RESEARCH ARTICLE

BIOSORPTION STUDIES FOR REMOVAL OF METALS FROM AQUEOUS SOLUTION USING IMMOBILIZED FRESHWATER ALGA *SPIRULINA PLATENSIS*

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ABSTRACT

The main aim of this study is to screening the potential of freshwater alga *Spirulina platensis* to remove Cr, Fe, Mn and Se from aqueous solution. The effects of initial metal concentrations, pH and algal biomass dose on metal biosorption were investigated. The results shows that the increase in an initial concentrations of Mn, Fe, Cr and Se led to decrease in the percentage of removal for all the metals, also it was observed that as the dose of algal biomass increases the amount of metal removed decreases. The maximum uptake for Mn, Fe, Cr and Se was found to be at 0.025 gm of algal biomass dose. The initial pH of the metal solution is an important parameter affecting the biosorption of metal ions. The effect of pH on metal biosorption has been studied, and the results indicated that the pH value of solution could significantly influence the biosorption. A trend of increasing metal ion binding with increasing pH could be observed for all the metals. The optimal removal efficiencies of all the metals were observed at the pH 6. The maximum uptake at pH 6 and initial metal concentration of 5 mg/L, for Mn, Fe, Cr and Se using immobilized cells of *Spirulina platensis* observed as 4.97 mg/L, 4.99 mg/L, 4.99 mg/L and 4.98 mg/L after 24 hours of incubation period.

INTRODUCTION

Water is one of the most essential substances in our life. Next to air, it is most essential for life processes. In fact, the origin and evolution of life on planet Earth is most intimately linked with water .Water is the most abundant and essential compound in all the living systems. All living organisms on our planet are well connected with water, that life on the Earth is believed to have evolved in and around water. It is the most essential ingredient in living organisms. Its amount in living organisms, excluding spores and inert seeds, fluctuates between 60 and 99.7 percent by weight. Potable water is one that is safe to drink, pleasant in taste and suitable for domestic purpose. Drinking water must be free from major type of water pollutants which can be classified into microorganisms, organic wastes, plant nutrients, sediments or silts, inorganic chemicals, acids and bases, heat, radioactivity, heavy metals, pesticides and other industrial chemicals (Saini, 2006). It is reported that, by international standards, about 70 percent of fresh water available in India is polluted. The sacred Ganga River is highly polluted in several regions and a major action plan to clean the river is in progress. In addition, the Central Pollution Control Board (CPCB) of India has identified several grossly polluted river stretches (Sastri, 1995). Several living organisms including algae, according to their nutrient need, are used to evaluate most of the environmental characters. Algae were considered good bioindicators to

several qualitative and quantitative characters of aquatic environment (Al-bergoni *et al*., 1980; Favero *et al*., 1996). Their widely distribution enhanced their use in monitoring program through simple models (Rachlin *et al.,* 1983; Piccinni and Gutierrez, 1995). Many investigations have been carried out for biosorption of heavy metals by the other important divisions of algae Green algae (*Chlorophyta*) and Red algae (*Rhodophyta*) (Handy, 2000). Green algae *Cladophora fascicularis* was to be an effective and economical biosorbent material for the removal of heavy metal ions (Dend *et al*., 2006). *Spirulina* is tiny blue - green algae in the shape of a perfect spiral coil. Biologically speaking, it is one of the oldest inhabitants of the planet. Spirulina is being developed as the "food of the future" because of its amazing ability to synthesize high-quality concentrated food more efficiently than any other algae. Most notably, Spirulina is 65 to 71 percent complete protein, with all essential amino acids in perfect balance. In the present study, the removal of heavy metal ions such as Cr, Fe, Mn and Se by immobilized freshwater alga *Spirulina platensis* was investigated. The effect of initial metal concentration, pH and algal biomass dose was studied.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals used were of analytical reagent grade. All the metal solutions were prepared by dissolving appropriate

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quantities of respective metals. Double distilled water was used throughout the experiment.

