of uranium by actinomycetes was determined. The uptake of uranium by *Actinomyces levoris* and *Streptomyces viridochromogenes* reaches a plateau 2 min after addition of uranium and does not increase thereafter (Fig. 3). This rapid uptake of uranium by actinomycetes is similar to the rate of uptake observed with *Chlorella regularis* (Horikoshi et al. 1979a).



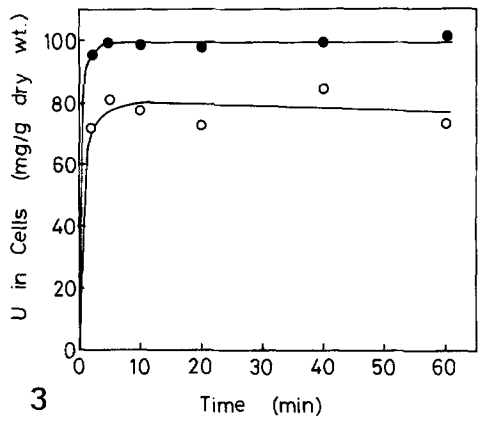
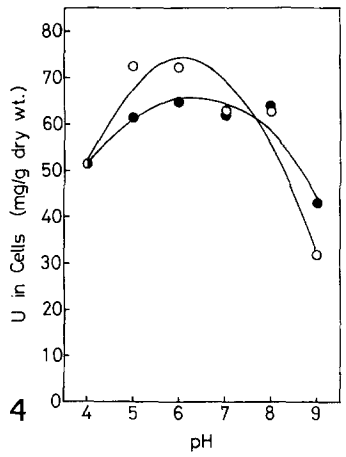
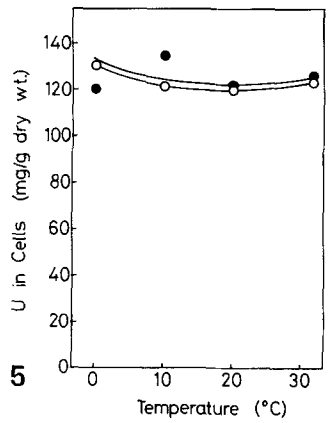


Fig. 2. Effect of culture time on the uptake of uranium by *Streptomyces viridochromogenes*. Uptake experiments were carried out by suspending the cells (dry weights, 12.7–14.7 mg) in 200 ml uranium solution (10 ppm, pH 6) for 30 min

Fig. 3. Time course of the uptake of uranium by actinomycetes. ○, *Actinomyces levoris* and ●, *Streptomyces viridochromogenes* were treated essentially as described in the legend to Fig. 2. Dry weights of the cells used were 13.5–19.7 mg (*Actinomyces*) and 17.9–20.0 mg (*Streptomyces*)





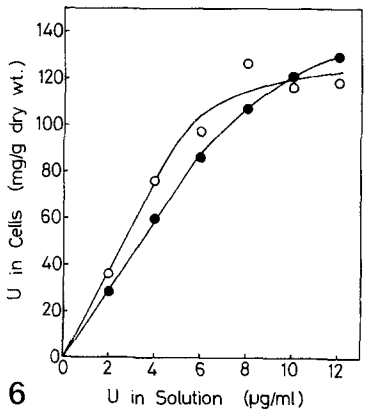


Fig. 4. Effect of pH on the uptake of uranium by actinomycetes. Cells of, ○, *Actinomyces levoris* and ●, *Streptomyces viridochromogenes* were suspended in 100 ml uranium solution (10 ppm) for 30 min. Dry weights of the cells used were; 12.9–15.9 mg (*Actinomyces*) and 13.9–16.2 mg (*Streptomyces*)

Fig. 5. Effect of temperature on the uptake of uranium by actinomycetes. ○, *Actinomyces levoris* and ●, *Streptomyces viridochromogenes* were treated as described in the legend to Fig. 2. Dry weights of the cells used were; 11.1–11.4 mg (*Actinomyces*) and 8.7–10.8 mg (*Streptomyces*)

