Biosorption of heavy metals from waste water using *Pseudomonas* sp.

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Biosorption experiments for Cr(VI), Cu(II), Cd(II) and Ni(II) were investigated in this study using nonliving biomass of different *Pseudomonas* species. The applicability of the Langmuir and Freundlich models for the different biosorbent was tested. The coefficient of determination (R^2) of both models were mostly greater than 0.9. In case of Ni(II) and Cu(II), their coefficients were found to be close to one. This indicates that both models adequately describe the experimental data of the biosorption of these metals. The maximum adsorption capacity was found to be the highest for Ni followed by Cd(II), Cu(II) and Cr(VI). Whereas the Freundlich constant k in case of Cd(II) was found to be greater than the other metals. Maximum Cr(VI) removal reached around 38% and its removal increased with the increase of Cr(VI) influent. Cu(II) removal was at its maximum value in presence of Cr(VI) as a binary metal, which reached 93% of its influent concentration. Concerning to Cd(II) and Ni(II) similar removal ratios were obtained, since it was ranged between 35 to 88% and their maximum removal were obtained in the case of individual Cd(II) and Ni(II).

The presence of heavy metals in aquatic environments is known to cause severe damage to aquatic life, beside the fact that these metals kill microorganisms during biological treatment of wastewater with a consequent delay of the process of water purification. Most of the heavy metal salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation.

Physico-chemical methods, such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment, evaporative recovery, filtration, ion exchange, and membrane technologies have been widely used to remove heavy metal ions from industrial wastewater. These processes may be ineffective or expensive, especially when the heavy metal ions are in solutions containing in the order of 1-100 mg dissolved heavy metal ions/L (Volesky, 1990a; Volesky, 1990b). Biological methods such as biosorption/ bioaccumulation for the removal of heavy metal ions may provide an attractive alternative to physico-chemical methods (Kapoor and Viraraghavan, 1995).

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Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Shumate and Strandberg, 1985; Andres et al. 1992; Fourest and Roux, 1992; Hussein et al. 2001; Hussein et al. 2003). Feasibility studies for large-scale applications demonstrated that, biosorptive process are more applicable than the bioaccumulative processes, because living systems (active uptake) often require the addition of nutrients and hence increase biological oxygen demand (BOD) or chemical oxygen demand (COD) in the effluent. In addition, maintenance of healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors. In addition, potential for desorptive metal recovery is restricted since metal may be intracellulary bound, metabolic products may be form complexes with metals to retain them in solution and mathematical modeling of a non-defined system is difficult (Brown and Lester, 1982; Ajmal et al. 1996; Dilek et al. 1998).

The use of adsorbents of biological origin has emerged in the last decade as one of the most promising alternatives to conventional heavy metal management strategies (Shumate and Standberg, 1985; Eccles, 1990; Macaskie, 1990; Tsezos and Deutschmann, 1992). Because of the absence of rational method for a priori prediction of the biosorption potential of a microorganism, the only method for identifying and developing newer and efficient biosorbents is the sustained screening of microbes (Muraleedharan et al. 1995).

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial waste streams. Most studies of biosorption for metal removal have involved the use of either laboratory-grown microorganism or biomass generated by the pharmacology and food processing industries or wastewater treatment units (Tsezos and Volesky, 1981; Townsley et al. 1986; Rome and Gadd, 1987; Macaskie, 1990; Costa and Leite, 1991; Rao et al. 1993). Many aquatic microorganisms, such as bacteria, yeast and algae can take up dissolved metals from their surroundings onto their bodies and can be used for removing heavy metal ions successfully *(*Asku et al. 1991). Equilibrium studies, that give the capacity of the adsorbent and the equilibrium relationships between adsorbent and adsorbate are described by adsorption isotherms which is usually the ratio between the quantity adsorbed and the remaining in solution at fixed temperature at equilibrium. Freundlich and Langmuir isotherms are the earliest and simplest known relationships describing the adsorption equation (Muhamad et al. 1998; Jalali et al. 2002). Accordingly, this study aimed to investigate the continuous bisorptive potential of *Pseudomonas* species isolated from contaminated waste treatment plant.