Preparation of synthetic metal solution

All the working metal solutions are prepared by dissolving the respective metals in double distilled water. For preparation of iron working solution, the electrolytic iron wire was cleaned with fine sand paper to remove any oxide coating. Then 100 mg cleaned wire was placed in 100 ml volumetric flask and dissolved in 20 ml 6N-H_2SO_4 and diluted upto 100 ml mark by double distilled water. For preparation of chromium working solution 283 mg dried potassium dichromate was dissolved in small quantity of double distilled water and diluted to 100 ml with double distilled water. For manganese working solution 288 mg potassium permanganate was dissolved in 25 ml diluted H_2SO_4 (20%). The permanaganic acid by dropwise addition of hydrogen peroxide until it becomes colourless and diluted to diluted to 100 ml with double distilled water. For selenium working solution 2.190 gm sodium selenite was dissolved in small quantity of double distilled water containing 10 ml conc. HCl and diluted to 100 ml with double distilled water.

Algal biomass cultivation

The starting culture was obtained from the National Chemical Laboratory (NCL), Pune. *Spirulina sp.* in log phase used in the experiment was inoculated in the Spirulina medium for mass multiplication. The medium was sterilized by autoclaving at 121°C for 15 minutes. Medium was stored at 4°C until inoculated. Culture was grown in liquid media in 2 L glass Erlenmeyer flasks and incubated at 25°C in a growth chamber with a light and dark cycle of 8 h and 16 h and 3000 – 3500 lux, light intensity provided by cool white day light fluorescent tube lamps.

Table1. Composition of Spirulina medium for the growth of *Spirulina platensis*

NaHCO3	10.0 g
NaNO3	2.5 g
NaCl	1.0 g
K2HPO4	0.5 g
K2SO4	1.0 g
MgSO4.7H2O	0.2 g
CaCl2	0.04 g
FeSO4.7H2O	0.01 g
Distilled water	1.0 L
Agar (Difco)	20.0 g

NaHCO$_3$ and other salts were autoclaved separately at 15 lbs for 20 min and then mixed.

Preparation of biomass

The starting culture was obtained from the National Chemical Laboratory (NCL), Pune. *Spirulina platensis* in log phase used in the experiment was inoculated in the Spirulina medium for mass multiplication. The collected biomasses were washed with excess tap water and finally with distilled water to remove salt and particulate materials from its surface, then oven-dried at 60°C for 24 h. The dried biomasses were ground, sieved to particle size ranged from 0.25μm to 0.3μm and then stored in a dessicator at lab temperature for further use.

Preparation of biosorbent beads

The control Ca-alginate (CA) beads were prepared by injecting 10 ml of 4% Ca-alginate gel into 2% CaCl$_2$ solution, while the immobilized algal biomass (IAB) beads were prepared by mixing the desired weight of dried algal biomass with 10 ml of 4% Ca-alginate gel thoroughly, then injected into 2% CaCl$_2$ solution. The formed beads of (CA) or (IAB) were left in CaCl$_2$ solution for one hour, and then washed twice with double-distilled water. 10 ml of calcium alginate gel gives about 300 beads of (CA) or (IAB) having diameter 2.5-3.0 mm. The obtained beads were kept in refrigerator at 4°C for use.

pH adjustment of aqueous metal solutions

The adsorption of metals decrease at low pH values because of competition for binding sites between cations and protons, while at pH higher than 7, hydroxyl species of the metals can be formed and do not bind to the adsorption sites on the surface of the adsorbent. So, pH was adjusted with the range of 3-7 by adding the 0.1N HCl and 0.1N NaOH or acidic and basic pH respectively.

Biosorption experiments

About 100 ml of all the metal solutions of known concentration (5, 10, 15, 20 and 25 mg/L) was taken in a 250 ml conical flask and 10 ml of immobilized beads were added. The flask was shaken for 24 hours using orbital shaking machine. The samples were collected after 24 hours filtered through filter paper, centrifuged at 5000 rpm for 15 minutes and analyzed for residual metal concentration using UV-Spectrophotometer. The metal uptakes at different initial metal concentration, pH, and algal biomass were studied.

