Biosorption of Copper (II) from electroplating wastewaters by *Aspergillus terreus* and its kinetics studies

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Summary

In present investigation, samples from wastewaters of Electroplating industry were collected and analyzed for the concentration of Cu^{2+} heavy metal. For the bioremediation of heavy metal, *Aspergillus terreus* fungal strain was used. The fungal strain was isolated from soil and found that it biosorbed 7.77 mg/l Cu^{2+} heavy metal from solution within 1 hour. Biosorption was sensitive to variations in pH and in the range investigated; metal binding was optimum at 4.0. A wastewater with a pH of 3.0 or above can be effectively treated for metal ion removal and the wastewater with pH lower than 3.0 would need pH adjustment. Biosorption isotherm data fitted better into Langmuir isotherm implicated monolayer adsorption existed for the experimental conditions used. Based on the Langmuir isotherm plots the maximum biosorption capacity (Q_{max}) value was calculated to be 19.46 mg/g at pH 4 for fungal biomass.

Introduction

The presence of heavy metals in the environment is of major concern because of their toxicity, bio-accumulating tendency, and threat to human life and environment. The current pattern of industrial activity alters the natural flow of materials and introduces novel chemicals into the environment. The anthropogenic sources of heavy metals include wastes from the electroplating and metal finishing industries, metallurgical industries, tannery operations, chemical manufacturing, mine drainage, battery manufacturing, leather tanning industries, fertilizer industries, pigment manufacturing industries, leachates from landfills and contaminated ground water from hazardous waste sites (Faisal et al. 2004, Jackson et al. 1991, Huang et al. 1984, Mclaughlin et al. 1996). Wastewaters from these industries include metal ions having permanent toxic effect. Feasible and useful treatment methods have been developed to purify industrial wastewaters. Removal and recovery of heavy metals are very important with respect to environmental and economical considerations (Aksu et al. 1996, Nurba et al. 2002). There are several chemical methods for detection and remediation of these heavy metals. The designed physical and chemical methods for removal of metal ions from effluents are commercially impractical, because of high operating cost

and difficult techniques. Currently, there is a growing need to develop environmentally benign processes that do not use toxic chemicals in the protocol. Hence, innovations in integrated biological systems are coming up with low cost and easy methodologies for bioremediation. The removal of heavy metals from our environment especially wastewaters is now shifting from the use of conventional adsorbents to the use of bio-sorbents. Biosorption or bioremediations consists of a group of applications, which involve the detoxification of hazardous substances instead of transferring them from one medium to another by means of microbes and plants. This process is characterized as less disruptive and can be often carried out on site, eliminating the need to transport the toxic, materials to treatment sites (Gavrilescu, 2004). In recent years, many low cost sorbents such as algae, fungi, bacteria and lingo-cellulosic agricultural by-products have been investigated for their biosorption capacity towards heavy metals. Algae, fungi, yeast and bacteria remove heavy metals from wastewaters through functional groups such as ketones, aldehydes, carboxyls; on their cell walls (Tanja et al. 2001, Euef et al. 1991). But for this purpose, fungi possess some advantages over bacteria. The first is that most filamentous fungi have a high tolerance towards metals, and a high wall-binding capacity, as well as intracellular metal uptake capabilities (Volesky et al. 1995). In addition, they are easy to culture on a large scale, especially by the thin solid fermentation method, thus making it possible to easily obtain enough biomass for processing. The third one is that the fungus could grow on the surface of an inorganic vector during culture, which could distribute particles in a more efficient way as a catalyst. Biosorption considerably depends on the experimental conditions, e.g. pH value and metal concentration (Say et al. 2001). Mycelia of *Rhizopus* and *Absidia* species are effective biosorbents for lead, cadmium, copper, zinc and other metals. Even a loading with several metals of up to 25% of the dry weight is reported (Volesky and Holan, 1995). *Aspergillus niger* biomass after boiling in 0.1 N NaOH for 15 min was able to bind 7.24 mg lead per g (Kapoor et al. 1999). Removal of heavy metals by an *Aspergillus terreus* strain immobilized in a polyurethane matrix by Dias et.al. 2002. Microorganisms respond to heavy metals in different ways depending on the nature of the microorganism and on the concentration of the heavy metal in the environment. They accumulate metals by a number of different processes such as uptake by transport, biosorption by cell walls and entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Venkateswerlu et al. 1970, Subramanyam et al. 1983, Schumate et al. 1985, Brady et al. 1993, Akhta et al. 1995). In present investigation, biosorption of Cu^{2+} ions from wastewaters of electroplating industries of Agra by *Aspergillus terreus* fungus was investigated.

