Radionuclide Biosorption by Bacterial Biomass

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Abstract

Bacterial isolates from uncontaminated garden soil were found to be good biosorbents of radionuclides. Among the strains, a copper resistant Pseudomonas was identified as a potent accumulator of uranium (VI) and thorium (IV). Radionuclide (U) accumulation by the bacterium was found insensitive to culture age (lag, log and stationary) so also in presence of carbon/energy source (glucose) and metabolic inhibitor (sodium azide), suggesting it as passive biosorptive uptake. Bacterial cells grown in peptone rich enriched medium or in synthetic minimal medium showed no significant difference in U accumulation at lower U concentration (100 mg l⁻¹). However, at higher concentration range (1000 mg l⁻¹) minimal medium grown cells showed a significantly high metal loading. Such biosorptive uranium and thorium uptake capacity of the bacterium was characterized using metabolically inactivated lyophilized biomass. Radionuclide binding by the test bacterium was rapid, achieving >90% within 1-10 minutes of contact and the equilibrium was attained within two to four hours. Initial solution pH significantly affected U and Th sorption with its optimum at pH 5.0 for U and pH 4.0 for Th. Equilibrium isotherms for both the metals indicate a high affinity, efficient and high capacity uranium and thorium biosorption with the maximum loading of 541 mg U g⁻¹ dry wt. or 430 mg Th g⁻¹ dry wt. Experimental sorption data showing good conformity to Langmuir model suggests a monolayered metal binding. Sorption in presence of several interfering cations and anions indicates a specific U and Th binding by the Pseudomonas with significant antagonism offered only by the iron (III). Studies are going on to decipher the bacterial radionuclide binding mechanism using various physico-chemical approaches and to exploit its potential in continuous removal/recovery system.

Introduction

Radionuclide and heavy metal pollution by various industrial activities is of significant environmental concern (Barkay and Schaefer, 2001; D’Souza, 1999a; D’Souza et al. 2001a; D’Souza, 2002a). Microbial bioremediation of such toxic compounds are increasingly being considered as a potential alternative with high capacity and efficient metal removal from diluted effluent with almost no secondary waste generation. (Taxier et al. 1999 and Lloyd and Macaskie 2000). Although considerable studies are made on microbial removal of heavy metals, radionuclides in this regard remained little explored. Among the several microbial processes that determine the environmental fate of metallic toxicants viz. reductive or enzymatic precipitation, solubilization, etc., biosorptive accumulation of heavy metals and radionuclides is of recent interest (Gadd 2000, Hu et al. 1996, Dhami et al. 1998, Sar et al. 1999, Sar and D’Souza 2001a, b). Bioremediation of radionuclides, heavy metals and organic waste has been a major recent activity in the authors laboratory (D’Souza, 1999a; Bhainsa and D’Souza 1999; Joshi and D’Souza, 1999; Sar and D’Souza 2001a,b; Bhainsa and D’Souza, 2001a,b; Sar et al, 2001a,b; Sangurdekar et al. 2001, Melo and D’Souza, 2001; D’Souza, et al., 2001a,b; Kazy, et al, 2001; Shanmugam et al., 2001; Sar and D’Souza 2002; Kazy et al 2002; Panchapakesan et al,2002; Irani et al, 2002). Compared to the conventional treatment methods, these biomass based systems are more acceptable in being cost effective, with high efficiency of detoxification of even very dilute effluents and minimizing the disposable sludge volume. It also offers the flexibility for developing non-destructive desorption techniques for biomass regeneration and/or quantitative metal recovery.
Metal accumulation by microorganisms is either of energy driven active bioaccumulation or metabolism independent passive biosorption. The latter process of microbial metal removal by purely physico-chemical processes seems more appropriate for bioremediation with cation sequestration mainly regulated by the characteristics of the microorganism, the targeted metal and the solution microenvironment. Since, it is the chemical composition of the cell wall and other surface materials responsible for cation sequestration, cell viability or other metabolic activities that do not interfere with such characteristics effectively have no impact on biosorption. In some cases, pregrowth conditions, particularly, the growth medium ingredients also show significant influence on biosorptive metal uptake.