Fig. 6. Effect of the extracellular uranium concentration on the uptake of uranium of actinomycetes. ○, *Actinomyces levoris* and ●, *Streptomyces viridochromogenes* were treated essentially as described in the legend to Fig. 2. Dry weights of the cells used were; 8.8–11.1 mg (*Actinomyces*) and 12.9–13.7 mg (*Streptomyces*)

Effect of pH on the Uptake of Uranium

The effect of pH on the uptake of uranium by actinomycetes was determined between pH 4 and 9. As shown in Fig. 4, the maximum amount of uranium was absorbed by both *Actinomyces levoris* and *Streptomyces viridochromogenes* at around pH 6. Thus the uptake of uranium by actinomycetes was markedly affected by the pH of the solution which is similar to the result obtained with *Chlorella regularis* (Horikoshi et al. 1979a).

Effect of Temperature on the Uptake of Uranium

The uptake of uranium by actinomyces was studied between 0° and 30 °C. As shown in Fig. 5, the amounts of uranium by both *Actinomyces levoris* and *Streptomyces viridochromogenes* were constant between 0° and 30 °C. The uptake of uranium by actinomycetes is thus presumably independent of temperature and therefore not directly mediated by metabolic processes.

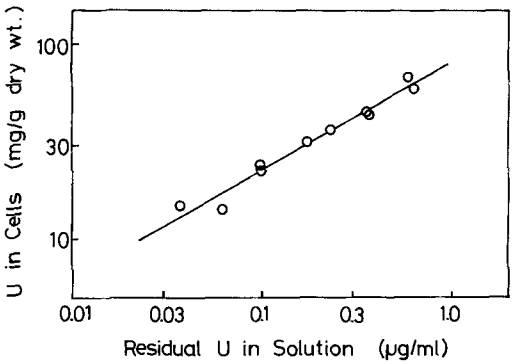


Fig. 7. Relationship between the concentration of residual uranium in the solution and the amounts of uranium absorbed by *Actinomyces levoris*. Uptake experiments were carried out by suspending the cells (dry weights, 11.6–12.5 mg) in 200 ml uranium solution (pH 6) containing 1–4 ppm of uranium for 30 min

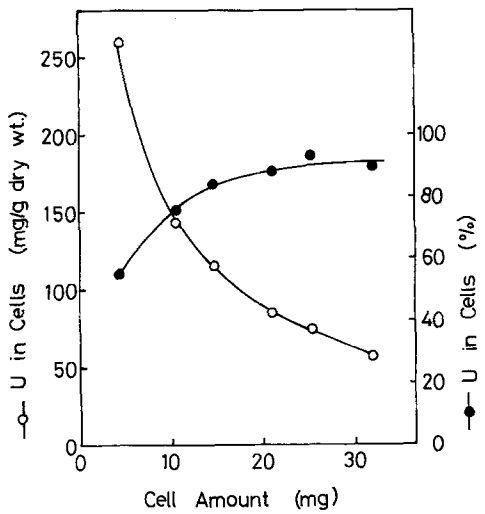


Fig. 8. Effect of the cell concentration of *Actinomyces levoris* on the uptake of uranium. The experimental conditions were as described in the legend to Fig. 2

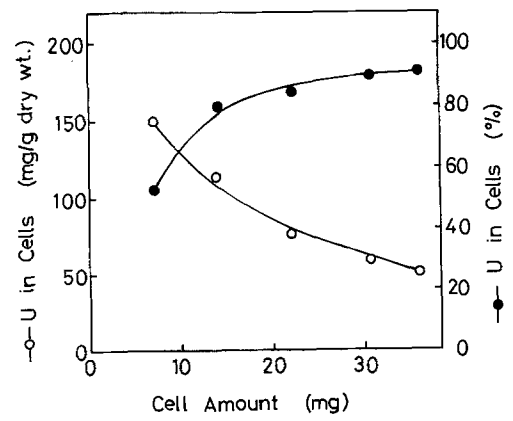


Fig. 9. Effect of the cell concentration of *Streptomyces viridochromogenes* on the uptake of uranium. The experimental conditions were as described in the legend to Fig. 2