Materials and Methods

Isolation of Fungus

Fungal strains were isolated from soil samples, collected from Khandari farm house, Agra. Enumeration of microbes present in the soil was done by Serial dilution-agar plating method (Nigam 1965). Serial dilution of soil suspension was prepared up to 10^{-6} dilution. 1 ml of suspension from dilutions 10^{-3} to 10^{-6} was transferred to the petridishes containing Czapek's-dox (CD) agar (g/l Na_2NO_3, 2; K_2HPO_4, 1; $MgSO_4$, 0.5; KCl, 0.5; $FeSO_4$, 0.01; Sucrose, 30; Agar, 15; pH 6.5) medium at 23 ± 2°C for 6-8 days and growth was observed after two days. Experiment was set up in triplicate to minimize the deviations.

Purification, identification and Maintenance of cultures

The fungi isolated on culture medium from soil were purified by spore suspension and streak method (Kathiresan et al. 2005). The cultures were routinely (every 6-8 days) transferred onto fresh Czapek's-dox agar plates by streaking. Before fungal cultures were used for inoculation of liquid growth medium, the fungus was subjected to three transfers on Czapek's-dox agar plates by Direct agar transfer method (Anonymous 1960). Identification of fungi was made by following Gilman 1967, Raper & Fennel 1965, Smith 1950, Subramanian 1975, Barnett and Hunter 1972. The pure cultures were maintained on Czapek's dox agar medium. The cultures were sealed by wax and kept at 4±1°C temperature in refrigerator.

Preparation of Biosorbent

Fungal biomass was cultivated in liquid medium using the shake flask method. This growth medium has the following composition (g/l): $FeSO_4$, 0.01; $MgSO_4.7H_2O$, 0.2; $CaCl_2.2H_2O$, 0.1; NaCl, 0.05; K_2HPO_4, 0.7; Na_2HPO_4, 0.1; $(NH_4)_2\ SO_4$, 0.5 and 1 ml of micronutrient solution [(%): $CuSO_4$, 0.7 mg; H_3BO_3, 0.5 mg; $CoSO_4$, 5 mg; Na_2MoO_4, 0.2 g; $MnSO_4.H_2O$, 0.5 g; $ZnSO_4$, 1.1 g] supplemented with malt extract (0.05%), designated as MMG. pH of the medium was adjusted to 5.0 using 1N HCl / 1N NaOH before autoclaving. Spores and mycelium from the Czapek's-dox spread plate cultures were transferred to 250 ml Erlenmeyer flasks containing 100 ml growth medium. Flasks were incubated at room temperature on a rotary shaker at 150 rpm for 5 days. The cultures grew as discrete pellicles. Harvesting of the biomass was done by filtering the cultured medium in the shake flask through a 150 µm glass microfiber filter. After harvesting, this is called as "live biomass".

Physicochemical Characterization of Effluent Waters

Samples of effluent wastewater were collected from the Electroplating Industry from a small-scale industry at Dhakran, Agra. Effluents used in this study were filtered with Whatman filter paper 44 (Himedia) to eliminate the suspended matter and then filtered with the 0.45µm nylon membrane filter (Millipore) after a partial lime treatment to pH 6 and then samples were kept at 4°C. Pollution load of effluent wastewater was estimated by measuring pH, electrical conductivity, dissolved oxygen and total dissolved solids of the effluent by Analytical Water Testing Kit (Systronics). The concentration of heavy metal in the effluent water was determined by atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 400).

