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Communication

Practical Removal of Radioactivity from Sediment Mud in a Swimming Pool in Fukushima, Japan by Immobilized Photosynthetic Bacteria

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About 90% of the radioactive Cs in the sediment mud of a school's swimming pool in Fukushima, Japan was removed by treatment for 3 d using the alginate immobilized photosynthetic bacterium *Rhodobcater sphaeroides* SSI. Even though batch treatment was carried out 3 times repeatedly, the activity of immobilized cells in removing Cs was maintained at levels of about 84% (second batch) and 78% (third batch). Cs was strongly attached to the sediment mud because, even with HNO_3 treatment at pH of 2.00–1.60 for 24 h, it was not eluted into the water. Furthermore, more than 75% of the Cs could be removed without solubilization with HNO_3. This suggests that the Cs attached to the sediment mud was transformed into immobilized cells *via* the Cs^+ ion by the negative charge of the immobilized cell surface and/or the potassium transport system of the photosynthetic bacterium.

Key words: radioactive cesium; immobilized photosynthetic bacteria; water containing sediment mud; nitric acid treatment; Fukushima

The devastating March 2011 nuclear accident at the Fukushima Electric Power Plant in led to a serious spread of radioactive materials into the area's soil, water, and sediment mud. Consequently, the soil and water must be purified, and treatment of contaminated materials must be expedited to recover fully from the disaster in the Tohoku area of Japan.

Approaches to removing radioactive materials have relied mainly on physical adsorption technologies using zeolite, clay, and chemicals.[1,2] However, even after the adsorption of Cs is accomplished, storage facilities available for radioactive Cs-contaminated waste are insufficient. Therefore, technologies that reduce the amount of waste under treatment are also required.

For biotechnological removal of Cs, adsorption by plants such as sunflowers has been proposed.[3] However, several months of growth are necessary before harvest, and the removal efficiency of phyto-remediation for radioactive Cs has not been fully elucidated. On the other hand, Cs adsorption by microorganisms was reported more than 25 years ago.[4–9] For example, Harvey *et al.*[4] studied ^{137}Cs and ^{85}Sr uptake by freshwater filamentous green algae. Haselwandter and Berreck[6] investigated radiocesium accumulation by the fungus, *Paxillus involutus*. Avery *et al.*[7] reported Cs accumulation by the cyanobacterium *Synechocystis* PCC 6803. Tomioka *et al.*[8] reported Cs accumulation by *Rhodococcus erythropolis* and *Rhodococcus* sp. However, no practical means of removing Cs has been firmly established.

We have been studying this issue more than 10 years, and recently we reported the simultaneous removal of such radionuclides as uranium, strontium, cobalt, and cesium by porous-ceramic-immobilized photosynthetic bacteria using alginate.[10,11] Almost 100% of the non-radioactive Cs in 5 mg/L of water was removed by treatment with an immobilized SSI strain of photosynthetic bacterium, *Rhodobacter sphaeroides*, within 2–3 d but we have no results for radioactive Cs. We carried out experiments on the removal of radioactivity (radioactive Cs) in swimming pool water of school A in Fukushima. In this practical attempt to remove radioactive Cs, the same results were obtained as in laboratory experiments using non-radioactive Cs.[10,11] In addition, we investigated reduction of biomass by relatively low-temperature drying and slow incineration as a solution to the problem of reducing the built-up stock of treated wastes containing radioactivity.

A photosynthetic bacterium *Rhodobacter sphaeroides* SSI (SSI strain), was used because it can adsorb radionuclides and heavy metals on the surfaces of cells by accumulating extra-cellular polymeric substances (EPSs).[10,11]

Cultivation of the SSI strain was carried out with a modified GM medium[10,12] in which the total amount of potassium in the medium was cut in half to make potassium-deficient cells. This was done because the uptake of Cs is active under a low-potassium condition with the SSI strain (less than 7 mg/L, unpublished results).

