Short Communication

Photosynthetic biomineralization of radioactive Sr via microalgal CO₂ absorption

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Highlights

• Photosynthetic biomineralization of ⁹⁰Sr was performed by microalgae.
• Sr was crystallized as strontianite (SrCO₃) at the biomass/liquid interface.
• Chlorella vulgaris carried out the highest ⁹⁰Sr removal ever reported.
• Microalgal carbonating process is one of innovative ways to effectively remove Sr.

Article Info

Article history:
Received 17 July 2014
Received in revised form 1 September 2014
Accepted 4 September 2014
Available online 16 September 2014

Keywords:
Radiostrontium
Microalgae
Biomineral
Chlorella vulgaris
Photosynthesis

Abstract

Water-soluble radiostrontium (⁹⁰Sr) was efficiently removed as a carbonate form through microalgal photosynthetic process. The immobilization of soluble ⁹⁰Sr radionuclide and production of highly-precipitable radio-strontanite (⁹⁰SrCO₃) biomineral are achieved by using Chlorella vulgaris, and the biologically induced mineralization drastically decreased the ⁹⁰Sr radioactivity in water to make the highest ⁹⁰Sr removal ever reported. The high-resolution microscopy revealed that the short-term removal of soluble ⁹⁰Sr by C. vulgaris was attributable to the rapid and selective carbonation of ⁹⁰Sr together with the consumption of dissolved CO₂ during photosynthesis. A small amount of carbonate in water could act as Sr²⁺ sinks through the particular ability of the microalga to make the carbonate mineral of Sr stabilized firmly at the surface site.

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1. Introduction

Current growing interest in nuclear power as a potential solution for global energy may also raise serious environmental and health concerns due to unanticipated nuclear accidents. An enormous amount of radioactive nuclides was released during the accidents of Chernobyl and Fukushima, and some of these reached the stratosphere and caused widespread contamination of the environment (García-León and Manjón, 1997; Bolsunovsky and Dementyev, 2011; Shimura et al., 2012). Of these radioactive nuclides, radiostrontium (⁹⁰Sr) and radiocesium (¹³⁷Cs) are important because of their long half-life (around 30 years) and high water solubility (Howard et al., 1991; Robison and Stone, 1992; Dupré de Boulois et al., 2008; Kuwahara et al., 2011; Thorpe et al., 2012). Water-soluble radioactive ⁹⁰Sr can contaminate aquatic ecosystems (Yablokov and Nesterenko, 2009). Radiocesium (¹³⁷Cs) is also an important radionuclide because of its ability to transfer to living organisms through food chains (Avery, 1996). Thus, developing an effective and economical method for removing the radionuclides from aquatic ecosystems and radioactive wastewater has become an increasingly important issue.

Conventional methods employed to remove the radionuclides by mostly chemical precipitation and ion exchange, but they are costly and/or ineffective for low concentrations with significant large volume. Therefore, there is an urgent and indispensable need for development of innovative but low cost processes to efficiently remove ⁹⁰Sr and ¹³⁷Cs.

There has been rare study for concentrating soluble radionuclides into hard materials (e.g., SrCO₃) using living organisms for...
the strategy of radioactive wastewater remediation (Krejci et al., 2011; Achal et al., 2012). Especially, the microalgal-driven radiostrontium ($^{90}$Sr) sequestration by biominal carbonation ($^{90}$SrCO$_3$) process has not been reported. The present study focuses on the elimination and mineralization of $^{90}$Sr utilizing microalgae, especially at environmentally relevant strontium concentrations and in relation to their activity.

Microalgae (~μm in size) represent an important group in the aquatic environment given their abundance and broad occurrence in fresh and sea water as well as in soils and extreme habitats (He et al., 2013; Ji et al., 2014). Chlorella is unicellular green microalga ubiquitously present all over the aquatic world. They are autotroph and proliferate well in waters containing low concentrations of inorganic nutrients. Thus, these species would exhibit great potential in wastewater treatment.

2. Experimental

2.1. Microalgae culture

Chlorella vulgaris (UTEX 26, UTEX Culture Collection of Algae) was cultured in 1.5% Modified Basal Medium (MBM) (Shi et al., 1997) agar plate for 1 month with continuous light at 30 °C. Chlorella sorokiniana ANA9 was cultured in 1.5% YPG media (peptone, 10 g; yeast extract, 5 g; glucose, 10 g) agar plate (Yoshida et al., 2006) for 3 days with continuous light at 30 °C. Each strain was washed 3 times with 3 mM of NaHCO$_3$ to remove and minimize any residual media and trace elements for subsequent experiments. Finally, cells were resuspended in 3 mM of NaHCO$_3$. Cell viability and pH stability of cell suspension for a certain period of time, 14 days for example, were investigated, confirming that cells could survive in simple sodium bicarbonate solution after several days. However, in fresh and sea water as well as in soils and extreme habitats (He et al., 2013; Ji et al., 2014).