MATERIALS AND METHODS

Biomass preparation

Four *Pseudomonas* strains, showing a good ability to resist and accumulate different metal ions, namely, Cr(VI), Cu(II), Cd(II), and Ni(II) isolated from western Alexandria sewage treatment plant, Alexandria, Egypt. The strains were characterized and identified as *Pseudomonas fluorescens* that resists to Cr(VI), three other strains from the species *P. putida,* resistant to Cu(II), Cd(II) and Ni(II). (Hussein et al. 2003). The strains were grown in casamino acid media (CAA), composed of casamino acid (Oxoid) 5 g/L; K_2HPO_4 (Merck) and 0.25 g/L $MgSO_4$ (Merck). The pH of the medium was adjusted at the predetermined optimum growth pH (5.5) (Hussein et al. 2003). Unless otherwise indicated, precultures were performed in 100 cm^3 Erlenmeyer flasks containing 20 ml of sterile CAA medium and incubated on a rotary shaker at 200 rpm at 30ºC.

Bacterial cells of each metal resistant strain harvested by centrifugation at 25ºC and 7959 g for 15 min and washed twice with distilled water. The cells were suspended in deionised water to a final concentration of 5 g/L.

Metal solutions

Different metal concentrations were prepared by dissolving of $CuCl_2$, $CdCl_2$, $NiSO_4$ and $K_2Cr_2O_7$ salts in deionised water to have metal concentrations of 1, 2, 5 or 10 mmol/L from each metal. Binary metal solutions were prepared by the use of the four metal concentrations at equimolar ratios. All glassware washed with 0.1 M HCl before and after each experiment to avoid binding of the metal to it.

Biosorption process

The metal solution was stored in a glass vessel and a peristaltic pump regulated the flow rate of metal solution to the reactor. On the other hand the biomass for metal adsorption was supplied from a storage vessel and a continuously operating peristaltic pump transferred the biomass from the storage vessel to the reactor. The pump regulating the inflow of biomass and metal solution to the reactor were calibrated in order to permit control of the actual flow rate. Samples were collected after 20 min and metal determinations were carried out. The experiment set up is shown in Figure 1 the reactor consists of 0.2 L glass vessel. A pH electrode connected to a pH meter was used to monitor the pH of the solution. Mixing was achieved with the use of a magnetic stirrer with stirring rate of 700 rpm.

Determination of metal concentration in the supernatant

The heavy metal concentration was determined by the use of atomic absorption spectrophotometer, Perkin Elmer Analyst 300. determination of copper, chromium, cadmium and nickel was done by using its specific lamp for each metal and at a specific wavelength.

Data evaluation

The amount of metal bound by the biosorbents was calculated as follows:

$Q = v(C_i-C_f)/m$

Where Q is the metal uptake (mg metal per g biosorbent), v the liquid sample volume (ml), C_i the initial concentration of the metal in the solution (mg/L), C_f the final (equilibrium) concentration of the metal in the solution (mg/L) and m the amount of the added biosorbent on the dry basis (mg).

Sorption models were chosen for comparison with experimental data:

The Langmuir model, $Q = Q_{max} bC_f/ 1+bC_f$

Where Q_{max} is the maximum metal uptake under the given conditions, b a constant related to the affinity between the biosorbent and sorbate.

Linearized Langmuir model $1/Q= 1/Q_{max} (1/b\ C_f +1)$

The Freundlich model, $Q = k\ C_f^{(1/n)}$

Where k and n are Freundlich constants, which correlated to the maximum adsorption capacity and adsorption intensity, respectively.

Linearized Freundlich equation

$Log\ Q = Log\ k +1/n\ Log\ C_f$

RESULTS

In the biosorption of the tested metals by the different *Pseudomonas* species, most of the metal ions were sequestered very fast from solutions within the first 10 minutes and almost no increase in the level of bound metals have been occurred after this time interval (data not shown). The comparison of the sorption performance of the different biosorbents was achieved under the same environmental conditions (*i.e.* pH, temperature, agitation speed, etc.). Biosorption equilibrium isotherms were plotted for the metal uptake Q against the residual metal concentrations in solution. The Q versus C_f sorption isotherm relationship was mathematically expressed by linearized Langmuir and Freundlich models. The higher the values of k and n and the lower the value of b, the higher the affinity of the biomass (Asku et al. 1991; Jalaliet al. 2002).

