

Biosorption of Copper (II) by *Aspergillus flavus* (ED4)

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Abstract: *The biosorption ability of Cu (II) using Aspergillus flavus and initial biomass were also studied. Results showed that the biosorption process was strongly affected by the pH value and initial concentration for both the metals. The optimum pH value for Cu (II) was observed in the range of 5. Maximum absorption was observed for Cu (II) when the initial concentration was maintained at 1mg ml⁻¹ and 29° C. Cu (II) shows increasing biosorption trend when the initial biomass concentration was increased from 1mg ml⁻¹ to 10 mg ml⁻¹. Desorption studies shows that Cu (II) were successfully recovered up to 80% using 0.1 N NaOH and 0.1 N HNO₃ respectively.*

Keywords: Biosorption, waste water, *Aspergillus flavus*, Cu (II)

1. Introduction

The electroplating process generate heavy metals like arsenic, cadmium, chromium, copper, lead, mercury, nickel, silver and zinc [1], [2], [3]. These metal which find many useful applications in our life, are very harmful if they are discharged into natural water resources and many pose finally a serious health hazard [4],[5],[6]. They can exert a harmful effects in many ways depending on environmental factors and metal species [7], [8], [9]. Copper like the majority heavy metals is toxic but it has been widely used in metallurgical and tanning industries. The toxic effects of copper on microorganisms are well documented and many reports exist of copper uptake by microorganism [10], [11], [12]. Heavy metal pollution is spreading throughout the world and also a major environmental concern in many countries. Industrial waste waters contain high loads of heavy metals and in order to avoid water pollution treatment is needed before disposal. The effluent removal of heavy metals from waste waters and industrial wastes still remains a major topic of present research [13].

Copper is a common useful metal but poses serious environmental problems as well. Undesired amounts of copper ions are released by several industries, e.g. dying, paper, petroleum, copper and brass plating [14]. The removal of copper from industrial waste waters is a problem of increasing concern that has been mostly solved by chemical and physical methods of treatment. Nevertheless, these processes are expensive in which some technological problems exist especially when applied to diluted metal solutions [15]. Therefore, the search for clean and competitive technologies is strongly recommended.

Heavy metals pollution is spreading throughout the world with the expansion of industrial activities, nickel and copper are known to be commonly used heavy metals [16] are released from industrial operations such as electroplating, steel manufacturing, wood preservation, tanning, glass manufacturing and chemical processing which are eventually

accumulating and circulating throughout the food chain posing a severe problems to the humans, animals and environment [17], [18]. Accumulation of such heavy metals in soils and environment may leads to reduction in soil fertility through their adverse impact on microbial communities which inhabit the soils. They are in the food chains are identified as causative agents for their toxic effects on plant and human health [19].

On the other hand, municipal and industrial sewage treatment plants releases toxic wastes, which may also persist in the sludges and by-products of these treatment plants which is a burden on the techno-economic feasibility of treatment process [20]. Conventional methods for heavy metal reduction such as chemical precipitation, coagulation, reverse osmosis, ion exchange and solvent extraction [21] often generates chemicals during the process and having inadequate efficiencies at lower concentration of metals. Cost effective technologies for the treatment of metal contaminated waste streams are desirable for the removal processes [22]. Alternative metal removal or recovery methods such as biological origin are being considered as they are economically viable when it's come from nature or any waste material [23].

Microorganisms such as bacteria, actinomycete, algae, fungi and yeasts have the ability to accumulate heavy metals from the toxic environment. Among recovery methods, heavy metal reduction using biosorption gained a lot of attention during the past decade. Biosorption is process which involves heavy metal binding to living and non-living biomass from an aqueous solution [24]. Biosorption uses various inexpensive dry biomasses for the reduction of heavy metals from the industrial effluents. The biomass used for biosorption can be composed of algae, fungi, bacteria, and different plant species. Microbial biosorption technique offers promising alternative method for the treatment of industrial effluents, mainly because of its low cost and high metal binding capacity in the toxic environment.

The speciation, transport, behavior and crucial fate of heavy metals in instinctive ecosystems are mainly depending on the sorption ability to the surface functional groups of microbial communities [25]. The different types of biomass which was capable of accumulating heavy metal ions was efficiently studied [26]. Among the different microbial populations, fungal biomass was found to be the efficient biosorbents utilized for heavy metal removal and detoxification in recent experimental studies [27]. In order to identify and study the complex process of biosorption, extensive studies targeting cell surface architecture, chemical functional group identification, and acid–base characteristics of the biomass are equally important for predicting metal biosorption behavior.

