Biosorption of copper using mucilaginous seeds of *Ocimum basilicum*

Arpita Gupte, Manal Karjikar, Jagdish Nair

Department of Biotechnology and Bioinformatics, Padmashree Dr. D.Y. Patil University, CBD Belapur, Navi Mumbai, Maharashtra 400614, India; Email: garpita@gmail.com

ABSTRACT

A viable and cost-effective technology was explored in the present study for removal of copper from aqueous solution using mucilaginous seeds of *Ocimum basilicum*. Pretreatment of the seeds were found to alter the metal binding capacity. Distilled water imbibed seeds showed higher adsorption of Cu^{2+} in comparison to seeds given pre-treatment with acid, alkali, alcohol and boiling. Biosorption of Cu(II) was found to be pH dependent with maximum uptake at 5.0. The maximum adsorption capacity as calculated from the Langmuir Isotherm was 400 mg Cu/g dry seeds.

Keywords: biosorption, copper, Langmuir isotherm, mucilaginous seeds, *Ocimum basilicum*.

INTRODUCTION

The presence of heavy metal ions from the transition series, viz., Cu, Fe, Ni, Pb, etc. in the environment is of major concern due to their toxicity to many life forms. Unlike organic pollutants, the majority of which are susceptible to biological degradation, metal ions do not degrade into harmless end products [1]. Several methods are being used for the removal of heavy metal ions from aqueous wastes (chemical precipitation, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, etc.) [2]. New cost-effective technologies for the removal of heavy metals from wastewaters have been directed towards biosorption, which is known for the last few decades. Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents, due to metal sequestering properties and can decrease the concentration of heavy metal ions in solution [3]. In recent years, agricultural by-products have been widely studied for metal removal from waste water. These include peat, wood, pine bark, banana pith, soybean and cotton seed hulls, peanut shells, hazelnut shell, rice husk, saw dust, wool, orange peel, compost and leaves [4]. *Ocimum basilicum* seeds can serve as a naturally immobilized source of agriculturally-based exopolysaccharides. The seeds swell upon wetting and once swollen consist of a hard core with a porous swollen outer layer. The mucilaginous layer of the swollen seeds is a pectinous matrix, consisting of considerable amounts of unesterified galacturonic acid with a large capacity for hydration [5].

In this work, mucilaginous seeds of different plant varieties were screened for copper metal uptake. *O. basilicum* seeds were further used to study the effects of biosorbent dose, effects of various pretreatments, concentration of copper solution, and pH on the efficiency of copper removal at ambient temperature under batch conditions at fixed shaking speed.

MATERIALS AND METHODS

Seeds of *Lapidum sativum*, *Linum ustitatissimum*, *Ocimum sanctum* and *Ocimum basilicum* locally known as *halim*, *flax, tulsi* and *sabja* respectively were purchased from the local market. The plants producing these seeds are extensively grown in Maharashtra, India. All the chemicals used were of analytical grade unless otherwise mentioned and purchased from SRL Pvt. Ltd., Mumbai. Stock solution of copper metal (1000 ppm) was prepared by dissolving 100 mg of $CuSO_4.5H_20$ in 100 ml of distilled water. Fresh dilutions of the stock solution were used for each set of experiments. The required quantities of seeds were allowed to swell in 100 ml distilled water for 30 min. The swollen (mucilaginous) seeds thus obtained were washed with distilled water and they were directly used as biosorbent.

