



# Biosorption of heavy metals and uranium by starfish and *Pseudomonas putida*

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## ABSTRACT

Biosorption of heavy metals and uranium from contaminated wastewaters may represent an innovative purification process. This study investigates the removal ability of unit mass of *Pseudomonas putida* and starfish for lead, cadmium, and uranium by quantifying the adsorption capacity. The adsorption of heavy metals and uranium by the samples was influenced by pH, and increased with increasing Pb, Cd, and U concentrations. Dead cells adsorbed the largest quantity of all heavy metals than live cells and starfish. The adsorption capacity followed the order: U(VI) > Pb > Cd. The results also suggest that bacterial membrane cells can be used successfully in the treatment of high strength metal-contaminated wastewaters.

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## 1. Introduction

Contamination of soils and groundwater with mixed wastes, which are a mixture of radionuclides and heavy metals, is of great concern for government, industry and communities [1]. These metals and radionuclides have been introduced into the environment from the industrial activities and the processing of ore mining [2].

Biosorption of metals is one of the possible innovative technologies involved in the removal of toxic metals from industrial wastes and subsurface environment [3]. Biosorption involves the accumulation of metals by biological material either by metabolically mediated methods or by purely physio-chemical means. Unlike physical and chemical treatments, biosorption can reduce the operational costs and many potential sources of biological material are cheaply and readily available [3]. Several recent studies sought to quantify the adsorption of heavy metals onto microorganisms [4,5]. The starfish have been tested as a biosorbent to remove the toxic metals since it is inexpensive, abundant, and contain calcium oxide compounds that are capable of precipitating and sorbing significant quantities of metals. The toxicity and mobility of heavy metals and radionuclides between biosorbent and adsorptive depends on the pH, the chemical nature of the metal species, the stability of metal complexes, the binding power of the functional groups, and the ionic strength [6].

More research is needed, however, to better understand the interactions of these contaminants with biosorbent in the contam-

inated soil and water. The present understanding in the ability of bacteria to remove various these metals related to microorganisms is incomplete, and it is still not known which microorganisms or other biosorbents, like starfish, are the most effective to remove these metals in the subsurface environment.

The objectives of the experiments were to determine the ability of *Pseudomonas putida* and starfish to uptake heavy metals and uranium. Results from this study should be useful in understanding bioavailability and further in the remediation of subsurface media polluted with mixed wastes.

## 2. Materials and methods

### 2.1. Bacteria and starfish

*P. putida* was obtained as single specie from American Type Culture Collection (ATCC 17484) and used as a bacterial strain. The bacterium was grown until the stationary phase for 24 h at 30 °C on the rotary shaker (150 rpm) in 50 mL of nutrients broth (Difco 0001, Difco Laboratory, Detroit, MI). One milliliter of the culture was used as an inoculum for 1 L of the medium. The cells were collected by centrifugation at 4 °C (15 min at 7000 rpm) and washed twice with distilled water. We determined the dry weight of *P. putida* suspension by drying them for 24 h at 60 °C. Dead cells were obtained by treatments suggested by Kurek et al. [7]. The dead cells were also washed with distilled water. The bacterial cells were suspended at 0.02 mg (dry weight) mL<sup>-1</sup>. Cell suspensions without heavy metals and uranium were prepared as a control.

Starfish collected from a local beach area of East Sea in Korea was air dried and ground in a crushing mill to a particle size <1 mm.

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**Table 1**  
The composition of starfish analyzed by X-ray fluorescence spectrometer (mass%)

Na <sub>2</sub> O	0.61
MgO	2.97
Al <sub>2</sub> O <sub>3</sub>	0.48
SiO <sub>2</sub>	1.02
P <sub>2</sub> O <sub>5</sub>	1.25
SO <sub>3</sub>	2.51
K <sub>2</sub> O	0.28
CaO	45.29
TiO <sub>2</sub>	0.08
MnO	0.11
Fe <sub>2</sub> O <sub>3</sub>	0.00
Others	0.48
LOI <sup>a</sup>	44.56

<sup>a</sup> Loss on ignition.

After washing with ultra-pure water at three times, the ground starfish was air dried again. The composition of starfish is analyzed by X-ray Fluorescence Spectrometer (Rigaku, ZSX100E) and given in Table 1.

