Immobilization of cesium-resistant bacterial cells by radiation polymerization and their bioremoval efficiency

Rita Singh, Kirti Shitiz and Antaryami Singh

ABSTRACT

Biological approaches for the removal of heavy metals and radionuclides from contaminated water are reported. The present study was carried out with the objective of identifying bacterial strains for the uptake of cesium that could be used for bioremediation. Polymer carriers prepared by radiation polymerization were used for the immobilization of bacteria and the efficiency of free cells and immobilized cells for the removal of cesium was evaluated. Thirty-five bacterial isolates were screened for resistance to cesium and five bacterial isolates based on resistance to cesium (BR-3, BR-6, BR-21, BR-39, BR-40) were selected for immobilization. Polymer carriers were prepared using 10, 20, 30, 40 and 50% acrylamide at different doses of 1 to 5 kGy gamma radiation. The polymer carriers prepared using 30% and 40% acrylamide at 5 kGy were found to be suitable based on gel fraction and absorption capacity for the immobilization of bacterial cells. Bioremoval of cesium by free and immobilized bacterial cells was evaluated. Significant reductions of 76–81% cesium were observed with bacterial cells immobilized by radiation polymerization.

Key words | acrylamide, bioremoval, cesium, gamma radiation, immobilization, polymerization

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INTRODUCTION

Industrial and nuclear wastes containing heavy metals and radionuclides released in the environment pose potential risk to human health. Cesium as a radionuclide (cesium-137) has been paid considerable attention due to its harmful gamma radiation, high solubility in water and long half-life of 30 years. It has been reported in various studies that hazardous amounts of radioactive cesium will remain in the environment for a long time, posing severe threats to human health and organisms (Tomioka *et al.* 1998; Jalali-Rad *et al.* 2004). Radioactive isotopes of cesium are present in the soil in their cationic form, and there are no general differences between the radioactive and stable isotopes with respect to their chemistry or their behavior in the environment. Radioactive cesium exhibits a high degree of mobility and bioavailability due to the chemical properties of Cs^{+}. Being an analog of potassium, cesium is easily taken up by organisms. Direct accumulation of cesium from the environment takes place readily in plants, microorganisms and lower organisms, while cesium accumulation in animals occurs mainly through consumption of contaminated food and water (Abdel-Razek *et al.* 2015). Radioactive cesium thus recirculates in living systems for centuries.

A number of methods have been reported for the removal of heavy metals and radionuclides from contaminated water (Munter 2013; Liu *et al.* 2014). Removal of cesium has been achieved using physical approaches like adsorption by natural or synthetic ion exchangers, clay and chemicals (Wu *et al.* 2009; Mandal & Lahiri 2011). A disadvantage associated with the use of ion exchangers is that the presence of other competitive monovalent cations like sodium and potassium in radioactive effluents can block cesium adsorption (Avery *et al.* 1991). Chemical treatment and adsorption by chemicals like iron ferrocyanide has also been proposed but these methods are not practically feasible due to high cost, safety and complexity (Seko *et al.* 2012). Therefore, more efficient, convenient, safe and economic technologies must be developed for the removal of cesium from contaminated waste waters.

Recently, research has been focused on the use and development of biological techniques for the removal of cesium owing to their performance, availability in large quantities and low cost. Bioremediation is based on the natural abilities of microorganisms and plants to metabolize, sorb, oxidize or reduce organic and inorganic compounds.

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Also, the biomass volume used in bioremediation can be simply reduced by drying and incineration (Sasaki *et al.* 2012). Various types of microorganisms and plants have been explored for the bioremoval of cesium (Mashkani & Ghazvini 2009; Vinichuk *et al.* 2013). Phytoremediation technology using the aquatic plants *Eichhornia crassipes* and *Ludwigia stolonifera* have been shown to have high efficiency for the accumulation of cesium and other toxic elements (Saleh 2012; Saleh *et al.* 2017, 2019). Removal of cesium by sunflower plants has also been carried out, though removal efficiency was poor (Inaba 2011) and another drawback of using plants is the time taken for their growth. Immobilization of radioactive biomass by inert materials such as cement is also reported (Saleh 2014; Bayoumi & Saleh 2018). Microbial decontamination has an edge over conventional radionuclide cleanup methods. Some microorganisms, such as cyanobacterium (Avery *et al.* 1991) and fungi (Kirchnera & Daillant 1998), have been reported to accumulate cesium.

Immobilization of microbial cells has received increasing interest in the field of waste treatment (Ahmad *et al.* 2012). Various methods for the immobilization of microbial cells, such as flocculation, adsorption, immobilization by cell entrapment, cross-linking and covalent bonding, have been reported (Woodward 1988; Datta *et al.* 2013; Mohamad *et al.* 2015). Bacteria and yeast cells, due to their small size, low density, poor mechanical strength and rigidity, require some support for their effective use in bioremediation (Tsezos *et al.* 1987). A suitable way to enhance the mechanical strength of microbial biomass is immobilization or physical entrapment in a suitable polymer matrix. Immobilized biomass has several advantages over free cells, such as high biomass, enhanced cell stability, high metabolic activity, strong resistance to toxic chemicals, easy separation of biomass and effluent, and reusability of biomass (Cai *et al.* 2011; Liu *et al.* 2012). Entrapment of microbial cells in various polymer matrices using radiation polymerization has been extensively investigated (D'Souza 2002). Radiation polymerization has an edge over chemical polymerization as there is no need to add chemical initiators (Carenza 1992). Preparation of matrices by radiation polymerization has several advantages – the products show good activity, stability and easy regeneration (Kawashima & Umeda 1974; Hoffman 1977).

