

Biosorption of Cu²⁺ from aqueous solutions by pretreated *Cladosporium sp.*

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Abstract: In this paper, adsorption properties of a pretreated of *Cladosporium sp.* for Cu²⁺ were studied. The fungi pretreated with some chemical reagents exhibited higher Cu²⁺ removal capacities than native biomass. The optimum chemical reagent was 0.2M NaOH. After 0.2M NaOH pretreatment, optimum conditions of biosorption were found to be pH 5.0, temperature 35°C, and stirring speed of 100rpm. Equilibrium isotherms were obtained from adsorption experiments and the biosorption maximum capacity obtained was at 28.31mg/g. The biosorbed metal ions were effectively eluted by 0.05M HNO₃ solution. After eluting, the biosorbed metal ions biomass was regenerated by washing with deionized water and then contacted with a solution containing 0.1M of Ca²⁺, Mg²⁺ ions before further adsorption tests. The pretreated fungi biomass could be used for three cycles: biosorption, elution of biosorbed ion, regeneration of biomass.

Key words: Biosorption, *Cladosporium sp.*, Mycelial pellets, Pretreated, Cu²⁺.

Introduction

Biosorption using the biomass of microorganisms is an effective and economic technology for the removal and recovery of Cu²⁺ and other heavy metal ions from wastewater streams. Different types of biomass have been investigated for the biosorption properties of Cu²⁺ and other heavy metal ions. These include bacteria (Fry *et al.*, 1992), yeasts (Stoll and Duncan, 1996), bark (Freer *et al.*, 1989), peat moss (Chaney and Hundemann, 1979), fungi (Tobin *et al.*, 1984; Fourest *et al.*, 1992; Kapoor *et al.*, 1999) and marine algae (Volesky, 1994; Matheickal and Yu, 1996). Many of these materials are available in large quantities either as industrial waste by-products or from the culture.

However the fungal biomass has been mostly used in the native forms, which generally are difficult to separate from aqueous solutions. They have low mechanical strength and biological stability. Many scholars utilized immobilized cells to remove heavy metals (Nestle and Kimmich, 1996; Spinti *et al.*, 1995). However, little is reported about fungi on its pretreated mycelial pellet.

In the present work, we have studied the effect of pretreated mycelial pellet with some chemical reagents on uptake capacity of Cu²⁺. The effect of the experimental parameters on biosorption capacity of the biomass was studied as well as adsorption isotherms of Cu²⁺ on the pretreated biomass with the Langmuir model. Elution of biosorbed heavy metal from pretreated biomass and regeneration of pretreated biomass were also examined.

Materials and Methods

Mycelial pellets and pretreatment: *Cladosporium sp.* was isolated from electroplating sludge and wastewater in Wenzhou's galvanization factory. All mycelial pellets used in this study were obtained from cultivation using Czapek's medium and incubated at 30°C on a rotary shaker (100rpm) for

2 days. The mycelial pellets were harvested after cultivation and then used in the following pretreatment.

5g (wet weight) *Cladosporium sp.* (biomass sample) was pretreated respectively with 50ml dilute acids (HCl: 0.05, 0.10, 0.20, 0.50 mol/l) or alkali (NaOH: 0.05, 0.10, 0.20, 0.50 mol/l) or salt (CaCl₂: 0.05, 0.10, 0.20, 0.50 mol/l) for 40min or heat pretreatment for 5min. Pretreated biomass was then washed with generous amounts of deionized water till the pH of the wash solution was near to neutral range (7.0-7.2).

Adsorption experiment: All adsorption properties for pretreated biomass were measured with standard equilibrium experiment. A series of vials contained 5g of biomass and 100ml of heavy metal solutions of known concentration and the contents were shaken at 20°C for 4hr in a rotating shaker (100rpm). After experiment, mycelial pellets were filtered through gauze, and the supernatant liquid was used for metal analysis, by atomic absorption spectrometer.

Experimental parameters: In order to evaluate the effect of pH, temperature and speed of shakerbed on metal uptake, pH of the solution was adjusted to be in the range between 2.5 and 7.5 before mixing biomass. pH was adjusted to the required value with 0.1M HNO₃ or 0.1M NaOH. Experiments were performed at 20°C-40°C. The speed of shakerbed ranged between 50rpm to 250rpm.

