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Comparison of Biosorption of Cadmium (II) from Aqueous Solution, by *Bacillus* Sp and *Pseudomonas Aeruginosa*

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Abstract

Heavy metal contamination of various water resources is of great concern because of the toxic effect on human being, other animals and plants in the environment. Concern over this problem has led to the development of alternative technologies for effecting the removal of these pollutants from aqueous effluents. Biosorption of cadmium (II) ions from aqueous solutions has been studied in a batch system by using a Bacillus sp. AEJ-89 (gram-positive) and Pseudomonas aeruginosa (gram-negative). Langmuir equation was based on the maximum rate of uptake of Cd (II) in Bacillus and Pseudomonas. The effects of various physicochemical factors on Cd (II) biosorption such as initial metal concentration, pH, and contact time were studied. This study has shown Cd (II) biosorption, equilibrium time of about 5 minutes for Pseudomonas and Bacillus about 10 minutes, respectively and the adsorption equilibrium data were well described by Langmuir equation. The maximum capacity has been extrapolated to 0/45, 0/85 mmol/g for Bacillus and Pseudomonas respectively. The gram positive bacterium showed more cadmium biosorption compared to the Gram-negative bacterium.

Keywords: *Cadmium, Pseudomonas Aeruginosa, Biosorption, Bacillus Sp. AEJ-89, Iisotherms*

1. Introduction

Comprising over 70% of the Earth's surface, water is undoubtedly the most precious natural resource that exists on our planet. Without this invaluable compound, the life on the earth would not exist. Although this fact is widely recognized, pollution of water resources is a common problem being faced today. Lakes, rivers and oceans are being overwhelmed with many toxic contaminates. Among toxic substances reaching hazardous levels are heavy metals (Table 1).

Heavy metals are defined as metals with a specific weight usually more than 5.0 g cm^{-3}, which is five times higher than water. The toxicity of heavy metals occurs even in low concentrations of about 1.0-10 mg/L. Heavy metals contaminate the soil and the environment from various anthropogenic sources such as industrial wastes, automobile emissions, mining activity and agricultural practices [1].

High concentration of cadmium is highly corrosion resistant, and is widely used for

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plate metal parts used in general industrial hardware as well as in automobiles, electronics, marine and aerospace industries. Cadmium is known to bind with essential respiratory enzymes causing oxidative stress and cancer. Most heavy metals are highly toxic to ecosystems even at very low concentration [2].

Available conventional methods (precipitation, flocculation, ion exchange, and membrane filtration) for removal of this metal from water at low concentrations are claimed to be expensive and inefficient. For these reasons, recent research efforts have concentrated on recovery of heavy metals using different biosorbents [3]. Among the various biosorbents, *Pseudomonas* and *Bacillus* were widely used in many reports for their good heavy metals biosorption performance [4, 5].

Some confusion has prevailed in the literature regarding the use of the terms "bioaccumulation" and "biosorption" based on the state of the biomass. Herein, therefore bioaccumulation is defined as the phenomenon of living cells; whereas biosorption mechanisms are based on the use of dead biomass [6]. Compared with conventional technologies, biosorption has advantages such as high efficiency in detoxifying many diluted effluents and low operating cost. Feasibility studies for large-scale applications demonstrated that, biosorptive process is more applicable than the bioaccumulation processes, because living systems often require the addition of nutrients and hence increase biological oxygen demand or chemical oxygen demand in the effluent[7].

In addition, potential for desorptive metal recovery is restricted since heavy metal may be intracellulary bound, metabolic products may form complexes with metals to retain them in solution and mathematical modeling of a non-defined system is difficult [8]. Metal bioaccumulation by marine organisms has been the subject of considerable interest in recent years because of serious concern that high levels of metals may have detrimental effects on the marine organisms and may affect marine food and through them, humans. Algae, bacteria and fungi have proved to be potential metal biosorbents [9]. Generally, microorganisms interact with toxic metals by three process including biosorption, bioaccumulation and enzymatical reduction.

Table1. Classification of heavy metals based on toxicity [10].

Fe, Mo, Mn	Low Toxicity
Zn, Ni, Cu, V, Co, W, Cr	Average Toxicity
As, Ag, Sb, ***Cd***, Hg, Pb, U	High Toxicity

This study aimed to investigate the potential of gram positive and gram negative *Bacillus sp* and *P. aeruginosa* for the biosorption of cadmium (II) from solution. The effect of initial metal ion concentration was studied and the relationship between pH and removal capacity was also analyzed. The pseudo first order kinetic models were used in the next step of data analysis.

