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# *Oscillatoria anguistissima*: A Promising $Cu^{2+}$ Biosorbent

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**Abstract.** *Oscillatoria anguistissima* rapidly adsorbs $Cu^{2+}$ from aqueous solution. The adsorption of $Cu^{2+}$ followed Freundlich Isotherm, and the amount of $Cu^{2+}$ removed from solution increased with increasing $Cu^{2+}$ concentration. The adsorption is pH dependent, and maximum $Cu^{2+}$ removal occurs at pH 5. Of the various pretreatments, HCl treatment of the biomass increased the capacity for $Cu^{2+}$ removal. Presence of $Mg^{2+}$ and $Ca^{2+}$ resulted in decline in the $Cu^{2+}$ adsorption capacity of *Oscillatoria* cells. This species could also effectively remove $Cu^{2+}$ from mine water containing 68.4 μg/ml of $Cu^{2+}$ at pH 3.45.

Microorganisms can selectively take up various heavy metal ions from aqueous systems and are, therefore, important in regulating environmental pollution.

These organisms remove metals mainly by three phenomena: (i) biosorption [13, 14, 29], (ii) extracellular precipitation [27], and (iii) binding by purified biopolymers [18]. Among these, the use of microbial cells [2, 6] as biosorbents for heavy metals offers a potential and cost-effective alternative to conventional methods for decontamination and/or recovery of heavy metals from a variety of industrial aqueous process streams.

Various types of microbial biomass including both photoautotrophs [7–10] (algae and cyanobacteria) and heterotrophs [2, 4] (bacteria and fungi) have been reported to possess these metal-binding properties.

There are a number of reports on the feasibility of developing technology for removal of metals from polluted water [24] with phototrophs. These phototrophs are generally less resistant/tolerant to heavy metals for growth, but can be grown cheaply on minimal nutritional medium without sugars. Recently, Alga SORB™ (Bio Recovery Systems, Las Cruces, USA), a potential algal biosorbent, has been developed with *Chlorella vulgaris* for waste water treatment. This could efficiently adsorb metals leading to decontamination [1].

The present work deals with copper biosorption by a filamentous, non-heterocystous cyanobacterium, *Oscillatoria anguistissima*. The biosorption capacities of treated and untreated biomass have been compared. The potential for its use in decontamination of mine effluent sample has also been examined.

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## Materials and Methods

**Organism and growth conditions.** *Oscillatoria anguistissima* culture was obtained from National Facility for Blue Green Algal Collections, (IARI, New Delhi, India). It was grown at 25° ± 2°C under 1100 lux light intensity in BG11 minimal medium [22]. The medium composition (g/L) is $NaNO_3$ 1.5, $K_2HPO_4$ 0.04, $MgSO_4 \cdot 7H_2O$ 0.075, $CaCl_2 \cdot 2H_2O$ 0.036, citric acid 0.006, ferric ammonium citrate 0.006, EDTA (disodium salt) 0.001, $Na_2CO_3$ 0.02, and trace metal mix 1 ml/L. The composition of trace metal mix in g/L is $H_3BO_3$ 2.86, $MnCl_2 \cdot 4H_2O$ 1.81, $ZnSO_4 \cdot 7H_2O$ 0.222, $Na_2MoO_4 \cdot 2H_2O$ 0.39, $CuSO_4 \cdot 5H_2O$ 0.079, $Cu(NO_3)_2 \cdot 6H_2O$ 0.0494.

The pH of the medium was adjusted to 7 ± 0.2. The filaments were harvested in exponential phase after 10 days of growth by centrifugation at 6000 rpm for 10 min. Thereafter, the filaments were washed thoroughly with deionized distilled water and then used for metal uptake experiments.

**Dry weight determination.** The dry weight of cells was determined by pelleting a known volume of cell suspension and drying the pellet at 80°C for 48 h until a constant weight was obtained.

**Preparation of treated cells.** (a) *High temperature treatment*. The cells equivalent to 4 mg dry wt were incubated at 60°C and 100°C in a water bath for 10 min and subsequently washed thoroughly by deionized water and collected by centrifugation.