Although, biosorptive uptake of several heavy metals is well documented, such studies on radionuclide sorption are relatively less. Particularly, with respect to uranium, a variety of living and non-living biosorbents composed of fungi and bacteria have been reported to bind the cation considerably, whereas reports on thorium sorption are not impressive. Previous studies on microbial metal sorption by our group have identified the strains of *Pseudomonas* as a potent accumulator of metals and radionuclides (Sar et al. 1998, Sar et al. 1999, Kazy et al. 1999, Sar et al. 2001a, Sar and D’Souza 2001a, Sar and D’Souza 2002, Kazy et al 2002). The present study was undertaken to evaluate the uranium (VI) and thorium (IV) biosorption capacity of a *Pseudomonas* soil isolate. Equilibrium sorption behavior of the lyophilized biomass was characterized employing the Freundlich and Langmuir adsorption isotherm models. The effect of solution pH on the chemistry of binding sites of both biosorbent types and also the metallic species in solution was assessed. Effect of bacterial culture age and presence of energy sources or metabolic inhibitor on uranium uptake was also studied. In view of a multimetallic composition of realistic waste, U and Th sorption in presence of other interfering cations and anions was investigated.

**Materials and Methods**

**Microorganism, growth medium and culture conditions**

*Pseudomonas* sp., a garden isolate was grown and maintained in Tris-minimal medium (Kazy et al. 1999). Mid exponential phase cells were collected by centrifugation (12000 × g, 30 min), washed thoroughly with distilled water, dried and used for biosorption experiments.

**Uranium and thorium biosorption experiments**

All biosorption experiments were done using dry *Pseudomonas* biomass. Nitrate salts of Uranium and Thorium was used (UO₂(NO₃)₂·6 H₂O or Th (NO₃)₂·5 H₂O, Merck, Germany). Other experimental details were same as described previously (Sar and D’Souza 2001, Sar and D’Souza 2002). Dissolved uranium and thorium was determined either by the Arsenazo III method or by inductively coupled plasma atomic spectrometry (ICP-AES).

The biosorption equilibrium uptake (q, mg metal g⁻¹ biomass dry wt.) for each sample was calculated according to the mass balance on metal ion expressed as:

\[ q = \frac{V(C_o - C_e)}{M} \]  

(1)

Where \( V \) is the sample volume (l), \( C_o \), the initial metal ion concentration (mg l⁻¹), \( C_e \), the equilibrium or final metal concentration (mg l⁻¹) and \( M \), the biomass dry weight (g).

Adsorption isotherm data were also fitted to the classical Freundlich and Langmuir isotherm equations (De Rome and Gadd 1987).

The linearized form of isotherm equations used are:

Freundlich Equation : \[ \log q = \log k + \frac{1}{n} \log C_e \]  

(2)

Where \( q \) is the equilibrium metal uptake capacity and \( C_e \), the residual metal concentration at equilibrium. The constant 'k'
is a measure of adsorption capacity and '1/n', the intensity of adsorption.

Langmuir Equation: \[ \frac{1}{q} = \frac{1}{q_{\text{max}}} \cdot \frac{b}{C_e} + \frac{1}{q_{\text{max}}} \] (3)

The constant 'q_{\text{max}}' represents the maximum specific metal uptake and 'b' the ratio of the adsorption/desorption rates related to energy of adsorption through Arrhenius equation.

**Time course of metal biosorption**

For sorption kinetic studies, bacterial biomass (0.5 mg ml\(^{-1}\)) was mixed with uranium or thorium solution (100 mg l\(^{-1}\)), samples withdrawn at timed intervals were centrifuged and dissolved U/Th was estimated.

**Effect of pH on biosorption**

The effect of solution pH on U and Th sorption was studied by adjusting the initial pH of the contact solution (100 mg Th l\(^{-1}\)) over the range pH 2.0-8.0. For pH adjustment, 1.0 M NaOH or 1.0 M HNO\(_3\) was used.

**Interference of cations and anions on uranium and thorium biosorption**

Uranium and thorium sorption in simultaneous presence of other interfering ions was tested in bi metallic combinations, by adding equimolar concentrations of uranium or thorium (430 \(\mu\)M Th or 420 \(\mu\)M U; equivalent to 100 mg U or Th l\(^{-1}\)) and test cation or anion. Details are same as described earlier (Sar and D'Souza 2001a, Sar and D'Souza 2002)

**Results and Discussion**

**Selection of uranium accumulating strain**

Initial study on selecting a good uranium accumulating strain was done using four soil isolates, three belonging to *Pseudomonas* sp and one to *Bacillus coagulans*. All these bacterial strains were selected based on their superior metal tolerance capacity. A *Pseudomonas* sp. 2 which showed optimal biosorption was selected for further study on biosorption of uranium (VI) and thorium (IV).