Table 1, Table 2, Table 3 and Table 4 describe summaries of linear regression data for Langmuir and Freundlich isotherms for Cr(VI), Cu(II), Cd(II) and Ni(II) biosorption using nonliving biomass of different *Pseudomonas sp*. The values of $1/C_f$ was plotted against the values of $1/Q$ yielding straight line relationships for each of Cr(VI), Cu(II), Cd(II) and Ni(II) as individual metal ions and also as binary mixtures. Similarly the values of Log C_f were plotted against the values of Log Q, also giving straight lines for all four metals. The Q_{max} (maximum adsorption capacity) as derived from the Langmuir isotherm and the Freundlich constant k were obtained from the linear equations of both models.

As indicated from the Tables, the coefficients of determination (R^2) of both models were more or less greater than 0.9 and in case of Ni(II) and Cu(II) their coefficients were close to one, indicating that both models adequately describe the experimental data of theses metal biosorption experiments.

The data presented in Table 1, revealed that the values of Q_{max} and k in the Cr(VI) biosorption process indicated that, the presence of any binary metal with Cr(VI) have a strong antagonistic effect in the Cr(VI) uptake, while the presence of the four metal ions together has a relatively lower antagonistic effect.

In Table 2, and from the values of Q_{max} and $k,$ it is clearly shown that the Cu (II) biosorption is strongly affected by the presence of Cr(VI), since the presence of Cr(VI) as a binary metal ion has a strong synergistic effect. This can be explained in terms of the partial oxidation and deprotonation of the carboxylate and phosphate groups in the outer membrane lipopolysaccharide by the $K_2Cr_2O_7$ which then increases the net of negatively charged functional groups that enhances the biosorption of Cu(II) to the biomass (Geiger 1996; He and Tebo, 1998).Whereas the presence of the other metal ions with or without Cr in the Cu (II) biosorption mixture have a significant antagonistic effect in the Cu (II) biosorption.

For Cd(II) and Ni(II) biosorption, the presence of any metal ion in their biosorption mixture as binary or as a mixture of all has a strong antagonistic effect on their biosorption processes.

From all the obtained results, it was concluded that Cd(II) and Ni(II) could be bounded to their *Pseudomonas* species as much as 500 and 556 mg/g biomass, respectively. While there was a considerable variation in the extent of maximum metal uptake in the other species. The extent of exhibited Cu(II) and Cr(VI) uptake values ranged between 8.9 to 238 mg/g biomass.

The metal removal studies were illustrated graphically in Figure 2, Figure 3, Figure 4 and Figure 5 which showed that their removals differed with the difference of metal and with the different operating conditions. Maximum Cr(VI) removal was found to be around 38%, its removal increases with the increase of Cr(VI) influent. Generally Cr(VI) removal was ranged between 16 to 38% of metal influent. In other hand, Cu(II) removal reached its maximum value in presence of binary Cr(VI) metal, which reached 93% of its influent concentration. The Cu(II) removal increased gradually with the increase of the metal influent. The percentage Cu(II) removal ranged between 50 and 93%.

In case of Cd(II) and Ni(II) similar removal ratios were obtained since it was ranged between 35 to 88% and its maximum value was obtained for each of individual Cd(II) and Ni (II) metal ions.