Metal stock solutions

Effluents of wastewaters were taken as metal solutions. Solutions were adjusted to desired pH with 0.1 M NaOH and 0.1M HNO_3 by using digital pH meter (Hanna Instruments, Italy). Initial concentrations of metals were measured at the beginning of experiment with Atomic Absorption Spectrophotometer. All chemicals used are of analytical grade obtained from Hi-media Company.

Metal sorption experiment

Biosorption experiments were conducted in 125 ml Erlenmeyer flasks by inoculating 50 ml of electroplating industry effluent solution with 0.1 gm of *Aspergillus terreus* biomass on a rotary shaker (125 rpm) at temperature (25 ± 2°C). All the experiments were conducted in duplicate for quality control and mean values were used in the statistical analysis.

Effect of pH on Biosorption

pH values of effluents were adjusted to 2.0, 4.0 and 6.0 with 1 M NaOH and 0.1M HNO_3. Then the effluents were incubated with biomass (0.1 g) on a rotary shaker (125 rpm) at temperature (25 ± 2°C). The reaction mixture pH was not controlled after the initiation of the experiments. Sample was filtered with 0.45µm polycarbonate filter and analyzed for metal concentrations using atomic absorption spectrophotometer.

Effect of time on Biosorption

When biomass is placed in a solution of effluent containing metal ions and kept on shaker for an adequate time, biosorption of metal ions occurs. The metal ion concentration decreases from an initial value to an equilibrium value provided the contact time is sufficient. The time needed to reach equilibrium is defined as "equilibrium time". Batch kinetics studies were conducted to determine the equilibrium time for biosorption of copper at various pH values. Biomass (0.1 gm) was added to metal solutions (50 ml) on a rotary shaker. Samples were collected at 5, 10, 15, 20 min and filtered through 0.45µm polycarbonate filters and analyzed for dissolved metal concentrations.

Metal estimation

Aliquots were withdrawn, filtered and analyzed by Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 400) for final metal concentration and percent sorption was calculated.

Data evaluation

Adsorption Quantity Assay:
Metal sorbed by the tested cells (mg metal/g biosorbent) will be calculated (Dias et al. 2002) as:

(1) $$Q = \frac{[(Ci - Cf)]}{X}$$

Where: Q is the specific heavy metal uptake (mg metal/g biosorbent), Ci is the initial concentration of metal in the solution (mg/l), Cf is the final concentration of metal in the solution (mg/l) and X is the amount of the added biosorbent on the dry basis (g).

Sorption models were chosen for comparison with experimental data:

The Langmuir model,

$$Q = \frac{Q_{max} b C_f}{1 + b C_f}$$

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,

(2) $$\frac{1}{Q} = \frac{1}{Q_{max}}\left(\frac{1}{b} C_f + 1\right)$$

The Freundlich model,

$$Q = k C_f^{\frac{1}{n}}$$

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

Linearized Freundlich equation,

(3) $$LogQ = Logk + \frac{1}{n} LogC_f$$

Table 1: *Water Analysis results of Effluent wastewaters*

S.No.	Parameters	Standard Max. Acceptable Limit*	Electroplating Wastewater
1.	pH	6.5 to 8.5	1.2
2.	TDS (ppm)	2000	1,83,800
3.	Conductivity (mS/cm)	3.1	5.38
4.	Copper (mg/l)	3.0	22.57

*United States Environmental Protection Agency (USEPA, 1986)

Results & Discussion

Physicochemical Characterization of the Effluent Water

The results of the physicochemical analysis of the main polluting heavy metals found in the effluent water generated in the electroplating Industry at Agra are shown in Table 1.

The hydrogen ion level of water, as measured by the pH was very low from the recommended range of 6.5 to 8.5 by the CPCB and also the United States Environmental Protection Agency (USEPA, 1986). Water acidity is known to influence the solubility, availability and toxicity of metals in the aquatic ecosystems. Electrical conductivity (E.C) gives a measure of water conductivity as well as an indication of the level of inorganic constituents in water. Typical conductivity values recommended for surface water ranged from 50 to 1500 $mScm^{-1}$.

In Industrial effluent heavy metal analysis, electroplating industry found to contain 22.57 mg/l Cu^{2+} that is higher than the recommended limits of < 3.0 mg/l. Toxic heavy metal affects environment due to bioaccumulation.