Effect of growth medium, culture age, energy source and metabolic inhibitor on uranium biosorption

Metal and radionuclide biosorption by microbes is strongly influenced by the nature, availability and arrangements of various cellular binding ligands sequestering cationic metallic species. Among the others factors, growth medium ingredients are often found to regulate the synthesis of these metal binding moieties. In the present study *Pseudomonas* cells were pre-grown in synthetic minimal- and peptone containing enriched- medium and their U sorption was compared. At low uranium concentration (100 mg l\(^{-1}\)), comparable metal sorption was observed by both enriched (63 mg g\(^{-1}\) dry wt.) and minimal (60 mg g\(^{-1}\) dry wt.) medium grown cells. However, improved (1.9-fold) uranium loading was found for minimal medium grown cells (245 mg g\(^{-1}\) dry wt.) at higher uranium concentration (1000 mg l\(^{-1}\)). Although Chang et al. 1995 reported a significant enhancement of copper adsorption by growing the culture in peptone containing medium, the present data corroborate very well with U sorption by *P. aeruginosa* CSU strain (Hu et al. 1996). The latter investigators also showed an improved uranium sorption following the use of synthetic defined medium instead of nutrient broth.

To elucidate the role of bacterial culture age on uranium sorption, cells collected before, during and after mid exponential growth phase was compared for their U accumulation capacity. Although in certain case (Friis and Myers-Keith, 1986) culture age has shown a strong effect on bacterial metal uptake, the present *Pseudomonas* biomass did not show any significant difference in metal loading for the cells of different growth phase.

Metal accumulation by bacteria could be a metabolism -dependent active uptake or an -independent passive biosorption. In the present investigation, such energy
dependency of U uptake by Pseudomonas biomass was tested by adding glucose (as carbon/energy source) or sodium azide (as metabolic inhibitor) in uranium uptake solution. Metal removal was also compared using live, lyophilized and autoclaved cells. Noticeably, no significant difference in U removal was observed, suggesting the metabolic independency of the test biomass in sequestering uranium.

**Time course of uranium and thorium sorption**

The kinetics of uranium and thorium sorption by lyophilized Pseudomonas biomass is shown in Fig 1. For both the radionuclides, the test biomass exhibited a rapid cation uptake with more than 90% of equilibrium was reached within one (for Th) or ten (for U) minutes and the process saturates after 2 (for U) or 4 (for Th) hours. The rapid cation uptake has been suggested as being essential for any good biosorbent as it allows short solution-sorbent contact time and would result in the use of much shallower contact beds of sorbent materials in column application (Tsezos and Volesky 1982, Volesky 1990, Andres et al. 1993, Hu et al. 1996).

**Effect of pH on uranium and thorium biosorption**

Initial solution pH is the most critical parameter for metal sorption as it influences both the bacterial surface chemistry as well as the solution chemistry of soluble metal ions. Uranium and thorium sorption by lyophilized *Pseudomonas* biomass was studied at a range of pH 2-8 and pH 2-6, respectively. As shown in Fig 2, initial solution pH significantly affected the equilibrium U and Th loading capacity. Over the range tested, extreme acid condition (pH 2.0) did not favour sorption of both the cations. As the pH increased, sorption of U and Th also increased and the maximum loading for thorium and uranium was attained at pH 4.0 and pH 5.0, respectively.