Lapidum sativum, *Linum ustitatissimum*, *Ocimum sanctum* and *Ocimum basilicum* seeds were screened for Cu metal uptake. The seeds (1 g each) were imbibed in distilled water for 30 min. Seeds were then washed with distilled water and used for the uptake studies. Swollen seeds were taken in 100 ml Erlenmeyer flask containing 50 ml of copper solution (100 ppm). Flasks were placed on a rotary shaker and shaken at 100 rpm at 25ºC. Kinetics of biosorption was studied out for 24 h. The aqueous phase was used to estimate copper. The concentration of unadsorbed copper in the solution was determined spectrophotometrically at 540 nm using the diphenylcarbazide reagent [6] on a UV-1700 PharmaSpec UV-VIS Spectrophotometer (Shimadzu Corp., Japan). The hydration capacity of seeds was determined by the swelling ratio (Q). Dry seeds were allowed to swell in water for 30 min. At saturation condition, the ratio of the weight of swollen seeds (Ws) to dry weight of seeds (Wd) is called as swelling ratio [7]. Mucilage content of the seeds was determined by the method of Anjaneyalu and Tharanathan [8].

To study the effect of different biomass concentration on uptake of copper, *Ocimum basilicum* seeds of different weight (0.1 g, 0.25 g, 0.5 g and 1 g) were imbibed in distilled water for 30 min and then the swollen seeds were used for uptake studies as detailed above. To observe the effect of different pre-treatments on the seeds to alter its surface chemistry, *O. basilicum* seeds (0.1 g) were given the following imbibing treatment for 30 min: (a) distilled water control (untreated); (b) boiling in distilled water; (c) 1 M NaOH; (d) 1 M HCl; (e) 1 M H_2SO_4; (e) 1 M $HClO_4$ and (f) 50 % CH_3CH_2OH. Treated seeds were thoroughly washed in distilled water thrice and used for uptake studies. To study the effect of pH on uptake of copper, swollen *O. basilicum* seeds obtained by soaking 0.1 g seeds in distilled water were incubated in copper solutions adjusted to various pH between 1 - 8 using 1 M NaOH/1 M H_2SO_4 and the uptake studies were carried out. Copper solutions of different concentrations such as 25 ppm, 100 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm were used to study the effect of metal concentration on the uptake process. The uptake (q) for plotting an isotherm was calculated using the equation: $q = (Ci - Ceq) \times V / M$ where, Ci is the initial metal concentration, Ceq is the equilibrium metal concentration, V is the volume of the metal solution in liter and M is the mass of the biomass in grams [7].

RESULTS AND DISCUSSION

Seeds of different plant varieties, viz., *Lapidum sativum*, *Linum ustitatissimum*, *Ocimum sanctum* and *Ocimum basilicum* that are known to produce mucilage were screened for uptake of copper (Figure 1). *Ocimum sanctum* and *Ocimum basilicum* were found to uptake copper to a level of > 90 % within 24 h. *Ocimum basilicum* was chosen as the biosorbent for further studies on copper uptake under batch conditions. *O. basilicum* seeds swell when steeped in water (Figure 2). Average size of the seeds before and after swelling was 2 and 3.5 mm respectively. The swelling ratio Q for *O. basilicum* seeds was found to be 24. The mucilage consisted of thread-like microfibrillar structures with a large area as observed under microscope. Mucilage content of the seeds was found to be 19

%. Effect of adsorbent dose was studied by varying the amount (weight) of seeds soaked in distilled water. Copper uptake was found to decrease with increase in the biosorbent dosage. Up to 90 % of copper was adsorbed by 0.1 g seeds within 2.5 h (Figure 3). The higher biomass concentration caused screen effect of dense outer layer, blocking the binding sites for metal ions, resulting in lower metal removal per unit biosorbent dose [9]. Therefore optimum biomass dosage was chosen as 0.1 g for the subsequent experiments.

