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

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Abstract: An attempt was made to isolate bacterial strains capable of removing strontium biologically. In this study ten different water samples collected from Neydasht spring in the north of Iran and then the bacterial species were isolated from the water samples. The initial screening of a total of 50 bacterial isolates resulted in selection of one strain. The isolated strain showed a maximum adsorption capacity with 55mg strontium/g dry wt. It was tentatively identified as *Bacillus* sp. according to the morphological and biochemical properties, and called strain GT-83. Our studies indicated that *Bacillus* sp. GT-83 is able to grow aerobically in the presence of 50mM $SrCl_2$, but its growth was inhibited at high levels of strontium concentrations. The biosorption capacity of *Bacillus* sp. GT-83 depends strongly on the pH solution. Hence the maximum strontium sorption capacity of *Bacillus* sp. GT-83 was obtained at pH 10, independent of absence or presence of $MgCl_2$ of different concentrations. Strontium-salt biosorption studies were also performed at this pH values. The equilibrium biosorption of strontium was elevated by increasing the strontium concentration, up to 250mg/l for *Bacillus* sp. GT-83. The maximum biosorption of strontium was obtained at temperatures in the range of 30-35˚C. The *Bacillus* sp. GT-83 biosorbed 97mg strontium/g dry wt at 100mg/l initial strontium concentration without $MgCl_2$. When $MgCl_2$ concentration increased to 15%(w/v), these values dropped to 23.6mg strontium/g dry wt at the same conditions. Uptake of strontium within 5 min of incubation was relatively rapid and the absorption continued slowly thereafter.

Keywords: ***Bacillus sp. GT-83, Biosorption, Neydasht Spring, Radionuclide, Strontium, Wastewater***

1-Introduction

Heavy metals and radionuclide contaminations are generated due to; industrial activities, mill tailing, nuclear power testing, nuclear waste disposal and accidents from nuclear power generation. An accident of this nature occurred at the Chernobyl Nuclear Power Station on April 26, 1986. Contamination of soil with typical fission radionuclides product, such as ^{90}strontium, has persisted far longer than was originally expected. ^{90}Strontium has been a serious concern because of the long half-life and the high water solubility. Thus, hazardous quantities of the ^{90}strontium will remain in the environment for centuries and the living organisms will easily absorb the ^{90}strontium mistaking it for harmless essential cations [1].

Strontium compounds that are water-insoluble can be water-soluble as a result of chemical reactions. Water-soluble forms of strontium have the opportunity to pollute the drinking water. People can be exposed to small levels of (radioactive) strontium by breathing air or dust, eating food, drinking water, or by contact with the soil that contains strontium. In most cases, the strontium uptake will be moderate. The only strontium compound that considered to be a danger to human health, even in small quantities, is strontium chromate. The toxic chromium that it contains mainly causes this danger. Strontium chromate is known to cause lung cancer, but the risks of exposure have been greatly reduced by safety procedures followed by companies, so that it is no longer an important health risk. When the strontium uptake is extremely high, it can cause disruption of bone development. But, this effect can only occur when strontium uptake is in the range of thousands of ppm. The radioactive strontium poses greater health risk than the stable strontium. When the uptake is very high, it may even causes anemia and reduce the oxygen levels in blood, and at an extremely high concentration is known to cause cancer because of the damage to the genetic material in the cells [2, 3].

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Although clean-up is necessary to prevent any further discharge of contaminated wastes into the environment, a proper cost effective technique is required for industry to be used. Methods, that have been traditionally employed for wastewater remediation, consist of removal of metals by filtration, flocculation, activated charcoal and due to high cost of applying these methods, development of a more cost effective remediation system is necessary. So far a great attention has been paid to the use of biological systems for removal of heavy metals from aqueous solutions. Algae, fungi, yeast and bacteria absorb heavy metals from wastewaters through functional groups such as ketones, aldehydes, carboxyls into their cell walls. Microbial accumulation performs well in comparison to the sorption on the commercial ion exchange resins, activated carbon and metal oxides [4-10].

