

Biosorption of Cu (II) by Metal Resistant *Pseudomonas* sp.

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Received 10 March 2008;

Revised 15 Aug. 2008;

Accepted 25 Aug. 2008

ABSTRACT: Bioremediation of heavy metals remains a major challenge in environmental biotechnology. Biosorption is a novel technique for decreasing metal ion content in the waste water. Biosorption experiments for Cu (II) were investigated in this study using bacteria isolated from the industrial effluent. The results showed that the dead cells of *Pseudomonas* sp. were an efficient adsorbent of Cu (II). For analysis, Langmuir and Freundlich adsorption isotherm were considered. Both model fitted to the experimental data however the Langmuir model fitted the experimental data better than the Freundlich model for copper. Adsorption was influenced by various parameters, such as the initial metal concentration, pH, and contact time etc. Studies pertaining to the assessment of the best adsorption parameters and quantitative analysis of metal uptake revealed that maximum biosorption for Cu (II) obtained was 74.2 % at pH 7. Citric acid was used as desorbing agents in order to recover heavy metal ions from the adsorbent.

Key words: Adsorption, Desorption, *Pseudomonas*, Isotherm, Citric acid

INTRODUCTION

Environmental pollution is the major concern faced by the society today. The pollutants generally include organic wastes and heavy metals. Heavy metal removal is important for survival of environment. One of the more important toxic metals, copper, finds its way to the water stream from industries like electroplating, mining, electrical and electronics, iron and steel production, the non-ferrous metal industry, the printing and photographic industries and metalworking and finishing processes (Brauckmann, 1990; Singanan *et al.*, 2008;). Copper is not acutely toxic to humans but its extensive use and increasing levels in the environment may cause serious health problems in particular possible liver damage with prolonged exposure (Sanchez, *et al.*, 1999).

Conventional methods for removing dissolved heavy metal ions from waste waters include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical

treatment and evaporative recovery. These techniques have significant disadvantages including incomplete metal removal, the need for expensive equipment and monitoring systems, high reagent or energy requirements or generation of toxic sludge or other waste products that require disposal (Aksu, *et al.*, 1999; Murugesan *et al.*, 2008).

Biosorption is considered as an alternative process for the removal of heavy metals, metalloid species, compounds and particles from aqueous solution by biological materials (Artola, *et al.*, 1997). Among various agents, the use of microbial biomass for the biosorption of metals from industrial and municipal waste water has been proposed as a promising alternative to conventional heavy metal management strategies in past decades. Microbial biomass can be used to decontaminate metal bearing wastewaters as well as to concentrate metals. The nature of biological surfaces is such that different functional groups form complexes with metal ions (Huang, *et al.*,

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1991), resulting in chemical complex as an uptake mechanism. Accumulation of metals in microbial biomass also proceeds by different processes such as uptake by transport, entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Brady and Duncan, 1994). The use of bacteria and fungal biomass as biosorbents should be of special interest to industries in undeveloped countries where pollution generators cannot afford to install costly high-performance treatment facilities. Even indigenous biomaterials like *Tridax procumbens* (*Asteraceae*) have been used as a bioadsorbent for removing heavy metal from synthetic wastewater (Signanan, *et al.*, 2008). To enhance the applicability of biosorption in wastewater treatment, it is important to identify microbial strains that could uptake metals with high efficiency and specificity as well as to design better bioprocesses that effectively remove or recover heavy-metals from aquatic systems. The objective of the present study was to investigate the use of metal resistant *Pseudomonas sp.* biomass as a bio-sorbents for the removal of Cu (II) from an aqueous solution. The optimum biosorption conditions were determined as a function of initial pH, initial metal ion concentration and time.