## 2.2. Batch experiments

MINTEQA2 [8] was used to determine the upper concentration limit to avoid the supersaturation of the metals in this study. Since the chemical speciation of these metals and uranium have a different selective affinity to biosorbent and toxicity to microorganisms [9], all experiments were conducted at pH 6.0 on the basis of the result from modeling.

All adsorption experiments were conducted at room temperature. Adsorption isotherms were constructed for starfish and bacteria (live and dead cells) by equilibrating them with increasing Pb, Cd, and U concentrations. The range of contaminant concentrations [as PbNO<sub>3</sub>, CdNO<sub>3</sub>, and UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O] and the solid to solution ratio, were set up based on the result of preliminary adsorption tests [10], to get measurable and statistically significant measurements. The solutions contained Pb, Cd, and U were placed in 50 mL tubes and 25 mL of NaNO<sub>3</sub> (0.05 M) was used as a background solution. Initial pH of the solution was adjusted to 6 by adding small amounts of 1 M HNO<sub>3</sub> or 1 M NaOH. They were shaken for 48 h at 200 rpm (orbital shaker) and then centrifuged at 10,000 rpm for 20 min. Supernatants were analyzed for Pb and Cd using Atomic Absorption Spectrophotometer (AAS, Varian 240 FS), and for U with a kinetic phosphorescence analyzer (CHEM-CHECK Inst. Inc., Model KPA-11). All experiments were conducted in triplicate.

The pH edge studies of adsorption were carried out by mixing each 1.0 mg L<sup>-1</sup> PbNO<sub>3</sub>, CdNO<sub>3</sub>, and UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub>·6H<sub>2</sub>O with 0.1 g *P. putida* or 0.1 g starfish to 30 mL of 0.05 M NaNO<sub>3</sub> solution, and pH values were adjusted from 2 to 12 by adding small amounts of 1 M HNO<sub>3</sub> or 1 M NaOH. The samples were equilibrated for 24 h at 200 rpm (orbital shaker) and then centrifuged at 12,000 rpm for 20 min. The final pH of the supernatant was measured using a pH meter (Fisher, Model Accumet 25).

Adsorption kinetic experiments were carried out with the same mass used on adsorption experiment: total concentration of 1 mg L<sup>-1</sup>, final pH of 6.0, and ionic strength of 0.05 M NaNO<sub>3</sub>. The samples were placed on a reciprocating shaker table and agitated for designated time periods. After the desired time, the samples were centrifuged and filtered to remove particle larger than 0.2 μm.

The uptake percentages were calculated from the difference between the initial and final concentrations after equilibrating for 5 days. Blank samples without the metals were prepared to verify no contribution from the original material.

**Table 2**  
Parameters of pseudo-second order adsorption kinetics

Sorbent	Chemicals	$q_e$ (mg g <sup>-1</sup> )	$k$ (g mg <sup>-1</sup> min <sup>-1</sup> )	$r^2$
Starfish	Pb	0.1495 ± 0.0053	0.2203 ± 0.0047	0.9688
Live cell	Pb	0.1315 ± 0.0047	0.3379 ± 0.0053	0.9650
Dead cell	Pb	0.2383 ± 0.0013	0.3620 ± 0.0011	0.9980
Starfish	Cd	0.1197 ± 0.0055	0.3162 ± 0.0039	0.9459
Live cell	Cd	0.1010 ± 0.0047	0.6703 ± 0.0036	0.9345
Dead cell	Cd	0.2020 ± 0.0036	0.4525 ± 0.0032	0.9893
Starfish	U	0.1745 ± 0.0053	0.3124 ± 0.0051	0.9730
Live cell	U	0.1529 ± 0.0043	0.2653 ± 0.0031	0.9789
Dead cell	U	0.2599 ± 0.0024	0.3629 ± 0.0010	0.9971

## 2.3. Pseudo-second order kinetic model

In order to examine the controlling mechanism of the biosorption process, kinetic models are used to test the experimental data. The pseudo-second order kinetic equation is widely used by many researchers to express the kinetic of metal ion biosorption on biological materials because it always provided a more appropriate description than the first order equation [11,12]. It can be expressed in a linear form:

$$\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{t}{q_e} \quad (1)$$

where  $q_t$  is the amount of sorbate on sorbent at time  $t$  (mg g<sup>-1</sup>),  $k$  is the equilibrium rate constant of pseudo-second order sorption kinetics (g mg<sup>-1</sup> min<sup>-1</sup>), and  $q_e$  is the equilibrium uptake (mg g<sup>-1</sup>).