The present study reports on the screening of bacterial strains for resistance to cesium and their immobilization in polyacrylamide hydrogels using the radiation polymerization technique. Further, the efficiency of immobilized bacterial cells was evaluated for the bioremoval of cesium.

MATERIALS AND METHODS

Screening of bacteria for resistance to cesium

Thirty-five bacterial strains isolated from water samples collected from different sites of Jaisalmer and Pokhran area of Rajasthan state were screened for resistance to cesium. Nutrient agar supplemented with 1, 2, 3, 4, 5, 10, 15, 20 and 25 μg/ml of cesium (cesium chloride) was prepared and poured into plates. Each plate was divided into eight sectors and streaked with freshly grown bacterial cultures. The plates were incubated at 32 ± 2 °C for 48 hours. The maximum concentration of cesium which permitted growth and beyond which there was no growth was considered as the maximum resistance of the test strain.

Preparation of polymer carriers for immobilization of bacterial cells

Polymer carriers for the immobilization of bacterial cells were prepared by the radiation polymerization technique. Acrylamide in concentrations of 10, 20, 30, 40, and 50% in water was exposed to gamma radiation for gel formation. Irradiation was carried out at the doses of 1, 2, 3, 4, and 5 kGy.

Determination of gel fraction

The gel fraction of polymer gels prepared using 10, 20, 30, 40 and 50% acrylamide at different doses of 1 to 5 kGy gamma radiation was determined. The samples were dried to a constant weight at 50 °C in an oven (W_i), then soaked in distilled water for 24 hours up to a constant weight (W_s) in order to remove the unpolymerized parts and solvent. The gels were dried again at 50 °C in an oven and weighed (W_d). The gelation percentage was then calculated using the following equation (Equation (1)):

$$\text{Gel fraction} = W_d/W_i \times 100 \quad (1)$$

W_d = Weight after washing and drying
W_i = Initial weight after drying

Determination of degree of absorption of polymer carriers

The water absorption capacity of polymer gels prepared using 10, 20, 30, 40 and 50% acrylamide at different doses of 1 to 5 kGy was determined. The polymer gels after

washing were dried and weighed. The preweighed samples were immersed in distilled water at room temperature and weighed periodically up to 144 hours until the gel reached the equilibrium state of swelling. The water on the surface of the swollen gel was removed with tissue paper and immediately weighed after 1, 2, 3, 6, 7, 24, 30, 48, 72, 96 and 144 hours. The degree of swelling was calculated by the following equation (Equation (2)):

$$\text{Absorption} = (W_s - W_d)/W_d \times 100 \quad (2)$$

W_d = Weight after washing and drying
W_s = Weight of the swollen gel.

Immobilization of bacterial cells

Five bacterial strains BR-3, BR-6, BR-21, BR-39, BR-40 showing resistance to cesium were selected for immobilization using polymer matrices prepared by gamma irradiation of acrylamide. The bacterial inocula were prepared as a suspension representing 10^4 CFU/ml. Bacterial cell suspensions were dispersed in acrylamide solution and irradiated at 5 kGy for polymerization at ambient temperature. The activity of immobilized cells was tested for 20 continuous cycles.

Determination of bioremoval ability of free and immobilized bacterial cells

The ability of bacteria to remove cesium was tested. Five bacterial strains named as BR-3, BR-6, BR-21, BR-39 and BR-40 were selected based on their higher resistance to cesium for evaluating bioremoval efficiency. The free and immobilized bacterial cells at initial concentrations of 10^4 CFU/ml were added to broth supplemented with 10 μg/ml cesium and incubated at $32 \pm 2\,^{\circ}C$ with shaking. The amount of metal removed by bacterial cells was determined by measuring the metal content in the solution periodically after 0, 3, 5, 7 and 10 days. The bacterial cell growth in the presence of cesium was also estimated by the plate count technique.

Determination of cesium content

Samples containing cesium treated with free and immobilized bacteria BR-3, BR-6, BR-21, BR-39 and BR-40 were collected at periodic intervals of 0, 3, 5, 7 and 10 days. The samples were filtered using 0.45 μm pore size Millipore membrane filters to remove bacterial cells. The cesium content in the filtered samples was determined by atomic absorption spectrophotometer (AAS) novAA 400, Analytik Jena, Germany at a wavelength of 852 nm. Polymer carriers containing no bacteria was used as the negative control. The assays were performed in triplicate and the data are presented as the mean ± SD.