From the obtained results, it was observed that the concentrations of copper heavy metal, total dissolved solids were higher than the levels permitted by the environmental legislation (Article 4 of CONAMA 20).

Metal sorption capacity of Microbes

The isolates from soil were identified as *Aspergillus terreus* (NCIM # 1202) by National Chemical Laboratory, Pune. These were used as biosorbents for Cu^{+2}. The biomass of *Aspergillus terreus* is seen to sorb 7.77 mg / l Cu^{+2} within one hour of experiment. The highest biosorption of Cu^{+2} was determined at 4.0 pH and temperature at 27 °C. Biomass show different metal removal capacities at different pH as shown in table 2.

Adsorption potential of *Aspergillus terreus* for copper metal ion is shown in Figure 1.

Effect of pH on Biosorption

The pH of medium affects the solubility of metals and the ionization state of the functional groups (carboxylate and phosphate groups) of the fungal cell wall (Tobin et al., 1994). It is reported that chitin, a cell wall component of the fungus, is also responsible for metal sorption. In all filamentous fungi, excluding *Oomycetes*, chitin (poly-n-acetylglucosamine) was found to be a ma-

Table 2: *Heavy metal removal capacities of biomass at different pH are shown*

Biomass	At pH 2	At pH 4	At pH 6
Aspergillus terreus	3.7%	77.7%	61.7%

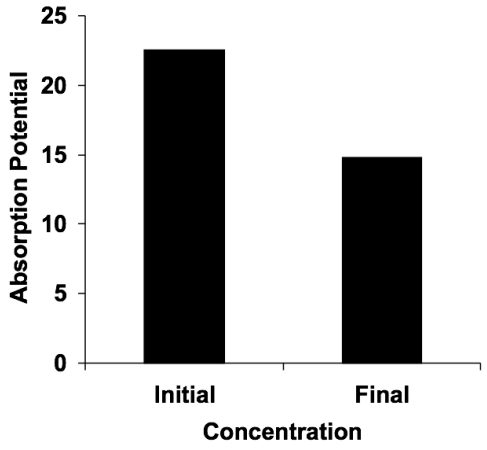

Figure 1: *Adsorption Potential of fungi* Aspergillus terreus *for* Cu^{2+} *metal.*

jor constituent of microfibril (Tzezos et al., 1982). Chitosan (deacetylated poly-N-acetyl glucosamine) is thought to be responsible for biosorption due to the nitrogen site of the chitosan amine group (Guibal et al., 1995). The carboxyl and phosphate groups carry negative charges that allow the fungal cell wall components to be potential scavengers of metal ions.

Little or no biosorption of Cu^{+2} metal ions was observed for pH less than 4.0 and almost no biosorption was observed below 2.0 pH. The heavy metal removal capacity increased very sharply with an increase in pH at 4.0 as shown in Table 3.

A sudden increase in sorption with a slight increase in pH is often referred to as an "absoroption edge". This effect of pH on adsorption of heavy metals has also been observed for activated carbon. (Reed et al. 1994, Huang et al. 1978). Beyond pH 4.0 removal of metal ions increased with pH. At higher pH values, the experiments were not conducted to avoid formation of solid Cu^{+2} hydroxides. The low metal ions removal at pH 3.0 has been attributed to the competition that metal ions face from hydrogen ions for the available biosorption sites (Huang et al. 1988). The data indicated that biosorption started to increase for samples with final pH value of 4. The results showed that pH is an important parameter affecting the biosorption of heavy metals. At low pH (3.0) heavy metal removal was inhibited, possibly as a result of a positive charge density on metal binding sites due to a high concentration of protons in solution. With an increase in pH, the negative charge density on the cell wall surface increases due to de-protonation of the metal binding sites and thus increases biosorption (Kapoor et al. 1999). Measurement of final pH represents that final pH is less than initial pH of solutions, therefore confirming the ion-exchange to be one of the biosorption mechanisms. Other studies with seaweed and fungal biomass have indicated ion-exchange as the dominant mechanism of biosorption (Ahuja et al. 1999, Fourest & Roux 1992, Schiewer & Volesky 1996, Volesky 2001).

