

Bioaccumulation of ^{137}Cs by Immobilized Bacterial Species Isolated from Radioactive Wastewater

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Abstract The increases of environmental concern over the accumulation of the long term impact radioactive nuclides e.g. ^{137}Cs encourage the isolation of bacterial species resistant to radioactive nuclides and could accumulate such nuclides. Bacterial species isolated from hazardous liquid wastes at Hot Laboratories Centre were investigated for their removal of ^{137}Cs from waste solutions. The biosorption capacities of the free and immobilized biomass were studied using batch experiments at optimum conditions. Different immobilization matrices namely; calcium alginate (CA), chitosan (CTS), chitosan-alginate (CTS/CA) and polyvinyl alcohol-alginate (PVA/CA) were examined for use in the biosorption system. The effects of the immobilized weight, beads numbers and initial ^{137}Cs activity on the removal efficiency were studied. Although, the results indicated that control CA and PVA/CA gel beads had nearly the same and the higher removal efficiency, the CA-immobilized beads showed higher removal percent than that of PVA/CA-immobilized beads. The immobilized system achieved maximum biosorption capacities at ^{137}Cs solution activity of 15000 Bq/ml, where 62.2, 66.5 and 46.9 KBq/g dry weight were removed by CA and CA-immobilized *Bacillus pumilus* and *Bacillus licheniformis* beads, respectively. Reused experiments for the control CA and CA-immobilized bacteria beads were studied for three cycles. The elution percent increased after the second cycle followed by increase in the removal percent. The studied CA-immobilized system could be used for more than one cycle with removal efficiency of about 50 % of the first cycle.

Keywords: biosorption, ^{137}Cs , immobilization, *B. pumilus*, *B. licheniformis*, radioactive wastewater

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1. Introduction

As an abundant fission radionuclide, ^{137}Cs has been paid considerable attention due to its gamma radiations, a long half-life of 30 years, high water solubility and lability. It has been shown that hazardous quantities of ^{137}Cs will remain in the environment for centuries, with long lasting severe harm for human health and organisms [1,2,3]. The fate of radioactive cesium in the environment has heightened considerably in recent years in the wake of the Chernobyl accident in 1986 [4]. The distribution of released ^{137}Cs and ^{134}Cs is related to the chemical properties of Cs^+ , which generally dictate a high degree of mobility and bioavailability. The partitioning of cesium between abiotic (i.e. soils, sediments, water) and biotic components of terrestrial and aquatic ecosystems is complex and dependent on a number of factors, e.g. inorganic mineral contents of the solid substrates and the abundance of monovalent cations. Direct biological cesium accumulation from the environment occurs readily in lower organisms and primary producers, e.g. microorganisms and plants. Cesium accumulation by higher animals results predominantly from consumption of contaminated food stuffs, although direct inhalation

absorption from the environment may also occur. Thus radioactive cesium continues to be re-circulated in biological systems, for many years, following a pulse of contamination [5,6].

Natural or synthetic ion exchangers, such as zeolites, montmorillonites, silicotitanate, molybdophosphates, phosphotungstates, hexacyanoferrates and others have been used for large scale separation of cesium from low and intermediate level radioactive waste effluents [7-12]. However, one disadvantage for the application of ion-exchangers relates to the competitive interactions of other monovalent cations, in particular Na and K in waste effluents, which can considerably block Cs adsorption [5]. Hence, sorbents with better selectivity are required for Cs removal from solutions containing large amounts of Na and K. Recently, more and more attention has been paid to the use of biological technologies for the removal of Cs radioisotopes from radioactive waste effluents, owing to their good performance, low cost and large available quantities. Several types of microorganisms and plants have been applied for the removal of Cs [2,5,6,13-18]. An adsorption capacity up to 195mg/g was achieved by *Azolla filiculoides* as previously reported. It has been noted that in a biological metal removal process using living cells, the low toxicity of Cs was regarded as an advantage, even though the radiolytical effect for both

radioisotopes (^{134}Cs and ^{137}Cs) might be an important drawback of this type of approach.