2. Materials and Methods

2.1 Isolation of Metal Resistant Fungi

Effluent samples were collected from Wise park industrial area, Palakkad and the samples were serially diluted upto 10^9 dilutions using sterile saline and the diluted samples are plated on the sterile potato dextrose agar (PDA) plates amended with Potassium dichromate (1mg ml^{-1}) and copper sulphate (1mg ml^{-1}) using spread plate method[28]. The plates were incubated at 27°C for 4 to 7 days. Plates were examined and different isolates were further purified by repeated single colony isolation. The fungal isolates were identified using cultural morphology, cellular morphology and biochemical tests. Cultural morphology to determine the colony colour, shape and texture was studied on PDA medium [29].

2.2 Copper solution and AAS Measurements

Stock solution of Cu (II) was prepared by dissolving an appropriate amount (1000mg/L concentration) of analytical grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in double distilled water and other concentrations were prepared by dilution of the stock solution. At the end of each experiment, the metal containing solution was filtered through a $0.45\mu\text{m}$ cellulose acetate membrane and residual heavy metal concentration in solution was measured using an Atomic Adsorption Spectrometry (AAS 240). All tests were carried out in triplicates and a blank sample, replacing the biosorbent with deionized water, was also measured [30].

2.3 Preparation of Fungal Biosorbents

For biosorption study, the metal resistant fungi was inoculated in potato dextrose broth and incubated for 4 to 7 days at 27°C . [31]. After incubation, the completely grown fungi were killed by adding 0.5 N NaOH in a conical flask containing the fungi mat and kept in a water bath for 15 minutes. The mat was washed with distilled water for about 6-7 times till the pH reaches at 7. The mat was then transferred to a sterile Petri dish and placed in hot air oven for 24 hours. The dried dead fungus was powdered using mortar and pestle and stored in sterile container for further study. [32].

2.4 Determination of Biosorption percentage

The filtered solutions and the concentrations of metal ions were measured on an AAS 240 atomic absorption spectrophotometer. The sorption capacity (Q) (mg/g) of the metal ion was calculated from Equation (1),

$$Q = (C_i - C_f) V/W$$

where C_i is the initial concentration of metal ion (mg/L), C_f is the final concentration of metal ion (mg/L), V is the volume of metal ion solution (L), and W is the weight of the fungal biosorbents (g) used. [33].

2.5 Metal Biosorption Experiments

All the experiments (except the effect of temperature on biosorption efficiency) were conducted with a volume of 50 ml and at a constant temperature of $35 \pm 2^\circ\text{C}$ [31]. To study the biosorption ability, the dead fungal biomass (1 mg ml^{-1}) was mixed with a solution containing 0.1 mg ml^{-1} of Cu (II) separately at pH 2.0 at $35 \pm 2^\circ\text{C}$ for 24 hours and the most promising fungal biomass was selected and used for further optimization studies.

To study the effect of pH on the biosorption of Cu (II) was performed by varying the initial pH of the metal solution i.e. 1 to 10 using 0.1 N HCl or 1 N NaOH . In order to determine the optimum incubation time for biosorption of Cu (II) ions, different flasks containing the metal solution of 1 mg ml^{-1} with biomass concentration of 1mg ml^{-1} was incubated separately at different time intervals (3, 6, 9, 12, 15, 18, 21 and 24 hours) at 35°C . Effect of temperature on the biosorption of Cu (II) was analyzed using five different temperatures viz., 25°C , 27°C , 29°C , 31°C and 35°C and incubated for 18 hours. In order to determine the effect of initial Cu (II) concentration on biosorption ability, metal solutions containing various concentration of 0.1 to 10 mg ml^{-1} were used and the effect of initial biomass concentration on the Cu (II) removal were also studied by amending the fungal biomass in the metal (0.1 mg ml^{-1}) containing solution at a concentration from 1 to 10 mg ml^{-1} . The flasks were kept for agitation on a shaker at 120 rpm. The solution was sampled at regular intervals, filtered and the Cu (II) concentration of the filtrate was analyzed for the residual concentrations of the metal.

2.6 Desorption Studies

The regeneration of the biosorbent may be crucially important for keeping the process cost down. The desorption process should yield the metal in a concentrated form, restore the biosorbent close to the original state for effective reuse with undiminished metal uptake and physical changes or damages to the biosorbent [34]. The biomass was loaded with Cu (II) was treated with 0.1 N NaOH and 0.1 N HNO_3 at room temperature (27°C) with constant shaking for 2 hours [35]. The concentration of released metals was determined as described above.