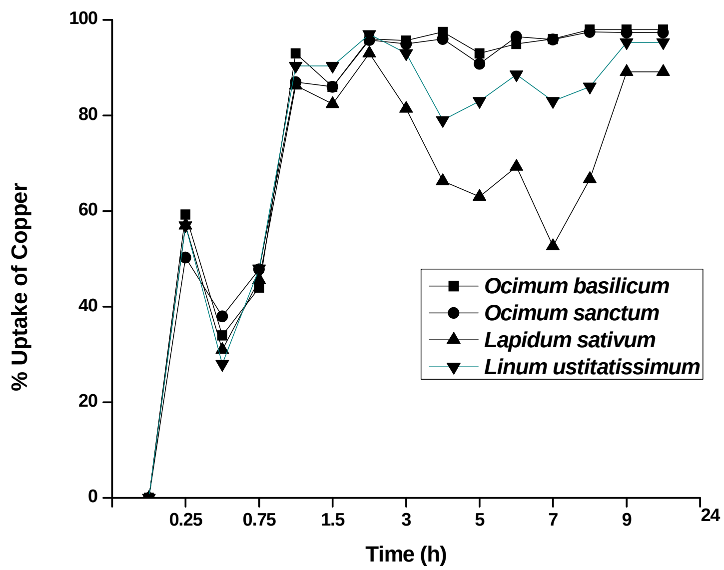
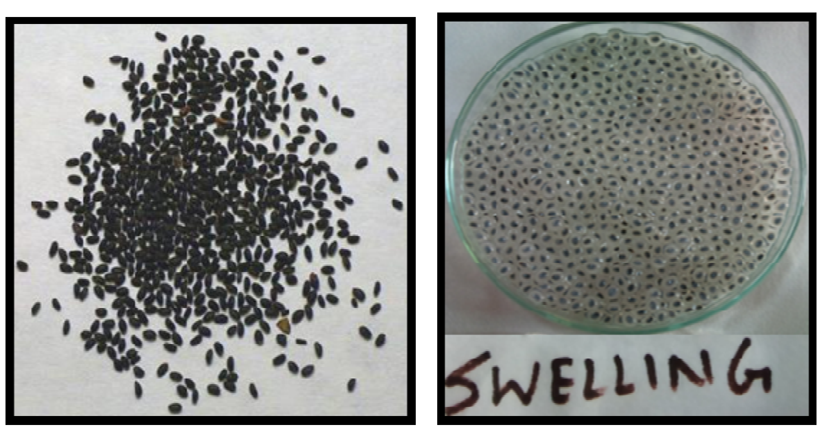


Figure 1. Screening of seeds of different plant varieties known to produce mucilage.



(a) (b)

Figure 2. Characteristics of *Ocimum basilicum* seeds: (a) normal, (b) soaked in distilled water for 30 min.

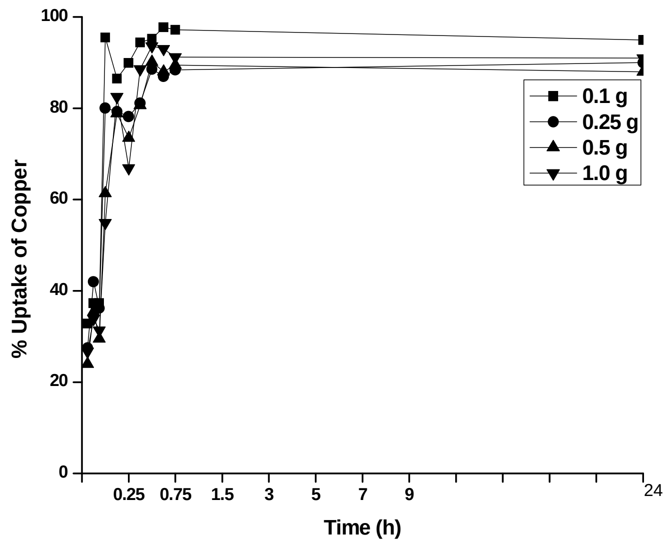


Figure 3. Effect of biosorbent dosage on copper uptake.