(b) *Dilute alkali/acid treatment*. The cells equivalent to 4 mg dry wt were treated with 0.01 N NaOH for 10 min and subsequently centrifuged, repeatedly washed, and collected. Similarly, the cells were treated with 0.01 N HCl and used for metal adsorption studies.

All the chemicals used in media preparation and the metal salt ($CuSO_4 \cdot 5H_2O$) were of analytical grade (BDH), and the HCl used was supplied by Merck (Mumbai, India). The standard solution of $Cu^{2+}$ (1000 ppm) for atomic absorption measurements were obtained from National Physical Laboratory, New Delhi, India.

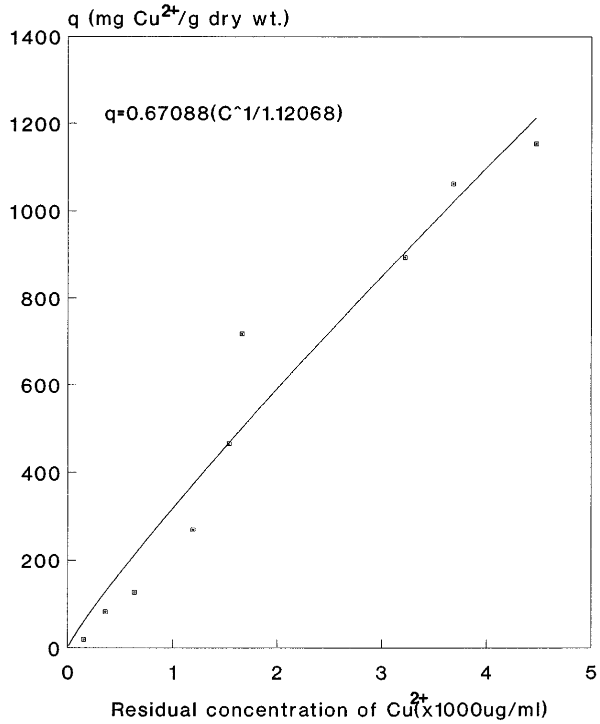


Fig. 1. Freundlich adsorption isotherm for copper at 0.08 mg dry wt/ml biomass concentration (pH 5.0).

**Analytical methods.** The desired concentrations of $Cu^{2+}$ (5 mg/L to 200 mg/L) were prepared by dissolving $CuSO_4 \cdot 5H_2O$ in deionized distilled water. Short-term metal uptake experiments were performed in 250-ml Erlenmeyer flasks containing 50 ml of $CuSO_4 \cdot 5H_2O$ solution of known initial concentration (pH 2–5) at 25°C for 2 h unless otherwise mentioned. In all experiments except for the effect of cell density, the biomass concentration was kept constant to 0.08 mg dry wt/ml. Metal-free and biomass-free blanks were used as controls. Biomass-free blanks were used to estimate the exact initial concentration (at 50 mg/L the exact initial concentration as estimated by Atomic Absorption measurements, Shimadzu AA-260 ranged from 48 to 51 mg/L) of $Cu^{2+}$ by dilution. Separation of biomass from metal-bearing solution was achieved through centrifugation at 6000 rpm for 10 min in Remi RC30 centrifuge. The supernatant was appropriately diluted and read for the remaining $Cu^{2+}$ content at 324.5 nm wavelength (Slit width 3.8A). All experiments were carried out in triplicate and repeated three times.

The metal uptake capacity in mg/g (q) was calculated from the initial concentration (Ci) and the final concentration (Cf) of the metal according to the following equation [28]:

$$q = V(Ci - Cf)/M$$

where V is the liquid sample volume and M is the biomass dry weight. The biosorptive metal uptake was evaluated and expressed by use of Freundlich adsorption model [5].

The general form of model is

$$q = kC^{1/n}$$

This can be linearized by taking natural logarithm in the form of $\ln q = \ln k + 1/n \ln C$.

where q = uptake of species (metal) and C = equilibrium (final/residual) concentration. The intercept lnk gives a measure of adsorbent capacity and the slope 1/n gives the intensity of adsorption.