The biosorption behavior and mechanism of ^{137}Cs on *Rhodospiridium fluviale* (*R. fluviale*) strain UA2 isolated from a stable cesium solution was investigated [19]. The biosorption was fast and pH-dependent, and Langmuir isotherm equation indicated that the biosorption of ^{137}Cs was a monolayer adsorption implied that *R. fluviale* strain UA2 adsorbed cesium ions by electrostatic attraction. The TEM analysis revealed that cesium ions were absorbed into the cytoplasm of *R. fluviale* strain UA2 across the cell membrane, not merely fixed on the cell surface, which implied that a mechanism of metal uptake contributed largely to the cesium biosorption process.

The removal of ^{137}Cs using marine green algae *Enteromorpha torta* collected from western Alexandria coast was studied [20]. The results showed that the maximum capacity was 4.32, 3.77, 3.51, 4.6, 10.55 and 12.6 m mol/g dry weights for control, 0.2, 0.15, 0.1, .05 and 0.025g of *E. torta*, respectively. The mean free energy was 18.9, 19.03, 21.13, 22.71, 23.47 and 23.59 KJmol⁻¹ for the same samples. It was observed that the equilibrium reached in three hours and the maximum biosorption of ^{134}Cs was occurred at pH 7 for all sorbents.

In a study on radioactive cesium ingestion by people in areas of Ukraine and Russia contaminated by the Chernobyl accident, a strong relationship was found between the extent of mushroom consumption and whole body radioactive cesium content at all study sites. Also, saprotrophic and mycorrhizal basidiomycetes can accumulate radioactive cesium and these organisms may form a major pool of radioactive cesium in soil; grazing of fruit bodies by animals may lead to radioactive cesium transfer along the food chain [21]. In the present study the bacterial species; *Bacillus pumilus* and *Bacillus licheniformis* isolated from low level radioactive waste solutions were examined for their removal efficiency of ^{137}Cs . Also, different gel matrices were examined for use in the biosorption system.

2. Materials and Methods

2.1. Materials

Chemicals:

All chemicals used were of analytical grades. Stock solutions were prepared by dissolving distinct amount in one liter distilled water, then other concentrations were prepared by dilution. Cesium-137 solution was prepared by labeling 100 ppm CsCl solution with certain volume of ^{137}Cs to obtain the desired activities. Sodium hydroxide and hydrochloric acid were used to adjust the pH of the solutions.

Apparatus:

Incubators

Binder incubator model WTB-720 was used for incubation of bacterial agar plates and slants. The biomass production was done using Lab Line Orbital Environmental Shaker model (3527-1).

Centrifuge

Refrigerator centrifuge (Hitach universal-32R) was used for biomass harvesting.

Multichannel Analyzers (MCA):

The activity of the collected hazardous liquid sample was determined using high pure germanium detector. The radioactive analysis of ^{137}Cs samples was done using MCA with well type NaI crystal.

Biolog:

The identification of bacterial isolates was carried out using Biolog (GEN III) instrument which is automated system for bacterial identification.

2.2. Methods

Isolation of bacterial species:

The water sample collected from low level radioactive waste solutions at Hot Lab Station was mixed thoroughly. In three Petri dishes, 1 ml of water samples was taken and mixed with 10 ml nutrient agar under sterile conditions. The plates were incubated at 37 °C for 24 h. The obtained colonies were purified using streak plate according to Benson [22]. The pure colonies were separated on nutrient agar slants for identifications.

Identifications of bacterial isolates:

The identification of bacterial isolates was carried out using Biolog GEN III in Cairo Mircen, Faculty of Agriculture, Ain-Shams University. The instrument analyze the ability of the cell to metabolize all major classes of biochemical's, in addition to determining other important physiological properties such as pH, salt, lactic acid tolerance, reducing power, and chemical sensitivity [23].

Biomass production:

The pure bacterial slants were eluted with sterilized 5ml distilled water and transferred to 500 ml conical flasks containing 150 ml nutrient broth, then incubated in environmental orbital shaker at 37 °C for 24 h. Each conical flask was used to inoculate three one liter conical flasks containing 200 ml of nutrient broth. The produced biomasses were harvested using cooling centrifuge, then washed with distilled water and kept frozen till use [24].

