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Removal of radioactive cesium from an aqueous solution *via* bioaccumulation by microalgae and magnetic separation

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We evaluated the potential sequestration of cesium (Cs^+) by microalgae under heterotrophic growth conditions in an attempt to ultimately develop a system for treatment of radioactive wastewater. Thus, we examined the effects of initial Cs^+ concentration (100–500 μM), pH (5–9), K^+ and Na^+ concentrations (0–20 mg/L), and different organic carbon sources (acetate, glycerol, glucose) on Cs^+ removal. Our initial comparison of nine microalgae indicated that *Desmodesmus armatus* SCK had removed the most Cs^+ under various environmental conditions. Addition of organic substrates significantly enhanced Cs^+ uptake by *D. armatus*, even in the presence of a competitive cation (K^+). We also applied magnetic nanoparticles coated with a cationic polymer (polyethylenimine) to separate ^{137}Cs-containing microalgal biomass under a magnetic field. Our technique of combining bioaccumulation and magnetic separation successfully removed more than 90% of the radioactive ^{137}Cs from an aqueous medium. These results clearly demonstrate that the method described here is a promising bioremediation technique for treatment of radioactive liquid waste.

The Fukushima Daiichi Nuclear Power Plant accident of 2011 released large amounts of radioactive nuclides into the environment. In particular, the release of radioactive cesium (^{137}Cs) was a major concern because of its long half-life (30.2 years), high water solubility, and rapid uptake by terrestrial and aquatic organisms due to its chemical similarity to potassium (K^+)[1,2]. This accident has thus led to the search for new methods that can prevent the adverse effects of pollution by radioactive nuclides, especially ^{137}Cs.

Researchers have previously examined the effects of many chemical and biological techniques for removal of Cs^+ and/or ^{137}Cs from wastewater effluents. Biological technologies have attracted intense interest because they appear to be less expensive and more ecologically friendly than non-biological methods[3]. The uptake of radioactive compounds by microorganisms can be a metabolism-independent process (biosorption, a physiochemical process that does not require cellular energy) or a metabolism-dependent process (bioaccumulation, uptake into the cytoplasm by use of cellular energy). Previous research indicated that energy-independent processes play a minor role in Cs^+ accumulation by microbes, because Cs^+ is a very weak Lewis acid and only has limited interaction with ligands[4]. However, microorganisms can actively take up Cs^+ *via* endogenous K^+ transport systems[5] because of the chemical similarity of Cs^+ and K^+. To the best of our knowledge, only a limited number of reports examined the use of microalgae for the bioaccumulation of environmental Cs^+. Conventional separation techniques, such as chemical precipitation and ion exchange, are well-developed, but are expensive and inefficient when the environmental concentration of Cs^+ is low. In general, contaminated environments have much lower concentrations of Cs^+ than other co-occurring and competing cations. Thus, a biological method that uses microalgae, which can efficiently accumulate low levels of Cs^+ in the presence of competing ions, is considered a promising approach[2].

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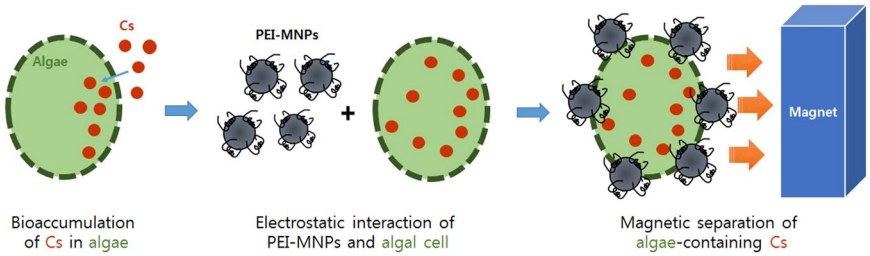

Figure 1. Overall process for removal of radioactive Cs *via* bioaccumulation by microalgae and magnetic separation.