Effect of the Extracellular Uranium Concentration on the Uptake of Uranium

The effect of the extracellular uranium concentration on the uptake of uranium by actinomycetes was studied (Fig. 6). The amounts of uranium absorbed by both *Actinomyces levoris* and *Streptomyces viridochromogenes* increased almost linearly with the concentration of uranium in the solution up to 8 ppm but thereafter the rate of increase slowed down.

The relationship between the concentration of residual uranium in the solution and the amounts of uranium absorbed was also investigated using *Actinomyces levoris*. As shown in Fig. 7 when the initial concentration of uranium in the solution is low, a linear relationship is observed, in a log-log scale, between the amounts of uranium absorbed and the residual uranium concentration. These results indicate that the uptake of uranium by actinomycetes obeys the following Freundlich isotherm: $C = KC_S^{1/n}$; where C is the amount of uranium absorbed per g dry cells, C_S is the residual uranium in the solution per ml, K is a constant and $n > 1$ and that the uptake of uranium by actinomycetes is almost dependent on adsorption at the cell surface.

Effect of the Concentration of Actinomycete Cells on the Uptake of Uranium

The effect of the cell concentration on the uptake of uranium by actinomycetes was studied. As the cell concentration of *Actinomyces levoris* was increased, the amount of uranium absorbed by each cell decreased while the total amount of uranium absorbed increased (Fig. 8).

A similar relationship was obtained between the uptake of uranium by *Streptomyces viridochromogenes* and the cell concentration (Fig. 9) which is a similar result to those found for the uptake of heavy metals (copper, uranium, manganese and cadmium) by other microalgae (Sakaguchi et al. 1977, 1979; Horikoshi et al. 1979a, b; Nakajima et al. 1979b).

Effect of Metabolic Inhibitors on the Uptake of Uranium

The uptake of uranium by actinomycetes is therefore very rapid, not affected by temperature and obeys the Freundlich isotherm which suggests that the uptake of uranium by actinomycetes depends on physico-chemical adsorption at the cell surface and not on a biological activity. To confirm this, the effects of the metabolic inhibitors *dinitrophenol* (DNP) and sodium azide (NaN_3), on the uptake of uranium were investigated. The cells of actinomycetes were stirred in 50 ml of a solution containing NaN_3 (10^{-4} or 10^{-3} M) or DNP (10^{-5} or 10^{-4}

M). After 30 min of exposure to the metabolic inhibitors, the cells were collected by centrifugation, washed thoroughly with deionized water and used for uptake tests. As shown in Fig. 10, the uptake of uranium by *Actinomyces levoris* and *Streptomyces viridochromogenes* was not affected by the treatment with metabolic inhibitors and thus the uptake of the uranium by actinomycetes is a non-metabolic process.