Equation (1) can be rearranged to obtain a hyperbolic equation [12]:

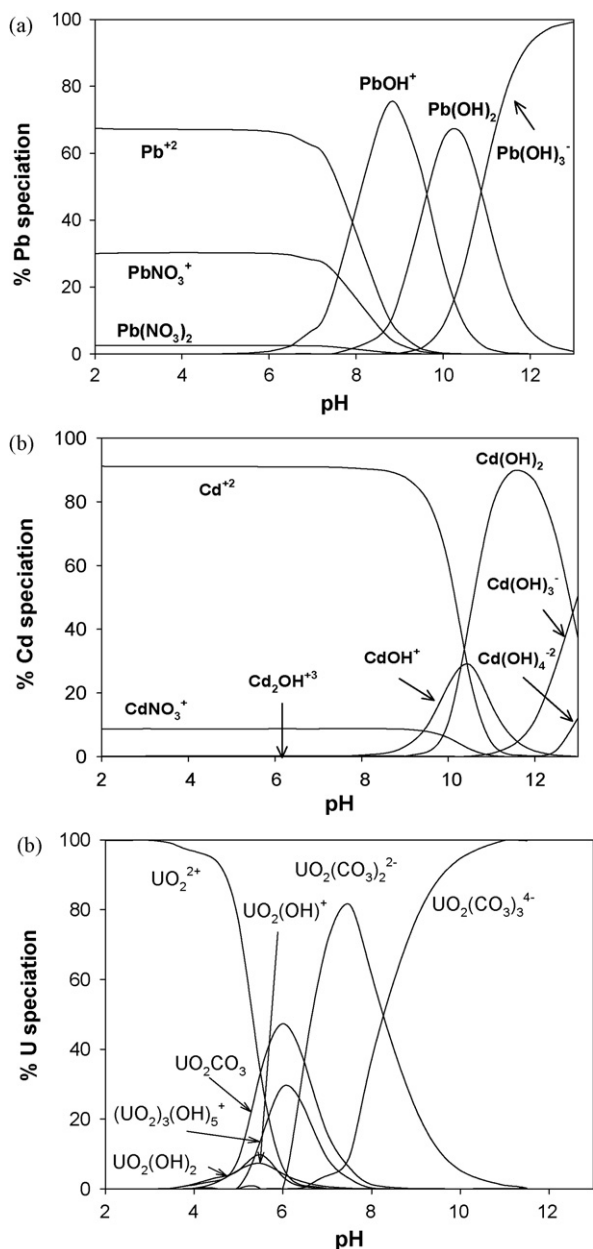
$$q_t = \frac{q_e t}{(1/kq_e) + t} \quad (2)$$

The parameters  $q_e$  and  $k$  were estimated by applying a nonlinear regression by least squares method performed with SigmaPlot software (see Table 2). The pseudo-second order kinetic equation shows how the adsorption capacity of adsorbate depends on time. If the equilibrium adsorption capacity of adsorbate and the rate constant  $k$  are known, then the adsorption capacity of adsorbate at any time can be calculated.

## 3. Results and discussion

The chemical speciation of metals may controls their mobility and adsorption [4,9]. The calculated speciation of chemicals changes with pH in the experimental system. Fig. 1 shows the speciation for heavy metals from pH 2 to 13, and clearly illustrates that below pH 6.0, both Pb and Cd do not complex with anions such as hydroxide ion in system. The speciation profile predicted that most of heavy metals are present as electrically positive and no precipitation with anions at pH 6.0. As pH increases in an open system, the concentration of hydroxide ions increases and heavy metals (Pb and Cd) may precipitate. Because the precipitation occurred in the solution at pH value above 7.0, we conducted all experiments at pH 6.0.

