Biosorption of Strontium from Aqueous Solution by New Strain *Bacillus* sp. GTG-83

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Abstract

Attempt was made to isolate bacterial strains capable of removing Sr biologically. In this study we collected ten different water samples from naturally radioactive spring Neydasht in Iran and bacterial strains samples isolated. Initial screening of a total of 50 bacterial isolates resulted in selection of one strain. The strain showed maximum adsorption capacity with 55 mg Sr/g dry wt. It was tentatively identified as *Bacillus* sp. according to morphological and biochemical properties and called strain GTG-83. Studies indicated that *Bacillus* sp. GTG-83 was able to grow aerobically in the presence of 50 mM $SrCl_2$ but showed severe growth inhibition at levels above that concentration. The biosorption capacity of *Bacillus* sp. GTG-83 strongly depends on solution pH, and the maximum Sr sorption capacity of *Bacillus* sp. GTG-83 were obtained at pH 10 independent of the absence or the presence of increasing concentrations of salt ($MgCl_2$). Sr-salt biosorption studies were also performed at this pH values. Equilibrium uptakes of Sr increased with increasing Sr concentrations up to 250 mg/l for *Bacillus* sp. GTG-83. Maximum biosorption of Sr was obtained at temperatures in the range of 30-35 ˚C. *Bacillus* sp. GTG-83 biosorbed 97 mg Sr/g dry wt at 100 mg/l initial Sr concentration without salt medium ($MgCl_2$). When salt concentration ($MgCl_2$) increased to 15% (w/v), these values dropped to 23.6 mg Sr/g dry wt at the same conditions. Uptake of Sr within 5 min of incubation was relatively rapid and the absorption continued slowly thereafter.
Key words: *Bacillus* sp. GTG-83, Biosorption, Radioactive Spring Neydasht, Radionuclide, Strontium, wastewater.

Introduction

Heavy metals and radionuclides contaminations are the result of industrial activities, mill tailing, nuclear power testing, nuclear waste disposal and accidents resulting from nuclear power generation. An accident of this nature occurred at the Chernobyl Nuclear Power Station on April 26, 1986. ^{90}Sr is the most widespread radionuclide in the environment. Contamination of soils with typical fission product radionuclides, such as ^{90}Sr, has persisted for far longer than was originally expected. ^{90}Sr has been matter of serious concern because of long half-life and high water solubility. Thus, hazardous quantities of ^{90}Sr will remain in the environment for centuries and living organisms easily absorb ^{90}Sr mistaking these for harmless essential cations [1].
Although cleanup is necessary to prevent any further discharge of contaminated wastes into the environment, a technology needs to be developed that is cost effective for industry to use. Methods traditionally employed for wastewater remediation consist of removal of metals by filtration, flocculation, activated charcoal and ion exchange resins. However, because of the high cost of these methods, development of a more cost effective remediation system is necessary. There has been a tremendous amount of attention given to the use of biological systems for removal of heavy

metals from aqueous solutions. Algae, fungi, yeast and bacteria remove heavy metals from wastewaters through functional groups such as ketones, aldehydes, carboxyls on their cell walls. Microbial accumulation performs well in comparison to sorption on commercial ion exchange resins, activated carbon and metal oxides [2-6].
We report here the biosorption of Sr by a bacterium isolated from the naturally radioactive spring Neydasht in Iran. Furthermore, we present the effects of various environmental parameters in the removal of Sr.

Materials and methods

Ten water samples were collected from the naturally radioactive spring Neydasht in Iran. One ml of each sample was cultured in an Erlenmeyer flask containing 25 ml trypticase soy broth (Difco) and incubated at 30 ^{o}C in a shaking incubator (150 rpm) for 7 days. In order to obtain separate colonies, the above culture was diluted 10-10000 folds in sterile distilled water and plated on trypticase soy agar (Difco) and was incubated at 30 ^{o}C for 7 days. In this way, 50 bacterial isolates were purified. Thereafter, bacterial masses were harvested by centrifugation at 10000 × g for 10 min at 4 ^{o}C and washed three times with distilled water and wet weight of the cells equivalent to 0.8 g dry weight/l was used in the experiments.