![Fig 2: Effect of pH on U (*) and Th (•) sorption (100 mg l⁻¹) by *Pseudomonas* biomass](image)

An increase in pH beyond the optimum caused decline in sorption of respective cations. The reduced sorption at low pH could be attributed to (i) the hydrolysis of biomass metal binding groups resulting in an increased competition by H₃O⁺, and (ii) the increased solubility and consequent reduced adsorptivity of thorium ions (Beas and Mesmer 1976). Furthermore, compared to the Th⁴⁺ and Th(OH)₂²⁺ ions formed at low pH that have been identified as a poor sorbate (Tsezos and Volesky, 1982), the higher uptake at pH 4.0 could be correlated
to the predominance of $[\text{Th}_2(\text{OH})_3]^{5+}$ and other polymerized species possessing a greater binding affinity thus facilitating faster and enhanced metal sorption (Gadd and White 1989). For uranium, the observed trend with regard to pH may be explained by an increasing binding affinity of monovalent uranyl species ($\text{UO}_2^{2+}$, ($\text{UO}_2)\text{(OH)}_3$) formed at higher pH (pH 4.0-5.0) over the divalent ($\text{UO}_2^{2+}$) at low pH (pH 2.0).

Biosorption Isotherm

The biosorptive U and Th uptake by *Pseudomonas* biomass was quantitatively evaluated by equilibrium sorption isotherms over a concentration range of 0-1200 mg l$^{-1}$ (Fig 3). Representative isotherm curves for both the cations exhibited very efficient metal binding even at low residual concentration and a high saturation loading at equilibrium. The maximum sorption values obtained are 541 mg uranium g$^{-1}$ dry wt. and 430 mg thorium g$^{-1}$ dry wt. at equilibrium concentration of 359 mg U l$^{-1}$ and 885 mg Th l$^{-1}$, respectively. Such impressive U and Th binding by the test biomass significantly surpasses the economic threshold level (15% dry wt. basis) for practically usable biosorbents and so also the previous values on Th (*R. arhizus* (185 mg g$^{-1}$ dry wt.) (Tsezos & Volesky 1981) or *P. chrysogenum* (388 mg g$^{-1}$ dry wt.) (Gadd & White 1989)) and U (*R. arhizus and Penicillium chrysogenum* (both 180 mg g$^{-1}$) (Tsezos & Volesky 1981) *P. aeruginosa* CSU (110 mg g$^{-1}$) (Hu et al. 1996), and *M. smegmatis* (44.5 mg g$^{-1}$) (Andreas et al. 1993)) uptake.

Table 1: Freundlich and Langmuir constants for uranium and thorium sorption by *Pseudomonas* biomass

<table>
<thead>
<tr>
<th></th>
<th>Uranium</th>
<th>Thorium</th>
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</thead>
<tbody>
<tr>
<td><strong>Freundlich</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>199.00</td>
<td>159.20</td>
</tr>
<tr>
<td>$1/n$</td>
<td>0.206</td>
<td>0.176</td>
</tr>
<tr>
<td>r</td>
<td>0.931</td>
<td>0.973</td>
</tr>
<tr>
<td><strong>Langmuir</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>q$_{max}$</td>
<td>555.5</td>
<td>476.19</td>
</tr>
<tr>
<td>B</td>
<td>0.0027</td>
<td>0.0009</td>
</tr>
<tr>
<td>r</td>
<td>0.997</td>
<td>0.998</td>
</tr>
</tbody>
</table>

The relationship between equilibrium metal uptake capacity (q) and residual metal ion concentration (Ce) was further described using the model equation of Freundlich and Langmuir. Although linearized sorption isotherm for both the metals showed a reasonably good fit to both the models, the maximum correlation coefficient (r) was obtained with Langmuir equation. Values of respective sorption constants and correlation coefficients (r) are presented in Table 1. The better fitting of Langmuir model suggest a monolayerd U and Th binding on to the biomass with homogeneous surface energy and no interaction between sorbed metals (Langmuir 1918). The asymptotic maximum adsorption capacity as predicted by the Langmuir constant 'q$_{max}$' gives a very high value for U and Th while a desirable high affinity of the biomass for test metals are evident from the low values of other constant 'b'.