MATERIALS & METHODS

Bacterial strains were isolated from equalization tank containing industrial effluent from G.E.C.S.C.L (Green Environment Services Co-operative Society Limited), G.I.D.C (Gujarat Industrial Development Co-operation), Vatva, Ahmedabad. The isolates were cultivated on Luria Bertani medium at 32°C and identified through biochemical and morphological studies as per standard procedures (David and John, 1994). The metal solutions of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ were selected in varying concentration ranging from 50 to 1200 mg/L. Stock solutions of metal salts were prepared in sterile water and added to Luria Agar in varying concentration and spot inoculated with different isolates. The plates were incubated at 32-35°C for 24 h (Malik and Jaiswal 2000; Aleem, *et al.*, 2003). Bacteria showing resistance to highest concentration for given metal ion were subjected to subsequent biosorption studies.

Biosorption experiments were carried out using batch equilibrium technique. After screening

procedure, microorganisms were cultured on Luria- Bertani medium without metals. Cells were harvested by centrifugation at 8000 rpm for 10 min. Harvested cell (biomass) were then washed twice with de-ionized distilled water, autoclaved and dried in oven at 80°C for 48 h. The dried powdered biomass was used for sorption studies. Biosorption experiments were conducted at different initial Cu (II) concentrations (50 and 100 mg/L) until the equilibrium is reached. At the end of each experiment the mixture was centrifuged (8000 rpm for 5 min) and the remaining concentration of metals in the supernatant was determined. The experimental data were processed via Langmuir and Freundlich isotherms.

Adsorption of metal ion by dried bacterial biomass was checked at regular interval of time. 50 mL of metal solution ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) was added with appropriate amount of bacterial biomass into each flask. Biosorption was studied for the given time interval by taking out 2 mL of solution and reading the absorbance and extrapolating biosorption using a standard curve (Ikram and Malik, 2007).

Several flasks for each metal ion concentration were prepared. The metal ion concentration was selected on the basis of maximum biosorption as mentioned. pH of the solution was adjusted to 5.0, 6.0, 7.0, 8.0 and 9.0 by using 2 M NaOH and 1 M H_2SO_4 as required. Biosorption in each tube containing 50 mL of metal solution were studied by procedure described. Lower pH was avoided as it might cause precipitation of metal salts. For each set, a blank of metal solution without any inoculation was performed in parallel to avoid confusion between biosorption and possible metal precipitation. Following the metal adsorption batch experiment, metal laden pellet were taken out and suspended into 5mL of the eluant solutions (citric acid (0.1M)). The metal ion is slowly released into eluant. At regular time interval, 1 mL of the sample was withdrawn to perform metal analysis. Each experiment was continued until equilibrium condition was reached where no further change in ion concentration was observed. The unloaded biomass was regenerated, washed twice with distilled water and re-suspended in new metal solution for another cycle of biosorption-desorption

process. This biosorption-desorption experiment were continuously performed 2 to 3 times.

The capacity of an adsorbent can be described by its equilibrium sorption isotherm, which is characterized by certain constants whose values express the surface properties and affinity of the adsorbent. Langmuir and Freundlich isotherms were used to quantitatively describe metal sorption by test bacteria. The theoretical basis of Langmuir equation relies on the assumption, that there is a finite number of binding sites which are homogeneously distributed over the adsorbent surface, having the same affinity for adsorption of a single molecular layer and there is no interaction between adsorbed molecules. The mathematical description of the equation is:

$$q = \frac{Q_{max} b C_e}{1 + b C_e}$$

Where q is the amount of metal ion adsorbed; C_e is the residual metal concentration in solution (mg/L); Q_{max} the maximum specific uptake corresponding to sites saturation (mg g⁻¹), and b the biomass–metal binding affinity (Donmez and Aksu, 2002). The Freundlich equation is the empirical relationship whereby it is assumed that the adsorption energy of a metal binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied. This empirical equation has the form:

$$q = K C_e^{1/n}$$

where, K and n are constants indicating adsorption capacity and adsorption intensity, respectively (Bayramoglou, *et al.*, 2005). The amount of metal ion adsorbed was obtained by using following expression:

$$q = (C_i - C_e) V/m$$

where C_i and C_e are the initial and final metal ion concentration (mg/L), respectively. V is the volume of the medium (L) and m is the amount of the biomass (g). Estimation of copper was done by diethyldithiocarbamate method. Sodium diethyldithiocarbamate reacts with a weakly acidic or ammoniac solution of copper solution in low concentration to produce a brown colloidal suspension of the copper diethyldithiocarbamate. The suspension is extracted with the organic

solvent like chloroform and colored extract analyzed spectrophotometrically by measuring absorbance at 435 nm (Snell and Snell, 1959).