Estimation of metals

Standard procedures were used for quantitative estimation of metals Cr, Fe, Mn and Se by UV-Spectrophotometer. Chromium estimation was done by s-Diphenylcarbazide method at 540 nm, iron by thiocyanate at 510 nm, manganese by persulphate at 545 nm, selenium by 3,3$^/$ Diaminobenzidine method at 420 nm. All analysis were done in triplicate.

Data treatment

The removal efficiency was obtained by using the following expression

$$R\ (\%) = \frac{(C_0 - C_e) * 100}{C_o}$$

R is the percent removal efficiency of metal ions. C_o and C_e are the initial and equilibrium concentrations of the metals (mg/L) after biosorption, respectively.

RESULTS AND DISCUSSION

Effect of Initial Metal Ion Concentration on Biosorption

Figure 1 represents the effect of different initial concentrations of Mn, Fe, Cr and Se (5 – 25 mg/L) using immobilized cells of *Spirulina platensis*.
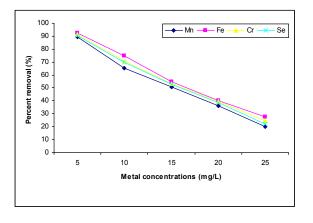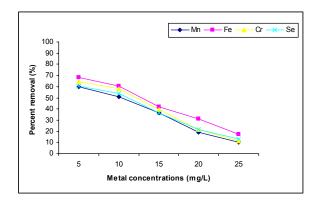
Fig. 1. Effect of initial metal concentration on percent removal from aqueous solution (pH - 6, algal biomass dose – 0.025 gm).

The results shows the increase in initial concentrations of Mn, Fe, Cr and Se led to decrease in the percentage of removal for all the metals due to saturation of the sorption sites on adsorbents, the maximum uptake for Mn, with initial concentrations, 5, 10, 15, 20 and 25 mg/ L at pH 6.0 and 0.025 gm of algal biomass dose were obtained as 89.40%, 65.20%, 50.60%, 36.35% and 20.20% respectively. The same for Fe, with initial concentrations, 5, 10, 15, 20 and 25 mg/ L at pH 6.0 and 0.025 gm of algal biomass dose were obtained as 92.40%, 74.80%, 54.80%, 40.20% and 27.52% respectively. In case of Cr, the maximum uptake, with initial concentrations, 5, 10, 15, 20 and 25 mg/ L at pH 6.0 and 0.025 gm of algal biomass dose were obtained as 91.60%, 70.80%, 53.46%, 39,70% and 24.48% respectively. For Se, the maximum uptake, with initial concentrations, 5, 10, 15, 20 and 25 mg/ L at pH 6.0 and 0.025 gm of algal biomass dose were obtained as 90.60%, 70.10%, 52.73%, 38.45% and 21.96% respectively. While figure 2 represents the percent metal removal from aqueous solution at various concentrations of Mn, Fe, Cr and Se (5 – 25 mg/L) by using calcium alginate beads at pH - 6. The result shows that the maximum metal uptake by CA beads was found at 5 mg/L initial metal concentration for all the metals. The maximum uptake for Mn, Cr, Fe and Se at pH 6 was 60.12%, 68.16%, 64.96% and 60.77%.

Fig. 2. Percent metal removal from aqueous solution at various concentrations by using calcium alginate beads at pH - 6.

By increasing initial concentration, the uptake was decreased. This was due to the saturation of the sorption sites on adsorbents. The results of present investigation revealed that the biosorption of metals decreases with increasing the initial metal concentration. The effect of initial metal concentration on the ability of adsorption by duckweed. It took many different concentrations at a range of (10 - 50) ppm for the mixture of metal ions. The reduction of removal metal ions on duckweed was found with the of increase initial concentration from 10 to 50 ppm. This was due to the increase of metal ions concentration which was completed on the effective site, Also this may be a limited active site for adsorbent material. Therefore, more metal ions were left unabsorbed in solution at higher concentration levels (Dhabab, 2011).