Preparation of control and immobilized beads:

• Calcium alginate (CA) beads:

Sodium alginate gel (4%) was prepared by dissolving 4 g in 100 ml distilled water with gentle stirring overnight. The gel was injected by peristaltic pump into the 100 ml caustic solution of 2% CaCl₂. The formed beads were left in CaCl₂ solution for one hour with gentle stirring, then washed thoroughly with distilled water and kept in refrigerator for use [20].

• Polyvinyl alcohol - Calcium alginate (PVA/CA) beads:

A mixture of PVA and sodium alginate gel was prepared where; 6 g of PVA was dissolved in 100 ml distilled water at 90 - 110 °C with gentle stirring, then 4 g of sodium alginate was added and left overnight with stirring at 60 - 70 °C. The gel mixture was injected into 100 ml caustic solution composed of 2 % CaCl₂ and saturated boric acid with ratio (1:1). The beads were washed with distilled water and then kept in refrigerator for use [25].

• Chitosan (CTS) beads:

In 250 ml conical flask 2.5 ml of glacial acetic acid was added to 2.5 g of chitosan flaks, and then distilled water was added to complete 100 ml. The mixture was gentle stirred at room temperature overnight. 0.25 ml of 0.5% Glutaraldehyde was added to 5 ml of CTS gel with gentle stirring for five minutes (cross linking), and then kept in

refrigerator for ten minutes. The gel injected into the caustic solution (0.5 N Na OH) and left for five minutes with gentle stirring. The formed beads were transferred to 100 ml 0.1 M phosphate buffer solution with gentle stirring for 20 minutes, then washed with distilled water and kept in refrigerator for use [26].

• Chitosan - Calcium alginate (CTS/CA) beads:

Chitosan gel was prepared as previously mentioned, then 5 ml of the CTS gel was injected into cross linking and caustic solution composed of 25% Glutaraldehyde, 4% sodium alginate and 0.1 M phosphate solution with ratio 10 : 20 : 45 and left for 35 minutes. The beads were washed thoroughly with distilled water and kept in refrigerator for use [27].

The immobilized biomass beads:

The immobilized biomass beads were prepared by mixing the desired biomass weight thoroughly with the certain gel volume before cross linking and injection into the caustic solution, then the same above previous steps were followed.

Determination of the activity:

20 ml from the hazardous liquid were used. 1ml was taken on glass watch, then dried under IR lamp. The process was repeated until the 20 ml were dried, then the glass watch was measured using High Pure Germanium Detector. The activity of ^{137}Cs solutions was determined by counting 1ml sample for 300 seconds using Multichannel Analyzer (MCA) at channel of 662 Kev. The instruments were first calibrated with point source and each measurement was done in triplicate.

Biosorption experiments:

In batch experiment the free biomass or the exact gel volume of control or immobilized with studied bacteria was mixed with 10 ml ^{137}Cs solution in 25 ml conical flask. The experimental conditions were adjusted with pH value of 6 - 6.5, the temperature was $25 \pm 3^\circ\text{C}$ and stirring was 120 rpm. Samples of 1ml were taken at different contact times 15, 30, 60, 120 minutes, to determine the solution activity, and then the removed ^{137}Cs could be calculated.

Calculations:

-The activity of ^{137}Cs solution was determined from the equation:

$$A = \frac{\text{count} - \text{background}}{E \cdot I} \quad (1)$$

Where; (A) is the activity, (E) is the efficiency of system and (I) is the intensity of element.

- The uptake percent of ^{137}Cs at different contact times:

$$\text{Uptake\%} = A_0 - A_t / A_0 \times 100 \quad (2)$$

Where, A_0 the initial activity, A_t is the activity at time (t).

-The activity (Bq/ml) removed by g dry weight:

$$Q = A_0 - A_t / M \quad (3)$$

Where, (Q) is the biosorption capacity, (M) is the dry weight (in g) of free biomass, control gel beads or immobilized biomass beads.