3. Results and Discussion

A total of 8 different fungi were isolated from the effluent sample as metal resistant fungi for Cu (II). Among the 8 different fungi, *Aspergillus flavus* (ED4) dead biomass shows maximum biosorption efficiency which was chosen for further optimization studies. Based on the morphological characteristics the isolated of fungal isolates were identified as *Aspergillus flavus* (ED 4) by using A Manual of Soil Fungi [29]. The effects of different parameters like pH, incubation time, temperature on the biosorption efficiency of and Cu (II) ions using *Aspergillus flavus* (ED4) dead biomass was studied.

3.1 Effect of pH

The effect of pH on the removal of Cu (II) from aqueous solutions is shown in Fig. 1. The sorption ability was clearly affected by pH, the amount of metal removed increasing with decreasing pH. The maximum uptakes for Cu (II) was 24.6 mg/g biosorbents at around pH 5.0. Heavy metal ions biosorption onto nonspecific and specific biosorbents is pH-dependent [36]. It is observed that variations in the pH could affects the characteristics and availability of metal ions in solution as well as the chemical nature of the functional groups responsible for the biosorption process [37]. Optimum biosorption of Cu (II) was observed at pH value of 5 shows higher biosorption ability. The cell wall is made up of several components such as carboxyl, carbonyl, alcoholic and amino groups which determines the biosorption ability based on its protonation or unprotonation nature. The increase in sorption capacity with pH could be due to metal speciation as well as to the degree of ionization of the active groups of the fungal biomass. Earlier studies shows pH value has been observed as one of the important parameters which controls the heavy metal biosorption [38], [39], [40].

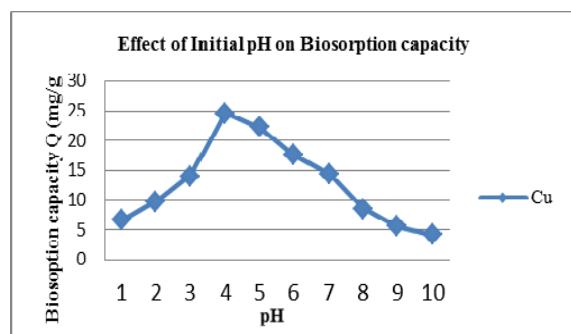


Figure 1: Effect of Initial pH on Biosorption capacity

3.2 Effect of incubation time

To study the optimal incubation time for the removal of Cu (II), biomass samples with metal solutions were incubated at different time intervals. The kinetics of adsorption describing the contact time in the removal of Cu (II) is one of the characteristics defining efficiency of biosorption rate. The effect of the contact time (6 to 24 h) in the biosorption of Cu (II) by *Aspergillus flavus* is shown in Fig. 2.

The biosorption capacity of the biosorbent for the maximum removal of metallic species occurred in 18 h where the uptake was 25.2 mg /g for Cu (II). After this period, the equilibrium was reached, further adsorption was less

significant. Similar results were observed in many studies using different biosorbents on the uptake of different heavy using *Aspergillus versicolor* [41], *Rhizopus oligosporus* [42], *Tectona grandis* L.f [43] and olive stone waste [44].

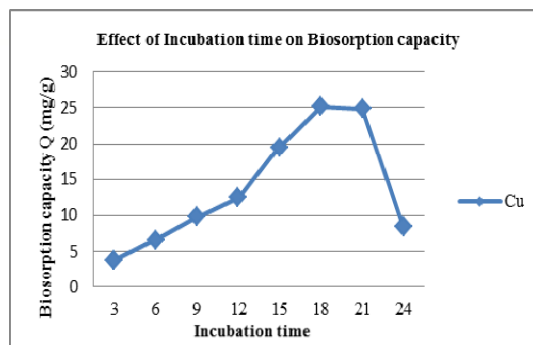


Figure 2: Effect of Incubation time on Biosorption capacity

3.3 Effect of temperature

Temperature plays a major role in biosorption of ions at 27°C given at Fig 3. The increase in temperature have improved the Cu (II) biosorption rate and decreased the contact time required for heavy metal removal [31]. The temperature of the adsorption medium considered to be an important for energy dependent mechanisms in metal removal using biosorbent. The biosorption ability for maximum removal of Cu (II) was found to be 26.2 mg/g, when the temperature was maintained at 31 °C in the adsorption medium. Temperature is known to affect the stability of the cell wall components, its configuration and cause ionization of chemical moieties. Energy-independent mechanisms are less likely to be affected due to temperature changes since the processes responsible for removal are largely physiochemical in nature [45]. Similar results have been reported in the bioaccumulation of Cu (II) by Cr (VI) by *S. equisimilis* and *Aspergillus sp.* [46], [47]. Fig.3