Selection of the strain

To select the strain with higher capacity of removing Sr from solution, 0.8 g dry weight/l of the bacterial strains were added into 30 ml aliquots of the Sr^{2+} solutions ($SrCl_2$) (100 mg/l Sr, pH 5.0) in 150-ml Erlenmeyer flasks and were incubated at 30 ^{o}C on a shaker (150 rpm) for 90 min. The experiments were conducted in triplicates and Sr-free solutions were used as control. The cells were centrifuged at 10000 × g for 10 min at 4 ^{o}C and the pellet digested in a mixture of nitric acid and chlorhydric acid (3:1, v/v). The amount of Sr in supernatant and solution of digested biomass were measured by inductively coupled plasma-mass spectroscopy (ICP-MS). Subsequently, an isolate was identified which adsorbed Sr in a solution containing 100 mg/l.

Optimization for the Sr uptake by the strain GTG-83

The experiments were conducted in 150 ml Erlenmeyer flasks containing 30 ml of Sr or Sr-salt ($MgCl_2$) mixture-bearing synthetic solutions at desired level of each component at the beginning of the adsorption. The flasks were agitated on a shaker at a 150 rpm constant shaking rate for 360 min to ensure equilibrium was reached.
In batch experiment, pH profile, salt ($MgCl_2$) concentration, temperature, contact time and adsorption capacity studies of *Bacillus* sp. GTG-83 biomass were conducted. About 0.03 g of *Bacillus* sp. GTG-83 biomass was allowed to contact with Sr solution in the pH range 5-10 under the shaking condition and room temperature. The metal sorption ability of the *Bacillus* sp. GTG-83 biomass at varying temperatures was determined by estimating residual metal concentration in the solution. In another batch experiment, *Bacillus* sp. GTG-83 biomass was allowed to contact with Sr solution for a certain period of time of 5.0, 10, 15, 30, 45, 60, 90, 120, 240 and 360 min under the latter favorable pH condition. The favorable pH condition and saturated time of *Bacillus* sp. GTG-83 biomass to adsorb Sr were then applied to the adsorption capacity study by varying the concentration of Sr up to 300 mg/l.

Determination of MIC

The MIC of Sr was determined with Petri dishes containing Muller-Hinton (MH, Merck) agar supplemented with different concentrations of Sr at pH 7.0. The metal solutions were sterilized by using 0.45 μm pore-size sterile filters. Analysis of Sr resistance was performed by mid-log phase cultures in 5 ml of liquid medium. Cells were streaked on MH agar plates containing different concentrations of Sr. Growth was recorded after three days of incubation at 30 ˚C. The lowest concentration of metal that completely prevented growth was termed the minimal inhibitory concentration (MIC).

Metal Solutions

Sr solution with different initial concentrations were prepared by dissolving $SrCl_2$ (Fluka) in distilled de-ionized water. All glassware for the biosorption experiments was routinely rinsed with HNO_3 and washed extensively with distilled deionized water to prevent interference by contaminants. The pH of each solution was measured by a digital pH meter and adjusted by the addition of 0.1M HNO_3 or 0.1M NaOH solutions.

Results and Discussion

A total of 50 bacterial isolates were isolated from water samples taken from naturally radioactive spring Neydasht in Iran. Based on gram reaction and KOH test 70% gram negatives and 30% gram positives were identified (mostly *Bacillus* spp.) among a total of 50 isolates.

According to the Sr uptake capacity the isolates were categorized as ([0.0-9.99], [10.0-19.99], [20.0-29.99], [30.0-39.99], [40.0-49.99] and [50.0-59.99] mg Sr/g dry wt). Prevalence of these groups were 27%, 44%, 15%, 10%, 3% and 1%, respectively. Among the isolates, only one strain showed maximum uptake capacity (55 mg Sr/g dry wt) which was selected for further investigation and optimization of conditions for the Sr removal from $SrCl_2$ solution. The strain was identified as *Bacillus* sp. according to Bergey's Manual of Systematic Bacteriology and called GTG-83. Morphological and biochemical characteristics of the isolate are presented in Table 1.