Reused Experiments:

The reusability for both control CA and CA-immobilized *B. pumilus* and *B. licheniformis* beads was studied. The accumulated beads (control or immobilized) were washed with 10 ml (0.1 N) HCl for 10 min and then with distilled water [28]. The activity of washing solution (HCl and dist. H_2O) was determined by counting 1 ml using MCA to calculate the elution percent. The washed beads were added to new ^{137}Cs solution to start the second cycle and the previous two steps were repeated to perform the third cycle.

$$\text{Elution \%} = A_w / A_e \times 100 \quad (4)$$

Where; (A_w) is the activity of the washing solution and (A_e) is the activity removed at equilibrium.

3. Results and Discussion

Radioactive analysis of the liquid waste:

The analysis of the hazardous liquid waste showed the presence of ^{60}Co in the liquid waste (Figure 1), so the isolated bacterial species will be radioactive resistant bacteria. The total activities of the sample for the two peaks of ^{60}Co were 9729 Bq and 12406 Bq at channels 1173 and 1332, respectively.

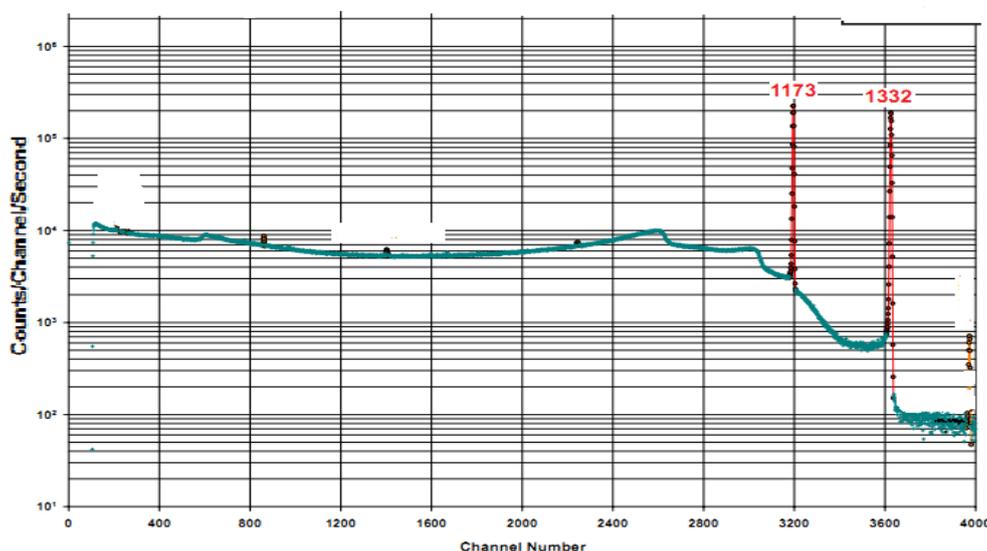


Figure 1. Gamma spectrum of the collected hazardous liquid waste

Identification of bacterial isolates:

The isolated bacteria were identified as; *Bacillus pumilus* and *Bacillus licheniformis*.

Uptake of ¹³⁷Cs by free bacterial cells:

The biosorption of ¹³⁷Cs by different weights; 0.25 and 0.5 g of the free bacterial cells was done under optimum experimental conditions using 10 ml ¹³⁷Cs solution. The results presented in (Figure 2) indicated that the increase in cells weight from 0.25 to 0.5 g at the same ¹³⁷Cs solution resulted in slightly increase in uptake percent for *B. licheniformis* (48.9 and 57.1%). However, the increase in free biomass weight of *B. pumilus* had no effect on the uptake percent, while the absorption rate increases. The biosorption capacity decreased with the increase in biomass weight for both bacterial strains [20].