Bioremoval efficiency (%) was calculated by the following equation (Equation (3)):

$$\%\ \text{Bioremoval} = (C_i - C_t)/C_i \times 100 \quad (3)$$

C_i = Initial concentration of cesium
C_t = Concentration of cesium at time t.

RESULTS AND DISCUSSION

Thirty-five bacterial strains were screened for resistance to cesium. Growth of bacterial isolates in the presence of 1, 2, 3, 4, 5, 10, 15, 20 and 25 μg/ml of cesium was analyzed. 9 bacteria were resistant to 20 μg/ml cesium. At the higher concentration of 25 μg/ml, 3 strains were resistant to cesium. The percentage of bacterial isolates showing resistance to 1, 2, 3, 4, 5, 10, 15, 20, 25 μg/ml cesium is shown in Figure 1. 89% of bacterial strains showed growth in the presence of up to 4 μg/ml cesium. At 5 μg/ml cesium concentration, 86% of bacterial isolates showed growth. At higher concentrations, the bacterial growth was reduced. Growth was observed in only 9% of the isolates at 25 μg/ml of cesium.

Microorganisms play an important role in environmental processes. Microorganisms have been successfully used for bioremediation at various contaminated sites, and can be used for bioremoval of heavy metals and radionuclides (Sepehr *et al.* 2005; Shanab & Essa 2007).

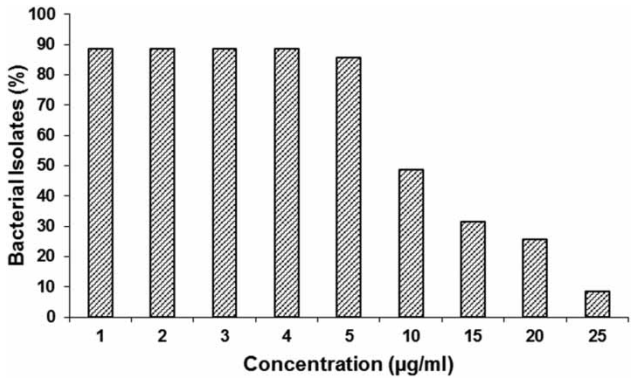

Figure 1 | Resistance of bacterial isolates to cesium.

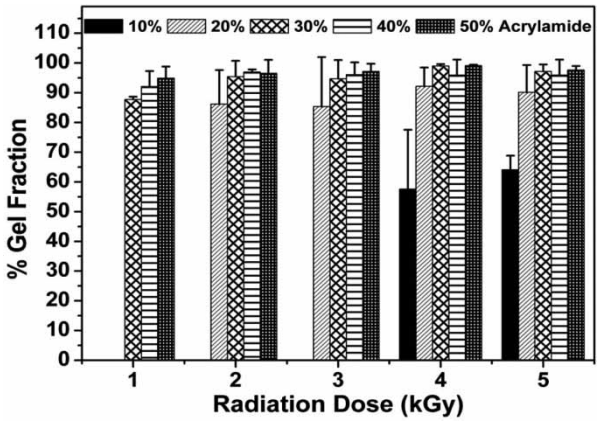

Figure 2 | Gel fraction of acrylamide gels at different doses of gamma radiation.

Their ability to flourish in a wide range of environmental conditions, rapid growth and ability to take up harmful metals and radionuclides render them suitable for bioremediation of contaminated water and soil. Removal of radiocesium from water has become an emerging issue after the Fukushima Daiichi Nuclear Power Plant Disaster, during which a total of approximately 3.3×10^{16} Bq Cs was released in the environment (Liu *et al.* 2014). In the present study, screening of microflora for identification of potential microbes for decontamination of cesium was carried out. Thirty-five bacterial strains were screened for their resistance to cesium. Cesium-resistant bacterial strains were immobilized and the bioremoval efficiency of free and immobilized bacterial cells was assessed.

Polymer carriers for the immobilization of bacterial cells were prepared using 10, 20, 30, 40 and 50% acrylamide. The gel fraction of acrylamide gels at different doses of 1, 2, 3, 4 and 5 kGy gamma radiation is presented in Figure 2. No gel formation was detected with 10% acrylamide at doses of 1, 2 and 3 kGy and 20% acrylamide at 1 kGy. 57–64% gel fraction was observed with 10% acrylamide at higher doses of 4 and 5 kGy. The gel fraction with 20% acrylamide was 85–92% at doses of 2 to 5 kGy. Higher gel fractions were recorded with increasing acrylamide concentration. The gel fraction percentage for 30% acrylamide was 88% at 1 kGy and 97% at 5 kGy. 92% and 95% gel fractions were observed with 40% and 50% acrylamide at the lowest irradiation dose of 1 kGy. Increases in the gel fraction percentage were observed at higher doses. The highest gel fraction of 99% was observed with 50% acrylamide at 4 kGy.

