Original Article

Optimization of conditions for removal of 137Cs by *Rhodococcus erythropolis* CS98 immobilized in agarose hydrogel

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Abstract

Several microorganisms reportedly accumulate cesium. Among these microorganisms, *Rhodococcus erythropolis* CS98 shows the highest accumulation. We previously showed that agarose gel was more suitable for immobilization of the CS98 strain than calcium alginate gel and poly(vinyl alcohol) gel. In this study, we optimized the conditions for removal of 137Cs from artificial contaminated water by the CS98 strain immobilized in agarose gel. The optimum ammonium acetate concentration, pH, temperature of the contaminated water, and cell density in the agarose gel were 5 mM, 8.5, 20-40 °C, and 3.6×10^9 cells per 2 mL of gel, respectively.

Key words

cesium, *Rhodococcus erythropolis* CS98, immobilized cell, agarose, wastewater treatment

1. Introduction

An accidental and serious release of radionuclides into the environment occurred at the Fukushima nuclear power plant in 2011 (Chino et al., 2011). The released radionuclides contaminated large quantities of soil and water (Kinoshita et al., 2011). In addition, the volume of contaminated water has increased because of continuous cooling of fuel rods by water in the power plant (Honda et al., 2012). Removal of the radionuclides from soil and water is an urgent issue for people in this area. Among the released radionuclides, radioactive 137Cs is important because of its long half-life of 30.1 years.

Radioactive cesium has mainly been removed using inorganic solid adsorbents such as zeolites (Sasaki et al., 2012). Because the available storage space for radioactive waste is limited, a new adsorbent that can be reduced in volume after adsorption of cesium is required. A promising candidate for this application is biomass from plants or microorganisms. A large reduction in the volume of biomass (> 90 %) can be achieved by drying and subsequent incineration (Sasaki et al., 2012). Although plants such as sunflowers have been used for removal of cesium, they did not remove sufficient quantities (Inabal, 1997). Therefore, we have focused on microorganisms for removal of cesium. To date, several microorganisms, including fungi (Haselwandter and Berreck, 1988) and cyanobacterium (Avery et al., 1991), that can accumulate cesium have been reported. Among these microorganisms, *Rhodococcus erythropolis* CS98 shows the best ability to accumulate cesium (Tomioka et al., 1992; 1994). Immobilization of *R. erythropolis* CS98 in a hydrogel matrix is essential for efficient removal of cesium because it allows for easy separation of cells from contaminated water, and reduces its susceptibility to contamination by foreign microorganisms. We previously found that agarose gel was more suitable for immobilization of the CS98 strain than calcium alginate gel or poly(vinyl alcohol) gel (Takei et al., 2014; 2015). However, the conditions for removal of 137Cs by the immobilized CS98 strain need to be optimized. Therefore, in this study, we examined the influence of the concentration of ammonium acetate as a carbon source for the strain, pH, temperature of the artificial 137Cs-contaminated water, and cell density in the agarose gel on an ability of the immobilized CS98 strain to accumulate 137Cs.

2. Materials and methods

2.1 Materials

Agarose (SeaPlaque® agarose) was purchased from Cambrex Bio Science Rockland Inc. (Rockland, ME). Radioactive 137CsCl was obtained from Eckert & Ziegler Isotope Products Inc. (Valencia, CA). For proliferation, the CS98 strain was incubated in a medium (1 g/L yeast extract and 2 g/L ammonium acetate) with shaking at 30 °C. The strain was harvested by centrifugation in the late exponential stage of growth, and then washed with distilled water three times.

2.2 Immobilization of the CS98 strain in agarose gel

Agarose powder was dissolved in distilled water by autoclaving (121 °C, 20 min). After cooling the polymer solution to 40°C, the CS98 strain was carefully dispersed in the solution. The cell suspension was then poured into glass petri dishes and cooled at 4°C for gelation. Rectangular gels (3 mm × 3 mm × 2 mm) were prepared by cutting the gel plate.