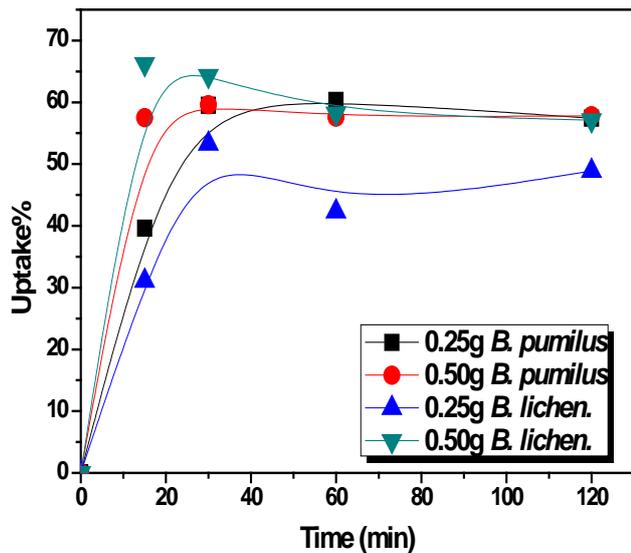


Figure 2. The uptake percent of ¹³⁷Cs by different free biomass weights of *B. pumilus* and *B. licheniformis*

Uptake of ¹³⁷Cs by the different gel matrices:

The support selection is one of the crucial decisions in preparation of the immobilization process [29]. The support criteria such as; physical characteristic, possibility

of microbial growth, biodegradability, solubility and increasing biosorption capacity are very important parameters [30]. The different gel beads were examined for their uptake of ¹³⁷Cs. The results presented in (Figure 3) showed that both CA and PVA/CA control gel beads had the higher and nearly the same removal percent of 72.4 and 72.3%, respectively. On the other hand, 24.9 and 17.2% were removed by CTS and CTS/CA beads, respectively. Results cleared that both CA and PVA/CA beads could be used in the biosorption system. CA-beads were used in the rest of the investigation experiments.

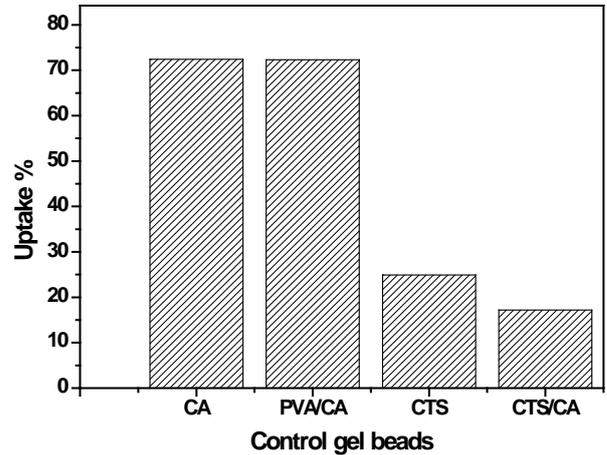


Figure 3. The uptake percent of ¹³⁷Cs by different immobilization matrices beads after two hours

The effect of beads numbers:

The effect of beads numbers in the activity removal at the same ¹³⁷Cs solution (volume and activity) was studied using 50 and 250 beads prepared using 0.25 g biomass / 5 ml gel. Results showed in (Figure 4) Indicated that the increase in beads numbers increased the uptake percent. This explained by increasing surface area for control and immobilized beads, in addition of increasing biomass weight for the immobilized beads [31].