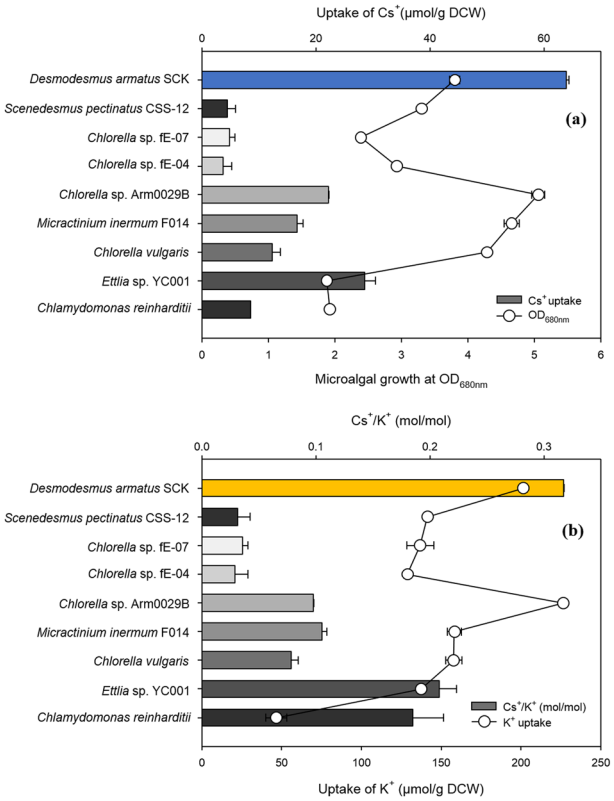

Figure 2. (**a**) Relationship of Cs^+ uptake (bars) and algal growth (circles) and (**b**) relationship of the Cs^+/K^+ ratio (bars) and K^+ accumulation (circles) by nine species of microalgae under constant illumination.

In this study, we examined the feasibility of using microalgae with a magnetic separation system to remove Cs^+ and ^{137}Cs from aqueous solutions. Thus, we initially examined the Cs^+-uptake capabilities of nine microalgae, including several newly isolated strains, under constant illumination to select the strain that best accumulates Cs^+. We then selected the best of these nine microalgae and examined the effect of different environmental factors on Cs^+ uptake and sequestration[6], including pH, initial concentrations of different ions (Cs^+, K^+, and Na^+), and different organic carbon sources (acetate, glycerol, and glucose). We also used polyethyleneimine (PEI)-coated magnetic nanoparticles (MNPs) to simultaneously recover the microalgae and ^{137}Cs from solution. As a cationic surfactant, polyethlylenimine (PEI), which is known for its high density of positive charge, was introduced onto the surface of Fe_3O_4 nanoparticles to synthesize PEI-coated magnetic nanocomposites[7]. The proposed overall approach is summarized in Fig. 1.

Results and Discussion

Screening of Cs^+-accumulating microalgae. We initially grew the nine different microalgae under constant illumination in Cs^+-containing TAP medium, following 3 days of growth in K^+-starved TAP medium (Fig. 2a). Measurements of OD_{680nm} indicated *Chlorella* sp. Arm 0029B had the highest cell density, followed by *M. inermum* F014, *C. vulgaris*, and then *D. armatus* SCK. Measurements of Cs^+ uptake by these microalgae indicated that *D. armatus* SCK removed the greatest amount of Cs^+ (63.9 μmol/g DCW), followed by *Ettlia sp*. YC001 (28.6 μmol/g DCW), *Chlorella* sp. Arm 0029B (22.2 μmol/g DCW), and *M. inermum* F014 (16.7 μmol/g DCWM)