2.3 Accumulation of cesium in the CS98 strain

Agarose gel (2 mL) containing the CS98 strain was added to 20 mL of artificial contaminated water containing tris(hydroxymethyl)aminomethan, ammonium acetate, and 137CsCl, and then shaken at 165 rpm. The concentrations of tris(hydroxymethyl)aminomethan and 137CsCl in the solution before addition of the gel were 25 mM and 10 Bq/mL, respectively. The radioactivity of the solution after addition of 2 mL of gel was 9.1 Bq/mL. At set intervals, the solution containing agarose gel was centrifuged and the radioactivity of the supernatant was determined using a gamma-ray spectrometer (SEIKO EG&G Co., Ltd., Tokyo, Japan). The cesium accumulation ratio was defined as follows:

- Amount of cesium accumulated in the cells within the measurement period = (initial radioactivity of contaminated water just after adding the gel (9.1 Bq/mL)—radioactivity of the supernatant at the measurement time) × total volume (22 mL) of contaminated water and gel
- Cesium accumulation ratio = amount of cesium accumulated in cells during the measurement period × 100/initial amount (200 Bq) of cesium in the water

2.4 Statistical analysis

All experiments were performed more than three times. Differences among more than three groups were analyzed using one-way analysis of variance with Scheffe's method.

3. Results and discussion

First, we investigated the influence of the concentration of ammonium acetate in the contaminated water on the ability of the CS98 strain immobilized in agarose gel to accumulate 137Cs. The pH, temperature, and the cell density were fixed at 8.5, 30 °C, and 3.6×10^9 cells per 2 mL of gel (cells/2 mL-gel), respectively. Ammonium acetate is reportedly a suitable carbon source for cesium accumulation in the CS98 strain (Tomioka et al., 1998). Figure 1 shows time course of cesium accumulation ratio. We previously confirmed that 137Cs adsorption to agarose gel was minimal (Takei et al., 2014). In the present study, with the highest concentration of ammonium acetate, accumulation of 137Cs with the immobilized CS98 strain was also minimal. Reduction of the ammonium acetate concentration increased 137Cs accumulation, and the maximum cesium accumulation ratio was achieved with 5 mM ammonium acetate. Further reduction of the ammonium acetate concentration decreased the cesium accumulation ratio. In previous research, free CS98 accumulated 137Cs and then released it though the mechanism was not clearly defined (Tomioka et al., 1992). This trend was maintained for the immobilized strain with an ammonium acetate concentration between 1 and 10 mM. In subsequent experiments, we fixed the ammonium acetate concentration and incubation time in contaminated water at 5 mM and 6 h, respectively.

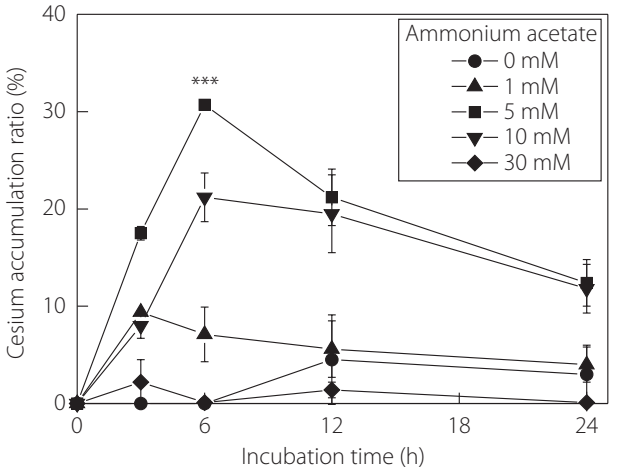

Figure 1: Relationship between ammonium acetate concentration in artificial contaminated water and the cesium accumulation ratio

Notes: $n = 3$, *** $p < 0.001$ vs. the other conditions at 6 h.

Next, we examined the influence of the pH of the contaminated water on the ability of the immobilized CS98 strain to accumulate 137Cs. The temperature of the contaminated water and cell density in the agarose gel were fixed at 30 °C and 3.6×10^9 cells/2 mL-gel, respectively (Figure 2). The lowest cesium accumulation ratio was obtained at pH 10.5. Reducing the pH increased the cesium accumulation ratio, and the maximum value was achieved at pH 8.5. Further reductions in the pH decreased the cesium accumulation ratio. Thus, in the following experiments, we fixed the pH at 8.5.