Adsorption isotherms: In order to obtain the sorption kinetics data, the metal uptake value (q) was calculated using the following equation:

$$q \text{ (mg metal/g of biomass)} = V (C_i - C_f) / 1000m,$$

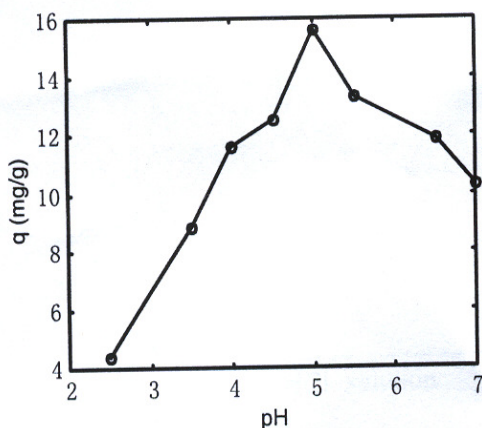
where V is the volume of metal solution (ml), C_i and C_f are the initial and final concentration of metal respectively, and m is the mass of the fungi.

The Langmuir sorption model was chosen for estimation of the maximum metal uptake (q_{max}):

$$q = q_{\max} b C_e / (1 + b C_e),$$

Table – 1: Adsorption capacity of native and pretreated *Cladosporium sp.*

Pretreated methods	Concentration (mol/l)	Biosorption capacity (mg/g)
No pretreatment		4.14
Pretreated by NaOH solution for 40min	0.05	12.05
	0.10	14.62
	0.20	15.64
	0.50	13.48
Pretreated by HCl solution for 40min	0.05	6.89
	0.10	5.88
	0.20	7.15
	0.50	7.03
Pretreated by CaCl ₂ solution for 40min	0.05	8.70
	0.10	12.37
	0.20	6.33
	0.50	4.42
Heated up to 80 °C for 5min		6.75

**Fig. 1:** Effect of pH on Cu uptake by pretreated *Cladosporium sp.*

where b is the Langmuir constant, q_{\max} the maximum metal uptake and C_e residual concentration at equilibrium.

Elution of metal ions biosorbed by pretreated biomass and regeneration of pretreated biomass: The metal loaded pretreated biomass after biosorption was contacted with 25ml of the elutants deionized water, 0.05N HNO₃, 0.1M CaCl₂, 0.1M MgSO₄, 0.1N Na₂CO₃, 0.1N NaHCO₃ and 0.1N NH₄Cl for one hour on a rotary shaker (100rpm) to study the removal of biosorbed metal ions. Metal concentrations were determined after separating the biomass from eluting agents by filtration through a 0.45 μ m polycarbonate filter. 0.05N HNO₃ solution was used to elute metal ions from pretreated biomass in subsequent experiments to study the reuse of biomass in biosorption. Use of 0.05N HNO₃ solution as an elutant deposits H⁺ ions on the biomass surface. Excessive amounts of H⁺ ions

could reduce the metal biosorption capacity of biomass. Therefore, reuse of the fungal biomass in biosorption after elution of biosorbed metal ions would require H⁺ ions to be removed from the biomass. Washing the biomass with deionized water could also remove H⁺ ions. Two methods were studied to regenerate the biomass after eluting the biosorbed metal ions. The first regeneration method involved washing the biomass with deionized water till the pH of the wash solution was in the range of 5.0-5.4. In the second regeneration method, biomass was washed by deionized water till the pH of the wash solution was in the range of 5.0-5.4, and then shaken in a solution containing 0.01M of Ca²⁺, Mg²⁺ for 30 min at 100rpm. The biomass regenerated by these two methods was air-dried and its ability to biosorb metal ions was examined. The biosorption-elution of biosorbed metal ion-regeneration of biomass cycle was repeated three times.