Microorganisms can be immobilized in different matrices, whether the cells are entrapped in the support material or an adsorption phenomenon occurs. A variety of polymers, both natural (alginate, carrageenan, agarose, chitin, chitosan) and synthetic (polyacrylamide, polysulfone,

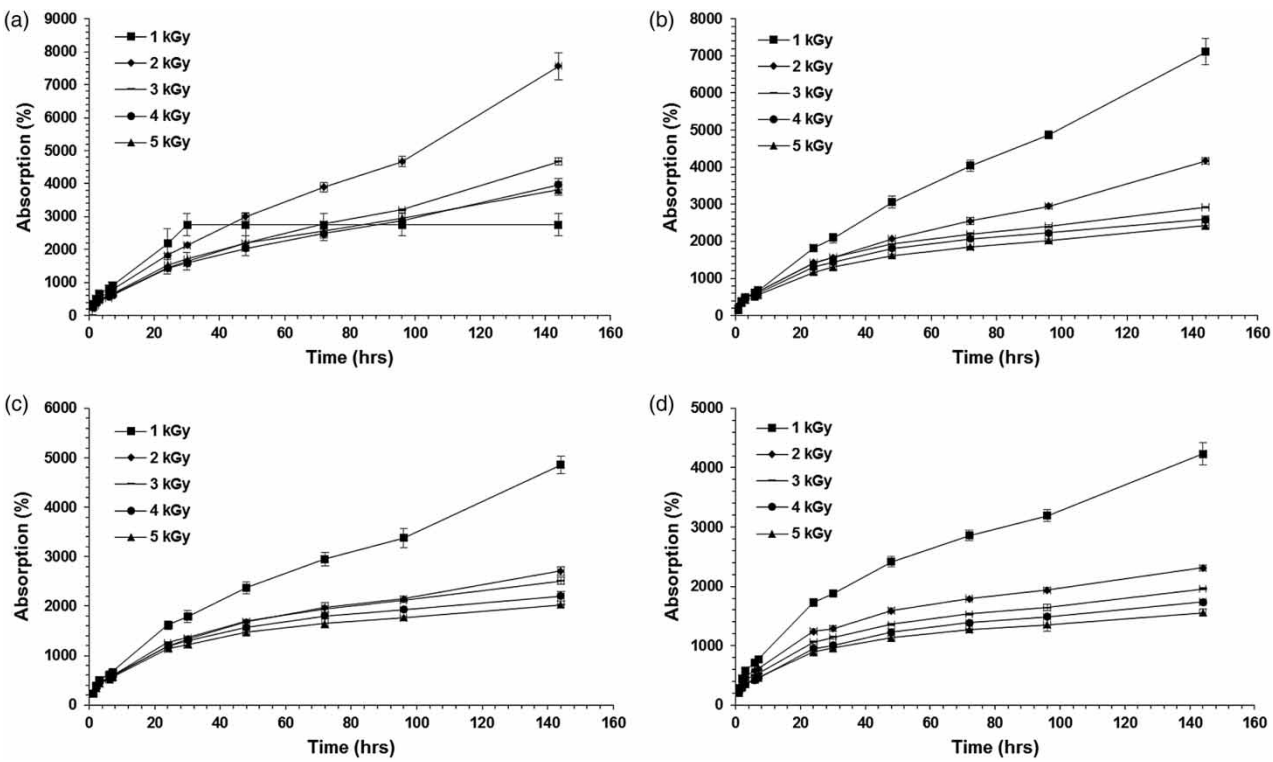

Figure 3 | Water absorption capacity of polymer carriers at different doses of gamma radiation prepared with different concentrations of acrylamide: (a) 20%, (b) 30%, (c) 40% and (d) 50%.

polyurethane, polyvinyl alcohol, etc.) have been used for the immobilization of microbial cells. Bacterial cells immobilized on porous ceramic support and inside sodium alginate polymeric beads have been efficiently used for the bioremoval of cesium (Sasaki *et al.* 2013, 2015). Cesium accumulation by bacterial cells immobilized in hydrogel matrices has been reported (Takei *et al.* 2014). Bioaccumulation of cesium by bacterial cells immobilized in different types of polymeric beads has also been demonstrated (Abdel-Razek *et al.* 2015).

In our study, 30% and 40% acrylamide hydrogels prepared at the 5 kGy dose were found suitable for the immobilization of bacterial cells for bioremoval. The process of entrapment and immobilization of cells was carried out by suspending bacterial cells in the polymer solution and exposing it to gamma radiation for polymerization. The gel fraction and immobilization efficiency was 97.18 ± 2.29% with 30% acrylamide and 95.91 ± 3.24% with 40% acrylamide at the gamma radiation dose of 5 kGy. Acrylamide has been widely used in the preparation of polymer carriers for the immobilization of bacterial cells. Different concentrations of acrylamide ranging from 10 to 40% alone or in combination with other polymers and varying doses of gamma radiation have been reported for the preparation of polymer carriers (Kawashima & Umeda 1974; D'Souza & Nadkarni 1980; D'Souza *et al.* 2006). In our study, more than 97% gel fraction was obtained for 30% acrylamide hydrogels irradiated at 5 kGy, which suggests this dose is sufficient to achieve complete gelation. It has been reported by Rosiak *et al.* (1983) that gamma irradiation of acrylamide at 2 kGy produces crosslinked polyacrylamide at ambient temperature. Saraydin *et al.* (2002) optimized a gamma radiation dose of 5.2 kGy for the preparation of acrylamide hydrogels and obtained complete gel formation without any residual monomer.

