Removal of Uranium from Aqueous Solution, Uranium Effluent and Seawater Using Various Microorganisms Isolated from Several Uranium Deposits

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Abstract

Attempts were made to remove and recover uranium (U) occurring in nuclear fuel effluents and mine tailings using bacteria isolated from U deposits in Canada, the United States, Australia, and Japan. To establish which microorganisms accumulate the most U, hundreds of strains of microorganisms were screened. Extremely high U accumulating ability was detected in some bacteria isolated from North American U deposits. *Arthrobacter* and *Bacillus* spp. Cells removed U from refining waste water with high efficiency and accumulated thorium with high efficiency. *Lactobacillus* cells isolated from Japanese U deposits could remove more U from seawater than could microorganisms that had superior U removal capacity in non-saline U solutions. Cells immobilized with polyacrylamide gel had excellent handling characteristics. These microorganisms from U deposits can be used as adsorbing agents for the removal of nuclear fuel elements which may be present in nuclear effluents, mine tailings, seawater, and other waste sources.

Key words: Uranium accumulation, microorganisms, *Arthrobacter, Lactobacillus, Bacillus,* immobilized microorganisms, thorium

Introduction

The recovery of nuclear fuel elements, such as uranium (U), from aqueous systems has become a focus of interest as a way to exploit undeveloped energy resources. The removal of radioactive elements and toxic heavy metals from contaminated sources is also a worthwhile priority for environmental protection initiatives. In this regard, research efforts have concentrated on accumulation of U by microorganisms, including bacteria, fungi and yeasts (for example Andres et al. 1993).

We have investigated U accumulation from aqueous systems using bacteria isolated from U mines, among which some strains were found to possess extremely high U accumulating ability (Sakaguchi et al. 1996). Microbial biomass may thus be considered for use as a removal agent for the recovery of U from metallurgical effluents, mine tailings, seawater and other waste sources.

In U deposits, some microorganisms with a high U accumulating ability presumably can co-exsit with different species that can leach U from ore in mine soil and aqueous systems. It would, therefore, be beneficial to isolate microorganisms that have an enhanced ability to accumulate U from mines.

Recently, we screened hundreds of types of microorganisms in U deposits located in North America, Australia, and Japan for their ability to accumulate significant quantities of U and identified new strains that do so, such as *Bacillus subtilis* in Australia, *Arthrobacter* and *Bacillus* spp. in North America, and *Lactobacillus* and *Bacillus* spp. in Japan (Sakaguchi 1998). We discuss new strains of bacteria found in these North American, Australian, and Japanese U deposits, especially *Arthrobacter, Lactobacillus,* and *Bacillus* spp., for their potential to remove nuclear fuel elements such as U from U refining wastewater and seawater.

Materials and Methods

Culture of Microorganisms

Microorganisms were grown in medium containing 3 g/l meat extract, 5 g/l peptone, and 5 g/l NaCl in deionized water. The cultures of microorganisms, maintained on agar slants, were grown in 300 ml of the medium in a 500-ml flask with continuous shaking (120 rpm) at 30^{O}C. In order to have a sufficient amount of the resting microorganisms after separation from growth medium, the cultures were grown for 72 h.

Cells were collected by centrifugation, washed thoroughly with deionized water, and then used in the following removal experiments.

Screening of Microorganisms Isolated from Japanese Uranium Deposits for Their Ability to Remove Uranium

U was supplied as $UO_2(NO_3)_2$. The pH of the uranium solution was adjusted to 5.8 with 0.1 M NaOH. For all studies in this screening, U removal experiments were conducted as follows. Resting microorganisms (15 mg dry wt. basis) were suspended in 100 ml of solution (pH 5.8) containing 84 μM U and the suspension was shaken for 1 h at room temperature. Microorganisms were then collected by filtration through a nitrocellulose membrane filter (pore size 0.2 μm). Control studies confirmed that free uranium was not adsorbed onto the filter.