2.2. Radioisotopes ($^{90}$Sr and $^{137}$Cs) uptake in cell suspension

For the kinetics study, aqueous media (3 mM of NaHCO$_3$) containing radionuclides ($^{90}$Sr and $^{137}$Cs) were prepared. Prior to the addition of radionuclides into the media, their stock solutions for $^{90}$Sr and $^{137}$Cs were prepared in 74,000 and 37,000 Bq/ml radioactivity, respectively. From the stock solutions, $^{90}$Sr and $^{137}$Cs were aseptically and separately injected as $^{90}$SrCl$_2$ (200 and 2000 Bq/ml) and $^{137}$CsCl (210 and 2100 Bq/ml). Microalgal cells were dispensed into the radioactive media (30 ml of NaHCO$_3$ 3 mM) to provide ca. 0.1–0.2 million cells/ml in a near-neutral pH condition. The cell suspensions were incubated at 30 °C for 6 days under illumination (1800 lux) of LED. Periodically, liquid radioisotopic samples were aseptically removed by syringe and needle through 0.2 μm cellulose acetate filters and analyzed to determine the change of radioactivity in the solution using beta or gamma-ray spectroscopy.

2.3. Radioactivity measurement and microscopic analysis

The radioactivity (Bq/ml) of $^{90}$Sr in the liquid sample was measured by a liquid scintillation counter (LSG, Perkin Elmer, Tri-Carb 2910 TM) in the range of 0–2000 keV for 600 s. A diluted solution that has liquid sample (3.9 ml) and distilled water (1.1 ml) in a vial was mixed with 15 ml of cocktail solution (Ultima Gold™). The prepared solution was kept in a cold and dark place for 24 h before a beta-ray measurement by the LSC. The $^{137}$Cs radioactivity in a diluted solution that was prepared by mixing liquid sample (1 ml) and distilled water (9 ml) was measured using a gamma-ray spectroscopy (Canberra, Ge detector GC2018) at 661 keV for 120 s. The change of cell numbers in the media with time was frequently measured using UV–Vis absorption spectrophotometer (Cary 300, Agilent Technologies) at 686 nm.

To microscopically identify the existence of radionuclides in and around the cellular body, Sr$^{2+}$ (as stable Sr(NO$_3$)$_2$) or Cs$^+$ (as stable CsCl) was added in the aqueous media to achieve 2.0 mM concentration. Solid samples (cell + nuclides) were frequently removed by syringe and needle, and then the samples were investigated by using X-ray diffraction and other microscopic analyzers. For X-ray diffraction (XRD) analysis, the solid phase separated from solution by centrifugation (4000 rpm for 10 min) was frozen and freeze-dried for 48 h by a freeze dryer (Bondiro, Ilshin Co). The XRD analysis was performed with a Bruker D8 Advance automatic horizontal goniometer diffractometer equipped with a scintillation counter and a Cu X-ray tube operating at 40 kV/30 mA in a continuous scan mode. For the electron microscopic observation, the microalgal suspension was centrifuged, frozen, and dried in the freeze-dryer. The microalgal sample was uniformly sprayed on a carbon tape pasted on the specimen holder. In order to avoid charging during observation, the sample was coated with a thin OsO$_4$ (~10 nm) layer. The sample was observed using field emission scanning electron microscopy (FESEM; S-4700, Hitach) to acquire its morphological and structural information in detail. Chemical analysis was also carried out using an energy dispersive X-ray spectrometer (EDS; EMAX, Horiba). HRTEM (high-resolution transmission electron microscopy) was used to examine the cell and its metabolic byproduct in detail, especially for the biogenic structure and crystallinity (Lee et al., 2014). The collected sample was fixed in 2% paraformaldehyde and 2% glutaraldehyde. After washing it with Na-cacodylate, it was gradually dehydrated by ethanol series and then infiltrated into Spurr’s resin (see Table S1 for the detailed procedure). Cured blocks of the resin sample were sectioned by the ultramicrotome diamond knife (MT-X RMC). Ultrathin sections (50–70 nm in thickness) were mounted on copper grids with Formvar support film coated with carbon. Samples were examined by the JEOL JEM 2100F high-resolution field emission TEM at 200 kV. An energy dispersive X-ray spectrometer (EDS) was also used to analyze a chemical distribution of isotopes.