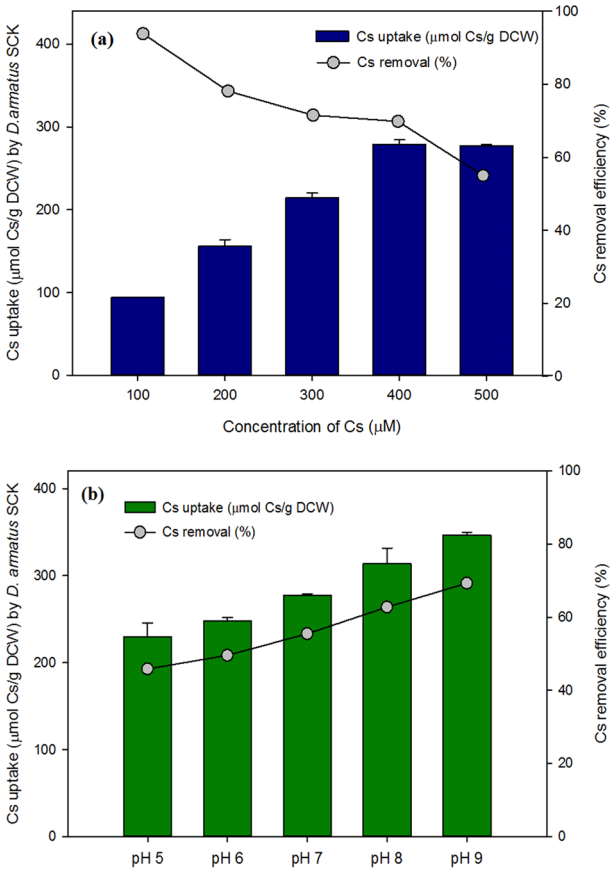

Figure 3. (**a**) Effect of initial Cs^+ concentration on Cs^+ uptake (bars) and removal efficiency (circles) at pH 7, and (**b**) effect of pH on Cs^+ uptake (bars) and removal efficiency (circles) at 400 μM Cs^+ by *D. armatus* SCK cells grown in TAP medium.

(Fig. 2a). We also examined the cellular uptake of K^+ from TAP medium without Cs^+ (Fig. 2b). It is well known that cells take up Cs^+ using their K^+ transport systems, such as the K^+/K^+ and K^+/H^+ exchanger[5,8,9].

However, our results indicated the uptake of K^+ was not always proportional to uptake of Cs^+. Based on uptake of K^+, *D. armatus* SCK had the greatest Cs^+ uptake at a Cs^+/K^+ molar ratio of 0.33. This indicated that uptake of Cs^+ does not necessarily correlate with uptake of K^+ or cell growth. Trans-membrane movement of monovalent cations like Cs generally occurs against a concentration gradient and thus energy is consumed to drive it. Additionally, it likely has to do with the plasma membrane-bound H^+-ATPase, which acts to generate a transmembrane electrochemical proton gradient[4,5]. It is therefore possible that cation transports are coupled with H^+ movements by either symport or antiport. Besides, the monovalent cation uptake may be mediated rather directly by K^+-ATPase[4,5]. All this led to a mechanistic hypothesis that an apparent Cs^+ uptake capacity is could be different for each cell type whose monovalent cation transport system has varied affinities toward Cs.

Previous studies reported that *Chlorella salina* and *Synechocystis* PCC 6803 accumulated Cs^+ in the range of 0.8 to 0.49 nmol per 10^6 cells when grown in BG-11 medium under constant illumination[10,11]. We found that *D. armatus* SCK sequestrated 2.08 nmol Cs^+ per 10^6 cells under our growth conditions, so this microalga appears to be a promising option for the removal of environmental Cs^+. Thus, all of our subsequent experiments focused on this species.

Effects of initial Cs^+ concentration and pH on Cs^+ removal. Cs^+ is a hard metal that is generally non-toxic to microorganisms because of its weak coordinating ability. Moreover, several hard metals are essential nutrients for microbial growth, and so they are readily accumulated[4]. Thus, we examined the effect of the initial Cs^+ concentration and pH on Cs^+ uptake by *D. armatus* SCK (Fig. 3). The results show that Cs^+ uptake increased with an increasing initial Cs^+ concentration, with an apparent saturation near 400 to 500 μM. The maximum equilibrium uptake of Cs^+ was 280 μmol/g DCW and the removal efficiency was 70% at 400 μM. Tomioka *et al.*[12] reported that several strains of *Rhodococcus* accumulated Cs^+ in the range of 98.3 to 395 μmol/g cells weight for initial Cs^+ concentrations of 0.01 to 1 mM, but that the Cs^+ uptake did not increase when the extracellular Cs^+ concentration was greater than 100 μM[12].