The amounts of U removed by the cells were determined by difference measurements of the initial and the final uranium content in the filtrate using an inductively coupled plasma quantometer (ICPS8000, Shimadzu Corporation, Kyoto).

Immobilization of Microbial Cells

Five grams of precultured Arthrobacter cells were suspended in 4.5 mL isotonic NaCl solution and 680 mg acrylamide monomer. A total of 34 mg N, N'-methylene-bis(acrylamide), 0.3 mL 3-dimethylaminopropionitrile solution (5%) and 0.34 mL potassium persulfate solution (2.5%) were added to the suspension. After solidification, the gel was crushed into small pieces (50-100 mesh), washed thoroughly with isotonic NaCl solution followed by deionized water and then used for adsorption experiments.

Removal of U from U wastewater by Batch System using Immobilized Microbial Cells

Resting cells (15.0 mg dry weight) were suspended in 100 ml of a solution (pH 6.0) of wastewater containing 21.0 M U for 1 h at room temperature.

Removal of U from U wastewater by Column System using Immobilized Microbial Cells

Uranium refining wastewater (100 mL, pH 6.0) supplemented with 2.1 mM of U were adsorbed on a column (bed volume 2 mL) of immobilized bacterial cells at a space velocity of 10 /h at room temperature.

Removal of U from seawater by bacteria

Resting cells (15 mg dry weight basis) were suspended in 100 ml solution (pH 8.0) supplemented with 4.2 μM of uranium for 1h at room temperature. To remove carbonate ions from natural sea water, natural sea water was acidified to pH 4 with 1N HNO_3, boiled for 10 min and then the pH value of the water was restored to 8 with 1N NaOH solution.

Results and Discussion

Screening of microorganisms isolated from U deposits for their U accumulating ability

To determine the ability of microorganisms isolated from U deposits in Canada, the United States, Australia, and Japan to accumulate U, we screened hundreds of strains of microorganisms.

Table 1. Accumulation of uranium using microorganisms isolated from soils or water at uranium deposits.

Strain Number	Uranium accumulated (%)	Strain Number	Uranium accumulated (%)	Strain Number	Uranium accumulated (%)	Strain Number	Uranium accumulated (%)
US-1	14.0	CAN-1	12.6	AUS-1	19.4	JPN-1	10.9
US-2	17.1	CAN-2	23.7	AUS-2	25.4	JPN-2	21.2
US-3	23.9	CAN-3	33.9	AUS-3	36.6	JPN-3	32.5
US-4	38.7	CAN-4	49.8	AUS-4	46.2	JPN-4	44.7
US-5	43.9	CAN-5	55.7	AUS-5	53.3	JPN-5	54.9
US-6	58.9	CAN-6	67.9	AUS-6	66.8	JPN-6	65.2
US-7	72.0	CAN-7	74.2	AUS-7	74.7	JPN-7	76.8
US-8	84.4	CAN-8	85.7	AUS-8	85.1	JPN-8	86.2
Bacilluss sp., US-9	95.9	CAN-9	95.4	AUS-9	96.9	*Bacilluss* sp., JPN-9	97.6
Arthrobacter sp., US-10	96.4	CAN-10	96.9	*Bacilluss* sp., AUS-10	98.3	*Lactobacillus* sp., JPN-10	97.8

The quantities of U that the cells accumulated ranged from a minimum of 10.9-% to a maximum of 98.3-% (Table 1). Of special interest is the wide range of effectiveness different microorganisms species exhibit in accumulating U.

Of the microorganisms tested, we identified extremely high U accumulating ability in *Arthrobacter* and *Bacillus* spp. found in the U.S., *Lactobacillus* and *Bacillus* spp. found in Japan, and *Bacillus* spp. found in Australia (Sakaguchi,1998), all of which accumulated large quantities of U per gram dry wt. of microbial cells within 5 min.

Removal of U from U refining wastewater by bacteria

As mentioned above, some microbial species have a high U accumulating ability, which suggests the possibility that they may be used for removal of U from U mine tailings, U refining wastewater and other waste sources.