Effect of interfering ions on uranium and thorium biosorption

Uranium and thorium sorption by *Pseudomonas* biomass was studied in presence of equimolar amount of several competing ions (Table 2). Selection of such ions are based on their likely occurrence in realistic effluent interfering biosorption of U and Th. Among the series of cations tested, a significant antagonism in U sorption was
Table 2: Effect of interfering ions on U and Th biosorption by Pseudomonas

<table>
<thead>
<tr>
<th>Control (only U or Th)</th>
<th>Percentage of sorption</th>
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<tbody>
<tr>
<td></td>
<td>Uranium</td>
</tr>
<tr>
<td>Control</td>
<td>100</td>
</tr>
<tr>
<td>Cations:</td>
<td></td>
</tr>
<tr>
<td>Na⁺</td>
<td>100</td>
</tr>
<tr>
<td>Ag²⁺</td>
<td>98</td>
</tr>
<tr>
<td>K⁺</td>
<td>98</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>98</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>98</td>
</tr>
<tr>
<td>Cd²⁺</td>
<td>96</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>78*</td>
</tr>
<tr>
<td>Al³⁺</td>
<td>82*</td>
</tr>
<tr>
<td>Fe²⁺</td>
<td>45*</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>20*</td>
</tr>
<tr>
<td>Th⁴⁺</td>
<td>37*</td>
</tr>
<tr>
<td>UO₂²⁺</td>
<td>-</td>
</tr>
<tr>
<td>Anions:</td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>100</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>100</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>100</td>
</tr>
<tr>
<td>CO₃²⁻</td>
<td>74*</td>
</tr>
</tbody>
</table>

*Initial U/Th concentration 100 mg dm⁻³, pH 3.5, biomass 0.5 mg cm⁻².

offered only by thorium (IV), iron (II and III), aluminium (III) and copper (II) while metals like cadmium (II), lead (II) silver (II) and anions like chloride, phosphate and sulphate had no effect. The order of inhibition to uranium binding by the cations was Fe³⁺ > Th⁴⁺ > Fe²⁺ > Cu²⁺ > Al³⁺. Iron (III), the cation considered as the most potent competitor of uranium in binding biosorptive sites (Hu et al. 1996), also caused a severe decline (80%) in U loading. Such inhibition to U sorption by Fe³⁺, is a common phenomenon e.g. P. aeruginosa CSU (Hu et al. 1996), R. arrhizus and S. levoris (Byerley et al. 1987). Although Al³⁺ and Ag²⁺ have also been found to inhibit U adsorption by P. aeruginosa CSU and R. arrhizus, the present Pseudomonas biomass remained insensitive with respect to Ag⁺, while Al³⁺ was significantly antagonistic. Noticeably, in case of thorium, except iron (III), no other test cation showed an inhibition more than 20%. Th sorption was insensitive to the presence of Na⁺, Ca²⁺, Cd²⁺, Pb²⁺, CO₃²⁻, SO₄²⁻ and Cl⁻, while the order of inhibition by other cations is UO₂²⁺<Co²⁺<Ni²⁺<Al³⁺<Ag⁺<Cu²⁺. The observed insignificant interference (only 8% inhibition) of U on thorium binding can be attributed to a higher oxidation state of Th along with additional bonding parameters (Andres et al. 1993). Although, uranium and thorium biosorption by the present biomass was fairly efficient in the presence of a range of cations, the role of Fe (III) in inhibiting U and Th binding imposes serious limitations in wastewater treatment by this biosorbent. Ideally, iron should be removed by pH adjustment or other methods prior to biosorption.

Fig 4: Transmission electron micrograph of control cell (a), after uranium accumulation (b) and after thorium accumulation (c)

Mechanism of biosorption and use of immobilized microbes in continuous column reactors

Some studies have been undertaken to study the mechanism of biosorption. Transmission electron microscopy of metal loaded cells
revealed an intracellular metal sequestration (Fig.4) with X-ray diffraction pattern ascertaining their phosphide nature. IR spectroscopy and NMR data suggests the role of cellular phosphoryl groups in radionuclide binding. For continuous use in a column the biomass was immobilized using various techniques (D’Souza 1999b; D’Souza 2001b; D’Souza 2002b) including entrapment in radiation polymerized acrylamide beads (Fig. 5). Scanning electron micrograph of immobilized bacterial biomass (Fig.6) indicated no cellular damage/distortion during the immobilization process. The biomass could be used for repeated sorption-desorption cycles in continuous column operation. More than 90% of biomass bound U and Th was recovered through elution using carbonates.

**Conclusion**

The overall study using the *Pseudomonas* strain suggests the present biomass as a potential candidate for developing biosorbent for uranium and thorium removal for wastewater remediation, however, a prior reduction of iron content is necessary. A clear insight on the biomass U and Th binding mechanism(s) and other technological parameters (currently underway) will substantially improve its feasibility in process application.

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**Reference**


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