## Results and Discussion

*Oscillatoria* sp. showed excellent copper biosorption (Figs. 1, 2), and the adsorption behavior of cells could be explained by the Freundlich isotherm model. A log-log plot between the amount of $Cu^{2+}$ in the solution and in the biomass followed a straight line with acceptable correlation coefficient ($r^2$). All previous studies of adsorption have been evaluated in terms of either Langmuir or Freundlich isotherms. The validity of the model was tested at the biomass concentrations viz biomass equivalent to 0.08 and 0.16 mg dry wt/ml. The high value of $r^2$, viz. 0.951 and 0.959 respectively, implies that the curve fits the data fairly well. The application of ‘‘t’’ test showed n to be highly significant for both biomass concentrations, but k was not significant.

*Oscillatoria* sp. could adsorb $Cu^{2+}$ even at lower concentrations, indicating a good affinity for the metal. The uptake was found to increase with the increase in concentration of $Cu^{2+}$. It could adsorb about 268.45 mg/g dry wt of $Cu^{2+}$ at a residual concentration of 23 mg/L, and it increased to about 1 g/g $Cu^{2+}$ adsorption at a residual concentration of 89.33 mg/L. *Oscillatoria* sp. showed a high sequestration of copper at lower equilibrium concentrations, as indicated by the steeper isotherm shown in Fig. 2, suggesting a strong binding for copper. Such high capacities for metal adsorption have been reported for few organisms [9, 16].

The effect of increasing concentration of biomass on $Cu^{2+}$ adsorption revealed that, although the amount of $Cu^{2+}$ adsorbed per unit dry weight decreased, the total amount of $Cu^{2+}$ adsorbed increased. *Oscillatoria* sp. could adsorb about 685 mg/g dry wt of $Cu^{2+}$ at 0.04 mg dry wt/ml of biomass, whereas the value decreased to 165 mg dry wt/ml at 0.2 mg dry wt/ml biomass density, but the percentage removal was greater in the latter (Table 1). This confirms the previous observations of adsorption in algae by Horikoshi et al. [10, 11] and Nakajima et al. [17]. Similar results have been obtained in a recent study [26] with *Arthrobacter* sp.; reduction in biomass concentration was shown to increase the specific uptake of copper. The dependence of adsorption on the cell density may be due to electrostatic interactions as more cations are adsorbed on the cell when the cell distances are great, as suggested by Itoh et al. [12].

The time course of uptake of $Cu^{2+}$ showed the adsorption to follow fast kinetics. It was found that 90% of adsorption was complete within the first 15 min of initial contact with the metal-bearing solution.

Adsorption of copper was found to increase with increase in temperature from 25°C to 45°C. *Oscillatoria* sp. showed maximum $Cu^{2+}$ adsorption at 45°C, 427.5

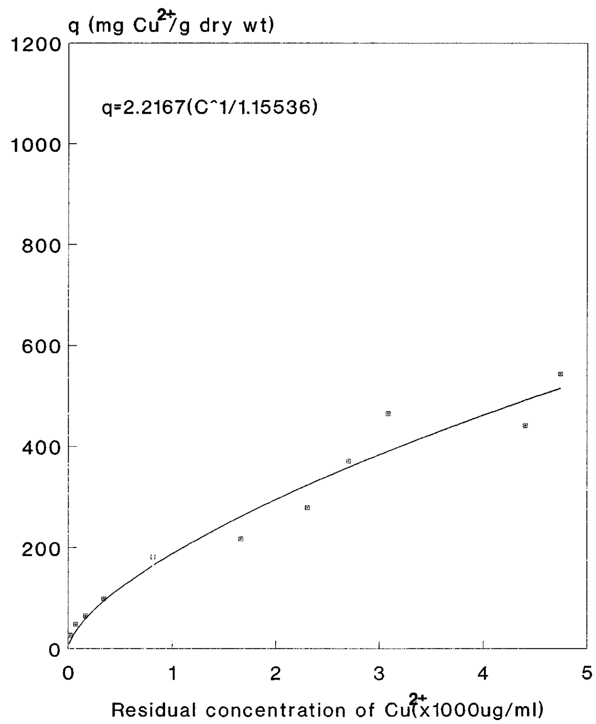


Fig. 2. Freundlich adsorption isotherm for copper at 0.16 mg dry wt/ml biomass concentration (pH 5.0).