The water absorption capacity of polyacrylamide gels prepared using 20, 30, 40 and 50% acrylamide at different doses of 1, 2, 3, 4, 5 kGy was studied. 10% acrylamide gels were not stable, hence their water absorption capacity could not be studied. The water absorption capacity of acrylamide gels prepared at varying doses of gamma radiation is presented in Figure 3. The absorption capacity of the polymer carrier with 20% acrylamide after 1 hour was 204 ± 23%, 233 ± 30%, 253 ± 43% at 3, 4 and 5 kGy respectively (Figure 3(a)). Increases in absorption capacity were observed with time. However, no significant differences with an irradiation dose of 3 to 5 kGy was observed. The water absorption capacity of 30% polymer gels after 24 hours was significantly higher at 1 kGy as compared to gels prepared by gamma radiation at 3, 4 and 5 kGy (Figure 3(b)). A similar observation was recorded after 144 hours. The absorption capacity of 40% acrylamide gels was 1,141–1,264% after 24 hours and 2,030–2,712% after 144 hours at doses of 2 to 5 kGy (Figure 3(c)). Higher absorption capacities were observed with gels prepared at the 1 kGy dose. Water absorption was 1,614% after 24 hours and 4,857% after 144 hours at 1 kGy for 40% gels. However, no significant differences in absorption capacity were observed with 2 to 5 kGy gamma radiation doses. The water absorption of the polymer carrier with 50% acrylamide is presented in Figure 3(d). Polymer carriers developed from 50% monomer concentration were comparatively stiff and showed lower absorption capacity as compared to polymer carriers prepared using 30% and 40% acrylamide. The polymer carriers prepared using 30% and 40% acrylamide at 5 kGy were found suitable based on gel fraction and absorption capacity for the immobilization of bacterial cells.

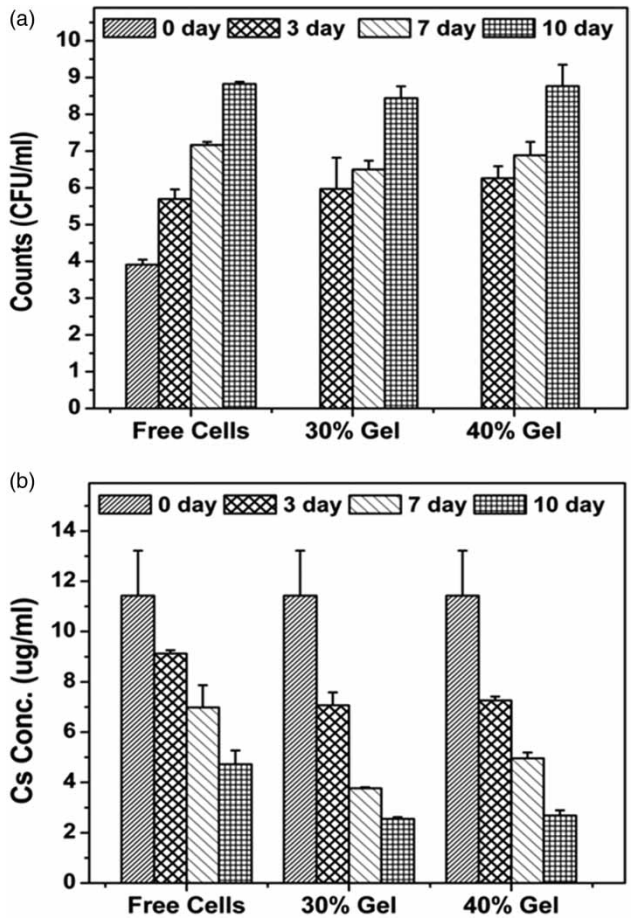

Figure 4 | Growth and bioremoval efficiency of free and immobilized bacterial strain BR-3: (a) cell counts, (b) reduction in cesium content.

The water absorption capacities of 30% and 40% acrylamide gels prepared at 5 kGy were almost similar, at around 1,100%, 1,200% and 2,100% after 24, 30 and 144 hours respectively. Similar swelling characteristics of acrylamide hydrogels have been reported by Saraydin *et al.* (2002) where the maximum absorption of the nutrient medium of around 1,400% was observed after 24 hours. A time-dependent increase in fluid absorption was observed in both the studies. For non-ionic hydrogels like acrylamide, fluid absorption is controlled by the hydrophobicity of the monomers/polymers.