Results and Discussion

Effects of biomass pretreatment: The comparisons for Cu²⁺ adsorption capacities of native *Cladosporium sp.* with pretreated *Cladosporium sp.* were shown in Table 1. It showed that all kinds of chemical pretreatment to fungi were beneficial to increase the adsorption ability on Cu²⁺. Especially, the biomass pretreated with NaOH solution has the maximum adsorption capacity, and the optimal concentration of NaOH solution is 0.2M. The reason could be that alkali pretreatment could remove the amorphous polysaccharide on the cell wall and change the structure of the dextran and chitin, so the biomass could adsorb much more Cu²⁺ on its surface. At the same time, NaOH could dissolve the inclusions in the cell which encumber adsorption, and expose much more active binding sites to improve the adsorption capacity. Furthermore NaOH makes H⁺ to be dissociated from cell wall, resulting in the increase of negative functional groups, then the improvement of the adsorption capacity. In the following experiments the biomass pretreated with 0.2M NaOH were used.

Effect of solution pH on Cu²⁺ biosorption: The results as depicted in Fig.1, clearly showed that the uptake of Cu²⁺ by biosorbent was significantly affected by the initial pH of the solution, which accorded with the results reported by others (Fourest and Roux, 1992; Matheickal and Yu, 1996). From this figure, with the increase in pH the removal of Cu²⁺ increases up to pH 5.0 and decreases when pH value was over 5.0. It shows the optimum pH value for uptake of Cu²⁺ was 5.0. The pH dependence of heavy metal uptake could be largely related to the various functional groups on the cell surface and also on the metal solution chemistry. The functional groups involved in heavy metal biosorption were generally weak acids, such as the carboxylic groups (Fourest and Roux, 1992), which were protonated at low pH values. At low pH values, the concentration of hydrogen ions was also high, so that hydrogen ions effectively competed for binding sites, resulting in a reduced uptake capacity. As the pH increased, more functional groups were dissociated and became available for Cu²⁺ binding, but as the pH increased further, Cu²⁺ began to deposit and refused to be absorbed.

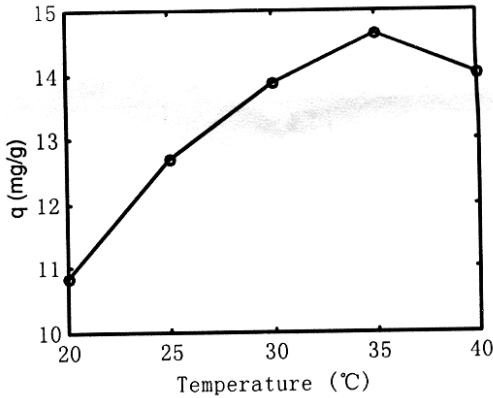


Fig. 2: Effect of temperature on Cu uptake by pretreated *Cladosporium sp.*

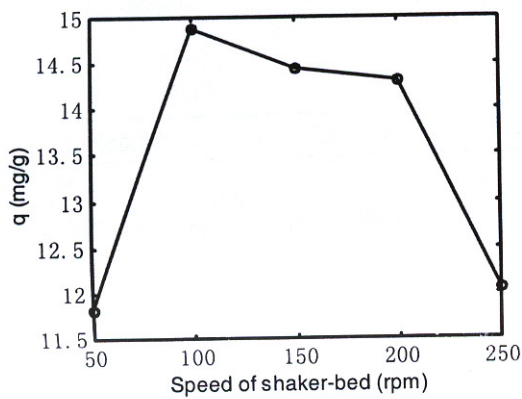


Fig. 3: Effect of speed of shaker-bed on Cu uptake by pretreated *Cladosporium sp.*

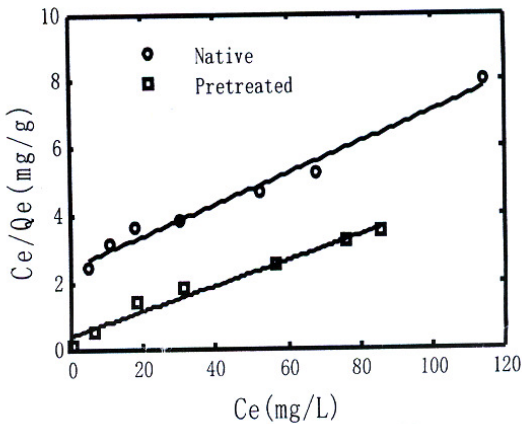


Fig. 4: Cu-sorption isotherms for *Cladosporium sp.*

Effect of temperature on heavy metal uptake: The effect of changes in the temperature on the metal uptake was shown in Fig. 2. When temperature was lower than 35°C, Cu²⁺ uptake increased with increasing temperature, but when temperature was over 35°C, the results were on the contrary. This response suggested a different interaction between the ligands on the cell wall and the metal. The fungi pretreated with low