RESULTS & DISCUSSION

Seventeen isolates were selected from the industrial effluent on Luria Bertani agar. Out of these, three isolates 15, 16 and 17 were found to resist upto 1200 mg/L of Cu (II) which were identified by metal inhibition assay. It has been reported that various parameters like time, pH, concentration of metal ions, loading biomass affects the biosorption process (Ozturk, 2007). All these parameters vary from species to species and ion under investigation.

For determining the sorption capacity of a biosorbent, it is necessary to generate the equilibrium sorption data at various metal solution C_i (initial concentration) values. These data are further necessary for modeling on the Langmuir and Freundlich adsorption isotherms, a fit which is usually used to interpret the efficiency of metal biosorption. For Cu⁺², biosorption potential of three isolates 15, 16, 17 was assessed at concentrations of 50 mg/L and 100 mg/L beyond which saturation was achieved. Isolate 15 showed maximum biosorption (73.8 %) at 50 mg/L metal concentration while further increasing the concentration to 100 mg/L decreased the potential to 67.1%. This indicated that 50 mg/L metal concentration can be used for further studies. In the case of lower concentrations, the ratio of initial number of metal ions to the available sorption sites was low and higher biosorption yields were obtained. At higher concentrations, the available sites of biosorption became fewer and the saturation of the sorption sites was observed. So biosorption yield was decreased (Arzu and Dursan, 2006).

Isolate 17 showed increased biosorption when concentration was raised to 100 mg/L however isolate 16 and 17 showed relatively less sorption when compared to isolate 15 (Fig. 1,2). So they were not selected for pH and further desorption studies. This experiment confirmed that equilibrium sorption capacity was highly dependent on the initial metal concentration. All the selected isolates 15, 16 and 17 showed maximum biosorption of Cu(II) at 6 h when measured at the interval of 2 h (Fig 1,

2). After 6 h, the isolate 15 showed maximum adsorption capacity. It was observed that effect of time was not dependent on concentration of metal ions of this study. Saturation time beyond which no further increase in biosorption observed may represent the time that allows complete occupation of all the binding sites on the cells which limits the cells from sequestration of any further ions due to lack of adsorption sites (Al-Garn, 2005). Hence, in the present experiment, 6 h was chosen as the equilibrium time.