Table 3. *Effect of initial values of pH on biosorption of* Cu^{2+}

C_i = 22.57 mg Cu^{2+} /l	
pH	Adsorption (mg Cu^{2+} /l)
2.0	0.37
4.0	7.77
6.0	6.17

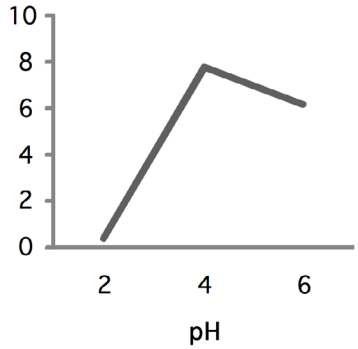

Figure 2: *Effect of pH on the biosorption of* Cu^{2+} *ions from aqueous solutions.*

Effect of time on biosorption

The changes in residual metal concentration with time at optimum pH values are shown in Figure 3. The copper adsorption capacity increased with the time during the first 15 min. and then decreased towards the equilibrium adsorption capacity. Adsorption rate was fast at high concentration of Cu^{2+}. The plot shows that kinetics of biosorption of heavy metal ions consisted of two phases; an initial rapid phase where biosorption was fast and contributed significantly to equilibrium uptake and a slower second phase whose contribution to the total metal biosorption was relatively small. The first phase of biosorption kinetics lasted for 30-45 min for pH 4.0 or more and was observed to last shorter for pH 2.0. This is due to the high complexation rate between the Cu^{2+} ions and metal complexing groups on the surface of biomass. Mass transfer limitations were overcome by the high driving force, which was the difference of Cu^{2+} concentration between the liquid and the biomass phases, in the case of high Cu^{2+} metal concentration.

Adsorption Quantity Assay

Aspergillus terreus shows adsorptive quantity of 77.7 mg Cu^{+2}/gm biomass within one hour.

Adsorption Isotherms

Equilibrium data, commonly known as adsorption isotherms, are basic requirements for the design of adsorption systems. Classical adsorption models (Langmuir and Freundlich) are used to describe the equilibrium between adsorbed metal ions on the fungal cell (*Q*eq) and metal ions in solution (*C*eq) at a constant temperature. The equilibrium established between adsorbed component on the biosorbent and un-adsorbed component in solution can be represented by adsorption isotherms. The most widely used isotherm equation for modeling equilibrium is the Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites which are homogeneously distributed over the sorbent surface.

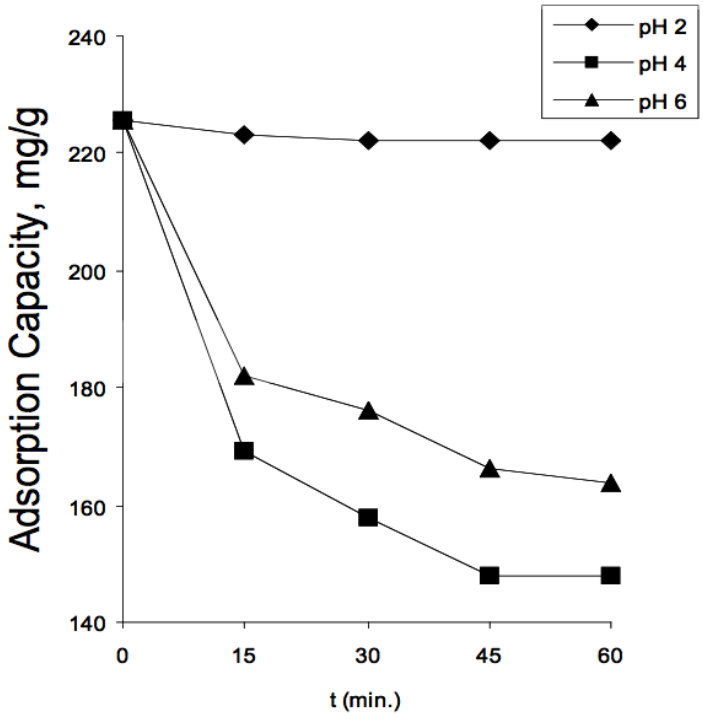

Figure 3: *Effect of time on Biosorption of Cu+2 from aqueous solutions.*

Langmuir constants, Q_{max} and *b* and correlation factor (R^2) are presented in Table 4. The agreement of the experimental data with the Langmuir model implied that monolayer adsorption existed for the experimental conditions used. The Langmuir model parameters obtained were statistically significant at all pH values studied at a confidence level of 95%. The values of Q_{max} for copper appear to be significantly higher at pH 4. This shows high adsorption capacity at this pH for the fungi.

