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STUDIES ON REMOVAL OF TOXIC METALS FROM WASTEWATER USING PSEUDOMONAS SPECIES

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ABSTRACT

Toxic trace metals can be hazardous even at very low concentrations. When they get into water supplies and aqueous environments the health of plants and animals, as well as humans, can be impaired. Toxic trace metals are commonly found in wastewater and removing them efficiently presents a unique challenge. The discharge of heavy metals into aquatic ecosystems has become a matter of concern in India over the last few decades. These pollutants are introduced into the aquatic systems significantly as a result of various industrial operations.Biosorption experiments for Chromium (Cr (VI)), Copper (Cu (II)), Cadmium (Cd (II)) and Nickel (Ni (II)) were investigated in this study using nonliving biomass of different Pseudomonas species. The Langmuir and Freundlich models for the different biosorbent were applied and tested. Maximum Cr (VI) removal reached around 40% 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 95% of its influent concentration. Concerning to Cd (II) and Ni (II) similar removal ratios were obtained, since it was ranged between 36 to 90% and their maximum removal were obtained in the case of individual Cd (II) and Ni (II).

Keywords: wastewater, toxic metals, biosorption, pseudomonas sp., freundlich isotherm, Langmuir isotherm.

INTRODUCTION

Many industries, such as the electroplating and mining companies, produce large amounts of waste water that contain hazardous amounts of mercury, lead, cadmium, silver, copper, and zinc ions. They are required by law to reduce the concentrations of these toxic metals in their waste water before it is discharged into sewers, lakes and streams. The search for new technologies involving the removal of toxic metals from wastewaters has directed attention to biosorption, based on metal binding capacities of various biological materials. Biosorption can be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Volesky, 1986). The major advantages of biosorption over conventional treatment methods include (Kratochvil and Volesky, 1998 a): Low cost; High efficiency; Minimization of chemical and biological sludge; No additional nutrient requirement; Regeneration of biosorbent; and Possibility of metal recovery. The biosorption process involves a solid phase (sorbent or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorbent for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorbent affinity for the sorbate determines its distribution between the solid and liquid phases. 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 will provide an attractive alternative to physico-chemical methods (Kapoor and Viraraghavan, 1995). 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 bio accumulative 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 modelling 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 are 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.

Biosorption mechanisms

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into:

a) Metabolism dependent; and
b) Non -metabolism dependent.

According to the location where the metal removed from solution is found, biosorption can be classified as:

a) Extra cellular accumulation/ precipitation;
b) Cell surface sorption/ precipitation; and
c) Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defence system of the microorganism, which reacts in the presence of toxic metal. During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988). In the case of precipitation, the metal uptake may take place both in the solution and on the cell surface (Ercole *et al*., 1994). Further, it may be dependent on the cell's' metabolism if, in the presence of toxic metals, the microorganism produces compounds that favour the precipitation process. Precipitation may not be dependent on the cells' metabolism, if it occurs after a chemical interaction between the metal and cell surface.

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 National Institute of Technology, Warangal sewage treatment plant, Warangal, India. 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). The strains were grown in casamino acid media (CAA), composed of casamino acid (Oxoid) 5 g/L; K_2HPO_4 and 0.25 g/L $MgSO_4$ .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 and biosorption process

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.

The metal solution was stored in a glass vessel and a peristaltic pump regulated the flow rate of metal solution to the bioreactor. 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 bioreactor. 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 consists the bioreactor 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.

The heavy metal concentration was determined by the use of atomic absorption spectrophotometer.
The amount of metal bound by the biosorbents was calculated as follows:

$$Q = v\,(C_i - C_f)/m \quad \text{---- (1)}$$

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,

$$Q = Q_{max}\, bC_f / 1 + bC_f \quad \text{------ (2)}$$

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

$$1/Q = 1/Q_{max}\,(1/b\,C_f + 1) \quad \text{------ (3)}$$

The Freundlich model,

$$Q = k\,C_f\,(1/n) \quad \text{------ (4)}$$

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

$$\text{Log Q} = \text{Log k} + 1/\text{n Log } C_f \quad \text{------ (5)}$$

RESULTS AND DISCUSSIONS

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 12 minutes and almost no increase in the level of bound metals have been occurred after this time interval .The comparison of the sorption performance of the different biosorbents was achieved under the same environmental conditions such as pH, temperature, and agitation speed. 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; Jalali *et 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 species. 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. The metal removal studies were illustrated graphically in Figures 1, 2, 3 and 4 shows that their removals differed with the difference of metal and with the different operating conditions.