Uranium(VI) exists as UO<sub>2</sub><sup>2+</sup> in acid environment. As pH increases in an open system, composite hydrolyzed ionic species predominate, the concentration of dissolved carbonate increases, and the degree of U(VI) complexation with carbonate increases as well. Mononuclear and multinuclear ions appear as hydrolysis products. It appears that loss of H<sup>+</sup> from coordinated H<sub>2</sub>O is followed by polymerization involving -OH-bridges and yielding species such as UO<sub>2</sub>OH<sup>+</sup>, (UO<sub>2</sub>)<sub>2</sub>(OH)<sub>2</sub><sup>2+</sup>, and (UO<sub>2</sub>)<sub>3</sub>(OH)<sub>5</sub><sup>+</sup>. Carbonate complexes, UO<sub>2</sub>CO<sub>3</sub> (aq) and UO<sub>2</sub>(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup>, are to predominate in the pH range 7–9. Additionally, competition between



**Fig. 1.** Speciation of  $1.0 \text{ mg L}^{-1}$  heavy metals and U as a function of pH: (a) Pb, (b) Cd, and (c) U. Calculation made with MINTEQA2 using the standard thermodynamic database. Calculations made for an open system with  $\log p\text{CO}_2 = 10^{-0.3}$  in  $0.05 \text{ M NaNO}_3$ .

U(VI) and carbonate ion to adsorption sites of adsorbent will also increase as the pH increases. In equilibrium with atmospheric  $\text{CO}_2$  (used in this study),  $\text{UO}_2\text{CO}_3$  (aq) and  $\text{UO}_2(\text{CO}_3)_2^{2-}$  becomes the predominant species and might contribute to adsorption of metals for starfish and *P. putida*. Though the effect of  $\text{CO}_2$  concentration on adsorption of three metals was not tested in this study, based on the model in predicting chemical speciation of three metals, adsorption experiments were conducted to determine the competitive uptake ability of metals by starfish and *P. putida*.

Adsorption of metals at low concentrations that are environmentally relevant can be well described [10] with Langmuir adsorption isotherm using nonlinear regression [10] (Table 3):

$$q = \frac{MKq^*}{1 + Kq^*} \quad (3)$$

**Table 3**  
Statistics for nonlinear regression of Langmuir model fit

Sorbent	Chemicals	$M$ ( $\text{mg g}^{-1}$ )	$K$ ( $\text{L mg}^{-1}$ )	$r^2$
Starfish	Pb	$0.73 \pm 0.07$	$1.91 \pm 0.13$	0.9886
Live cell	Pb	$0.48 \pm 0.09$	$2.84 \pm 0.26$	0.9835
Dead cell	Pb	$1.73 \pm 0.14$	$1.03 \pm 0.13$	0.9896
Starfish	Cd	$0.57 \pm 0.02$	$1.60 \pm 0.06$	0.9972
Live cell	Cd	$0.44 \pm 0.06$	$2.87 \pm 0.05$	0.9907
Dead cell	Cd	$1.16 \pm 0.12$	$1.91 \pm 0.14$	0.9741
Starfish	U	$1.14 \pm 0.21$	$3.61 \pm 0.24$	0.9901
Live cell	U	$0.53 \pm 0.09$	$2.63 \pm 0.22$	0.9858
Dead cell	U	$2.91 \pm 0.09$	$1.22 \pm 0.17$	0.9658

where  $q$  is the sorbed amount of adsorbate on the biosorbent ( $\text{mg g}^{-1}$ ),  $q^*$  is the equilibrium concentration in solution ( $\text{mg L}^{-1}$ ),  $M$  is the maximum sorbed amount of adsorbate on the biosorbent ( $\text{mg g}^{-1}$ ), and  $K$  is the Langmuir constant related to the binding strength. Nonlinear relationship was observed between heavy metal and U(VI) adsorptions (Fig. 2). Nonlinear adsorption is characteristic of decreasing biosorbent–adsorbate affinity with an increasing extent of adsorption [13]. Although the dependence of heavy metal adsorptions to all biosorbents was not steeper than that of U(VI), the percentage of metals adsorbed increased linearly and then reached near equilibrium. The experimental data for all biosorbents were shown in Fig. 3.

The starfish preferentially sorbed U(VI), followed by Pb, and exhibited the least preference for Cd. The order of selective affinity of metals on starfish is  $\text{U} > \text{Pb} > \text{Cd}$ , whose sequence did not exactly follow the order of Misono softness parameters suggested by Spósito [13] who defined that the tendency of metals to form covalent bonds on the basis of ionic radius and the ionization potential. McBride [14] mentioned that the electronegativity is an important factor in determining which of metal adsorbed with the highest preference.

The metal sorbed preferentially by live *P. putida* was U(VI), followed by Pb, and showed the least preference for Cd (Fig. 2). Most microbial cells exhibit colloidal characteristics similar to those of soil mineral oxides (pH-dependent charge sites) in the adsorption of metals or hydrolyzed metals. The adsorption sequence on live cells was found following as:  $\text{U} > \text{Pb} > \text{Cd}$ . This sequence did not exactly follow the order of the electronegativity of the metal ions suggested by Evans [15]. However, this selective affinity is in good agreement with the sequence presented by Schwertmann and Taylor [16].