The process of biosorption of strontium by a bacterium isolated from the Neydasht spring in Iran has been investiged and presented in this article. Furthermore, the effects of various environmental parameters in removal of the strontium have been explored.

2- Materials and methods

Ten water samples were collected from the Neydasht spring in the north of Iran. One ml of each sample was cultured in an Erlenmeyer flask containing 25ml trypticase soy broth (Difco) and incubated at 30^{o}C in a shaking incubator (150rpm) for 7 days. In order to obtain isolated colonies, cultures were 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. 50 isolated bacterial species were purified by this method. 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 0.8g dry wt/l was used in the experiments.

3- Selection of the strain

To select the strain with higher capacity of removing the strontium from solution, 0.8g dry wt/l of the bacterial strains were added into 30ml aliquots of the strontium solutions ($SrCl_2$) (100mg/l strontium, pH 5.0) in 150-ml Erlenmeyer flasks and were incubated at 30^{o}C on a shaker (150rpm) for 90 min. The experiments were conducted in triplicates and cell suspensions without metal ions and solution of metals without added cells were also incubated and served as controls. 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). In order to determine the strontium quantity present in the biomass, acid digestion of the biomass was carried out.

The amount of strontium in supernatant and solution of digested biomass were measured by inductively coupled plasma-mass spectroscopy (ICP-MS). Subsequently, a bacterial strain was identified which adsorbed strontium in a 100mg/l solution.

4- Determination of MIC

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

5- Optimization for the strontium uptake by the strain GT-83

The experiment was conducted in 150ml Erlenmeyer flasks containing 30ml of strontium or strontium $MgCl_2$ 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 150rpm with constant shaking rate for 360 min to ensure the desired equilibrium.

In the batch experiment, the pH profile, $MgCl_2$ concentration, temperature, contact time and the adsorption capacity studies of *Bacillus* sp. GT-83 biomass were performed. About 0.03g of *Bacillus* sp. GT-83 biomass was allowed to have contact with strontium solution in the pH range of 5-10 under the shaking condition at room temperature. The metal sorption ability of the *Bacillus* sp. GT-83 biomass at different temperatures was determined by estimating the residual metal concentration in the solution. In another batch experiment, *Bacillus* sp. GT-83 biomass was exposed to the strontium solution for periods of 5.0, 10, 15, 30, 45, 60, 90, 120, 240 and 360min, under the favorable pH condition. The suitable pH condition and saturated time of *Bacillus* sp. GT-83 biomass to adsorb strontium were, then applied for the adsorption capacity study by varying the concentration of strontium up to 300mg/l.

6- Metal Solutions

Strontium solution with various initial concentrations was prepared by dissolving $SrCl_2$ (Fluka) in distilled de-ionized water. All glasswares for the biosorption experiments were routinely rinsed with HNO_3 and washed extensively with distilled deionized water to prevent interference with 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.

7- Results and Discussion

Screening of bacteria from Neydasht spring leads to isolation of 50 bacterial strains. Based on gram reaction and KOH test, 70% of bacterial isolates were gram negatives and 30% gram positives (mostly *Bacillus* sp.).

Based on strontium 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 strontium/g dry wt). Prevalence of these groups were 27%, 44%, 15%, 10%, 3% and 1%, respectively. Among the isolates, only one strain showed a maximum uptake capacity (55mg strontium/g dry wt) which was selected for further investigations and optimization of conditions for the strontium removal from $SrCl_2$ solution. The strain was identified as *Bacillus* sp. according to Bergey's Manual of Systematic Bacteriology and is called GT-83 [11, 12]. Morphological and biochemical characteristics of the isolate are presented in Table 1.