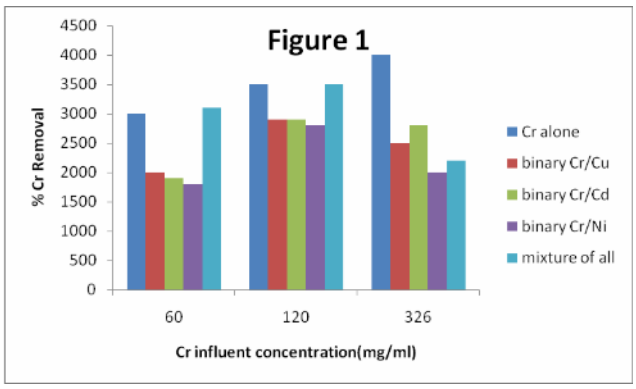

Figure-1. Comparison between continuous Cr (VI) uptake by nonliving pseudomonas fluorescens under different condition.

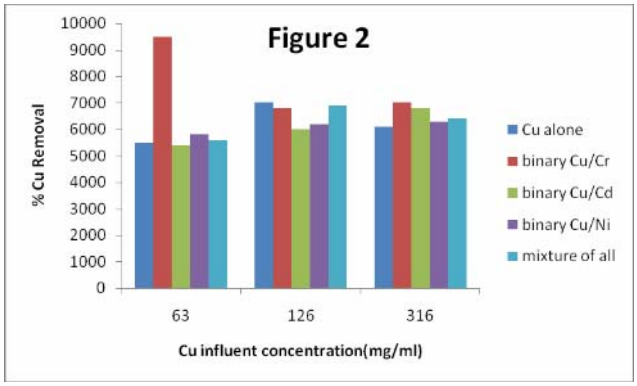

Figure-2. Comparison between continuous Cu (II) uptake by nonliving pseudomonas putida under different condition.

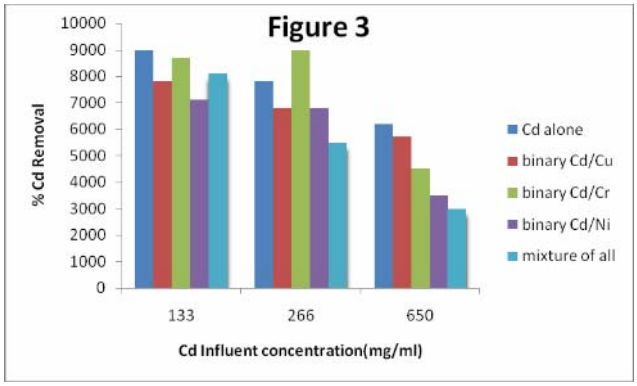

Figure-3. Comparison between continuous Cd (II) uptake by nonliving pseudomonas putida under different condition.

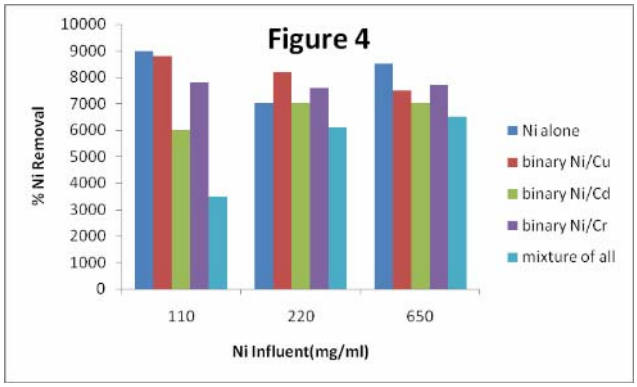

Figure-4. Comparison between continuous Ni (II) uptake by nonliving pseudomonas putida under different condition.

Maximum Cr (VI) removal was found to be around 40%, its removal increases with the increase of Cr (VI) influent. Generally Cr (VI) removal was ranged between 16 to 40% of metal influent. In other hand, Cu (II) removal reached its maximum value in presence of binary Cr (VI) metal, which reached 95% 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 95%. In case of Cd (II) and Ni (II) similar removal ratios were obtained since it was ranged between 36 to 90% and its maximum value was obtained for each of individual Cd (II) and Ni (II) metal ions.

From all the obtained results, it was concluded that Ni (II) and Cd (II) could be bounded to their Pseudomonas species as much as 556 and 500 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.

CONCLUSIONS

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).

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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	111.1	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)	R^2	k	n	R^2
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/Cr	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

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