3. Results and discussion

3.1. Uptake of radionuclides by microalgae

C. vulgaris and C. sorokiniana cultivated in MBM and YPG media, respectively, were collected by centrifugation and washed three times with NaHCO$_3$, 3 mM. The viability of microalgae interacted with intense radioactive $^{90}$Sr and $^{137}$Cs was evaluated in different levels of radioactive solutions for 6 days. As a result, their survival patterns were different from each other according to the radiation effect on the organisms (Fig. S1). With high radioresistance, C. vulgaris has doubled its biomass within 2 days in a malnurtite condition. When cultured under illumination, uptake of radiostrontium and radioceusim by the microalgae was observed with time after the addition of $^{90}$SrCl$_2$ and $^{137}$CsCl, and maximum removals of $^{90}$Sr and $^{137}$Cs by C. vulgaris were >90% and 70% of the total $^{90}$Sr and $^{137}$Cs, respectively (Figs. 1 and S2). Both the microalgal cultures containing C. vulgaris and C. sorokiniana demonstrated different efficiency for strontium uptake. These results indicate that the removal rates of strontium highly depend on microalgal strains. The removal of radiostrontium by C. vulgaris was very fast in 24 h, in which almost 80% of the aqueous $^{90}$Sr was removed from the solution. Even in 2000 Bq/ml radiation, the dissolved $^{90}$Sr decreased quickly and the $^{90}$Sr radioactivity was little detected in solution after several days. However, $^{90}$Sr sequestration by
C. sorokiniana was a little inefficient in higher \(^{90}\)Sr radioactivity with lower removal rate of \(^{90}\)Sr.

### 3.2. Photosynthetic biocrystallization of soluble Sr\(^{2+}\)

To probe the sequestered Sr kept by the cell, the centrifuged cell suspension interacted with soluble Sr was pretreated by fixation and dehydration and subsequent polymerization with resin for HRTEM investigation. A cross-section of the polymerized sample was made using a microtome to a thickness of 50–70 nm. A detailed procedure for the HRTEM sample preparation is described in Table S1.

The strontium trapped by C. vulgaris was overall examined using SEM-EDS, and a trace amount of Sr (3.47 wt%) was detected on the cell body (Fig. S3). A byproduct derived from the interaction between cell and Sr was grown to micrometer sizes, and it was found as inorganic particles including considerable contents of strontium (average 40 wt%) within them. The biologically induced byproduct had a characteristic feature of elliptical cone morphology with averages of 5 \(\mu\)m in length and 1 \(\mu\)m in thickness.

C. vulgaris successfully survived in the highly radioactive conditions of Sr, displaying excellent biomineralization of soluble Sr\(^{2+}\). It seems that the organism effectively alleviated high toxic \(^{90}\)Sr by preventing its inward diffusion and synthesizing a solid phase at the outer surface. To elucidate the biological removal of Sr, HRTEM was used to inspect the Sr concentration and distribution over the cell body at the subcellular scale. A nanoscale probe for the sectioned specimen has made possible to find numerous fine Sr particles outside rather than inside of the cell body (Fig. S4). The fine particles seem to be an indirect evidence that microalgae could dexterously nucleate soluble \(^{90}\)Sr to solid \(^{90}\)Sr aiming at outliving in the severe radioactive environment.

The exposed cell surface can function to provide proper sites to trap toxic Sr that diffuses into the cell body by combining strontium and carbonate. Cell walls are usually composed of heteropolysaccharides and offer metal-binding functional groups such as carboxyl, hydroxyl, sulfhydryl, phosphoryl and amino groups which cause a negatively charged or polar cell surface (Kalin et al., 2005; Bansal et al., 2012). The functional groups could afford a place for the mobile and toxic Sr ions to be captured and stored up efficiently, limiting the free-diffusion of Sr into the body.

A direct HRTEM probe on the Sr solid associated with the cell surface wall reveals a way of Sr crystallization facilitated on the surface as a biological pathway of Sr sequestration (Fig. S5). Sr particles that were observed on the cell wall showed a characteristic feature of multi-nucleated nanoparticles with typical lattice fringes of strontianite. HRTEM mapping for the strontium clearly showed that most of Sr was locally enriched around the cell wall, while a small fraction of Sr was only detected inside of the cytoplasm. This result implies that Sr was removed by the microalgae through two processes: firstly, Sr is rapidly and efficiently biosynthesized as a solid phase on the cell wall, and secondly, a part of Sr is taken up into the cytoplasm (Krejci et al., 2011).