MIC of Sr for *Bacillus* sp. GTG-83 was examined. Cell growth of *Bacillus* sp. GTG-83 isolated from naturally radioactive spring Neydasht in Iran was inhibited strongly by 60 mM $SrCl_2$ and inhibited completely by 70 mM $SrCl_2$. Bacterial sensitivity to metal toxicity is known to depend on their isolation site. In natural bacterial communities, the development of metal resistance is greatly enhanced by the horizontal dispersal of genetic information. Evolution of resistance via such transfer between natural bacterial isolates has been shown to occur in situ and also under laboratory conditions. This in part is related to other factors, such as, differences in the content of organic nutrients and the presence of other pollutants.

Table 1. Morphological and biochemical characteristics of *Bacillus* sp. strain GTG-83

Characteristic	**Result**	**Characteristic**	**Result**
Gram stain	+	Cytochrome oxidase test	+
Cell shape	Rod	Catalase test	+
Endospore formation	+ (Central)	Oxidation/fermentation (O/F)	F
Motility	+	Acid production from carbohydrates:	
Growth at pH	4-12	Glucose	+
Growth on NaCl (%)	3-15	Sucrose	+
Indol test	-	Mannose	+
Methyl Red test	+	Lactose	-
VP test	-	Mannitol	-
Citrate utilization	+	Arabinose	-
Nitrite reduction	+	Egg yolk reaction (Lecithinase)	+
H_2S production	-	Hemolysis	β
Starch hydrolysis	+		

Since contaminated water and industrial effluents are often found at different pH, studies were performed to determine the effects of pH upon Sr ion adsorption by *Bacillus* sp. GTG-83. Earlier studies on heavy metal biosorption have shown that pH was the single most important parameter affecting the biosorption process. To find the suitable pH for the effective biosorption of Sr ion by *Bacillus sp. GTG-83* in single and salt containing mediums, experiments were performed at different initial pH values (5-10) and at different initial $MgCl_2$ concentrations (0-15%). The relationship between pH and the amount of Sr adsorbed of biomass are shown in Fig.1. It can be observed that the favorable condition for *Bacillus sp. GTG-83* to adsorb Sr was around pH 10. The biosorption of Sr increased significantly with further increase in pH for all tested $MgCl_2$ concentrations. The presence of salt also interacted the biosorption antagonistically. At pH 10 the removal of Sr decreased from 97 to 23.6 mg Sr/g dry wt with increasing salt concentration up to 15% (w/v). Solution pH influences both cell surface metal binding sites and metal chemistry in water. As the pH was lowered, the overall surface charge of cell surface will become positive, whereas at higher pH values the overall surface charge will become negative, resulting in an increase of cationic Sr biosorption.

Salt concentration is proportional to the ionic strength of aqueous solution directly. Ionic strength, besides pH is also one of the important factors that influence the equilibrium uptake. Although the ionic strength or the salinity did not affect the optimum pH, adsorption decreased sharply with increasing ionic strength of the aqueous solution at all pH values studied as shown in Fig. 1.

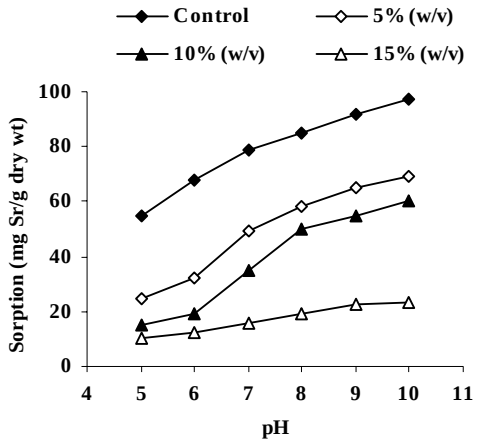

Fig. 1. Uptake of the Sr from solution by *Bacillus sp. GTG-83* at various pH and ionic strength values.