A 1.5-L Rhoux bottle (1 L of liquid) was used for static light cultivation of the SSI strain at 5 klux (20 W/cm^2) illumination on the surface of the vessel for 3 d.[10,12] We adjusted the harvested cells by centrifugation (10,000 × *g* 20 min), where $OD_{660} = 20$, and added a 4% sodium alginate solution (final 2%). After mixing

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this solution well, it was immobilized as relatively large beads (about 2 cm in diameter) using a specially shaped plate in the $CaCl_2$ solution to make gel beads.[13] Beads containing the SSI strain were used to remove radioactive Cs. The beads were put into a mesh bag (1.0 × 1.0 cm mesh, 15 cm in diameter and 30 cm long). We prepared nine mesh bags containing a total of about 1,900 beads and 90 g of dry cells of photosynthetic bacteria. After removing the Cs, the mass and weight of the radioactive contaminated beads (waste) can be reduced by drying and incineration. This is a key advantage as compared with zeolite and clay treatments.

The experiment on removing Cs used swimming pool water of school A, which had relatively high amounts of sediment mud from the a hedge surrounding the pool and algae growth during the summer. Water containing sediment mud with negligible amounts of radioactivity (1.2–1.3 μSv/h in the liquid) was put in a 1-ton vessel with a 1-L/ton chemical solution containing 0.5% kitosan solution (Fujiclean, Sapporo, Japan). After 24 h, sedimentation by kitosan treatment, concentrated sediment mud was obtained (dry solid 6.69 g/L, COD_{Mn} 3, 200 mg/L). The mud had relatively high radioactivity (13–15 μSv/h). Furthermore, radioactivity of 1.23–1.30 μSv/h was detected in the supernatant water of concentrated sediment mud. The concentrated sediment mud was used in the Cs-removal experiment using beads of the SSI strain. The concentrated sediment mud (14.4 μSv/h) showed radioactivity of 6,912 Bq of ^{134}Cs, 8,567 Bq of ^{137}Cs (as 15,479 Bq of Cs), and <2 Bq of ^{131}I. The radioactivity of the concentrated sediment mud in Bq units was measured with a Germanium Semiconductor Detector (Canberra, Meriden, CT, USA, adopted by the Department of Environment of the Radioactivity Monitoring Center, Fukushima City).

Fifty L of concentrated sediment mud (liquid condition) was put in a container (55 L) at a pH of 2.00 adjusted by concentrated HNO_3, and kept 24 h to transfer the Cs into water as Cs^+ ions (sediment mud solubilized by acid). After a pH adjustment to 7.0, nine mesh bags were placed in the sediment mud solubilized by acid. Then radioactive Cs was removed by aeration at 0.2–0.3 vvm, and 200 g of glucose and 7.5 g of pepton were added to the substrate of the SSI strain. The pH value was maintained at 6.0–7.5. The temperature was maintained 28.2–15.9 °C, corresponding to the poolside environment. The SSI strain is active under an aerobic dark condition in removing Cs and Sr without light irradiation.[10,11] The liquid was mixed well every day, and its radioactivity was measured. A liquid sample was filtrated with a cotton cloth until the filtrated liquid (water) became clear. The radioactivity of the residue's sediment and that of the clear water were measured separately.

Radioactivity was measured with an Aloka TGS121 survey meter (Aloka, Tokyo, Japan) and two different models of the Dose RAE2 (PRM-1200) (Rae System, Boston, MA, USA). In using the TGS121, a sensor was covered with a waterproof vinyl bag and soaked in 2–5 cm of water to measure radioactivity. The small (5 × 8 × 2 cm) RAE2 was also put in a waterproof vinyl bag and soaked in liquid, and radioactivity was again measured in 2–5 cm of liquid. The radioactivity of a

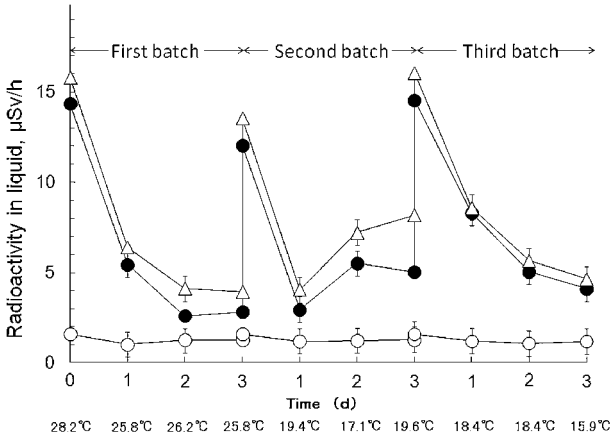

Fig. 1. Removal of Radioactive Cs from Sediment Mud Obtained from a School Swimming Pool Solubilized by Acid (HNO_3) by Repeated Batch Treatment with the Same Immobilized *R. sphaeroides* SSI.