It is suggested that the mechanisms for metal uptake process are mainly both microorganism- and metal-dependent because of specific surface properties of the microorganisms, cell physiology, and solution chemistry. Ledin et al. [17] found that the negative surface charge of live bacteria carried over the entire pH ranges and the negative charge of bacteria surface may electrostatically attract ions of opposite charge in non-specific way. They also found that the charged surface of bacteria was present with multitude of functional groups that form complexes with heavy metals in soil solution. Most heavy metals are abiotic factors in the cell, and there are indications that some living microorganism processes may inhibit the uptake of heavy metals. This may be dependent on the genetic constitution of the cells that can uptake heavy metals [18].

Dead cells of *P. putida* preferentially sorbed U(VI), followed by Pb, and exhibited the least preference for Cd being more than an order of magnitude greater than for adsorption on other biosorbents (Fig. 2). The adsorption selectivity sequences of dead cell are in keeping with the finding of Wang and Chen [19] that divalent cations formed the most stable complexes with microorganisms,

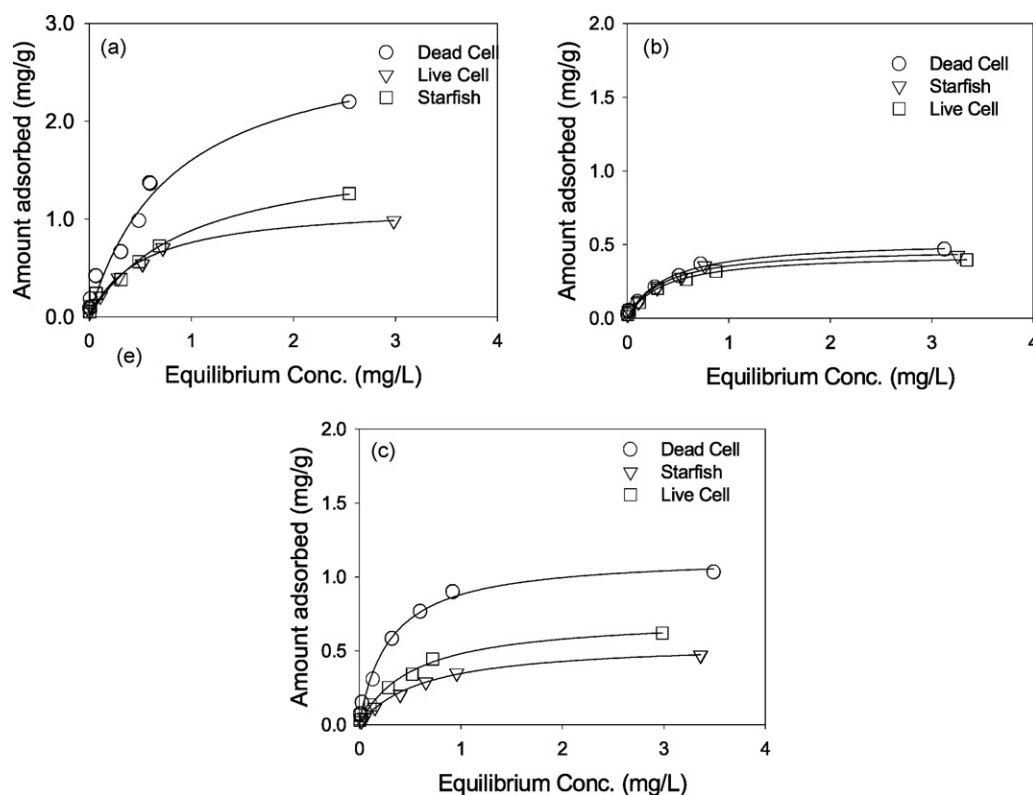


Fig. 2. Sorption isotherms of heavy metals and uranium on starfish and bacteria at pH 6.0 ( $\text{NaClO}_4$ , 0.05 M): (a) U, (b) Cd, and (c) Pb.

and the stability of complexes of U(VI) with *Pseudomonas* strain has been stressed by Sar et al. [20]. Bollag and Duszota [18] observed Cd adsorption on live and dead cells of several strains of bacteria using natural soils and waters. However, they did not test other heavy metals and uranium.