The obtained results are in good agreement with the previous results that showing that the total amount of metal biosorption in a multiple metal system is lower than that in a single metal system (Chang and Hong, 1994; Figueira et al. 1997; Utigikar et al. 2000) Liu et al. 2003 found that biosorption of Cu(II) reached a maximal capacity of 39.84 mgCu(II)/g dry cell weight of *Thiobacillus thiooxidans* at pH 5.0. Volesky et al. 1999 worked extensively with one of the best metal-sorbing biomass types, ubiquitous *Sargassum seaweed*. They compared three different species of non living *Sargassum biomass* for their equilibrium Cd and Cu uptakes from aqueous solutions by using experimental sorption isotherms. Uptakes of Cd optimum pH4.5 were q_{max} = 87 mg/g for *Sargassum vulgare*, 80 mg Cd/g for *S. fluitans*, and 74 mg/g for *S. filipendula*. Uptakes of Cu at pH 4.5 were q_{max} = 59 mg/g for *S. vulgare* 56 mgCu/g for *S. filipendula* and 51 mg Cu/g *S. fluitans*. Also, Kaewchai and Prasertsan, 2002 studied the Ni and Cd adsorption by dried cells of *E. agglomerans* SM 38 and found that at optimum pH their removal reached 25.2% and 32%, respectively. While for *B. subtilis* WD 90 their removal exhibited 27% and 25%, respectively.

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APPENDIX

Tables

Table 1. Linear regression data for Langmuir and Freundlich isotherms for Cr(VI) biosorption by non living *Pseudomonas fluorescens*.

Metal form	Langmuir parameters			Freundlich parameters		
	Q max	b(x100)	R^2	k	n	R^2
Cr alone	111.11	0.25	0.98	0.112	0.769	0.99
Binary Cr/Cu	11.90	0.30	0.89	0.028	0.850	0.88
Binary Cr/Cd	8.91	0.38	0.92	0.009	0.699	0.95
Binary Cr/Ni	10.16	0.31	0.90	0.019	0.804	0.92
Mixture of all	66.67	0.09	0.96	0.089	1.039	0.96

Table 2. Linear regression data for Langmuir and Freundlich isotherms for Cu (II) biosorption by non living *Pseudomonas putida*.

Metal form	Langmuir parameters			Freundlich parameters		
	Q max	b(x100)	R2	k	n	R2
Cu alone	163.93	0.56	0.95	0.41	0.76	0.97
Binary Cu/Cr	238.10	0.16	0.97	0.36	1.01	0.99
Binary Cu/Cd	32.26	0.55	0.97	0.06	0.73	0.99
Binary Cu/Ni	41.49	0.48	0.98	0.08	0.76	0.99
Mixture of all	33.11	0.56	0.96	0.07	0.73	0.99

Table 3. Linear regression data for Langmuir and Freundlich isotherms for Cd (II) biosorption by non living *Pseudomonas putida*.

Metal form	Langmuir parameters			Freundlich parameters		
	Q max	b(x100)	R^2	k	n	R^2
Cd alone	500.00	1.50	0.99	41.2	2.565	0.97
Binary Cd/Cu	90.09	1.40	0.99	6.7	2.475	0.97
Binary Cd/Cr	84.03	1.88	0.94	11.54	3.421	0.84
Binary Cd/Ni	60.61	2.35	0.94	10.76	3.808	0.83
Mixture of all	43.10	4.60	1.00	12	4.787	0.93

Table 4. Linear regression data for Langmuir and Freundlich isotherms for Ni (II) biosorption by non living *Pseudomonas putida*.