Seeds pre-treated with water only (normal) were used as a control. Of various pretreatments given to the seeds it was found that seeds imbibed in distilled water exhibited maximum adsorption of copper within 30 min and it was observed to be 98.1 % (Figure 4). Seeds treated with alkali showed biosorption capacity and copper uptake was about 90 %. Pre-treatment of microbial mass or other agricultural biomass with alkali has shown to enhance metal adsorption capacities [10]. Seeds treated with alcohol and boiled in water resulted in decrease in the uptake of copper and it was observed to be 88 % and 70 %. Alcohol treatment decreases uptake rate due to its dehydration effect on the mucilage [11], whereas plant derived mucilaginous polysaccharides lose viscosity with increasing temperature [12]. Acid treatments resulted in decrease in the biosorption rate due to hydrolysis of mucilage polysaccharide [11].

Metal uptake was found to depend on pH of the metal solution (Figure 5). Copper uptake was found to be maximum (78 %) at pH 5.0. The pH range from 3.0 - 6.0 was found to be favorable for copper uptake. The pH of the solution has a significant impact on the uptake of heavy metals, since it determines the surface of the adsorbent, the degree of ionization and speciation of the adsorbate. At lower pH, hydrogen ions compete with metal ions for sorption sites [13], whereas as the pH increases metal uptake is facilitated [14]. However, at a higher pH, copper ions precipitate as hydroxides decreasing the rate of adsorption and subsequent removal of metal ions [15].

Copper uptake kinetics by *O. basilicum* reached equilibrium within 60 min of incubation in the aqueous solution for all the different concentrations of copper tested (Figure 6). When the experimental data were fitted with a Langmuir Isotherm (Figure 7), a maximum uptake (qmax) of 400 mg Cu/g dry seeds was observed. The isotherm describes the equilibrium established between the adsorbed metal on a biomass in a monolayer [16]. An uptake in excess of 15 % dry weight has been calculated to have economical industrial importance [17], thus showing that *O. basilicum* seeds can be a potential industrial level biosorbent.

In the present study, copper uptake by mucilaginous seeds of *O. basilicum* was studied. The seeds are readily available in tropical countries like India. When used as biosorbents, the seeds

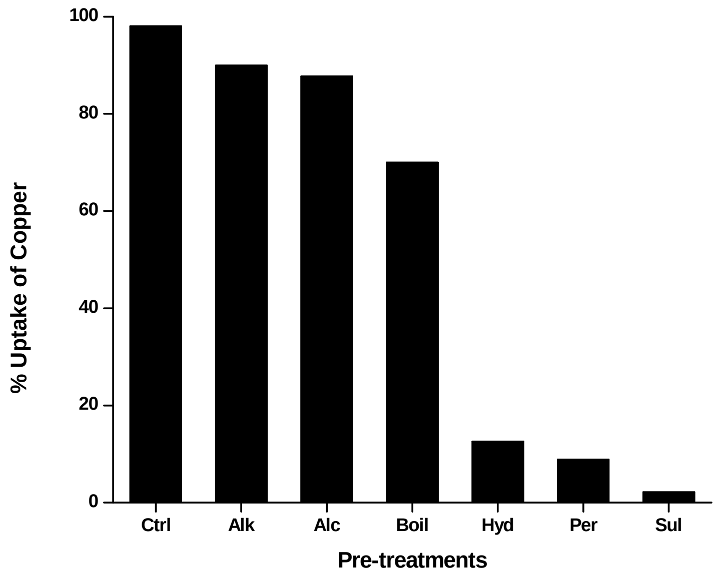


Figure 4. Effect of different pre-treatments on *Ocimum basilicum* seeds. Ctrl, Control; Alk, 1 M NaOH; Alc, 50 % CH_3CH_2OH; Boil, Boiling; Hyd, 1 M HCl; Per, 1 M $HClO_4$; Sul, 1 M H_2SO_4.