The pH of the growth medium can impact the bioaccumulation or adsorption of Cs^+. Our results show that the bioaccumulation of Cs^+ by *D. armatus* SCK was efficient at pH values between 5 and 9, and the greatest uptake and removal efficiency were at pH 9. This result is similar to those reported for cyanobacterial removal of Cs^+. In

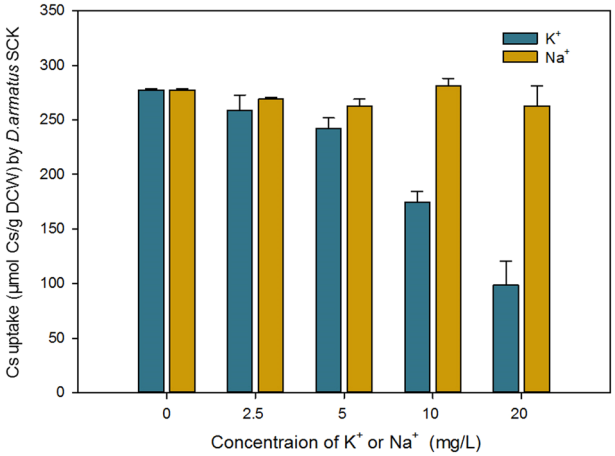

Figure 4. Effect of K^+ and Na^+ concentration on Cs^+ uptake by *D. armatus* SCK grown in TAP medium containing 400 μM Cs^+.

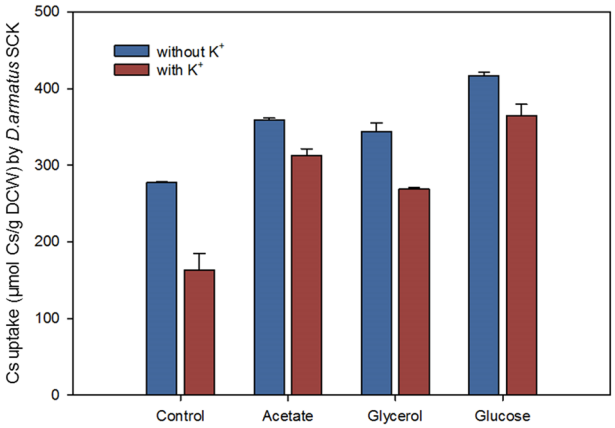

Figure 5. Effect of 500 μM K^+ and three different carbon sources on uptake of Cs^+ by *D. armatus* SCK when grown in liquid medium containing 400 μM Cs^+.

particular, optimal Cs^+ accumulation by *Synechocystis* PCC 6803 and *Rhodococcus* strain occurred under alkaline conditions (pH 9)[1,10], probably because plasma membrane depolarization at low pH inhibits Cs^+ uptake[10].

Effects of K^+ and Na^+ on Cs^+ removal. Cs^+ is an alkali monovalent cation that cells can transport because of its similarity to K^+. Thus, we examined the effects of K^+ and Na^+ concentration on Cs^+ uptake by *D. armatus* SCK (Fig. 4). The results show that the Na^+ concentration had little or no effect on Cs^+ uptake in the concentration range tested, but that Cs^+ uptake declined as the K^+ concentration was above 10 mg/L. Similar to other microbes, our results show that Cs^+ accumulation may occur through sharing K^+-transport channel, not Na^+-migration route[5]. Similarly, Cs^+ accumulation by *Synechocystis* sp. Strain PCC 6803 and *C. emersonii* declined as the concentration of K^+ increased[5,10]. These results also suggest that the K^+-transport system(s) of phototrophic microalgae, which are not specific to K^+, play an important role in accumulation of Cs^+[12]. The K^+ concentrations in natural fresh water and ground water systems typically range from 0.5 to 3 mg/L, levels that have no apparent impact on Cs^+ uptake by *D. armatus* SCK[2]. However, our results indicate that when this strain is used in laboratory or bioengineering studies, the K^+ concentration should be 10 mg/L or less.

Effects of different organic carbon sources on Cs^+ removal. We investigated the effects of three organic carbon sources (acetate, glycerol, and glucose; concentration: 1 g-Carbon/L) on Cs^+ uptake by *D. armatus* SCK with or without 500 μM K^+ (Fig. 5). The results show that each carbon source notably increased Cs^+ uptake in the presence or absence of K^+, and that glucose had the greatest effect. In agreement, a previous study also reported increased net K^+ uptake by heterotrophic *Rhodococcus* cells when glucose was added to the growth medium[13].