If metal adsorption was entirely electrostatic, metal ions of lower ionic radii would be more strongly adsorbed, and that would predict a different sequence, as follows: Pb (0.119) > Cd (0.095) > U(VI) (0.073), with values in parentheses being the ionic radii in nm. This discrepancy indicates that metal adsorption cannot solely be predicted by any given affinity sequence model.

The time dependence of metal uptake by three biosorbents at pH 6.0 is shown in Fig. 3. With the exception of adsorption kinetics by starfish and *P. putida* showed the approximately 80% uptake after 2 h of equilibrium (Fig. 3). The kinetics of heavy metal adsorption for starfish and dead cells were similar, whereas the live cells removed the added heavy metals slowly over the first 1–24 h and then at a very slower rate over the remainder of the experiment (Fig. 3). The results of kinetic experiments showed that contact time of 48 h was sufficient to achieve equilibrium. For all three biosorbents, after 24–48 h, the aqueous concentration changed by <5% over the next 96 h of the experiment. Although there is considerable scatter in the data, we conclude that the concentration of metals in the supernatant reached a constant value within the first few hours of reaction.

The pseudo-second order sorption kinetics was used to fit the experiment data in order to analyze the adsorption of metal ions on biosorbents. Good correlation between the experimental data and theoretical plots was observed from the graph, thereby implying that a pseudo-second order reaction is involved. Table 2 listed all adsorption systems included  $k$ ,  $q_e$ , and the coefficient of determination,  $r^2$ . The data illustrated good compliance with pseudo-second order rate law based on adsorption capacity because  $r^2$  were higher

than 0.93 for all the systems in this study. These results indicated that the external mass transfer and intraparticle diffusion together were involved in the adsorption process [19]. The order of accumulated metal ions at an equilibrium state was in following order: U(VI) > Pb > Cd. This result was in accordance with the findings of Choi and Park [4], who found that the uptake values of U(VI), Pb, Zn, Cd, and Ni onto immobilized *P. putida* increased in the order of U(VI) > Pb > Zn > Cd > Ni.

The results from the metal–biosorbent interaction study can be summarized in Fig. 4, showing the percentage metal uptake by each biosorbent at pH 6.0. The results clearly indicate that dead cell is a much stronger biosorbent of all metals than other biosorbents on a same mass basis. For example, at pH of 6.0 and initial conc.  $1.0 \text{ mg L}^{-1}$ , all metal uptake values by dead cells is above 70%. The relative order of metal uptake was the same for the dead cell: U(VI) > Pb > Cd. Maximum uptake of U(VI) was observed in dead cells (88.4%). Otherwise, the uptake percentage of all metals by live cells showed lower uptake (below 50%) than the other biosorbents. The result indicates that most heavy metals and uranium without known metabolic function are known to be toxic at very low concentrations, which may have contributed to the low uptake capacity of all metal ions.

A series of batch adsorption experiments was conducted with dead cells of *P. putida*, starfish with 0.05 M  $\text{NaNO}_3$  and three metals. The extent of adsorption at a given aqueous concentration increased with the pH of the three biosorbents (Fig. 5). Of the biosorbents, starfish exhibited narrow pH edges that has been observed for U(VI) on pure minerals such as hematite, goethite, quartz, and imogolite [21] in open system. The sharp increase in adsorption from pH 2 to 4 and the plateau in adsorption from pH 4 to 6, followed the dramatic decrease in adsorption from pH 6 to 9. As pH increases in an open system, the concentration of dissolved carbonate increases and the degree of U(VI) complexation with