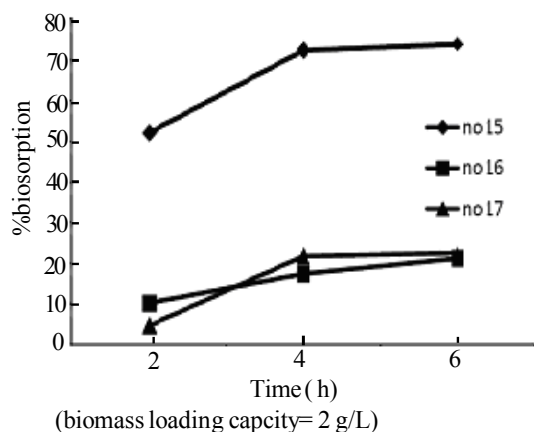


Fig. 1. Biosorption of copper (50 mg/L) by isolate 15

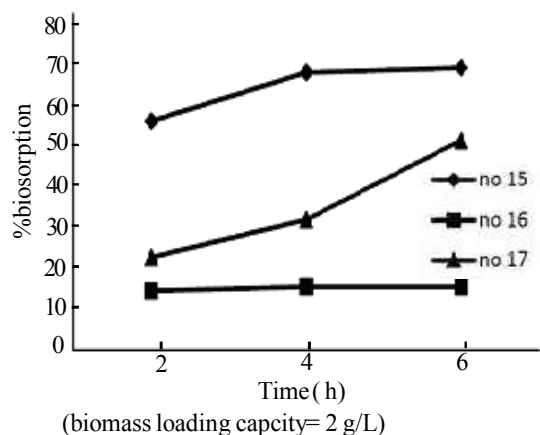
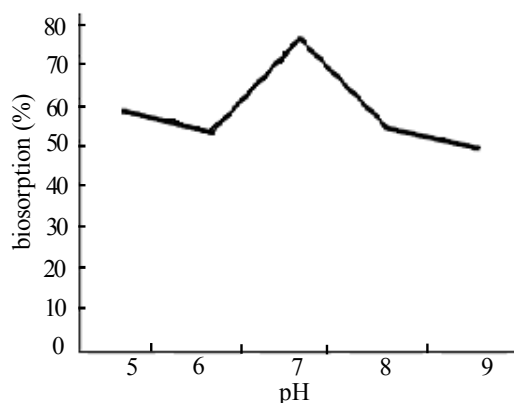


Fig. 2. Biosorption of copper (100 mg/L) by isolate 15

The inconsistency in literature regarding the influence of pH on biosorption seems to indicate that the way pH would alter the adsorption of metal ions to biomass varies with the type of adsorbent (biomass) and also the type of adsorbents (metal ions). The pH of the solution is perhaps the most important parameter for adsorption. The charge of the adsorbate and the adsorbent often depends on the pH of the

solution. Cu (II) is adsorbed in the form the divalent cation so higher protonation of the cell wall components decreased the metal uptake of the biomass, whereas on increasing pH, the negative charge density increases, due to the deprotonation of the metal binding sites. Maximum Cu (II) biosorption was recorded (74.2%) at pH 7 (Fig. 3). At low pH, the cell surface sites are closely linked to the H⁺ ions, thereby making these unavailable for other cations. However, with an increase in pH, there is an increase in ligands with negative charges which results in increased binding of cations. Higher pH values were not useful, since at higher pH values precipitation of the metal can occur by the formation of metal hydroxides (Pardo, et al., 2003).

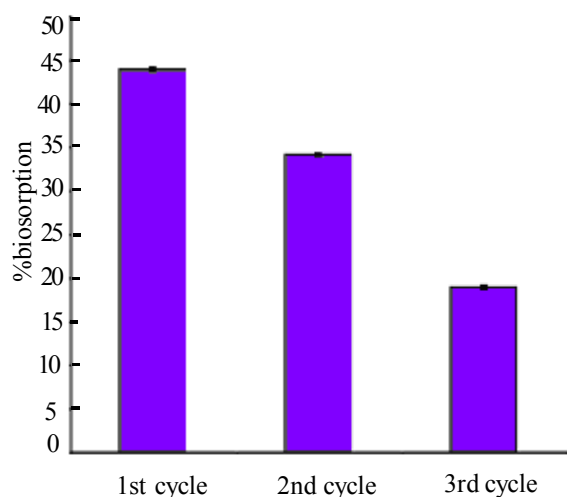


(biosorbent concentration = 2 g/L, metal concentration= 50 mg/L, contact time = 6 h)
 Bars indicate standard error at 5% significance
 Fig. 3. Effect of pH on copper biosorption by isolate 15

At pH values above the isoelectric point, there is a net negative charge on the cell wall components and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such, as to promote a reaction with metal cations. As the pH is lowered, however, the overall surface charge on the cells will be positive, which inhibits the approach of positively charged metal cations. It is likely that protons combine with metal ions for the ligands and thereby decrease the interaction of metal ions with cell components (Sag and Kutsal, 1996). Several researchers have also investigated the effect of pH on biosorption of metals by using different biomass and found similar results with this study. Investigation of the biosorption of Cu (II) ions by filamentous fungus *Phanerochaete chrysosporium* was done and for biosorption

optimum pH was determined as 6.0 (Say *et al.*, 2001).