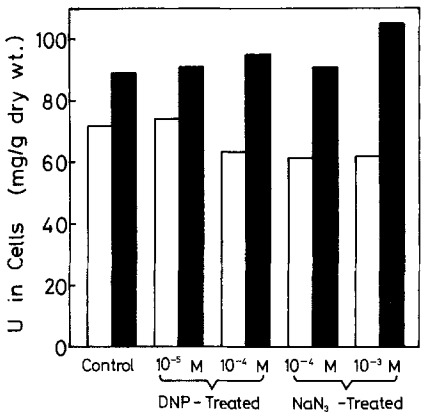


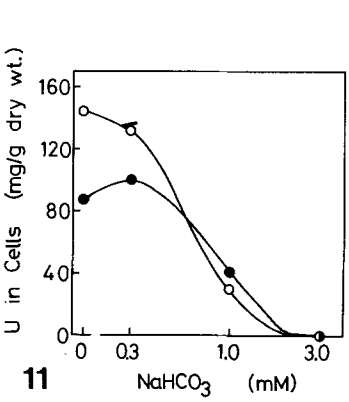
Fig. 10. Effects of metabolic inhibitors on the uptake of uranium by actinomycetes. *Actinomyces levoris* (□) and *Streptomyces viridochromogenes* (■) were treated with inhibitors by suspending the cells in 50 ml of a solution containing NaN_3 (10^{-4} or 10^{-3} M) or DNP (10^{-5} or 10^{-4} M) for 30 min with continuous stirring. The uptake of uranium by the cells treated with metabolic inhibitors was assessed as described in the legend to Fig. 2. Dry weights of the cells used for uptake experiments were 15.5–17.7 mg (*Actinomyces*) and 16.2–21.8 mg (*Streptomyces*)

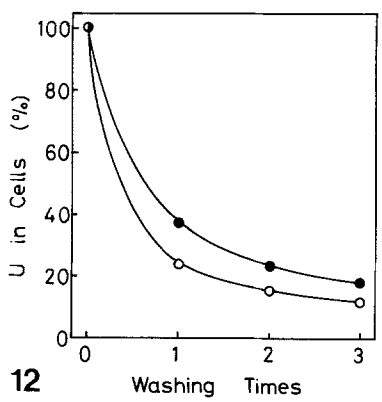
Effect of Carbonate Ion on the Uptake of Uranium

We have shown that the uptake of uranium by microalgae is hardly affected by the presence of cations such as Na^+, K^+, NH_4^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+}, but is markedly inhibited by carbonate due to the formation of the stable complex ions ($UO_2(CO_3)_2^{2-}$, $UO_2(CO_3)_3^{4-}$) which are not absorbed by microalgae (Sakaguchi et al. 1978; Nakajima et al. 1979a; Horikoshi et al. 1979b). We examined whether carbonate interferred with the uptake of uranium by actinomycetes by adding various concentrations of sodium hydrogencarbonate, pH 8 to the uranium solution. As shown in Fig. 11, 0.3 m*M* sodium hydrogencarbonate scarcely affects the uptake of uranium by actinomycetes, however, as the concentration of sodium hydrogencarbonate was increased from 0.3 to 3 m*M*, the amounts of uranium absorbed by both *Actinomyces levoris* and *Streptomyces viridochromogenes* decreased. 3 m*M* sodium hydrogencarbonate completely prevented uranium absorption by the cells.

The Distribution and Binding States of Uranium in Actinomycetes Cells

The distribution and binding states of uranium taken up by actinomycetes cells was investigated by washing cells which had taken up uranium with 10 ml of 10 m*M* EDTA (pH 4.9). Figure 12 shows that about 82–88% of the adsorbed uranium was released by 3 EDTA washes. These





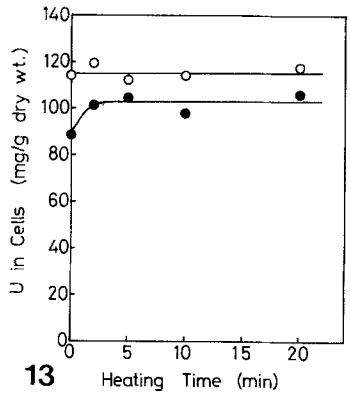


Fig. 11. Effect of carbonate ion on the uptake of uranium by actinomycetes. The uptake experiments were carried out by suspending the cells of *Actinomyces levoris* (○; dry weight, 6.1–8.9 mg) and *Streptomyces viridochromogenes* (●; dry weight, 13.1–14.1 mg) in 200 ml uranium solution (10 ppm, pH 8) containing various concentrations of sodium hydrogencarbonate