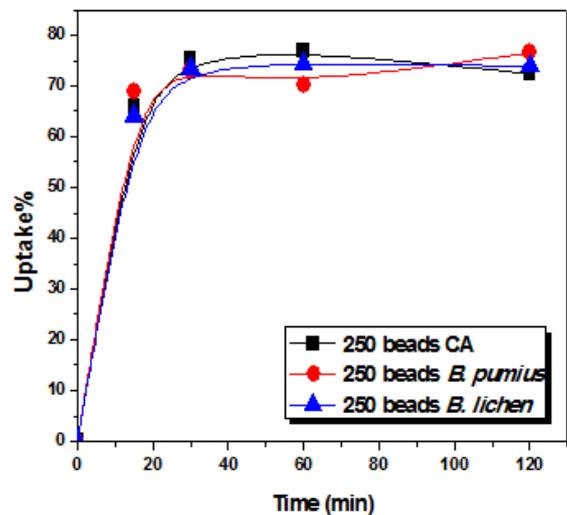
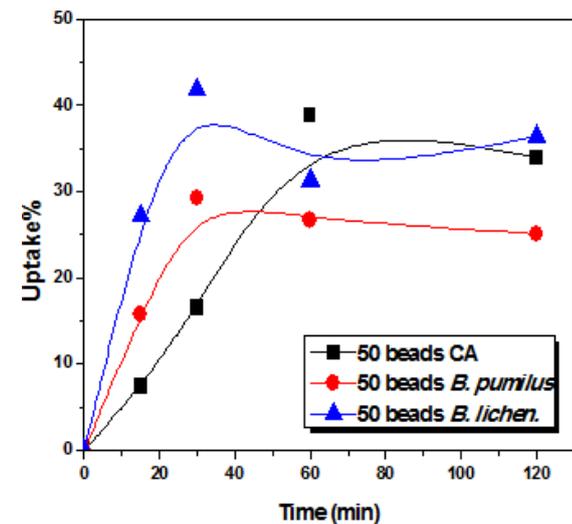


Figure 4. The effect of beads numbers on the uptake of ¹³⁷Cs using control CA and CA-immobilized *B. pumilus* and *B. licheniformis* beads

Effect of different immobilized weights:

Different weights 0.25, 0.5 and 1 g of *B. pumilus* and *B. licheniformis* were immobilized using 5 ml gel. The

results presented in (Figure 5) indicated that the increase in the immobilized weights from 0.25 to 0.5 g decreased the uptake percent from 76.6 to 67.7% for *B. pumilus*. On

the other hand, it was found that the increase in biomass weight from 0.25 to 0.5 g increased the uptake percent from 73.9 to 81.9% for *B. licheniformis*, and then further

increase in biomass decreased the uptake percent. However, the increase in the immobilized biomass decreases the uptake capacity of the immobilized system [20,32].

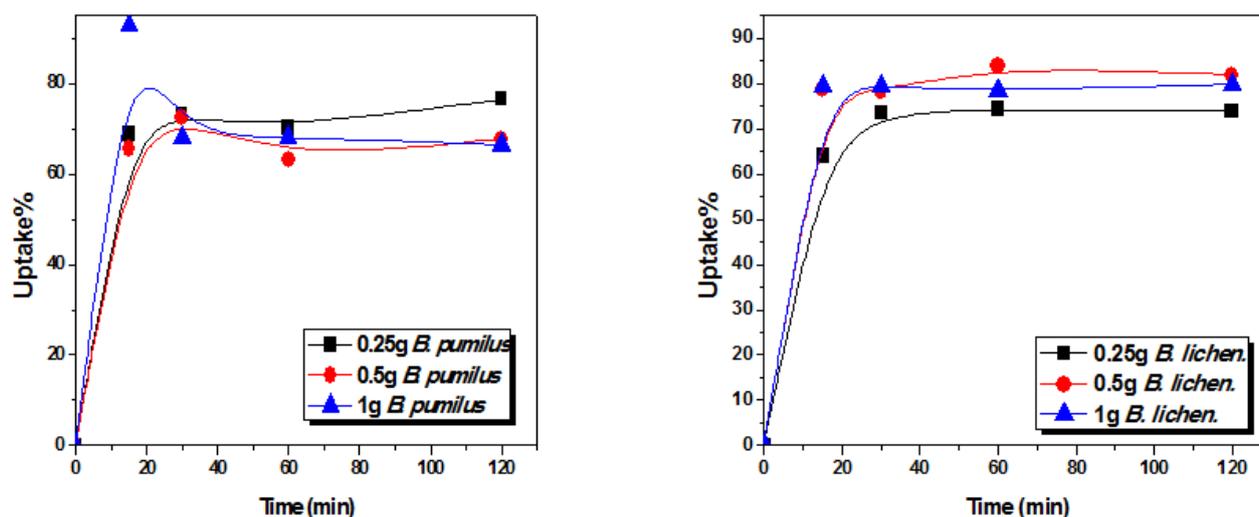


Figure 5. The effect of different immobilized weights of *B. pumilus* and *B. licheniformis* on the uptake of ^{137}Cs