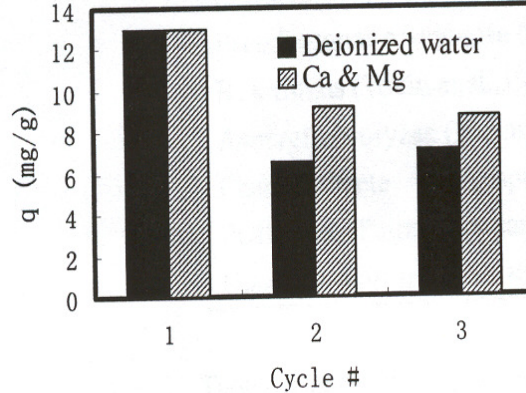


Fig. 5: Reuse of regenerated biomass Cu²⁺ biosorption.

concentration alkali were still active. Below 35°C, chemical adsorption mechanisms played a dominant role in the whole adsorption process, adsorption was expected to increase by increase in the temperature (Sag *et al.*, 1995). While at higher temperature, the fungi were in a nonliving state, and physical adsorption became the main process. Physical adsorption reactions were normally exothermic, thus the extent of adsorption generally decreased with further increasing temperature.

Effect of speed of shaker-bed on heavy metal uptake: The influence of the stirring speed on the biosorption capacity of the biomass showed that an optimum value was obtained at 100rpm (Fig. 3). At stirring speeds lower than 100rpm, the biosorption capacity for Cu²⁺ increases with increasing speed of shakerbed. The reason may be that at lower speed Cu²⁺ in the solution was restrained from diffusion, and took more time to reach equilibrium. For higher speeds the vortex phenomenon was encountered. This resulted in desorption of Cu²⁺ in the solution to some extent (Li Wei and wuJian, 2000).

Uptake of metal by native biomass and pretreated biomass: Cu²⁺ biosorption isotherms for *Cladosporium sp.* were depicted in Fig.4. Pretreated biomass demonstrated higher metal uptake than native biomass. A Langmuir sorption model was used to evaluate the sorption behavior of native and pretreated cells. The Langmuir parameters were summarized in Table 2. Metal uptakes at equilibrium residual concentrations of 10mg/l (q₁₀) were selected for comparison with other biosorbents. The q₁₀ was calculated from the Langmuir model. The Langmuir constants (b, l/mg) related to the affinity of the sorbent material for the metal sorbate were also shown in Table 2. When Cu²⁺ uptake by *Cladosporium sp.* was compared to the metal uptake by *Aspergillus* reported by other authors, it could be seen that the *Cladosporium sp.* was a better biosorbent. A q₁₀=13.19mg/g for *Cladosporium sp.* pretreated and q₁₀=3.44mg/g for *Cladosporium sp.* native biomass were calculated. The q₁₀ reported for Cu²⁺ biosorption by *Cladosporium sp.* (native biomass), under similar experimental conditions, was 3 mg/g (Volesky B., 1994). The q₁₀ value for Cu²⁺ uptake by *Cladosporium sp.* pretreated biomass was higher than the q₁₀

Table – 2: Langmuir parameters for Cu uptake by native and pretreated biomass of *Cladosporium sp.*

Biomass type	q ₁₀ (mg/g) ^a	q _{max} (mg/g)	b(l/mg)	r ²
Native biomass	3.44	21.48	0.019	0.98
Pretreated biomass	13.19	26.31	0.10	0.97

^aq₁₀: meattal uptake at the residual concentration of 10 mg/l; r: correlation coefficient.

Table – 3: Uptake capacities (Q_{max}, mg/g) for Cu²⁺ of various adsorbents.