We then investigated the influence of the temperature of the contaminated water on the ability of the immobilized CS98 strain to accumulate 137Cs. The cell density in the gel was fixed at 3.6×10^9 cells/2 mL-gel (Figure 3). Increasing the temperature from 10°C to 20°C greatly increased the cesium

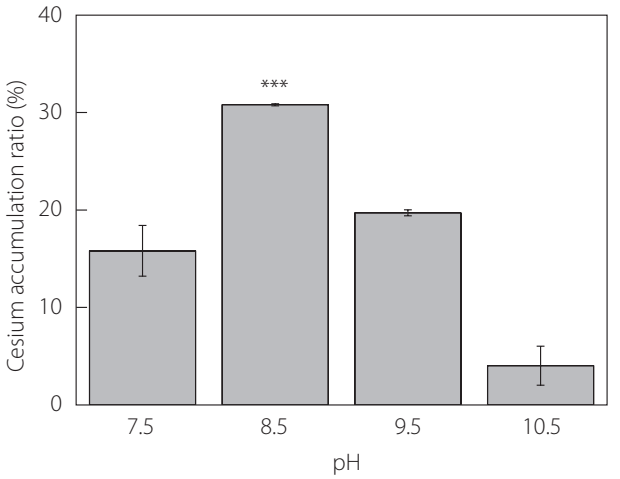

Figure 2: Relationship between the pH of artificial contaminated water and the cesium accumulation ratio

Note: $n = 3$, *** $p < 0.001$ vs. the other conditions.

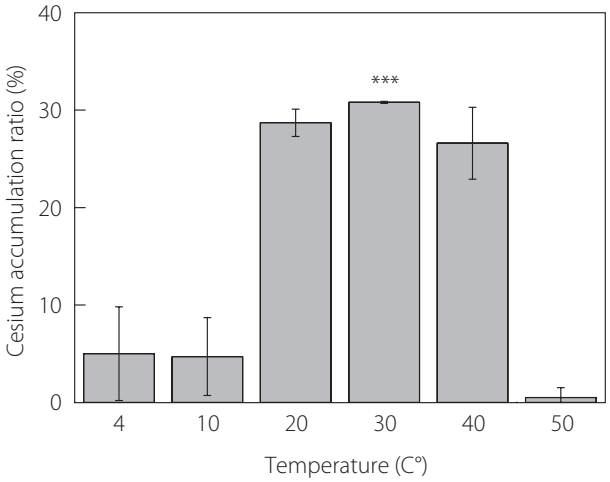

Figure 3: Relationship between the temperature of artificial contaminated water and the cesium accumulation ratio
Notes: $n = 3$, *** $p < 0.001$ vs. 4, 10, and 50 °C.

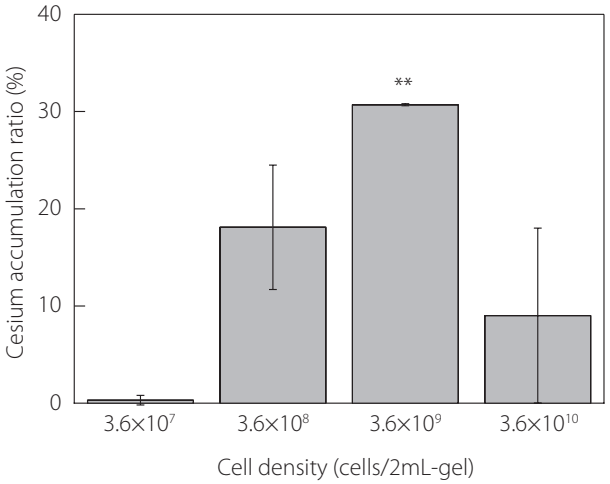

Figure 4: Relationship between the cell density of the CS98 strain in agarose gel and the cesium accumulation ratio
Notes: $n = 3$, ** $p < 0.01$ vs. 3.6×10^7 and 3.6×10^{10} cells/2 mL-gel.

accumulation ratio, and the highest ratios were obtained at temperatures between 20 and 40 °C. The differences among the results at 20, 30, and 40 °C were not statistically significant.