Table 1. Morphological and biochemical characteristics of *Bacillus* sp. strain GT-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	+		

MIC of strontium for *Bacillus* sp. GT-83 was examined. The cell growth of *Bacillus* sp. GT-83 isolated from Neydasht spring in Iran was inhibited strongly by 60mM $SrCl_2$ and inhibited completely by 70mM $SrCl_2$. The bacterial sensitivity to metal toxicity is known to be dependent 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 [13].

Since contaminated water and industrial effluents are often found at different pH [4], studies were performed to determine the effects of pH upon strontium ion adsorption by *Bacillus* sp. GT-83. The earlier studies on the heavy metal biosorption have shown that pH is the most important parameter affecting the biosorption process. To find the suitable pH for the effective biosorption of strontium ion by *Bacillus* sp. GT-83 in single and salt containing mediums, experiments were performed at different pH values (5-10) and at various initial $MgCl_2$ concentrations (0-15%). The relationship between pH and the amount of strontium adsorbed of biomass are shown in Fig.1. It was found that the favorable condition for *Bacillus* sp. GT-83 to adsorb strontium was around pH 10. The biosorption of strontium increased significantly with further increase in the pH for all tested $MgCl_2$ concentrations. The presence of salt also interacted with the biosorption antagonistically. At pH 10, the removal of strontium decreased from 97, to 23.6mg strontium/g dry wt with increasing $MgCl_2$ concentration up to 15%(w/v). The solution pH can influence on the cell surface metal binding sites and metal chemistry in water. As the pH was lowered, the overall surface charge of cell surface became positive, whereas at the higher pH values the overall surface charge became negative, resulting in an increase of cationic strontium biosorption. At pH values greater than 10, the strontium solubility decreased due to its precipitation as carbonate or sulfate [9-14 and 3]. Magnesium chloride concentration is directly proportional to the ionic strength of aqueous solution. The ionic strength, besides the pH, is also another important factor influencing the equilibrium uptake. Although the ionic strength or the salinity did not affect the optimum pH, the adsorption decreased sharply with increasing the ionic strength of the aqueous solution at all the pH values studies, as shown in Fig. 1 [14, 15 and 3].

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 strontium by *Bacillus* sp. GT-83. The maximum biosorption of strontium was obtained at temperatures in the range of 30–35˚C. The increase in metal uptake with increased temperature is due to either higher affinity of sites for metal or an increase in the binding sites on the relevant biomass [13, 14].

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 strontium will increase the biosorption rate. This effect was clearly demonstrated in Fig.3. The equilibrium sorption capacity of the *Bacillus* sp. GT-83 increased with increasing the initial strontium concentration. When the initial strontium concentration increased approximately from 25 to 250mg/l, the loading capacity increased

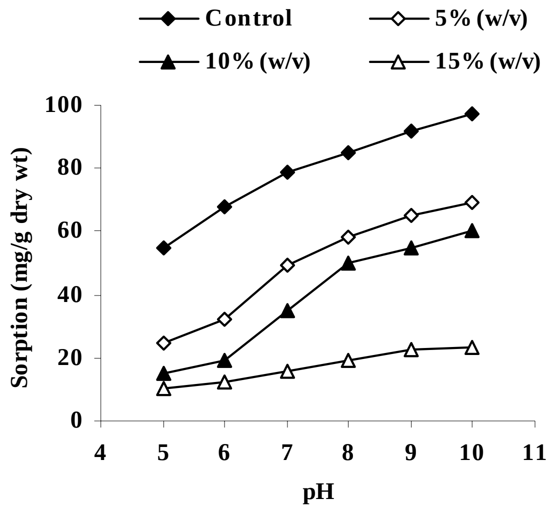

Fig.1. Biosorption of the strontium from solution by *Bacillus* sp. GT-83 at various pH and ionic strength values as $MgCl_2$.