During desorption studies, maximum desorption of Cu (44%) by isolate 15 was recorded during the first cycle which significantly decreased during subsequent cycles. These results indicated that after 3 to 4 cycles of repetitive biosorption-desorption, biosorption declined significantly (Fig. 4). This may indicate that cell components fade away due to such repeated treatment (Lu, *et al.*, 2006). The reuse of the biomass and desorbent is an important feature for its possible utilization in continuous systems in industrial processes. Isolate 15 was characterized and found to be of *Pseudomonas sp.* on the basis of standard morphological and biochemical tests (Table 1).



(Cu (II) concentration=50 mg/L, contact time= 24 h, biosorbent concentration = 2 g/L) Bars indicate standard error at 5% significance

Fig. 4. Desorption of copper with citric acid by isolate 15

Analysis of equilibrium data is important for developing an equation that can be used design a process. The Langmuir model makes several assumptions, such as monolayer coverage and constant adsorption energy while Freundlich equation deals with physicochemical adsorption on heterogeneous surfaces. The applicability of both Langmuir and Freundlich isotherms to metal-activated sludge biomass implies that both monolayer adsorption and heterogeneous surfaces conditions exist under the experimental conditions used. As seen in (Table 2), regression correlation

coefficients for the metal ion–bacterium systems are very high. The value of Q_{max} species [Langmuir adsorption constant (62.5 mg g^{-1})] appears to be significantly higher for the Cu(II). This Q_{max} value (calculated by fitting the Langmuir isotherm model to the experimental data) were very important in considering the suitability

Table 1. Identification of the isolated Cu resistant bacteria

No.	Test performed	Isolate 15
1	Fermentation	
	a) Dextrose	-
	b) Lactose	-
	c) Sucrose	-
	d) Maltose	-
	e) Mannitol	+
	f) Xylase	-
2	Starch hydrolysis	-
3	IMViC	
	1) Indole production	+
	2) Methyl red	-
	3) Voges-Proskauer	-
	4) Citrate utilization	+
4	Nitrate reductase	+
5	Oxidase	+
6	Gelatin hydrolysis	+
7	H ₂ S Production and Motility	+
8	Chitin hydrolysis	-
9	Caesin hydrolysis	+
10	Anaerobic growth	-
11	Catalase	+
12	Arginine dihydrolase	+
13	Lysine decarboxylase	-
14	Ornithine decarboxylase	-
15	Utilization of L-Arginine	+
16	Utilization of β -Alanine	+
17	Poly- β -hydroxybutyrate accumulation	-
18	Proverdin production	+
19	Pyocyanin production	+
20	Aesculin hydrolysis	-
21	Staining procedures	
	a) Gram test	-, rods
	b) Endospore staining	Non-sporing
	c) Flagella staining	+
22	Growth on <i>Pseudomonas</i> isolation Agar	+ (bluish green pigment)

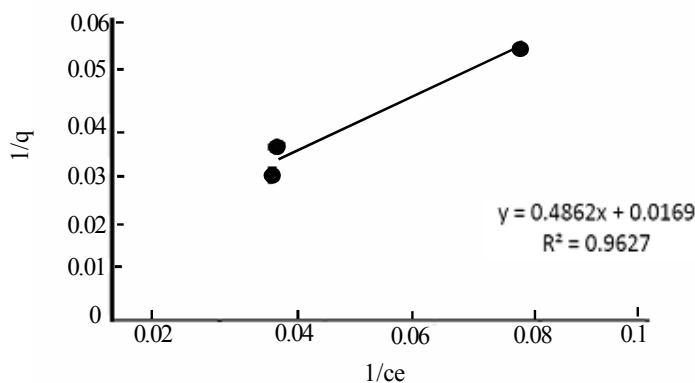
Table 2. Linear regression data for Langmuir and Freundlich isotherm for copper biosorption