Adsorbent	Q _{max}
Lignite (Allen and Brown, 1995)	6.35
S. fluitans (Kratochvil <i>et al.</i> , 1995)	60.96
Pseudomonas aeruginosa (Chang <i>et al.</i> , 1997)	19.05
R. arrhizus (Tobin <i>et al.</i> , 1984)	15.88
Aspergillus oryzae (Huang <i>et al.</i> , 1991)	4.45
Phanerochaete chrysosporium (Ridvan Say <i>et al.</i> , 2001)	26.55
Padina sp. (Kaewsarn, 2002)	50.80
Cladosporium sp. (This study)	26.31

Table – 4: The elution of biosorbed metals by various chemical reagents.

Chemical reagent	Recovery of biosorbed Cu (%)
Deionized water	11.2
0.05NHNO ₃	98.5
0.1M Ca ²⁺	50.1
0.1M Mg ²⁺	60.4
0.1N Na ₂ CO ₃	43.2
0.1N NaHCO ₃	41.0
0.1N NH ₄ Cl	19.8

value reported for fungal biomass (q₁₀=8mg/g for *Penicillium chrysogenum*) and wood sorbents (q₁₀=7mg/g for carboxylated sawdust) (Volesky and Holan, 1995).

In addition, the maximum adsorption capacities of the fungal biomass for Cu²⁺ were compared with those of other adsorbents, reported in literature as listed in Table 3, the adsorption capacity of *Cladosporium sp.* was relatively high when compared with other adsorbents.

Elution of biosorbed heavy metal from pretreated biomass and regeneration of pretreated biomass: The biosorbed metal ions were eluted from pretreated biomass using various elutants. Table 3 compared the removal efficiency of various chemicals in eluting biosorbed metal ions. Deionized water was able to elute biosorbed metal ions only to a limited extent, indicating the strong affinity that biomass possesses towards the metal ions. 0.05M nitric acid solution was able to effectively elute the biosorbed metal ions. The release of calcium, magnesium and potassium ions was observed during the biosorption of metal ions (Kapoor and Viraraghavan, 1997) indicating that ion exchange played an important role in

biosorption of metal ions. Table 4 also showed that Ca²⁺ and Mg²⁺ ions in solutions at a concentration of 0.1M were able to elute heavy metal ions, possibly due to ion exchange reactions. Other elutants tested were not as effective as nitric acid solution. The use of fungal biomass as a potential biosorbent depended not only on the biosorptive capacity, but also on how well the biomass could be reused. The biomass was used for three cycles of biosorption elution of biosorbed metal ions, regeneration of biomass to study the changes in metal biosorption with subsequent usage. Fig.5 showed that biomass lost a portion of its Cu²⁺ biosorption capacity after cycle #1, but was able to retain the residual Cu²⁺ biosorption for another two cycles. The biomass regenerated with deionized water and then contacted with a solution containing 0.01M Ca²⁺, Mg²⁺ ions exhibited higher metal biosorption capacity than the one regenerated using deionized water alone. These observations indicated that calcium, magnesium, lost from biomass as a result of biosorption and elution of biosorbed metals by 0.05M nitric acid, if replenished, was helpful in restoring the metal biosorption capacity of the biomass to a certain extent.

The present study evaluated the removal of Cu²⁺ from aqueous solutions using pretreated *Cladosporium sp.*. The effects on biosorption capacity of pH, temperature, speed of shakerbed have been examined. Biosorption of heavy metal on *Cladosporium sp.* was sensitive to pH. The pretreatment of biomass by 0.2M NaOH solution for 40min resulted in a significant improvement in Cu²⁺ removal in comparison with native biomass.

The fungal biomass didn't sorb ions such Ca²⁺, Mg²⁺ (Tobin *et al.*, 1984). Heavy metal removal by ion-exchange resins was sensitive to the presence of Ca²⁺, Mg²⁺ ions. Thus use of fungal biomass may be advantageous over the ion-

exchange resins when Ca²⁺, Mg²⁺ ions are present in wastewater at high concentrations. *Cladosporium* sp biomass was superior to activated carbon (F-400) in Cu²⁺ removal. Biosorbed Cu²⁺ metal ions were easily eluted and the biomass could be regenerated and used again. This research showed that pretreated fungal biosorption had a potential to be used in the removal of heavy metal ions from wastewaters.

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