After first and second batch treatments, fresh sediment mud solubilized by acid was placed in a 55-L container with glucose and pepton, and then aeration was continued. Under the figure, temperatures of the vessel at 10 am each day are shown. ●, radioactivity in broth; △, radioactivity of sediment residue after filtration; ○, radioactivity in filtrated water.

liquid is generally measured in Becquerel unit (Bq), but we were unable to use a large expensive equipment capable of expressing radioactivity in Bq units practically. Hence we expressed radioactivity in μSv/h units. One μSv/h can be expressed as 3.33 counts per second (cps). The background radioactivity in the air surrounding the pool was 1.0–1.3 μSv/h during the experiment.

The results of attempting to remove radioactive Cs in three repeated batch experiments are shown in Fig. 1. After 3 d radioactivity in the first batch had decreased by 10.6% (assuming background radioactivity of 1.20 μSv/h, $(2.60 - 1.20)/(14.35 - 1.20) \times 100$). This result is almost the same as that for removing the non-radioactive Cs with alginate- and ceramic-immobilized SSI cells, as previously reported.[10,11] It has been reported that alginate does not adsorb non-radioactive Cs.[10,11] But to investigate the adsorption of radioactive Cs by alginate, an additional experiment was carried out. Immobilized beads without cells (about 2 cm in diameter) were prepared and placed in a single mesh bag (210 beads) by the same experimental procedure shown in Fig. 1, and soaked in 10 L of sediment mud solubilized with acid. Aeration was carried out after the addition of nutrients (glucose and pepton), again using the procedure shown in Fig. 1. The temperature was kept to 20–25 °C. After 2 and 3 d, the initial radioactivity of the sediment mud solubilized by acid, 7.26 μSv/h changed to 7.19 and 7.23 respectively. Thus alginate also unable to adsorb radioactive Cs.

So far, no work has reported the successful removal of 90% (100–10.6%) radioactive Cs through practical microbiological treatment. In addition, when fresh sediment mud solubilized by acid was used for treatment (the second batch) with the same immobilized cells, after 1 d the radioactivity of the liquid decreased to 2.95 μSv/h. The immobilized cells remained active in the second batch, but the liquid's radioactivity rose to 5.51 μSv/h after 3 d. The temperature decreased (to 17.1 °C) and the cells became inactive, along with the

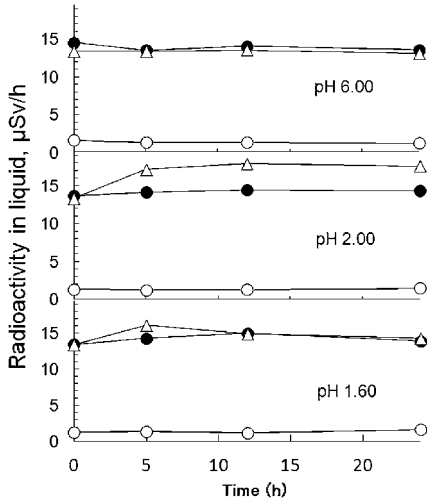

Fig. 2. Profiles of the Radioactivity of Cs during HNO_3 Solubilization of Concentrated Sediment Mud.

pH 6.00 indicates no HNO_3 addition, while pH 2.00 and 1.60 indicate conc. HNO_3 addition in concentrated sediment mud. No immobilized cells were added. ●, radioactivity of concentrated sediment mud broth; △, radioactivity in sediment residue after filtration; ○, radioactivity in filtrated water.