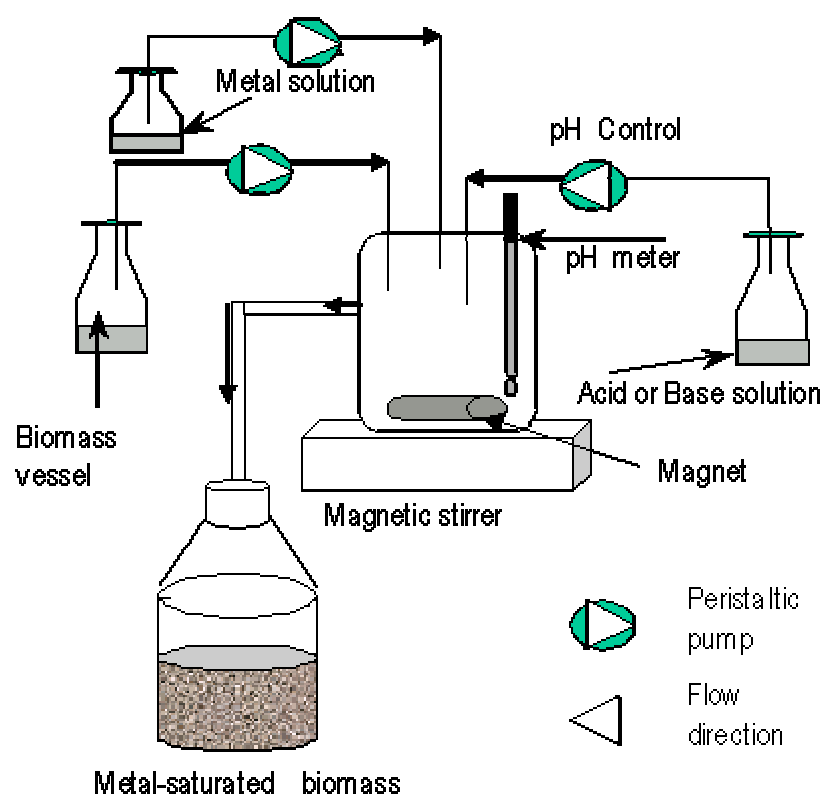| Metal form	Langmuir parameters			Freundlich parameters		
	Q max	b(x100)	R^2	k	n	R^2
Ni alone	556	0.34	1	1.04	0.62	1.00
Binary Ni/Cu	102	0.39	0.97	0.41	0.65	0.98
Binary Ni/Cd	48	0.51	0.91	0.11	0.56	0.95
Binary Ni/Cr	81	0.41	0.97	0.21	0.61	0.99
Mixture of all	24	0.59	0.85	0.03	0.48	0.93

Figures

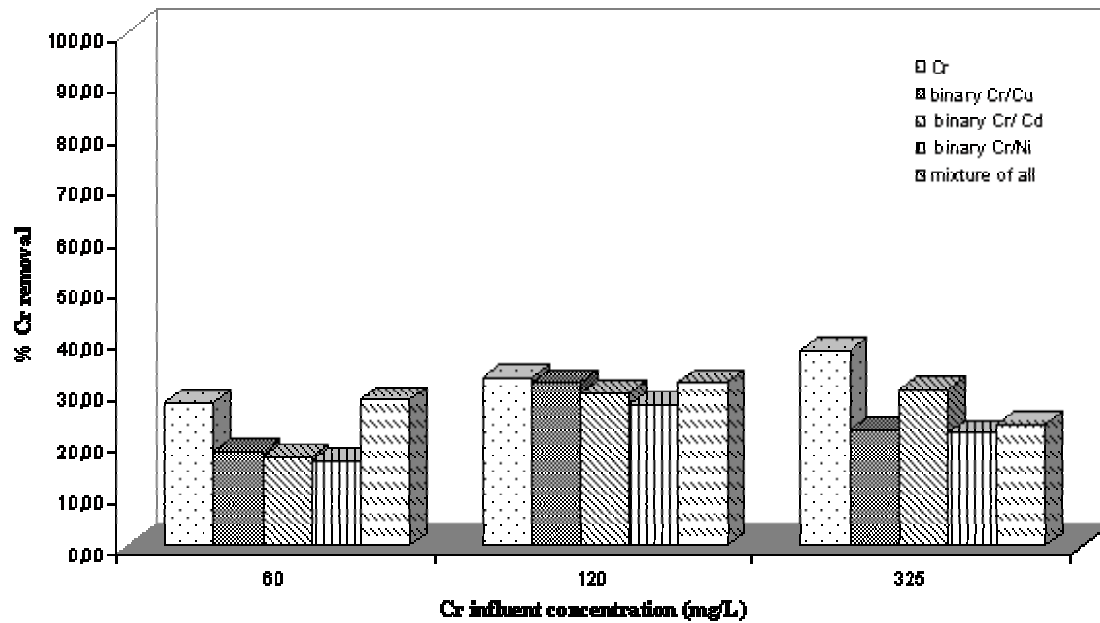**Figure 1. Experimental set up for the extraction of heavy metals from aqueous solutions.**