Temperature has not been studied as a relevant variable in biosorption experiments. Fig. 2 indicates that the rise in incubation temperature influenced very sharply the biosorption rates of Sr by *Bacillus sp. GTG-83*. Maximum biosorption of Sr was obtained at temperatures in the range of 30–35 ˚C. The increase in metal uptake at increased temperature is due to either higher affinity of sites for metal or an increase in binding sites on the relevant biomass.

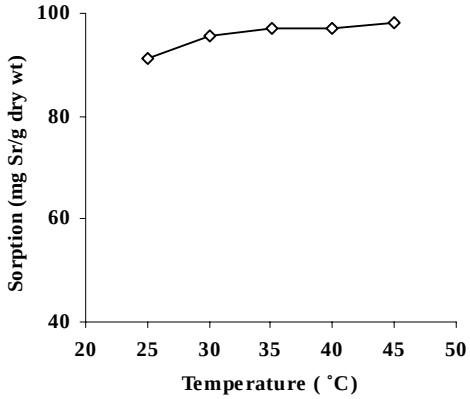

Fig. 2. Uptake of Sr from solution by *Bacillus sp. GTG-83* at various temperatures.

The initial concentration provides an important driving force to overcome all mass transfer resistance of metal ion between the aqueous and solid phases. Hence a higher initial concentration of Sr will increase the biosorption rate. Such an effect was clearly demonstrated in Fig. 3. The equilibrium sorption capacity the *Bacillus sp. GTG-83* increased with increasing initial Sr concentration. When the initial Sr concentration increased from 25 to 250 mg/l approximately, the loading capacity increased from 16.5 to 185.3 mg Sr/g dry wt due to the increase in the number of ions competing for the available binding sites in the biomass. The uptake of Sr by *Bacillus sp. GTG-83* reached a plateau at 250-300 mg/l showing the saturation of binding sites at higher concentration levels. The initial Sr concentration also influenced the biosorption yield significantly. Sr removal yield were the maximum at the 100 mg/l due to higher cell density attained than at higher concentrations of Sr.

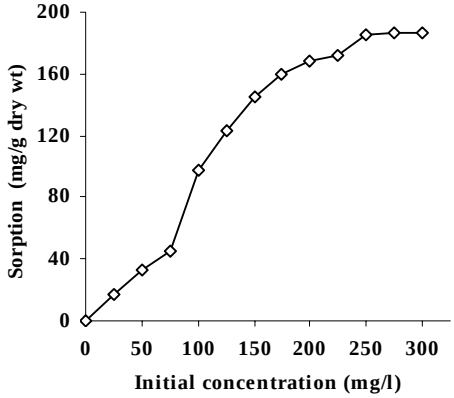

Fig. 3. Effect of initial concentration of Sr on the biosorption capacity of *Bacillus sp. GTG-83.*

The effect of contact time on the uptake of Sr ion by *Bacillus sp. GTG-83* is shown in Fig. 4. It was found that the biosorption was rapid during the first 5 min and reached saturation within 60 min. The rapid uptake of Sr from solution suggests that the binding sites are cell wall components and that the metal ions are not diffusing through the cell wall.

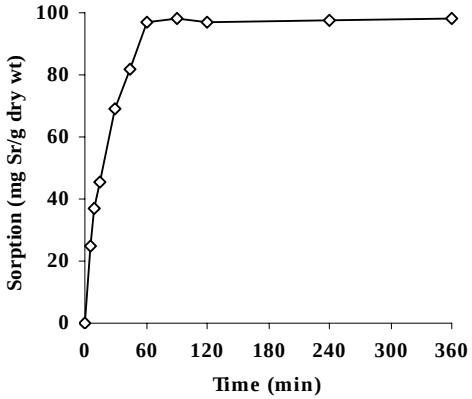

Fig. 4. Kinetics of Sr binding to the *Bacillus sp. GTG-83*.

These findings suggest that *Bacillus* sp. strain GTG-83 is highly efficient in Sr sorption when compared to all bacteria tested in this laboratory. The strain GTG-83 shows the potential applicability in removing Sr from environments containing considerable concentrations of the Sr.

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