Fig. 12. Release of uranium from actinomycetes cells, which had absorbed uranium, with EDTA. Cells of; ○, *Actinomyces levoris* and ●, *Streptomyces viridochromogenes* which had absorbed uranium from 200 ml solution (10 ppm, pH 6) for 30 min were washed 3 times with 10 ml of 10 m*M* EDTA. Dry weights of the cells used were 12.9–15.0 mg (*Actinomyces*) and 12.5–15.0 mg (*Streptomyces*)

Fig. 13. Uptake of uranium by heat-killed actinomycetes cells. The heat-killed cells of *Actinomyces levoris* (○) and *Streptomyces viridochromogenes* (●) were prepared by immersing the living cells in boiling water for 0–20 min. Uptake of uranium by the heat-killed cells was assessed as described in the legend to Fig. 2. Dry weights of the cells used for uptake experiments were 11.0–12.9 mg (*Actinomyces*) and 11.1–11.5 mg (*Streptomyces*)

results suggest that most of the uranium taken up by actinomycetes is coupled at the cell surface to ligands which are easily substituted with EDTA.

Uptake of Uranium by Heat-Killed Cells

The uptake of uranium by heat-killed cells of actinomycetes was studied as our previous results (Horikoshi et al. 1977, 1979a, b; Nakajima et al. 1979b; Sakaguchi et al. 1979) showed that the uptake of heavy metal ions (copper, uranium, manganese, and cadmium) by some microalgae could be greatly increased by heat treatment of cells. The heat-killed cells were prepared by boiling the cells in water for 0–20 min. As shown in Fig. 13, the uptake of uranium by actinomycetes was not increased by heat treatment. In *Chlorella regularis*, the amount of uranium taken up by heat-killed cells was 4 times that taken up by living cells.

Discussion

In the course of our study on the recovery of uranium from aqueous systems by living organisms it became appropriate to find more microorganisms which accumulate uranium efficiently. By screening several microorganisms, it was found that two species of actinomycetes, *Actinomyces levoris* and *Streptomyces viridochromogenes* accumulate uranium extremely well.

The uptake of uranium by these two actinomycetes is very rapid, is affected by environmental factors such as the pH of the solution and the presence of carbonate ion but is not affected by the length of preculture of the cells, temperature or metabolic inhibitors. The majority of the absorbed uranium is released by washing the cells with EDTA. These basic features of uranium uptake by *Actinomyces levoris* and *Streptomyces viridochromogenes* are essentially the same as those observed in *Chlorella regularis* (Horikoshi et al. 1979a; Nakajima et al. 1979a). These results suggest that the accumulation of uranium by actinomycetes depends on physico-chemical adsorption at the cell surface and not on a biological activity and that the uranium is coupled in the cells with ligands which are easily substituted with EDTA. The fact that the uptake of uranium obeys a Freundlich isotherm confirms this suggestion.

The uptake of uranium by actinomycetes is inhibited by the presence of a high concentration carbonate. A similar effect was observed with *Chlorella regularis* (Sakaguchi et al. 1978; Nakajima et al. 1979a) and *Synechococcus elongatus* (Horikoshi et al. 1979b). The depression of uranium uptake is thought to be due to the formation of stable complex ions such as $UO_2(CO_3)_2^{2-}$ and $UO_2(CO_3)_3^{4-}$ at pH 8 which these two species of actinomycetes cannot absorb.

The uptake of uranium by actinomycetes was not increased by heat treatment in contrast to our previous results (Horikoshi et al. 1977, 1979a, b; Nakajima et al. 1979b; Sakaguchi et al. 1979) which showed that the uptake of heavy metal ions (copper, uranium, manganese and cadmium) by other microalgae could be greatly increased by heat treatment of the cells. The explanation of the increased uptake by some microalgae but not by actinomycetes is unclear.

On the basis of these results, further studies will be undertaken to devise a practical approach to recover uranium from aqueous systems by microorganisms which accumulate uranium in large amounts.

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