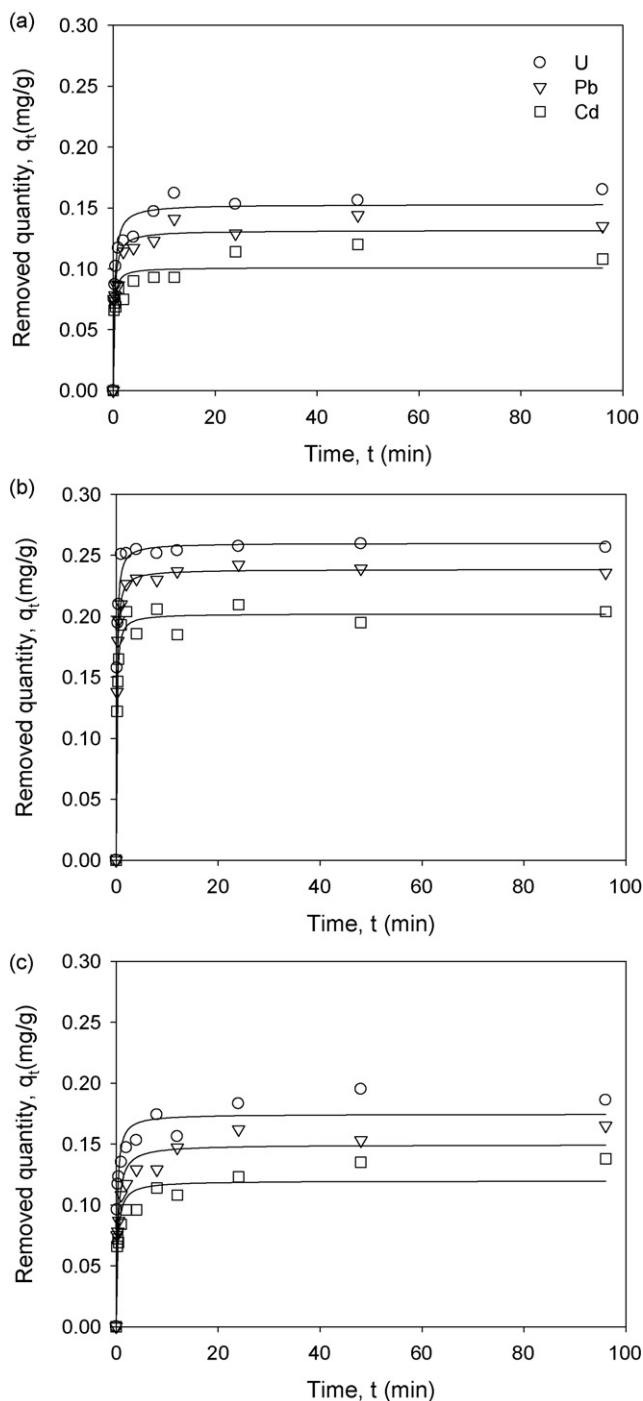


Fig. 3. Fitting of experimental data for removal of metal ions from aqueous solution onto biosorbents: (a) live cells; (b) dead cells, and (c) starfish.

carbonate increases as well. Since the U-carbonate species are neutral or anionic, electrostatic interactions with solid phase will be negligible. In addition, competition with U(VI) for surface sites from dissolved carbonate and bicarbonate anions will also increase as the pH and total carbonate concentration increase. However, an unusual phenomenon was observed at high pH. After a sharp decrease in adsorption from pH 6 to 8, the second adsorption edge reversed and began to increase again. One potential reason for the unusual phenomenon is the dissolved carbonate could not compete for U(VI) with a starfish surface. To verify this conclusion further studies are needed, which are based on comparisons with

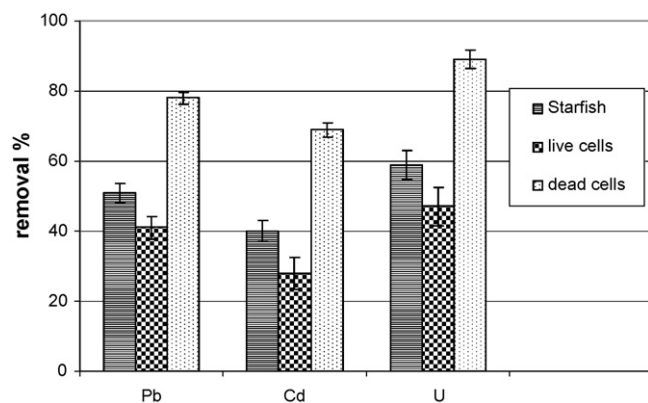


Fig. 4. Percentage of metal uptake by starfish, live cells, and dead cells after equilibrating for 5 days.

other biosorbents at different carbonate concentration, and which are competed with other inorganic cations and anions, and organic materials coexisting in nature.