Table 1. $Cu^{2+}$ biosorption at varying concentration of biomass by *Oscillatoria* sp.

| Biomass concentration (mg dry wt/ml) | mg $Cu^{2+}$/g dry wt |
|---|---|
| 0.04 | 685.0 ± 45.00 |
| 0.08 | 352.5 ± 13.20 |
| 0.12 | 234.9 ± 9.20 |
| 0.20 | 165.0 ± 8.70 |

pH 5.0; temperature, 25°C.

mg/g dry wt of $Cu^{2+}$, and minimum adsorption at 25°C. de Rome and Gadd [4] have also reported temperature-dependent $Cu^{2+}$ adsorption in filamentous fungi. Higher uptake capacities at increased temperature have also been reported for uranium by Shumate et al. [21], Strandberg et al. [23], and Tsezos and Volesky [25].

The adsorption of $Cu^{2+}$ was strongly affected by pH. The adsorption capacity of *Oscillatoria* sp. increased with increasing pH, to a maximum at pH 5.0 (327 ± 10.60 mg/g dry wt). Kuyucak and Volesky [14] also have similar reports in *Sargassum natans*, where they have found the optimum pH for biosorption of $Cu^{2+}$ to be 4–5. The experiments could not be conducted at higher pH values because of precipitation of copper hydroxide above pH 5.0.

At very low pH there is an increase in metal availability and mobility, but protons tend to compete [19] with the cations to bind to the sites, thus lowering the extent of biosorption.

The presence of some competing ions (particularly those present in hard water) generally affects the adsorption of heavy metal ions and can reduce the efficiency of metal removal [20]. The various groups implicated as participating in divalent metal ion binding according to Christ et al. [3] include the carboxylate, amine, imidazole, phosphate, sulfate, sulfhydryl, and other functional groups in cell surface proteins and sugars.

In the present study the divalent cations, viz. $Mg^{2+}$ and $Ca^{2+}$, were found to inhibit $Cu^{2+}$ adsorption. The $Cu^{2+}$ adsorption was not significantly affected by 100 mg/L of $Ca^{2+}$, but doubling of $Ca^{2+}$ concentration resulted in 21.2% decline in $Cu^{2+}$ adsorption capacity of *Oscillatoria* sp. Similar results were obtained with $Mg^{2+}$ ions, but the percentage decline in $Cu^{2+}$ adsorption was slightly more at 200 mg/L $Mg^{2+}$ (30.06%) than with $Ca^{2+}$. The inhibition by 100 mg/L $Mg^{2+}$ was found to be about 12%.

Pretreatment of biomass either by physical or chemical treatments [20] or crosslinking of biomass is known to alter the biosorption capacity of biomass [15]. Biomass of *Oscillatoria* sp. was differentially treated to observe the change in potency of biosorption for $Cu^{2+}$. Of the various treatments, only washing the biomass with 0.01 N HCl resulted in increased $Cu^{2+}$ adsorption to about 420 mg/g dry wt compared with 385 mg/g dry wt of the native biomass. Sampedro et al. [20] have reported an increased biosorption in a filamentous cyanobacterium *Phormidium laminosum* after NaOH treatment, but HCl treatment was ineffective. The observed results clearly indicate that some pretreatments do expose more metal-binding groups either by removing some of the masking groups or by a change in configuration [19]. Also, since the dried (dead) biomass could sequester copper to the same extent, the absence of an active energy-dependent mechanism is suggested.

*Oscillatoria anguistissima* was investigated for its $Cu^{2+}$ removal ability from effluent sample of Ghatsila Copper Mines (Bihar, India) containing about 68.4 mg/L $Cu^{2+}$ at pH 3.45. It was found that native biomass could adsorb about 202.5 mg/g dry wt. of $Cu^{2+}$ from mine water in batch experiments, and the total capacity for its $Cu^{2+}$ removal increased with doubling the biomass concentration, but the mg/g removal decreased to about 118.75.

The rapid, extremely high capacity of *Oscillatoria* sp. for Cu biosorption and removal of copper from mine effluents makes this fresh water cyanobacterium a promising future biosorbent. Further work is in progress in the direction of formulating *Oscillatoria* sp. to an economical biosorbent material and to achieve maximum desorption of $Cu^{2+}$ from the biomass for its repeated use in subsequent cycles.

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