Glucose-induced net uptake of K^+ and Cs^+ is likely due to stimulation of an electro-neutral ATP-dependent K^+/H^+ exchange, because glucose metabolism acidifies the cell interior. Ohunki *et al.*[14] also found that glucose stimulated the accumulation of Cs^+ in a fungal strain[14]. Our results also indicate that glucose and other carbon sources can act as energy sources that increase Cs^+ accumulation by microalgae under heterotrophic conditions. In contrast, other research indicated that *Chlorella* accumulated 2-fold less Cs^+ in chemoheterotrophic conditions

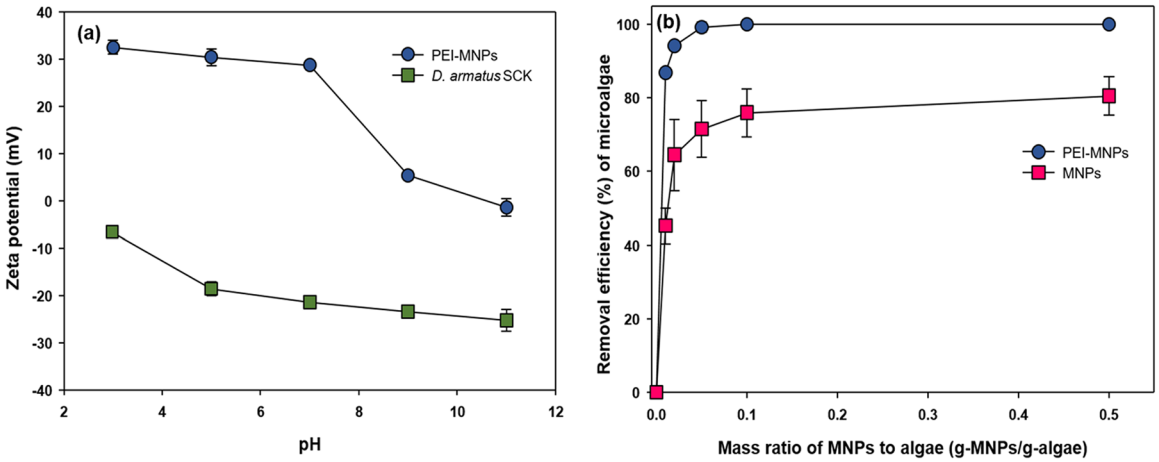

Figure 6. (**a**) Zeta potentials of PEI-MNPs and *D. armatus* SCK at different pHs, (**b**) Recovery efficiency of *D. armatus* SCK according to the mass ratio of MNPs to algae.

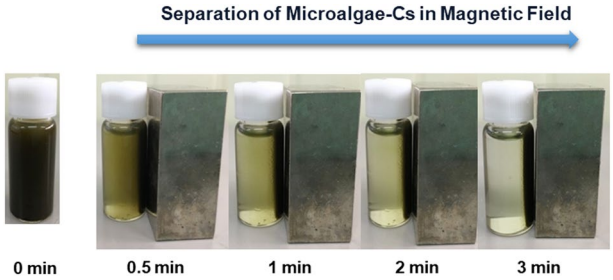

Figure 7. Time course of separation of Cs^+-containing microalgae using PEI-MNPs in a magnetic field.

relative to photoautotrophic conditions[5]. Few previous studies have examined the effects of other organic substrates, such as acetate and glycerol, on the uptake of Cs^+ by phototrophic microalgae. Our results (Fig. 5) indicate that acetate and glycerol significantly increased the uptake of Cs^+, although glucose had a stronger effect. Acetate is a volatile fatty acid that microalgae can directly convert into acetyl-CoA (an intermediate in the synthesis of cellular fatty acids) *via* the pyruvate pathway in the absence of glucose[15], so this may explain its effect (Fig. 5). Although glycerol was not as effective as glucose, it can be considered as an alternative to improve the accumulation of Cs^+ by *D. armatus* SCK. Therefore, from an economic perspective, these two low-cost organic carbon sources (acetate and glycerol) have potential for enhancing the accumulation of Cs^+ during the heterotrophic growth of *D. armatus* SCK.