Bacterial strains resistant to cesium were selected and evaluated for their bioremoval/biosorption ability. Five bacterial isolates BR-3, BR-6, BR-21, BR-39, BR-40 resistant to 20 μg/ml cesium were selected. Bacterial isolates were immobilized using polymer matrices prepared by gamma irradiation of 30% and 40% acrylamide. Free and immobilized bacterial cells were grown in the presence of cesium (10 μg/ml). The cesium content was tested periodically to evaluate bioremoval in the presence of free and immobilized bacterial cells. Adsorption by the polymer carriers without bacterial cells was also determined to evaluate the efficiency of bacterial cells.

The growth of free and immobilized bacterial cells in the presence of 10 μg/ml cesium are presented in Figure 4(a) (BR-3), Figure 5(a) (BR-6), Figure 6(a) (BR-21), Figure 7(a) (BR-39), and Figure 8(a) (BR-40). Counts were recorded to be log 8.65–8.93 CFU/ml for free cells after 10 days of incubation. Similar counts (log 8.44–9.20 CFU/ml) were recorded for immobilized bacterial cells.

The cesium content recorded for free and immobilized bacteria BR-3, BR-6, BR-21, BR-39 and BR-40 are presented in Figures 4(b), 5(b), 6(b), 7(b) and 8(b), respectively. Reduction in the cesium content was recorded after 3 days of incubation. Further reductions were observed with increasing time. The cesium content was recorded to be 3.87 to 5.24 μg/ml in the presence of free cells after 10 days of incubation. Lower cesium content was observed

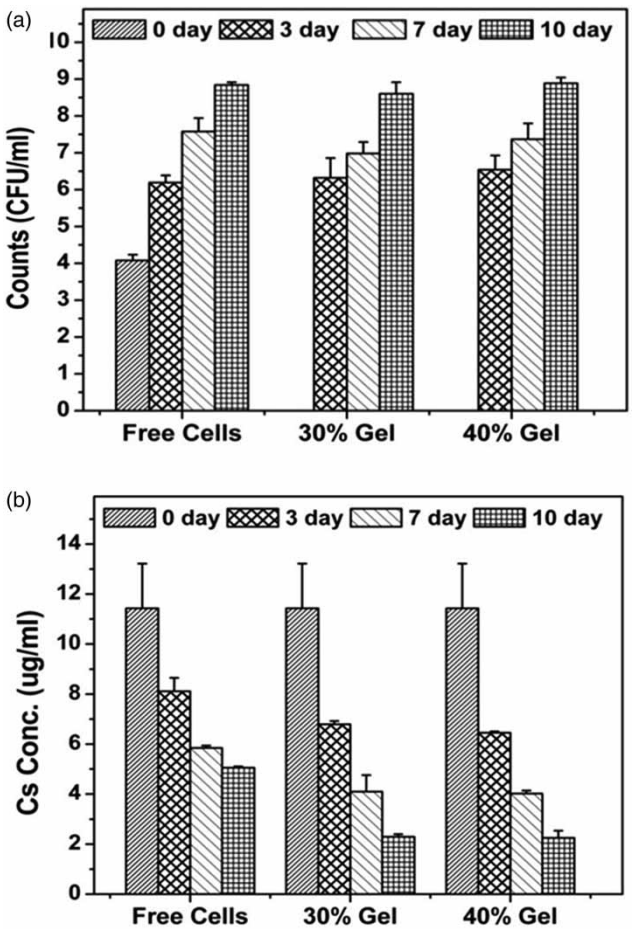

Figure 5 | Growth and bioremoval efficiency of free and immobilized bacterial strain BR-6: (a) cell counts, (b) reduction in cesium content.

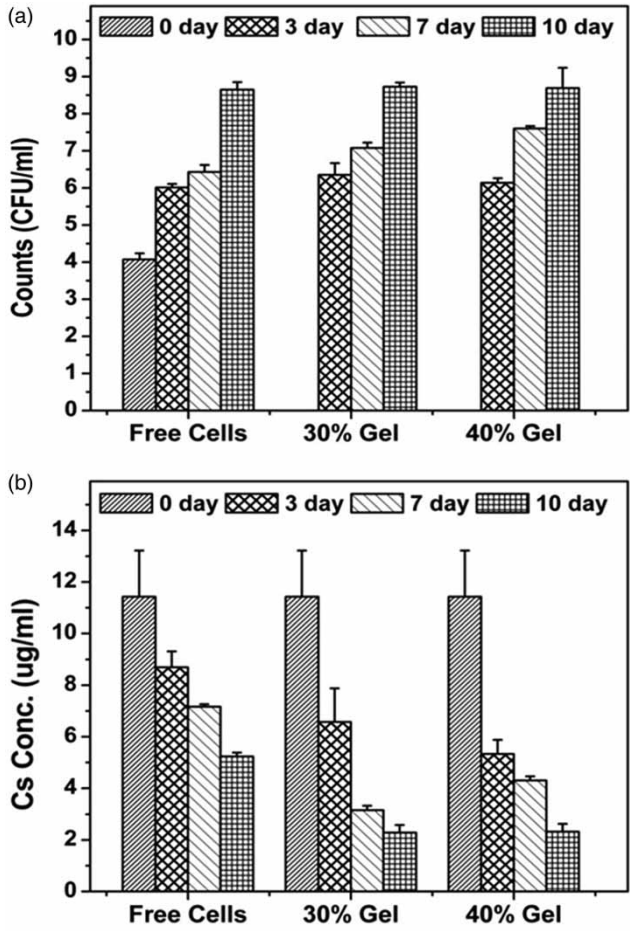

Figure 6 | Growth and bioremoval efficiency of free and immobilized bacterial strain BR-21: (a) cell counts, (b) reduction in cesium content.