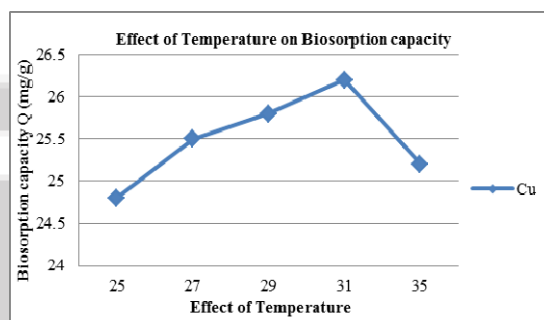


Figure 3: Effect of Temperature on Biosorption capacity

3.4 Effect of Initial Metal Ion Concentration

The metallic uptake as a function of the equilibrium concentration was studied by amending different initial metal concentrations on biosorption experiments using treated biomass. The rate of biosorption was decreased with increasing concentration of metal ions present in the solution as shown in the Fig 4. The maximum metal removal of 25.9 mg/g biosorbents was observed when the concentration of metal was maintained at 0.1 mg ml⁻¹ for Cu (II). It is important the metal sorption studies determine the capacity

of the sorbent [48] and metal concentration was considered as an important factor for effective biosorption process [49]. At lower concentration, the adsorption sites utilized the available metal more rapidly when compare to higher concentrations where the metal ions need to diffuse to the biomass surface by intraparticle diffusion [50].

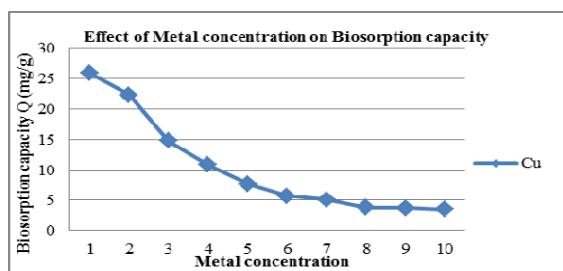


Figure 4: Effect of Metal concentration on Biosorption capacity

3.5 Effect of initial biomass concentration

The effect of biosorbent concentration on Cu (II) uptake *Aspergillus flavus* biomass is illustrated in Fig. 5. In view of the present results, it was clearly observed that the increasing the dose of biosorbent, the removal was increased. The increase in biosorbent concentration from 1 - 10 mg/L results in increase in the metal adsorption extensively. The increase of the adsorption surface area and the availability of free adsorption sites helps in removal of Cu (II). Maximum removal efficiency was observed at the biomass dosage concentration of 4 mg ml⁻¹ of biosorbent for Cu (II) of 26.27 mg/g. Further increase in biosorbent dosage concentration led to a decrease in the removal of Cu (II) ions. Similar reports available on this aspect also supported our results where increase in biomass concentration enhanced the amount of Cu (II) using *Cladonia rangiformis hoffm* [51].

The percentage desorption from Cu (II) adsorbed biomass was found to be 80% by using 0.1 N NaOH and 0.1 N HNO₃ respectively. Regeneration of biosorbent and heavy metals by desorption is a very important part in the application of the biosorption process which influences the cost of the whole process and also the possibility of metal recovering ability [52].

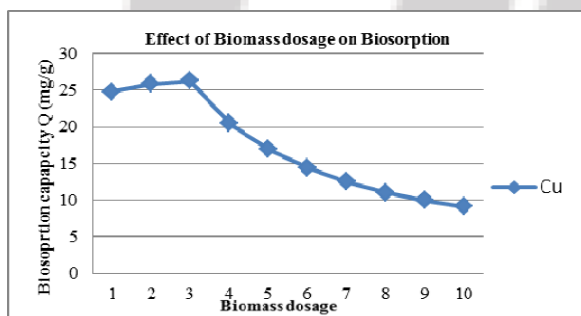


Figure 5: Effect of Biomass dosage on Biosorption capacity

4. Conclusion

In the present study, the biosorption ability of Cu (II) using *Aspergillus flavus* biosorbent has been investigated. Based on the present findings, it can be concluded that *Aspergillus flavus* is a potential biosorbent for the removal of Cu (II)

from aqueous solution which is also an effective low-cost material. It is also concluded that, pH affects the adsorption capability of the metals on the biosorbent. Results of desorption study also confirmed that there is a possibility to remove metal from biomass and regenerate and reuse biosorbent again. The overall findings of our laboratory studies are encouraging and can be applied to industrial waste waters biosorption.

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