Removal of ^{137}Cs using bioaccumulation and magnetic separation. We measured the separation of microalgae containing Cs^+ from liquid medium by use of PEI-MNPs. In an aqueous solution, the surface of metal oxide nanoparticles contains hydroxyl groups, which undergo pH-dependent protonation/deprotonation. Fe_3O_4 nanoparticles are generally negatively charged at pH 7[16,17]. Therefore, the negative surface potential of algal cells (*D. armatus* SCK: -21.4 ± 0.92 mV at pH 7) makes them strongly attracted to Fe_3O_4 nanoparticles that are coated with PEI, which have a high-density cationic charge ($+28.7 \pm 0.64$ mV at pH 7) (Fig. 6a). As reported in previous studies, the addition of cationic functional groups, such as PEI, to the surface of coated particles increases the effectiveness of separation[18–20]. As shown in Fig. 6b, the maximum recovery efficiency (~100%) was achieved when the mass ratio exceeds 0.05 g-PEI-MNPs/g-algae, which showed the improved magnetic separation than that of naked Fe_3O_4 particles. We also found that the complex of magnetic nanocomposites and microalgal cells were easily separated within 3 min in a magnetic field (Fig. 7). Thus, this magnetic harvesting method has potential for the efficient separation of microalgae containing ^{137}Cs because it is simple, rapid, and consumes very little energy.

We also examined the use of *D. armatus* SCK cells followed by magnetic separation for removal of ^{137}Cs from a medium with glucose (1 g/L) and 10 Bq/mL or 100 Bq/mL of ^{137}Cs (Fig. 8). Measurement of radioactivity in the liquid medium before and after treatment indicated these cells sequestered 91.4% and 97.1% of ^{137}Cs at 10 and 100 Bq/mL of ^{137}Cs, respectively (Fig. 8). These data are meaningful because low levels of ^{137}Cs are often difficult to remove using conventional methods in aqueous systems.

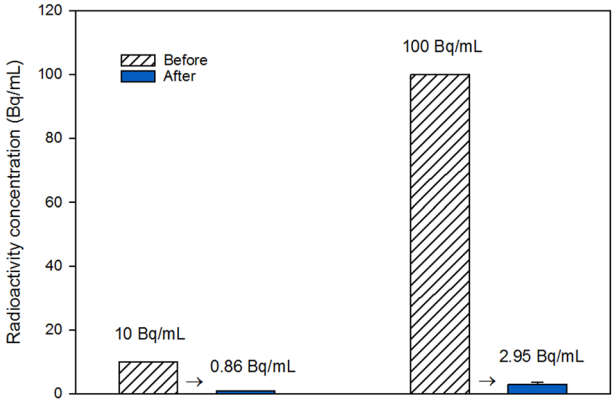

Figure 8. Change of ^{137}Cs concentration in the liquid medium following bioaccumulation by *D. armatus* SCK and magnetic separation.

Materials and Methods

Screening of Cs-accumulating microalgae. Four microalgae that accumulate Cs^+ were isolated from a local sewage treatment plant (*Desmodesmus armatus* SCK) and a lake (*Scenedesmus pectinatus* CSS-12, *Chlorella* sp. fE-04, and *Chlorella* sp. fE-07) in Daejeon, Republic of Korea. An additional five microalgae from the culture collections of the Korea Research Institute of Bioscience and Biotechnology (KRIBB) in Dajeon, Korea (*Chlorella* sp. ArM0029B, *Ettlia* sp. YC001, and *Chlamydomonas reinhardtii* CC124), Pusan National University in Pusan, Korea (*Micractinium inermum* F014), and University of Texas (UTEX), Austin, TX, USA (*Chlorella vulgaris* UTEX265). For screening of Cs-accumulating microalgae, each strain was grown in 1 L vessel in Tris-acetate phosphate (TAP) medium (pH = 7) with 100-μmol/L of CsCl and the following additional components: 25 mL of TAP salts, 0.375 mL of phosphate solution, 1 mL of Hunter's trace elements, 1 mL of glacial acetic acid, and 2.42 g of Tris[15]. The initial cell concentration was adjusted to an optical density of 0.2 at 680 nm. These algal strains were cultivated for 7 days in baffled hybrid flasks on a shaker (120 rpm) at 25 °C.

Effects of various parameters on Cs removal by microalgae. To evaluate the maximum uptake capability of Cs^+ by microalgae, cultivated cells were transferred to K^+-depleted TAP medium and maintained for 3 days (Supplementary Fig. S1). Then, cells in the early stationary growth phase were collected by centrifugation at 7000 rpm for 10 min, and the bio-pellets were suspended in a 20-mM Tris buffer solution containing CsCl, with the biomass adjusted to 1 g/L dry cell weight (DCW). Cell suspensions were then incubated at 25 °C for 24 h with rotary shaking (120 rpm) under continuous illumination. The effects of the initial concentrations of Cs^+, K^+ and Na^+, and various organic carbon sources (acetate, glycerol, and glucose) were examined at 25 °C under continuous illumination. The removal efficiency of non-radioactive Cs^+ was calculated as:

$$\text{Removal efficiency (\%)} = (C_i - C_f)/C_i \times 100 \quad (1)$$

where C_i and C_f are the initial (i) and final (f) concentrations of Cs^+.