2. Materials and methods

2-1. Biomass preparation

The bacterial strains were received from Iranian Research Organization for Science and Technology (IROST) Tehran, Iran. The nutrient broth was prepared in the prescribed growth medium containing beef extract 1.0 g, yeast extract 0.1 g, peptone 5.0g, sodium chloride 5.0g and distilled water 1.0 litre. The biomasses were separated from the growth medium by centrifugation at 6500 rpm for 10 min and washed thoroughly with distilled water. Test solutions containing cadmium ions were prepared from analytical grade chemicals $CdCl_2$ $6H_2O$. The concentrations of metal ions prepared from stock solutions ranged from 1to 25mg l^{-1}. Before mixing the micro-organisms, the pH of each test solution was adjusted to the required value by using 1M NaOH and HNO_3. All materials were from Merck Company.

2-2. Biosorption experiments

Biosorption experiments were carried out at different pH values, varying initial metal ion concentration and different biomass dosage to optimize the biosorption efficiency. A fully grown culture was centrifuged and different weights of the biomass ranging from 0.5 to 3.0g were obtained. These biomasses of varying weight from *Bacillus* and *Pseudomonas* were used for removal of Cadmium ions and were optimized. To determine the effect of optimum Cd (II) ion concentration batch equilibrium, study was conducted for equilibrium mixing time at a constant speed of 150 rpm to obtain the value of maximum biosorption. The effect of pH on the equilibrium biosorption of Cd (II) ion was analyzed over a pH range of 3 to 10.

Adsorption capacity was calculated by using the mass balance equation for the adsorbents:

$$q = \frac{(C_i - C_e)}{M} \times V \qquad (1)$$

where q is the amount of metal ions adsorbed on the biomass (mg/ g), C_i the initial metal ion concentration in solution (mg/l), C_e the final metal ion concentration in solution (mg/l), V the volume of the medium (l) and M is the amount of the biomass used in the reaction mixture (g).

The biosorption results were analyzed using intra-particle diffusion model (Weber-Moris). This is represented as [11]

$$q_t = k_{id} t^{0.5} + c \qquad (2)$$

where q_t (mg/g) is the amount adsorbed at time t (min), k_{id} (mg/g min$^{0.5}$) is the rate constant of intra-particle diffusion. C is the value of intercept which gives an idea about the boundary layer thickness, i.e. the larger the intercept, the greater the boundary effect. Concentrations of solutions were determined using the atomic absorption spectrophotometer (Chem Tech Analytical model CTA2000). All chemicals used in this study were of analytical-reagent grade.

2-3. Desorption of Cd (II) from bacterial cells

Desorption of Cd (II) from previously loaded *Pseudomonas* and *Bacillus* was studied by using 0.1 M HCl as eluent. For this purpose, 0.01 g of bacterial cells was added to 50 ml eluent in a 50 ml plastic tube. After 4 h of shaking, supernatants of centrifuged samples were analyzed for the Cd (II) concentration. Control experiments without biomass were carried out in order to determine the degree of the removal of Cd (II) from solution by the plastic tube.

2-4. Effect of contact time on biosorption

Contact time is one of the important parameters for successful biosorption application. Experiments for determining the kinetics of the process were performed using 50 mg/l on *Bacillus* sp. AEJ-89 and *pseudomonas* from the initial metal concentrations for Cd (II) ions in 20 ml of metal solution at a constant temperature (30◦C).

3. Results and discussion

3-1. Effect of pH

The interaction of metal cations with the electron-rich functional groups located on the biomass may be highly sensitive to the pH value of the environment. The most initial pH is the most important parameter affecting metal ion uptake. In synthetic solution, the effect of pH on the biosorption of cadmium ion onto *Bacillus* and *Pseudomonas* was done by varying the pH in the range of 2 to 9 as shown in Fig. 1. Experimental control tests indicated that the removal mechanism is purely biosorption. Due to hydrogen ion activity, the cadmium biosorption was observed to be high at a low pH of 2 in *Bacillus* and at a pH of 3 or *Pseudomonas*.As can be seen in Fig. 2, Cd (II) ions become precipitate in the form of Cu (OH) 2, because of the increasing concentration of OH− ions after the pH values 5.0 for Cd (II). However, if this precipitation contributed to the Cd (II) biosorption, the removal capacity should not have decreased and/or fixed at greater than these pH values. The increase in Cd (II) removal at low ph is attributed to less interaction between the negatively charged ions and the positively charged cell protonated functional groups.