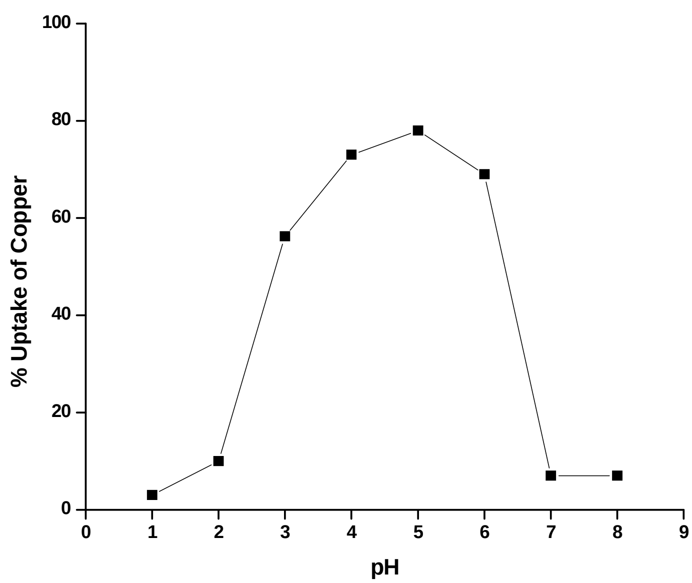


Figure 5. Effect of pH on biosorption of copper.

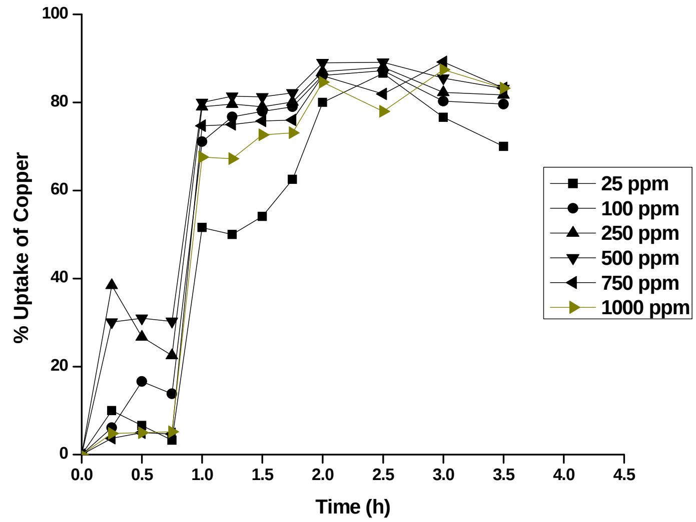


Figure 6. Effect of different concentrations of copper on the biosorption process.

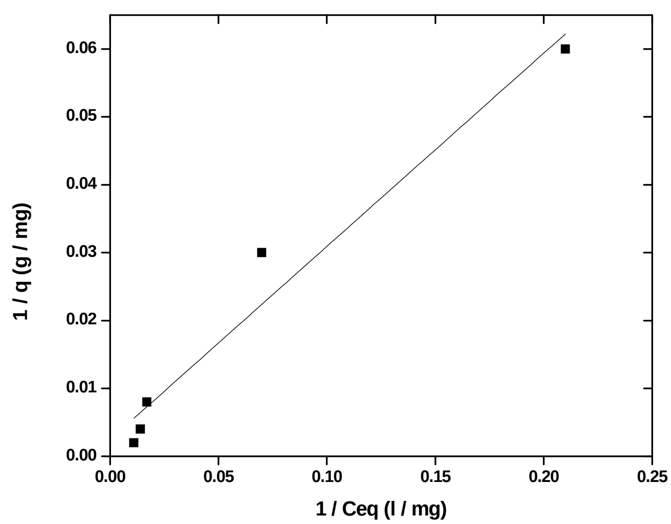


Figure 7. Langmuir Isotherm at adsorption equilibrium.

confer the advantage of microbial exopolysaccharides in terms of their ability to adsorb the Cu(II) and also provide a natural immobilized base which is a major cost factor in the process. It is a feasible green technology for effective removal of heavy metal ions from industrial wastewater.

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