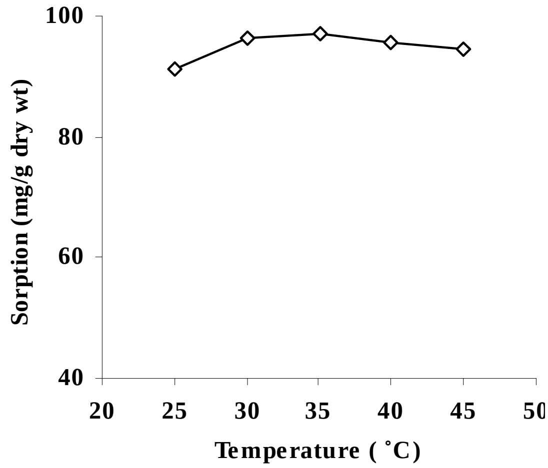

Fig.2. Biosorption of strontium from solution by *Bacillus* sp. GT-83 at various temperatures.

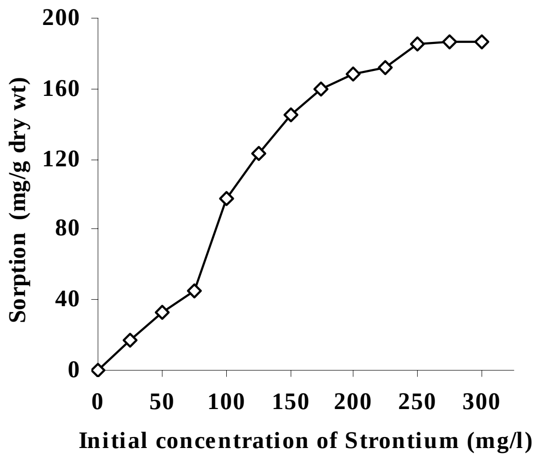

Fig.3. Effect of various initial concentrations of strontium on the biosorption capacity of *Bacillus* sp. GT-83.

from 16.5 to 185.3mg strontium/g dry wt due to the increase in the number of ions competing for the available binding sites in the biomass. The uptake of strontium by *Bacillus* sp. GT-83 reached a plateau at 250-300mg/l showing the saturation of binding sites at the higher concentration levels. The initial strontium concentration also influenced the biosorption yield significantly. Strontium removal yield reached a maximum at the 100mg/l due to higher cell density attained rather than at higher concentrations of strontium [10-13].

The effect of contact time on the uptake of strontium ion by the *Bacillus* sp. GT-83 is shown in Fig. 4. It was found that the biosorption was rapid during the first 5min and reached saturation within 60min. It has been suggested that the rapid sorption of strontium is due to prescence of binding sites on the cell wall components and that the strontium does not diffuse through the cell wall [14, 15].

These findings suggest that the *Bacillus* sp. strain GT-83 is highly efficient in strontium sorption when compared to other bacteria tested in this laboratory. The strain GT-83 shows the potential capability in removing strontium from environments containing considerable concentrations of the strontium and it may find an application in scavenging the strontium from waste water for waste water management.

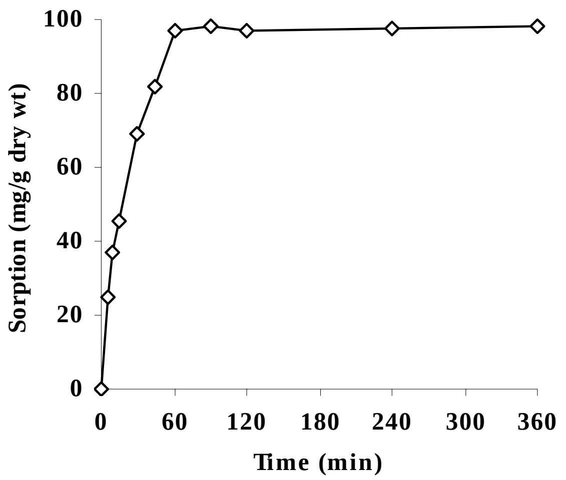

Fig. 4. Kinetics of strontium binding to the *Bacillus* sp. GT-83.

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