Figure 2. Comparison between continuous Cr(VI) uptake by nonliving *Pesudomonas fluorescens* under different condition.
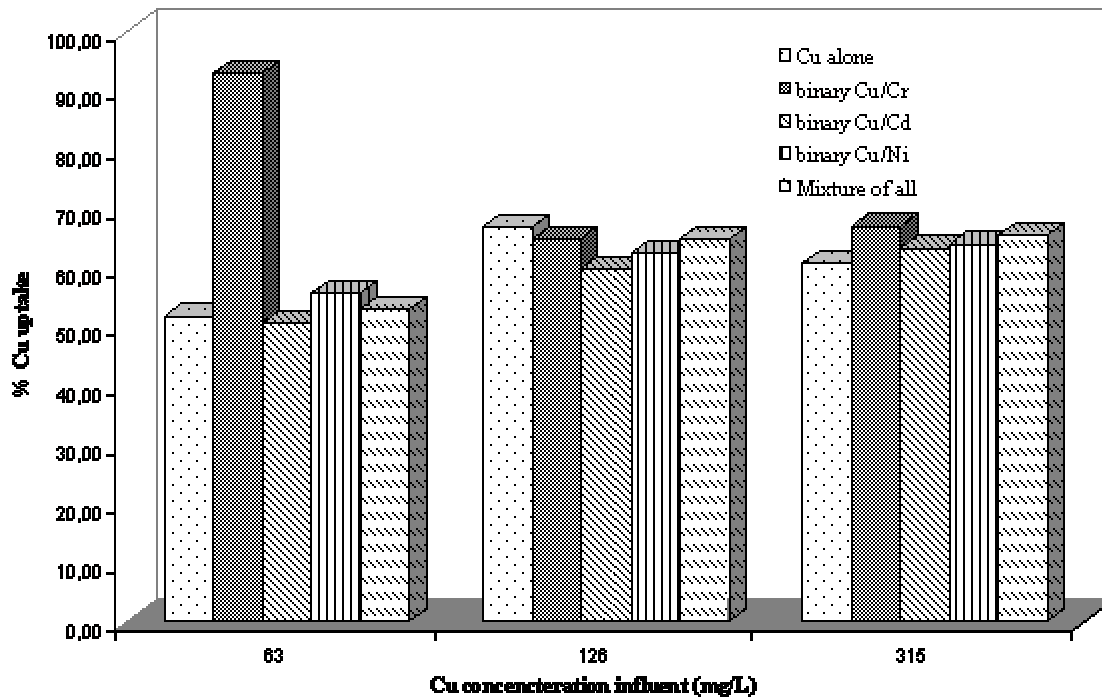