The Freundlich expression is an empirical equation based on sorption on a heterogeneous surface suggesting that binding sites are not equivalent and/or independent. The mechanism rate of adsorption is reported to be the function of 1/n and K were calculated from the Freundlich plot for biomass. K and 1/n are the measure of sorption capacity and intensity of sorption, respectively. In general, n values are less than unity indicating that the surface of the adsorbent is heterogeneous in nature.

The Freundlich parameters estimated were significant at all pH values studied at a confidence level of 95%. Sorption data of copper sorption system followed the Langmuir adsorption model with high coefficient of determination than Freundlich adsorption model for biomass concluded the surface sorption is dominant is sorption process.

Conclusion

The present study evaluated the removal of copper heavy metals from aqueous wastewater solutions using non-pathogenic fungus *Aspergillus terreus* isolated from Agra soil. A wastewater with a pH of 3.0 or above can be effectively treated for metal ion removal and the wastewater with pH lower than 3.0 would need pH adjustment. This could be explained by the increase in density of the negative charge on the cell surface, causing proton removal on the cell bonding sites, thereby increasing its biosorption capacity. The fungal biomass does not sorb ions such as Ca^{+2}, Mg^{+2} and K+ (Tobin et al. 1984). Heavy metal removal by ion-exchange resins is sensitive to the presence of Ca^{+2}, Mg^{+2} and K+ ions. Thus use of fungal biomass may be advantageous over the ion-exchange resins when Ca^{+2}, Mg^{+2} and K+ are present in wastewater at high concentrations. These biosorbed ions can be eluted and the biomass could be regenerated and used again. Thus, it represents an easy and cost effective technology for the abatement of pollution. The equilibrium sorption data are satisfactorily fitted with Freundlich and Langmuir equations. The calculated values of the dimensionless separation factor from the Langmuir constant confirm favorable sorption of Cu^{+2} onto *Aspergillus terreus*.

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Table 4: *Linear regression data for Langmuir and Freundlich isotherms for Cu^{+2} by Aspergillus terreus*

pH	Langmuir parameters			Freundlich parameters		
	Q max	b(x100)	R^2	k	n	R^2
at pH 2	9.52	1.05	1.05	$1.00e^{+104}$	0.013	0.9997
at pH 4	19.455	8.96	0.9882	$4.44e^{+4}$	0.4247	0.9969
at pH 6	12.05	7.54	0.9897	$6.054e^{+5}$	0.3045	0.9975

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Discussion with Reviewers

Anonymous Reviewer: Since heavy metals are toxic to fungi, is it not advisable to check the viability of the biomass during experimental runs?

Varshney, Bhadauria & Gaur: Yes, we have checked the viability of biomass cells by determining INT dehydrogenase activity after desorption of cells which showed cells subjected to copper metal exhibited 86% of their initial activity.

Anonymous Reviewer: Could the second slow phase in the adsorption time course shown in Fig 3 be due to this toxicity rather than to saturation effects?

Varshney, Bhadauria & Gaur: As the metal concentration is not toxic to the biomass cells, this slow phase is due to the saturation of biosorption.

Anonymous Reviewer: What is your explanation of why the biosorption of copper from aqueous solutions is significantly higher at pH2 than at pH4 shown in Figure 2, as this contradicts the adsorption data from wastewater? Also, what is the correct value of "k" at pH2 in Table 4?

Varshney, Bhadauria & Gaur: Yes, you are right. There is graphical error in Figure 2. The corrected graph has been included in the revised manuscript. The correct value of k at pH 2 is $1.00e^{+104}$. ■