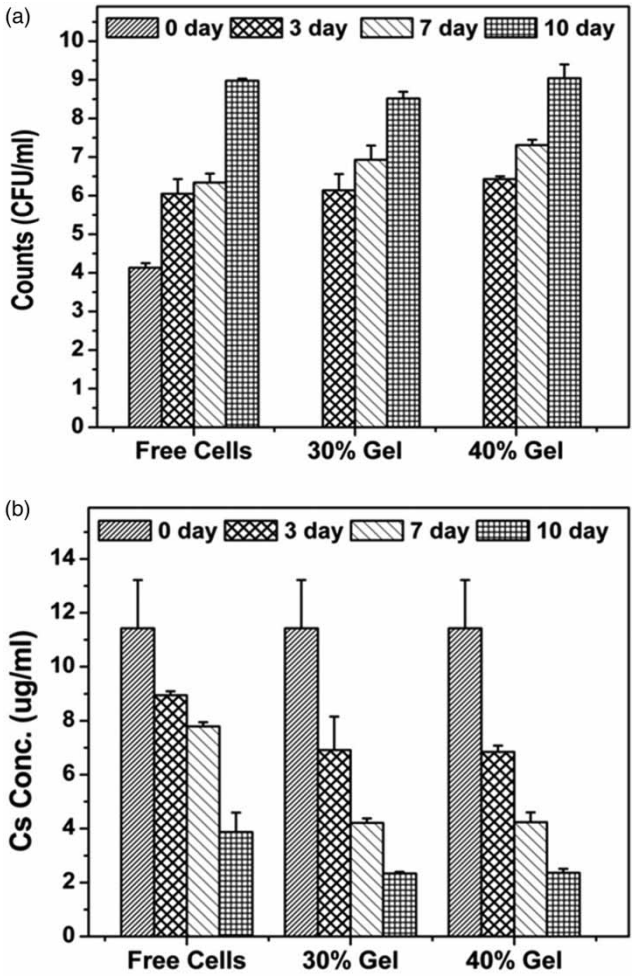

Figure 7 | Growth and bioremoval efficiency of free and immobilized bacterial strain BR-39: (a) cell counts, (b) reduction in cesium content.

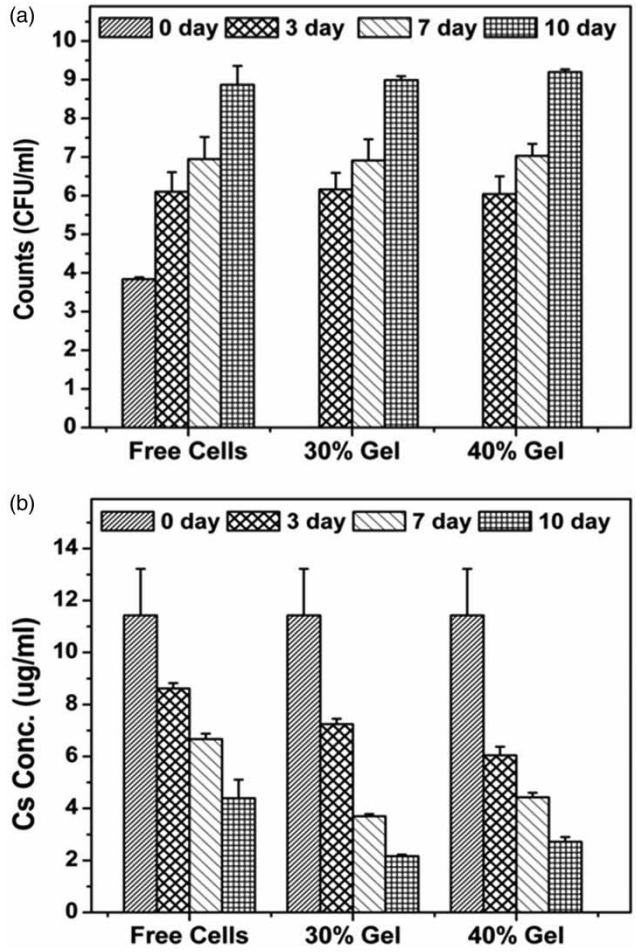

Figure 8 | Growth and bioremoval efficiency of free and immobilized bacterial strain BR-40: (a) cell counts, (b) reduction in cesium content.

with the immobilized cells as compared to free cells. Cesium content in the presence of bacterial cells immobilized using 30% acrylamide ranged from 2.16 to 2.55 μg/ml and 40% acrylamide ranged from 2.25 to 2.72 μg/ml. No significant differences in the bioremoval efficiency of cells immobilized using 30% and 40% acrylamide were observed. However, biosorption by immobilized cells was higher for all the five strains BR-3, BR-6, BR-21, BR-39, BR-40 than biosorption under free cell conditions after 3, 7 and 10 days of treatment.