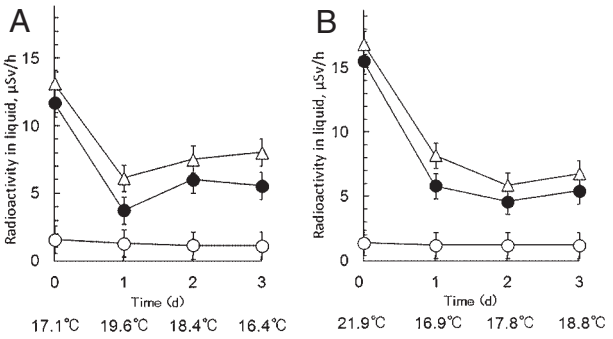

Fig. 3. Removal of Radioactive Cs from Concentrated Sediment Mud Directly (Without HNO_3 Treatment) (A) and Removal of Radioactive Cs from Sediment Mud Solubilized by Acid (With HNO_3 Treatment at pH of 2.00 for 1 D) Using Half Amount of Immobilized Cells (B).

Under the figure, the temperatures of the vessel at 10 am each day are shown. Symbols are identical to those used in Fig. 1.

release of Cs into the liquid, as adsorbed into or adsorbed onto the immobilized cells. Cs release has frequently been observed when cells become inactive due to substrate limitation and temperature decrease.[10] After three repeated batches, the SSI strain was still active in removing Cs, even at relatively low temperature. In the second and third batches, the removal rates were 83.9% and 78.3% respectively (Fig. 1) although the SSI strain was optimum for growth at 25–35 °C.

Surprisingly, Cs in the filtrated water was entirely absent (Fig. 1), and radioactivity was present in the sediment residue moiety. Cs appeared to be strongly bound to the sediment mud. Moreover, it was apparently removed by photosynthetic bacterial (SSI strain) activity.

For the elution of Cs from sediment mud by HNO_3 treatment, Cs did not elute into water even at a low pH of 1.60, as shown in Fig. 2. We confirmed that Cs was relatively strongly bound with the sediment mud which consisting of organic matter. Hashimoto[14] reported that organic muck consisting of humic and flubic acids strongly adsorbed cations, including Cs, at a level about 50 to 100 times higher than such clays as kaolinaite and mika. Kawamoto *et al.*[2] reported that at most 70% of Cs were released into water from polluted soil at 95 °C with 0.5 mol/L of HNO_3.

Removal of Cs from concentrated sediment mud (without HNO_3 treatment) was also carried out in batch treatment (Fig. 3A) using the same experimental procedure as that shown in Fig. 1, but almost the same results were obtained as that figure. The results shown in Fig. 3A suggest that radioactive Cs in sediment mud from a swimming pool can be removed by an SSI strain without acid treatment. This process is also applicable in removing radioactive Cs from soil that contains organic matter.

Based on these observations, our approach to removing Cs (Figs. 1 and 3A) starts to look interesting. A photosynthetic bacterium, the SSI strain appeared smoothly to transport Cs^+ from the sediment mud solubilized by acid because the radioactivity in the filtrated water was almost nil during the experiment. The total power to attract Cs^+ of the SSI strain appeared to be relatively strong as compared to the binding power of Cs^+ to sediment mud. The mechanism of Cs^+ attraction is assumed to operate as follows: First, a negative charge of EPS, produced on the surface of photosynthetic bacteria of the SSI strain adsorbs Cs^+ as a cation, as described elsewhere.[10,11] Next, the potassium transport system of the SSI strain is relatively active for Cs^+ uptake such as the cyanobacterium *Synecosystis* PCC 6803,[7] and by *Rhodococcus erythropolis* CS98.[8] Jasper[15] reported that a photosynthetic bacterium, *Rhodopseudomonas capsulata*, uptakes mainly Cs^+ by a potassium transport system. Hence, we speculate that the SSI strain strongly and smoothly transfers Cs^+ on and in immobilized cells by these two attractive powers (the negative charge of EPS and the potassium transport system) from sediment mud *via* the Cs^+ form. However, the details of the mechanism of removing Cs from sediment mud by the SSI strain are still under investigation.