The effect of different activities:

The effect of different activities was studied using various ^{137}Cs activities of 5000, 7500 and 15000 Bq/ml. Results showed in (Figure 6) indicated that the increase of activity from 5000 to 7500 Bq/ml increased the uptake percent and capacity for both CA-immobilized *B. pumilus* and *B. licheniformis* beads. Further increase in the activity to 15000 Bq/ml resulted in slightly increase in the uptake percent for CA-immobilized *B. pumilus* and decrease in the uptake percent of CA-immobilized *B. licheniformis*. The same pattern of uptake percent was observed for control CA-beads. The maximum uptake capacity for control CA and CA-immobilized beads of *B. pumilus* and *B. licheniformis* were 62.2, 66.5 and 46.9 KBq/ g dry weight, respectively. Previous studies [32] reported that, for control CA-beads and immobilized (live and dead) fungal biomass the uptake increased with the increase of activity from 100 to 250 Bq/ml. Also, the percent uptake by immobilized dead *Cunninghamella elegans* beads was slightly higher than alive one.

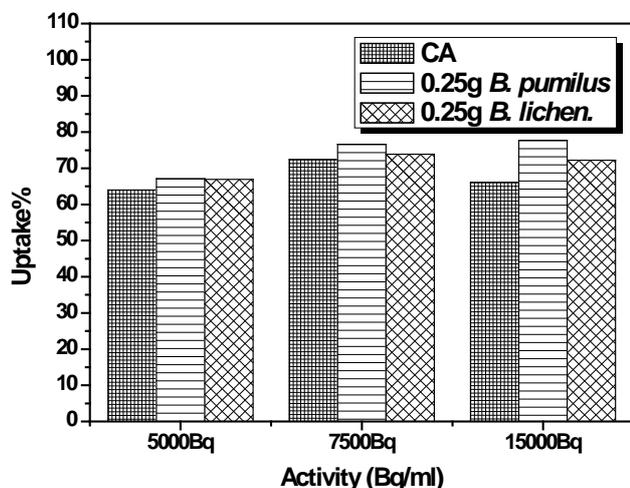


Figure 6. The effect of different activities on the uptake of ^{137}Cs using control CA and CA-immobilized *B. pumilus* and *B. licheniformis* beads

Biosorption of ^{137}Cs by PVA/CA immobilized biomass:

Control PVA/CA-beads showed nearly the same biosorption capacity of CA- beads, it was essentially to investigate the biosorption capacity of PVA/CA-immobilized biomass. Results presented in (Figure 7) indicated that PVA/CA-immobilized *B. pumilus* and *B. licheniformis* exhibited lower ^{137}Cs removal percent of 65.5 and 60.4%, respectively, as compared to CA-immobilized *B. pumilus* and *B. licheniformis*, they were 76.6 and 81.9%, respectively with ^{137}Cs activity of 7500 Bq/ml. This could be explained by low diffusion properties for PVA/CA-immobilized beads and / or less protection of the biomass by the gel.

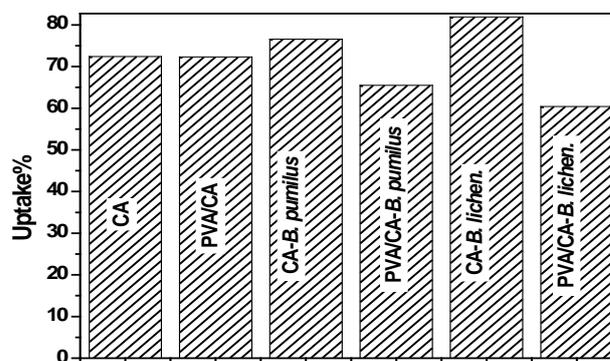


Figure 7. The uptake percent of ^{137}Cs by control and immobilized CA and PVA/CA beads