We attempted to remove U from U refining wastewater sampled at the Ningyotoge Environmental Engineering Center of the Japan Atomic Energy Agency using bacteria exhibiting a significant ability to accumulate U.*Lactobacillus* and *Bacillus* sp. isolated from Japanese U deposits removed 88.1 and 74.4% U, respectively (Table 2), when solution pH was adjusted initially to 6.0. Solution pH gradually decreased, with *Bacillus* cells being more adversely affected by pH change than *Lactobacillus* cells. However, both strains quantitatively removed U when the pH was maintained at 6.0. These species can thus remove U from U refining wastewater with a high efficiency.

As described above, bacteria such as *Arthrobacter, Bacillus,* and *Lactobacillus* sp. can accumulate large quantities of U from aqueous systems. However, the free cells of these bacteria are not reusable because of their mechanical instability and susceptibility to cell degradation. Furthermore, free cells are not suitable for use in column systems, because they cause plugging. To overcome these deficiencies with free cells, the cells of *Arthrobacter* sp. US-10 having high U accumulating ability were immobilized with polyacrylamide.

Attempt were also made to remove U from U refining wastewater using immobilized microorganisms having a high ability to accumulate U.

Immobilized bacterial cells isolated from U mines in the United States can also remove U from the U refining wastewater with high efficiency (Table 3).

Removal of U from seawater by bacteria

We also addressed the efficiency of these bacteria in removing U from seawater relative to their efficiency in non-saline solutions. To do so, we examined the removal of U from seawater supplemented with 4.2 μM U using the bacteria isolated from the U deposits. The concentration of carbonate in seawater is ~2.34 x 10^{-3} M (Ogata et al. 1971). The amount of U removed by *Chlorella* cells from solutions containing 1.196 x 10-3 M sodium hydrogen carbonate was less at pH values above 6 than at pH 5 (Nakajima et al.1979). The decrease in the amount of U removed from solutions containing carbonate was estimated from the amount of the UO_2CO_3 formed at pH 6 and of $UO_2(CO_3)_3^{4-}$ formed at pH values greater

Table 2. U removal from U refining wastewater using microbial cels isolated from Japanese uranium mine.

	Removed U (%)	
Strains		
	pH adjusted only	pH adjusted
	started at pH 6.0	continuously at pH 6.0
Lactobacillus sp.	88,1	99,5
Bacillus sp.	74,4	95,5

Table 3. U removal from U refinig wastewater using immobilized microorganisms isolated from U mines.

Strains	Adsorbed U (%)
Arthrobacter sp.	100
Bacillus sp.	100

than 7 (Nakajima et al.1979). As Table 4 shows, although the *Lactobacillus* sp. removed 36.2% of U from seawater, it removed almost twice as much (70.2%) when the seawater was decarbonated. *Arthrobacter* and *Bacillus* cells, which can remove large amounts of U from non-saline water, removed far less U from either seawater or decarbonated seawater compared to *Lactobacillus* cells. Thus, *Lactobacillus* cells have a greater potential in applications targetting removal of large amounts of U from seawater.

Conclusion

In U deposits located in Canada, the United States, Australia, and Japan, we isolated strains of bacteria such as *Arthrobacter, Bacillus* and *Lactobacillus* sp. having a significant ability to accumulate U. These species removed U from U refining wastewater with high efficiency. Lactobacillus also accumulated U from seawater more effectively than other microbial cells all of which have high accumulating capacities, from non-saline U solution.

These strains of *Arthrobacter, Bacillus* and *Lactobacillus* can be used as an adsorbing agent for the removal of nuclear fuel elements which may be present in nuclear fuel processing effluents, mine tailings, seawater and other environmental sources.

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Table 4. Accumulation of uranium using microorganisms isolated from uranium mines.

	Accumulated U (%)		
Solutions	*Lactobacillus* sp.	*Arthrobacter* sp.	*Bacillus* sp.
Uranium only solution (pH 8)	94,7	94,2	94,6
Natural sea water	36,2	0,8	0,9
Decarbonated sea water	70,2	6,1	6,0