Figure 3B shows treatment with half the amount of SSI cells. The only difference between Fig. 1 and Fig. 3B is that the cell amount was reduced by half. Although the amount of radioactive Cs removed was insufficient, Cs in sediment mud was removed even under low temperature with half the amount of immobilized cells. Accordingly, the cells needed to remove Cs can be reduced in consideration of shown practical costs, but, detailed optimum conditions must be elucidated. The results in Figs. 1 and 3 suggest the possibility of practical removal of radioactive Cs from water, organic sediment mud, and soil.

One advantage of alginate-immobilized SSI cells is a reduction in biomass due to drying and incineration. Harvested immobilized cells after three rounds of treatment (Fig. 1), which were easily performed with a mesh bag, were dried in an oven at 80–90 °C for 3 d with background radioactivity of the air in the oven at 0.12 μSv/h. Although alginate immobilizes cells (wet base), showing 1.3–2.0 μSv/h on the surface (sensor attached), no radioactivity was released into the air during drying, with a level of 0.12 μSv/h 1 d later, 0.13 μSv/h 2 d later, and 0.13 μSv/h 3 d later. The

radioactivity of the dried cells was also measured, but unfortunately only some of the wet immobilized cells (852 g) were dried. After drying (5.59 g), 11–45 μSv/h of radioactivity was detected on the surface of the dried residue, and the measured values fluctuated greatly depending on the precise position of the sensor, without showing a stable value. The amount of dried residue was not sufficient to cover sensor moiety completely, and thus the measurement did not accurate. However, high radioactivity was detected qualitatively on the residue of the dried immobilized cells. This suggests that the radioactive Cs in sediment mud was transferred to the immobilized cell moiety, because the immobilized cells were prepared in a low radioactivity environment (0.11–0.12 μSv/h).

The biomass was reduced to 97.2% (w/w) after drying from the original immobilized beads. After incineration, the biomass became ash, and the biomass weight was reduced by 99.3% from that of the original immobilized beads (wet base). Moreover, the mass of the biomass was reduced by 98.3% (v/v). Thus, biomass reduction achieved is also attractive from the viewpoint of reducing built-up stocks of radioactive waste materials.

The technology described in this paper can perpaps to be applied to the removal of Cs from radioactive soil.

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References

1) Asahi-Shinbun, p. 1, August 17, 2011.
2) National Institute of Advanced Industrial Science and Technology, http://www.aist.go.jp/aist_j/press_release/pr2011/pr2011083
3) Inaba M, "Textbook of NTS Seminar No. 6," NTS publisher, Tokyo, pp. 1–10 (2011).
4) Harvey RS and Patrick R, *Biotechnol. Bioeng.*, **11**, 449–456 (1967).
5) Plato P and Denovan JT, *Radiat. Bot.*, **14**, 37–41 (1974).
6) Haselwandter K and Berreck M, *Trans. Br. Mycol. Soc.*, **90**, 171–174 (1988).
7) Avery SV, Codd GA, and Gadd GM, *J. Gen. Microbiol.*, **137**, 405–413 (1991).
8) Tomioka N, Uchiyama H, and Yagi O, *Appl. Environ. Microbiol.*, **60**, 2227–2231 (1994).
9) Ohmura N, Watanabe Y, and Saiki H, "Report of Central Research Institute of Electricity," Central Research Institute of Electricity, Tokyo, pp. 1–31 (1996).
10) Sasaki K, Hara C, Takeno K, Okuhata H, and Miyasaka H, *Jpn. J. Wat. Treat. Biol.*, **46**, 119–127 (2010).
11) Sasaki K, Harada T, and Ohta M, *Chemistry*, **66**, 57–60 (2011).
12) Watanabe M, Kawahara K, Sasaki K, and Nopratnaraporn N, *J. Biosci. Bioeng.*, **95**, 374–378 (2003).
13) Yamaoka Y, Takeno K, Shinkawa H, Noparatnaraporn N, and Sasaki K, *Biosi. Biotechnol. Biochem.*, **72**, 1601–1603 (2008).
14) Hashimoto Y, "Textbook of NTS Seminar No. 5," NTS publishers, Tokyo, pp. 1–18 (2011).
15) Jasper P, *J. Bacteriol.*, **133**, 1314–1322 (1978).