### 3.3. Microalgae-driven Sr solid formation as radio-strontianite (\(^{90}\)SrCO\(_3\))

Conceptual model for the Sr enrichment at the outer surface of cell reveals that soluble and toxic Sr\(^{2+}\) ions are trapped by the cell surface, where \(^{90}\)SrCO\(_3\) nucleation and growth are facilitated rather than in normal aqueous solution (Fig. 2). The carbonate (e.g., CO\(_3\)\(^{2-}\)) ions can be directly incorporated into the framework of biologically-forming \(^{90}\)Sr-carbonate phase. This procedure will be termed “biological \(^{90}\)Sr synthesis” and the carbonate species that react with Sr\(^{2+}\) ions can be available from water-dissolved CO\(_2\) chemicals (HCO\(_3\)\(^{-}\) and CO\(_3\)\(^{2-}\)) during active photosynthesis (Nonova and Tosheva, 2014). There may be an unknown mechanism for promoting nucleation and/or facilitating crystal growth of Sr at the...
surface, which convert dissolved species to solid phases. For example, local supersaturation of carbonate and subsequent pH increase at the surface of cell could facilitate the growth of crystal nuclei and carbonate-binding process incorporating Sr as a solid phase. It is believed that a specialized photosynthetic Sr carbonating ability and membrane-bound protein of *C. vulgaris* are responsible for storing up strontium and carbonate and directing their surface assembly into high-ordered structure. Such a microalgae-driven crystallization is potentially important as a course of photosynthetic Sr carbonation to steadily remove $^{90}$Sr from radioactive wastewater.

Using X-ray diffraction technique, it was found that a residual solid obtained from the microalgae-free solution was not only a bit small in quantity but also a mixture of amorphous strontium-carbonate and sodium-bicarbonate (Fig. S6). However, the solid phase produced by *C. vulgaris* was identified as a pure and high dense SrCO$_3$ crystal. However, the solid phase produced by *C. sorokiniana* was a little amorphous with lower XRD intensity and larger background as compared with that by *C. vulgaris*. This result shows that the uptake and subsequent biocrystallization of Sr are much affected by an innate character of specific microalgae.

### 4. Conclusions

Living *C. vulgaris* had a potential to remove $^{90}$Sr to very low concentrations in highly radioactive conditions. The strontium trapped by *C. vulgaris* was confirmed as a carbonate solid phase, strontianite (SrCO$_3$). The $^{90}$Sr biocrystallization process that becomes structurally denser by microalgae is very important to effectively grasp soluble Sr ions and to prevent Sr resolubilization later on. This result manifests that the environment-friendly innovative method to sequester soluble radiostrontium as an immobile biominerial through the microalgae-driven carbonating process could play an important role to prevent radiostrontium spread to ecological environments.

### Acknowledgements

This research work was supported by the National Nuclear R&D Program (2012M2A8A4055325) and (2012M2A8A5025589) through the National Research Foundation (NRF) funded by The Ministry of Science ICT & Future Planning of Korea. The authors acknowledge the late Jungmin Kim for motivating us to initiate this work.

### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.biortech.2014.09.023](http://dx.doi.org/10.1016/j.biortech.2014.09.023).

### References


Supporting Information

Photosynthetic biomineralization of radioactive Sr via microalgal CO₂ absorption

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Fig. S1. Variations of living microalgal cell numbers of (a) C. vulgaris and (b) C. sorokiniana over time in radioactive solutions (200–2,100 Bq/ml radioactivity) with ⁶⁰Sr and ¹³⁷Cs.

Fig. S2. Aqueous ¹³⁷Cs (2,100 Bq/ml radioactivity) removal by C. vulgaris and C. sorokiniana for 6 incubation days.

Table S1. A detailed procedure for the HRTEM sample preparation.
**Fig. S3.** SEM photomicrograph and EDS spectra for the precipitable residues of (a) *C. vulgaris* and (b) its metabolic byproduct. Arrows indicate the microalgal-byproduct, strontium carbonate.

**Fig. S4.** A cross-sectioned HRTEM image of *C. vulgaris* cells that tightly keep Sr nanoparticles on the surface.

**Fig. S5.** HRTEM images of (a) a distinguished feature of fine Sr particles around the cell wall of *C. vulgaris*, (b) an enlarged view of (a) showing the Sr solid phase with multiple nm-seeds. (c) Lattice fringes of highly-ordered strontianite. (d) and (e) Mapping images for the cell exhibiting Sr enrichment coincided with the region of solidified phase.

**Fig. S6.** X-ray diffraction patterns for the residual solids precipitated from the aqueous media with microalga-free, *C. sorokiniana* or *C. vulgaris*. Asterisks for XRD peaks of the microalga-free sample indicate a small amount of sodium-carbonate phase that was coprecipitated with a trace quantity of strontium-carbonate.