Bioremoval of cesium by free and immobilized bacteria is presented in Table 1. After 10 days, removal of 54% to 66% was observed using the free cells; but when the cells were immobilized, the removal was increased: after 10 days, 76 to 81% bioremoval efficiency for cesium was recorded for bacterial strains immobilized using 30% and 40% acrylamide polymer carrier.

Bioaccumulation of radiocesium by various organisms, such as cyanobacteria, fungi, mosses and bacteria have been reported (Sugiyama *et al.* 2000; Wang *et al.* 2007). Cesium accumulation is assumed to be based on a potassium transport system (Bossemeyer *et al.* 1989; Avery *et al.* 1991). Biosorption of heavy metals via microorganisms involves transport across the cell membrane, complexation, ion exchange, precipitation and physical adsorption (Javanbakht *et al.* 2014). In the present study, the biosorption capacity of free and immobilized biomass was investigated. The highest bioremoval of 66% by free cells and 81% by immobilized bacterial cells was observed. There are various reports on the bioremoval of cesium using immobilized microbial cells. The immobilization method using hydrogel matrices is a promising method to improve bioremoval efficiency of microbial cells. Sasaki *et al.* (2013) investigated the practical removal of cesium using an immobilized photosynthetic bacterium, *Rhodobacter sphaeroides*. The biosorption capacities of free and immobilized bacterial cells for removal of radiocesium have also been investigated in

Table 1 | Bioremoval of cesium by free cells and cells immobilized in acrylamide gel

Treatment	Days	BR-3	BR-6	BR-21	BR-39	BR-40
Free cells	3d	20.12 ± 1.42	29.05 ± 6.65	23.97 ± 7.13	21.78 ± 1.74	24.67 ± 2.44
	7d	38.93 ± 12.75	48.91 ± 1.71	37.36 ± 1.31	31.85 ± 2.05	41.64 ± 3.15
	10d	58.71 ± 11.65	55.82 ± 1.19	54.16 ± 2.67	66.14 ± 18.60	61.59 ± 3.41
30% acrylamide gel	3d	38.15 ± 7.21	40.59 ± 1.47	42.52 ± 9.94	39.55 ± 17.94	36.66 ± 2.90
	7d	67.02 ± 1.22	64.13 ± 16.09	72.44 ± 3.17	63.17 ± 2.37	67.63 ± 2.70
	10d	77.69 ± 2.74	79.97 ± 4.80	80.05 ± 13.16	79.53 ± 2.56	81.10 ± 2.78
40% acrylamide gel	3d	36.48 ± 1.38	43.57 ± 0.77	53.37 ± 10.32	40.16 ± 3.51	47.16 ± 5.46
	7d	56.61 ± 4.64	64.83 ± 2.49	62.29 ± 2.32	62.90 ± 8.49	61.24 ± 3.84
	10d	76.55 ± 7.83	80.31 ± 12.89	79.70 ± 12.93	79.35 ± 6.35	76.20 ± 6.62

Values are means ± SD. Values indicate % removal of cesium.

other studies and it has been shown that immobilized cells possess higher bioremoval efficiency than free cells. Abdel-Razek *et al.* (2015) reported up to 57% cesium removal by free cells, ~76% by *Bacillus pumilus* cells and ~82% by *Bacillus licheniformis* immobilized in calcium alginate; whereas polyvinyl alcohol-calcium alginate immobilized bacterial cells exhibited lower cesium removal. Cesium accumulation by *Rhodococcus erythropolis* strain immobilized in agarose hydrogel matrices has been reported, with a maximum cesium accumulation of around 55% (Takei *et al.* 2014). Recently, removal of radioactive cesium from sediment mud and soil in Fukushima, Japan has been carried out using immobilized photosynthetic bacteria (*Rhodobacter sphaeroides*) in alginate beads, which led to a removal of around 82% radioactivity from contaminated sediment mud and 73% from soil (Sasaki *et al.* 2015). Previously, similar results were also observed for non-radioactive cesium with an alginate and ceramic immobilized *Rhodobacter* strain (Sasaki *et al.* 2013). Our results are in accordance with other studies, with comparable bioremoval efficiencies of 76 to 81% by immobilized bacterial cells after 10 days of treatment. The immobilized microbial system is an environmentally friendly approach with great potential for use in the removal of heavy metals and pollutants from the environment.

CONCLUSION

The results of the present study have demonstrated significantly higher efficiency of immobilized cells over free cells for the uptake of cesium. Bacterial cells resistant to cesium immobilized by radiation polymerization can be effectively used for decontamination. Further, studies on the effect of various environmental factors on bioremoval of cesium by immobilized cells would facilitate its field application.

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COMPETING INTERESTS

The authors declare no competing interests.

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