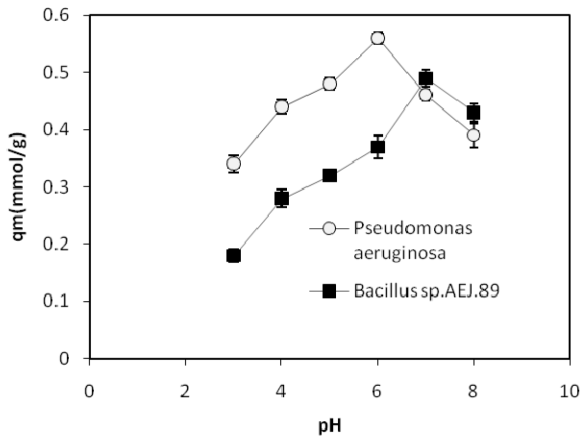

Figure 1. Effect of initial pH on the biosorption of Cd (II) ion (*Pseudomonas*, *Bacillus*, biomass dosage, 0.2 g, contact time 5 h, temperature 28 C, concentration, 100; g/l, agitation rate 150 rpm).

3-2. Desorption of Cd (II)

The possibility of loaded biosorbent regeneration is vital to keeping the process costs down and to opening the possibility of recovering the metal extracted from the liquid phase [9]. The experimental results of Cd (II) biosorption and desorption are reported in Table 2. It demonstrated that about 40% and 50% of the Cd (II) was actively taken up by *Pseudomonas*, *Bacillus* with the remainder being passively bound to the bacterium cells. Nearly 81.23% - 92.31% of bound Cd (II) by *Pseudomonas* could be desorbed with 0.1 M HCl. However, only 43.87%-71.98% of bound Cd (II) by *Bacillus* cells could be desorbed. This indicates that 0.1 M HCl can effectively desorb the bound

Cd (II) from Pseudomonas cells, but is not very effective for *Bacillus* cells. Desorption of Cd (II) by *Pseudomonas* is more effective than that of Cd (II) by *Bacillus* cells. It also may be because Bacillus cells uptake a part of Cd (II) through spore, since it is possible to remove metals from spore surfaces after biosorption.

Table 2. Desorption of Cd (II) from *Pseudomonas* and *Bacillus*.

Biosorbents	Biosorption Capacity (mg/g)	Desorption (mg/g)	Desorption (%)
Pseudomonas	95.54±0.5	73.49±0.5	77.36±0.5
Bacillus	50.58±0.5	22.82±0.5	45.12±0.5

3-3. Effect of time on biosorption

Fig. 2 shows that the rate of cadmium uptake increases rapidly in the first part within 5 min and 10 min of contact for *Pseudomonas* and *Bacillus* respectively. After that, the rate decreases till a constant value of cadmium concentration is reached after 30 min and then the uptake of metal ions changed significantly with contact time. This rapid initial uptake of metals may be an important parameter for practical application of biosorption in industrial wastewater treatment.

The data obtained from this experiment was further used successfully to evaluate the kinetics of the adsorption process. This rapid initial uptake is in accordance with the results given by other authors [12-14].

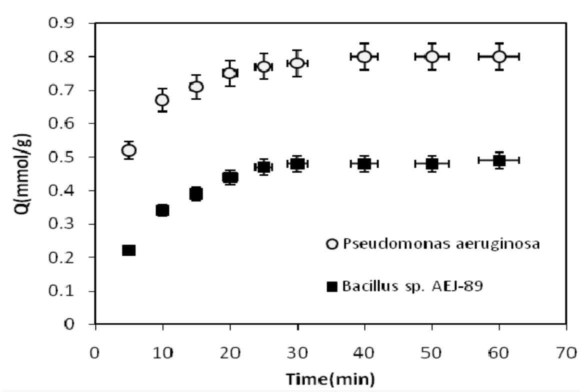

Figure 2. Kinetic of Cd (II) biosorption by *Pseudomonas* and *Bacillus*.

3-4. Langmuir adsorption Isotherm

The two most frequently used isotherm equations in the literature to reach that objective are the Langmuir and Freundlich models [15, 16]. Performance of cadmium uptake by *Bacillus* and *Pseudomonas* by Langmuir model are shown in Fig. 3. Measurement of sorption equilibrium is in the initial concentration of 1-25 mg l-1 for both metals in 30 minutes at 7.0 to 6.0 pH for *Pseudomonas* and *Bacillus*.The equilibrium adsorption isotherm obtained shows that metal uptake by bacterial biomasses was a chemically equilibrated and saturable mechanism. Therefore, there was an increase in metal uptake as long as binding sites were free. Experimental data were applied to adsorption models given by Langmuir, where its mathematical formula can be expressed as:

$$q_e = \frac{q_m b C_e}{1 + bC_e} \tag{3}$$

And its linear form is represented by the following equation:

$$\frac{1}{q_e} = \frac{1}{bq_m C_e} + \frac{1}{q_m} \tag{4}$$

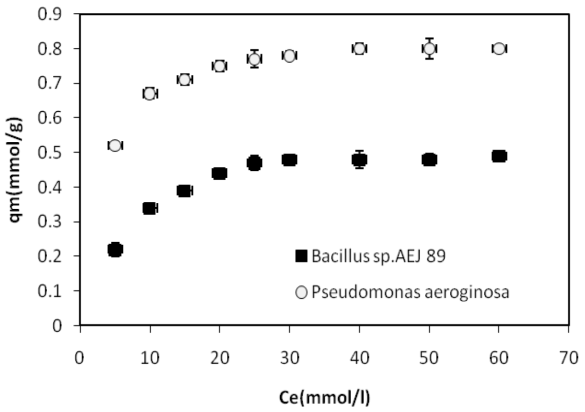

Figure 3. Biosorption equilibrium isotherm of Cd (II) in deionized water by *Pseudomonas* and *Bacillus.*

where q_e is the amount of metal ions adsorbed (mmol g^{-1}), C_e is the equilibrium concentration (mmol l^{-1}), q_{max} (mmol g^{-1}) is the maximum adsorption capacity and b (l $mmol^{-1}$) is an affinity constant (Fig. 4).

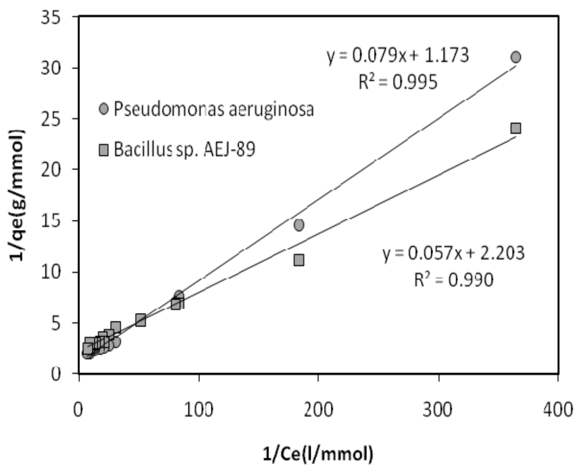

Figure 4. Sorption isotherm of Cd (II) in deionized water by Langmuir linear form.

The values of q_m (mmol g^{-1}) and b (l $mmol^{-1}$) were obtained from Eq. 3. The linear correlation coefficient was 0.995, 0.990 and q_m was 0.45, and 0.85 mmol g^{-1} for *Bacillus* and *Pseudomonas* respectively from calculation using the Langmuir equation (Table 3).

Table 3. Calculation parameters from Langmuir equation in deionized water.

Cadmium	q_m (mmol g^{-1})	b_L (l $mmol^{-1}$)	r^2
Bacillus sp. AEJ-89	0.453	38.986	0.990
Pseudomonas aeruginosa	0.852	14.892	0.995

4. Conclusions

Comparison of various literature studies of nickel removal by biosorptions is shown in Table 4. From the laboratory-based experiments, the following conclusions can be reached: The maximum biosorption capacities Cd(II) were 0.45 ,0.85 mmolg^{-1} for *P. aeruginosa* and *Bacillus* sp. AEJ-89 respectively. The adsorption equilibrium data fit the langmuir model well for metal ions in the studied concentration range. The mechanism of biosorption includes mainly ionic interactions and formation of complexes between metal cations and acidic sites in cell wall of *Bacillus* and *Pseudomonas.* The *Pseudomonas* biomass has greater affinity for Cd (II) than *Bacillus* biomass. The results demonstrate that bacterial biomass *Pseudomonas* and Bacillus could be used as promising biosorbents for the removal of Cd (II) ions from aqueous solutions.

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Table 4. Comparison of various literature studies of nickel removal by biosorption.

Biosorbent	pH	Ion	Capacity of Fixation(mmol.g^{-1})	References
Fucus vesiculosus	3.5	Cd^{2+}	0,65	[17]
Sargassum filipendula	4,5	Cd^{2+}	0,66	[18]
Chondrus crispus	6.0	Cd^{2+}	0,67	[19]
Palmaria palmate	6.5-7	Cd^{2+}	0,04	[20]
Codium vermilara	6.0	Cd^{2+}	0,19	[19]
Spirogyra insignis	6.0	Cd^{2+}	0,20	[19]
Bacillus licheniformis	3-9	Cd^{2+}	1.27	[21]

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