Reuse of CA-immobilized beads:

The ability of reusing the biosorption system and its efficiency through different cycles was studied for three cycles. Results showed that the biosorption percents in the three cycles for CA-immobilized *B. pumilus* were 77.9, 22.7 and 46.9% (Fig. 8), and the elution percents were 37.4, 100 and 100% for the three cycles. The results obtained for the CA-immobilized *B. licheniformis* showed the same pattern where, 81.9, 20.7 and 41.6% were removed for the three cycles and the elution percents were 39.4, 100 and 100%. The results indicated that the elution after the first cycle was lower than that after second and third cycles, consequently the biosorption of the second cycle was lower than the first and the third cycles.

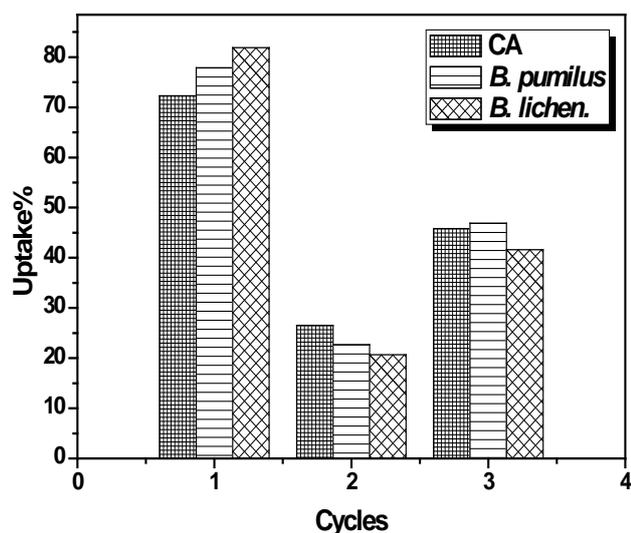


Figure 8. The uptake of ¹³⁷Cs by control CA and CA-immobilized *B. pumilus* and *B. licheniformis* beads, at different reused cycles

4. Conclusion

In the present study the ability of the free biomass to remove ¹³⁷Cs was investigated and the maximum biosorption capacities obtained at free biomass (0.25 g) were 173.8 and 148.1 KBq/g dry weight of *B. pumilus* and *B. licheniformis*, respectively. The different immobilization matrices were examined for ¹³⁷Cs biosorption capacities. The results showed that control CA and PVA/CA beads had nearly the same higher removal percent. However, CA-immobilized biomass beads showed better biosorption percent than PVA/CA-immobilized biomass for both the bacterial strains. Studies on the factors improving the biosorption of the immobilized system cleared that, the increase in the beads numbers for both control and immobilized CA-beads resulted in increase of ¹³⁷Cs uptake percent. Also, the effect of immobilized weight showed that 0.25 and 0.5 g per 5 ml sodium alginate gel were the best immobilized weights for *B. pumilus* and *B. licheniformis*, respectively, further increase in biomass decreased the uptake percent [20].

The effect of different activities on the biosorption systems indicated that the increase in radioactivity up to 7500 Bq/ml increased the biosorption percent and capacity for the control beads and biomass immobilized system [32]. However, the increase up to 15000 Bq/ml increased the uptake percent for *B. licheniformis* only. The reuse experiments indicated that elution after first cycle was lower than that after the second cycle which followed by decreasing in biosorption percent of the second cycle. However, increasing the elution percent after the second cycle improved the biosorption ability to about 50% of the first cycle.

The biosorption system of CA-immobilized *B. pumilus* and *B. licheniformis* showed high ability to remove ¹³⁷Cs from hazardous waste for more than one cycle.

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Conflict of Interest

The authors have no conflict of interest to declare.

List of Abbreviations

MCA: Multichannel Analyzer.
 CA: Calcium Alginate
 CTS: Chitosan.
 CTS/CA: Chitosan-Calcium Alginate.
 PVA: Polyvinyl alcohol.
 PVA/CA: Polyvinyl alcohol-Alginate.

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