Synthesis of PEI-Fe_3O_4 nanoparticles and magnetic separation. PEI (polyethyleneimine)-coated magnetic nanoparticles (MNPs) were synthesized according to a previously reported procedure[7]. First, iron salts (0.99 g $FeCl_2{\cdot}4H_2O$ and 2.7 g $FeCl_3{\cdot}6H_2O$) were dissolved in 100 mL of deionized water, and deoxygenated with nitrogen gas at 80 °C. Subsequently, 10 mL of NH_4OH (25% by wt.) was added and stirred for 0.5 h. After cooling to room temperature, the precipitated nanoparticles were separated with a magnet and washed four times with deionized water. Then, the Fe_3O_4 nanoparticles were added to a PEI solution (MW = 2 kDa) in phosphate buffer at pH 7.3 (10% by vol.). Finally, the PEI-MNPs were collected using a magnetic field and washed three times with deionized water. After synthesis of the nanoparticles, the zeta potentials of PEI-MNPs and prepared microalgae were measured by a Zetasizer instrument (Nano-ZS, Malvern, UK).

For the magnetic separation and removal of radioactive ^{137}Cs, *D. armatus* SCK cells were cultivated in a Tris buffer solution containing 10 or 100 Bq/mL of ^{137}Cs, and the PEI-MNPs were mixed with the cultures for 1 min. Then, the microalga-nanoparticle aggregates containing ^{137}Cs were separated from the medium using an external permanent magnet within 3 min. After separation, the radioactivity concentration of the solution was measured to calculate the removal efficiency, as described above.

Analytical methods. To quantify cell growth, the optical density of each sample was measured at 680 nm using a UV-visible spectrophotometer (UV-1800; Shimadzu, Japan). Cell counts were performed using an optical microscope (DM2500; Leica, Switzerland) with a hemocytometer.

After the Cs removal experiments, the supernatant was filtered through a PVDF membrane filter (0.2 μm), and the amount of non-radioactive Cs^+ remaining in the filtrate was quantified using inductively coupled plasma-mass spectrometry (ICP-MS; ELAN DRC II, Perkin-Elmer). Thus, the cellular uptake of Cs^+ was determined by measuring the change in Cs^+ concentration of the growth medium. The levels of K^+ and Na^+ were determined by ion chromatography (883 Basic IC plus; Metrohm AG, Switzerland) using an anionic column

(Metrosep A Supp 5–150/4.0; Metrohm AG, Switzerland). The ^{137}Cs concentration was determined using γ-spectrometry (Canberra, Genie 2000).

Conclusion

The aim of this work was to evaluate the ability of microalgae to remove Cs^+ and ^{137}Cs from aqueous solutions. Initial experiments indicated that a novel strain, *D. armatus* SCK, was the most effective of nine tested strains in the removal of Cs^+. Our results also showed that *D. armatus* SCK accumulated high levels of Cs^+ in the presence of competitive cations (Na^+ and K^+), that acetate and glycerol (inexpensive carbon sources) enhanced the uptake of Cs^+, and that uptake was greater at a higher pH. Use of *D. armatus* SCK for bioaccumulation and PEI-MNPs for magnetic separation of cells led to highly effective removal of ^{137}Cs from aqueous solutions. The use of microalgae-magnetic particles with an inexpensive organic substrate appears to have great potential for bioremediation of ^{137}Cs-polluted environments.

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Author Contributions

B.G. Ryu designed the research and supervised the experiments. I. Kim conducted the most of experiments and wrote the manuscript. H.M. Yang synthesized the PEI-Fe_3O_4 nanoparticles and assisted Ilgook Kim in performing magnetic separation tests. C.W. Park prepared radioactive cesium solution and assisted in analyzing data. I.H. Yoon, B.K. Seo and E.K. Kim gave B.G. Ryu a number of critical ideas and suggestions for the study.

Additional Information

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Competing Interests: The authors declare no competing interests.