Figure 3. Comparison between continuous Cu(II) uptake by nonliving *Pseudomonas putida* under different condition.
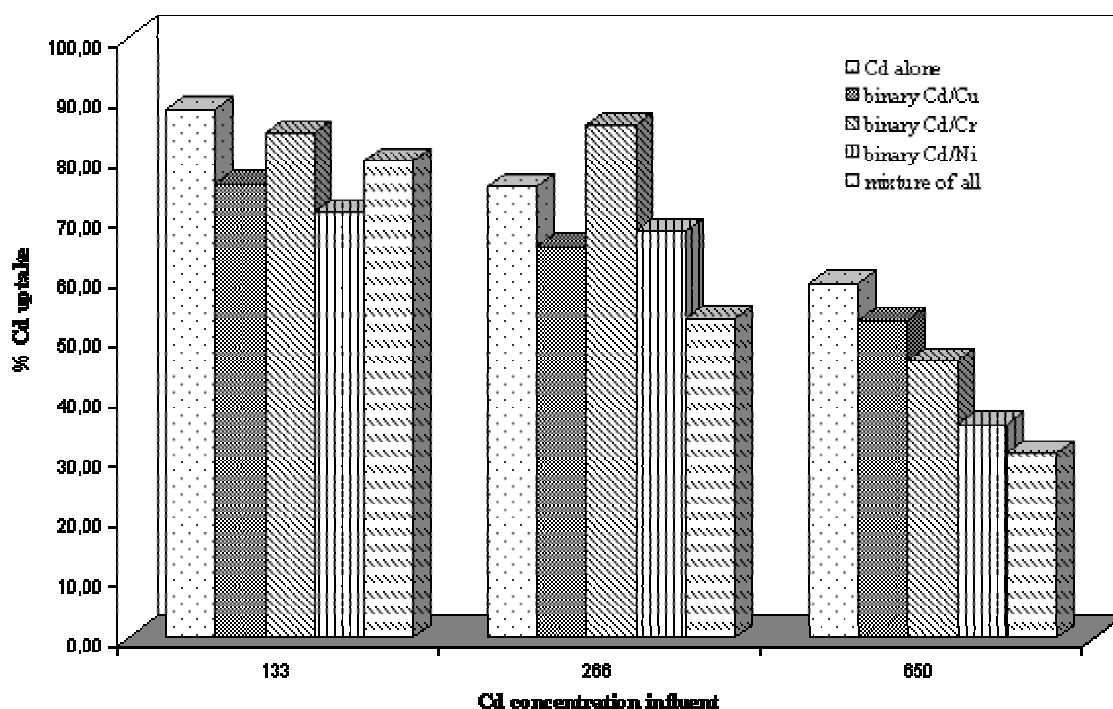
Figure 4. Comparison between continuous Cd(II) uptake by nonliving *Pseudomonas putida* under different condition.
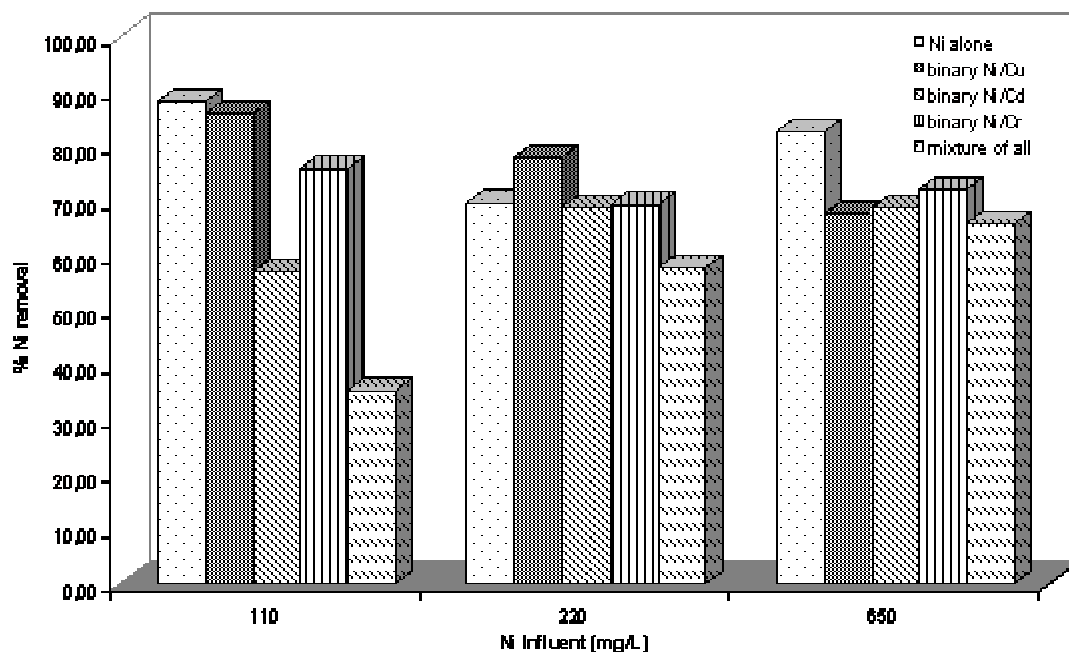

Figure 5. Comparison between continuous Ni(II) uptake by nonliving *Pseudomonas putida* under different condition.