Model	Langmuir model			Freundlich model		
	Qmax(mg/g) ^A	b ^B	R ² ^C	K ^D	1/n	R ^{2**}
Cu	62.5	0.033	0.962	4.197	0.574	0.959

A= Maximum adsorption capacity (mg/g),
 B= Langmuir adsorption constant,
 C= Correlation coefficient,
 D= Freundlich adsorption constant

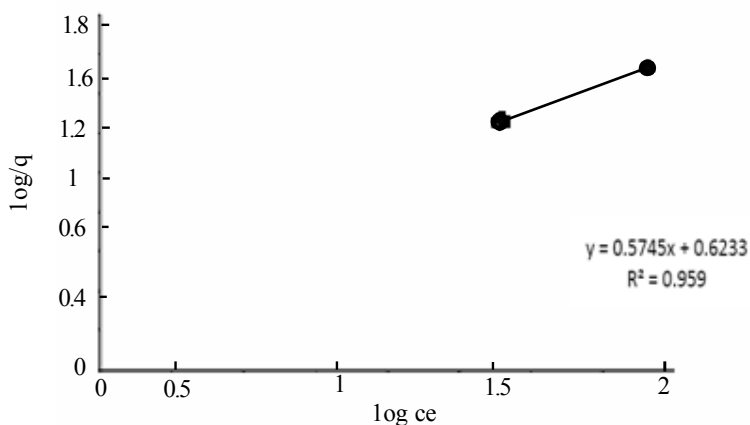
of materials for biosorption as they give an indication of the total number of binding sites that are available. The Langmuir isotherm model however makes a number of assumptions such as monolayer-based sorption. It also assumes that all the binding sites on the sorbent are free sites, ready to accept the sorbent from solution (Volesky, 2003). High values of Qmax observed for the sorption of

Cu (II) by test bacteria suggested the prevalence of Cu (II) binding ligands in them. The constant b, which is related to the affinity of a metal ion for binding onto the surface, was also generally the low for the sorption of Cu (II) (Fig. 5). The low value of b for Cu (II) in this case was suggestive of greater affinity of biomass for Cu (II). A good metal sorbent in general should have



(Ce is the residual metal concentration; q represents the metal uptake by the cells)

Fig. 5. Langmuir isotherm for copper biosorption



(Ce is the residual metal concentration and q represents the metal uptake by the cells)

Fig. 6. Freundlich isotherm for copper Biosorption

a high q_{max} as well as a low b (Kratochvil and Volesky, 1998). The Freundlich model should fit well for metal adsorption by a biomass because of heterogeneous nature of sites present on it (Zhou and Huang, 1998). The values of K and n (Freundlich constants) showed high adsorption capacity and easy uptake (Fig. 6). Since the values of n were higher than 1.0, the strength of metal adsorption by the biosorbent was quite intense (Ozer, *et al.*, 1994).

CONCLUSION

The aim of this work was to find the biosorption characteristics of selected metal resistant bacteria for removal of copper ion. Experiments were performed as a function of pH, initial metal ion concentration and time. The obtained results showed that *Pseudomonas* sp. was a good adsorbing medium for metal ions and had high adsorption yields for the treatment of wastewater containing copper ions. The Freundlich and Langmuir adsorption models were used for the mathematical description of the biosorption of copper to dried *Pseudomonas* and the isotherms constants were evaluated to compare the biosorptive capacity of dried inactive *Pseudomonas* sp. for metal ions. Moreover, the bacteria was isolated from metal contaminated water; it could also interact with the metals in water and influence their potential bioavailability and impact on the environment. So this bacterial isolate may be employed for metal remediation in simple reactors or even *in situ* condition. Furthermore, in view of the practical application of treatment of metal bearing streams, this preliminary study needs to be extended to additional experiments concerning in particular the influence of ionic strength and diverse constituents that are frequently found in actual industrial effluents, such as surfactants, complexing agents and other metal ions.

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