Otherwise U(VI) adsorption on dead cells of *P. putida*, the shape of adsorption curve (Fig. 5(b)) was fundamentally different in high pH range. The maximum U(VI) adsorbed and the pH edge increased relative to starfish. Although the adsorption edges shown for the

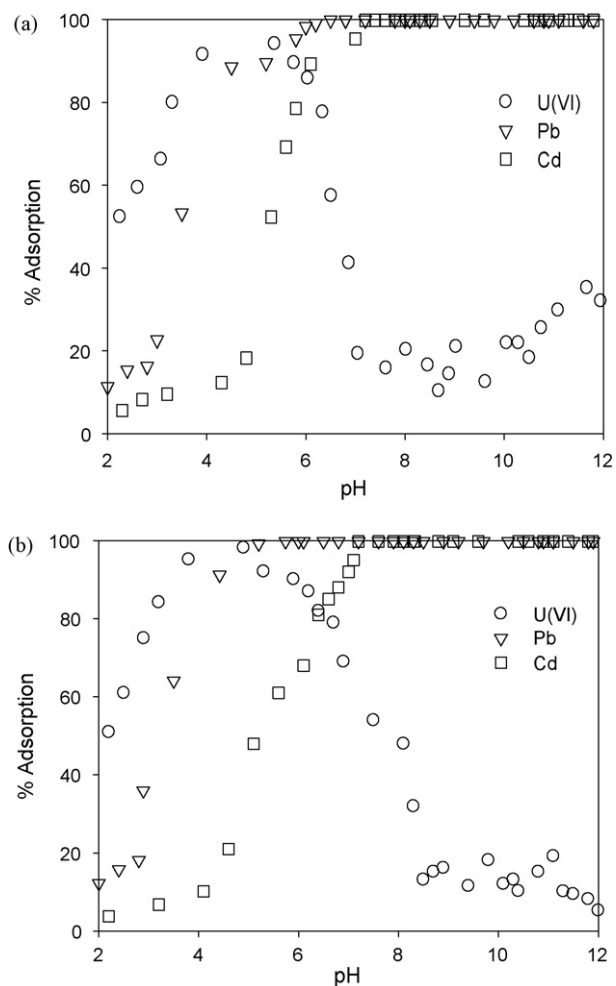


Fig. 5. Adsorption as a function of pH for (a) starfish and (b) dead cell of bacteria in open system with  $\log p\text{CO}_2 = 10^{-0.3}$  in 0.05 M  $\text{NaNO}_3$ . Total system concentration of each metals is  $1 \text{ mg L}^{-1}$ .

two biosorbents are not identical, maximum degree of adsorption is similar (above 95%). The dead cells are known to strongly interact with U(VI) [20]. They are also the dominant pH-dependent charge surface in these biosorbents. The similar pH-dependent U(VI) adsorption edges suggest the dominance of the dead cell in controlling U(VI) adsorption.

The difference observed in the adsorption of Pb and Cd in these biosorbents under same conditions of U(VI) pH-edge adsorption experiment exhibited a classic pH adsorption edge. The adsorption of Pb was sharply increased with increasing pH from 3 to 5 and reached maximum amounts of adsorption above pH 5. Similar trend of Cd adsorption were observed on both dead cells and starfish. The increase in Cd adsorption as pH increased was probably due to its complexation with hydroxide. The speciation profile predicted that most of heavy metals are present as electrically positive and no precipitation with anions at pH 6.0 (Fig. 1(a) and (b)). As pH increases in an open system, the concentration of hydroxide ions increases and heavy metals may precipitate.

The results shown in Figs. 1–5, can provide significant information of the on the interaction of U(VI), Pb, and Cd with starfish and *P. putida*. The results also suggest that these biosorbents could be used to remove hazardous or radioactive cations from contaminated subsurface environments.

#### 4. Conclusion

Three different types of biosorbents (dried starfish, live bacterial cell, and dead bacterial cell) were used in this study. The behavior of the biosorbents differs considerably in metal uptake ability. Dead cell was found to be more efficient than live cell and starfish in removing Cd, Pb, and U(VI). The metal removal by starfish and dead cells showed the approximately 80% uptake after 2 h, whereas the removal of metals by live cells took place slowly. The results of kinetic studies showed that biosorption of three metal ions by biosorbents could well be described by pseudo-second order kinetic model, which meant that the external mass transfer and intraparticle diffusion together were involved in the adsorption process.

The present results confirmed that dead bacterial cells can usually adsorb more metals than live ones and starfish, but the efficiency of metal removal from liquid medium did not depend on the only factor. To verify these conclusions further studies are needed, which are based on comparisons with other bacteria, different strains of bacteria, effect of storage, influence of age or incubation time into consideration. The desorption of bound metals should be further studied.

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