Optimum conditions for the ammonium acetate concentration, pH, and temperature of the contaminated water have been reported for cesium accumulation by free CS98 strain (Tomioka et al., 1994; 1998). The CS98 strain immobilized in agarose gel exhibited a higher cesium accumulation ratio at a higher concentration of ammonium acetate (5 mM) than the free strain (1-3 mM). By contrast, the optimum pH values for the free and immobilized strains were similar. However, when the pH was changed from 8.5 to 7.5, accumulation of 137Cs by the free strain decreased by 80 %, whereas accumulation by the immobilized strain only decreased by 50 %. For the temperature, the free strain showed good accumulation of 137Cs at temperatures between 10 and 35 °C, whereas 137Cs accumulation with the immobilized strain at 10 °C was much lower than that at 20 or 30 °C. When the CS98 strain is immobilized in hydrogel, it is compressed by the gel. It is widely known that the physical stimuli often induce changes in gene expression of immobilized microorganisms (Sun et al., 2007). This suggests the differences between the optimum conditions for the free and immobilized strains could be attributed to changes in gene expression.

We then examined the relationship between the cell density in agarose gel and the cesium accumulation ratio. The

Table 1: Cesium concentration factors of microorganisms

Microorganism	Concentration factor	Reference
Immobilized *R. erythropolis* CS98 at 3.6×10^7 cells/2 ml-gel	2.7×10^{3} [a]	This paper
Immobilized *R. erythropolis* CS98 at 3.6×10^8 cells/2 ml-gel	2.1×10^{4} [a]	This paper
Immobilized *R. erythropolis* CS98 at 3.6×10^9 cells/2 ml-gel	5.5×10^{3} [a]	This paper
Immobilized *R. erythropolis* CS98 at 3.6×10^{10} cells/2 ml-gel	1.9×10^{2} [a]	This paper
Free *R. erythropolis* CS98 [d]	6.2×10^{3} [a]	Takei et al., 2015
Pseudomonas aeruginosa	1.6×10 [b]	Strandberg et al., 1981
Saccharomyces cerevisiae	3.7×10 [b]	Strandberg et al., 1981
Microcoleus vaginatus	9.5×10^{2} [c]	Harvey and Patrick, 1967
Draparnaldia plumosa	1.5×10^{3} [c]	Harvey and Patrick, 1967
Navicula seminulum	1.6×10^{3} [c]	Harvey and Patrick, 1967
Euglena intermedia	7.1×10^{2} [b]	Williams, 1960
Chlorella pyrenoidosa	1.1×10^{2} [b]	Williams, 1960

[a] (mg of Cs/g of dried cells)/(mg of Cs/g of water)
[b] (cpm of ^{137}Cs/g of wet cells)/(cpm of ^{137}Cs/g of water)
[c] (cpm of ^{137}Cs/g of dried cells)/(cpm of ^{137}Cs/g of water)
[d] the data was obtained with a cell density of 3.6×10^9 cells/22 mL^{-137}Cs-contaminated water

concentration of ammonium acetate, pH, and temperature of the contaminated water were fixed at 5 mM, 8.5, and 30 °C, respectively. The maximum cesium accumulation ratio was achieved with a cell density of 3.6×10^9 cells/2 mL-gel (Figure 4). A reduction occurred in the cesium accumulation ratio at 3.6×10^{10} cells/2 mL-gel and would be associated with early depletion of ammonium acetate because of the large number of cells. Finally, we compared the ability of the immobilized CS98 strain to accumulate 137Cs at each cell density with the abilities of other microorganisms (Table 1). The concentration factor includes information about the amount of cesium accumulated in a cell (Tomioka et al., 1992). The maximum concentration factor was achieved with a cell density of 3.6×10^8 cells/2 mL-gel. By contrast, the maximum cesium accumulation ratio was obtained at 3.6×10^9 cells/2 mL-gel as shown in Figure 4. The concentration factors obtained with the immobilized CS98 strain at cell densities of 3.6×10^8 and 3.6×10^9 cells/2 mL-gel were higher than those of other microorganisms except for free CS98 strain. Reductions observed in the concentration factor at cell densities lower than 3.6×10^8 cells/2 mL-gel could be attributed to insufficient conditioning of the contaminated water by materials secreted from the CS98 strain because of the small number of cells. Reductions observed in the concentration factor at cell densities higher than 3.6×10^8 cells/2 mL-gel could be associated with early depletion of ammonium acetate because of the large number of cells as described above.

4. Conclusion

The purpose of this study was to optimize the conditions for removal of 137Cs from artificial contaminated water by the CS98 strain immobilized in agarose gel. The optimum ammonium acetate concentration, pH, temperature of the contaminated water, and cell density in agarose gel were 5 mM, 8.5, 20-40 °C, and 3.6×10^9 cells/2 mL-gel, respectively.

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