Some researchers studied that the percentage removal and adsorption capacity at different U (VI) concentrations are. When the initial metal ions concentration varied from 5 mg/L to 200 mg/L, the percentage of ions adsorbed at higher concentration levels showed a decreasing trend, whereas uptake of ions displayed the opposite trend. The maximum percentage removal of U (87.8%) was observed when the initial U concentration was 60 mg/L. The maximum U uptake capacity of this biosorbent was achieved at 98.4 mg U per g dry biosorbent with the initial U concentration of 200 mg/L. This may be due to the saturation of the adsorption sites and increase in the number of ions competing for the available binding sites in the biomass for complexation of U at higher concentration (Bai and Abraham, 2003). On the other hand, U (VI) adsorption increased with the increase of initial U (VI) concentration as higher concentration of metal ions enhanced the mass transfer driving force, and increased the metal ions adsorbed per unit weight of adsorbent at equilibrium (Aksu, 2002). In addition, increasing initial U concentration increased the number of collisions between U ions and the adsorbent, which enhanced the adsorption process (Bai and Abraham, 2001a, b). The results revealed maximum metal removal with lower initial concentrations. The percentage Pb uptake was 100% for metal solutions till 250 ppm metal. Later, an increase in initial concentration decreased percentage binding. The observations are the same for Zn (70%) and Cr (75.3%) at 250- ppm concentration. These observations can be explained by the fact that at very low concentrations of metal ions, the ratio of sorptive surface area to the total metal ions available is high and thus, there is a greater chance for metal removal. Thus, at low initial metal ion concentrations, the removal capacity is higher. When metal ion concentrations are increased, binding sites become more quickly saturated as the amount of biomass concentration remained constant (Sekhar, 2002).

Effect of Algal Biomass on Biosorption

Figure 3 represents the effect of different algal biomass dose on biosorption of Mn, Fe, Cr and Se (0.025 – 0.20 gm of *Spirulina platensis*). The results show that, as the dose of algal biomass increases the amount of metal removed decreases. The maximum uptake for Mn, Fe, Cr and Se was found to be maximum at 0.025 gm of algal biomass dose. The results for amount of metals removed were obtained maximum at 5 mg/L initial metal concentration and at pH 6 for Mn, Fe, Cr and Se were 4.47 mg/L, 4.62 mg/L, 4.58 mg/L and 4.53 mg/L. Thereafter, as the dose of algal biomass increases the there was a decrease in the amount of metal removed.
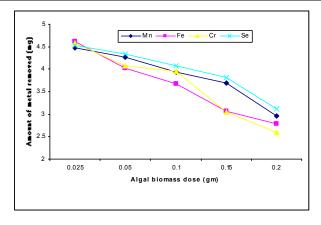
Figure 3. Effect of algal biomass dose on removal of metals from aqueous solution (initial metal concentration – 5 mg/L, pH - 6).

The weight of biomass to be immobilized is an important parameter in the immobilization process (Abdel-Hameed, 1994). For constant volume of Ca-alginate gel (10 ml), different dry weights (0.2, 0.15, 0.1, 0.05 and 0.025g) of algal biomass were mixed. The effect of immobilized biomass on the biosorption of 134Cs was studied at room temperature and pH 7.0, with initial cesium concentration of 10-5M. It is apparent that the removal of 134Cs increased as the immobilized weight decreased. Thus the optimum weight to be immobilized in 10 ml Ca-alginate gel was 0.025 g. This could be explained by the interaction between the active groups of the excess algal biomass and that of the gel, resulting in decreasing the free and available active sites on both the immobilized algal biomass and the gel. The preliminary experiments were carried out to assess the appropriate incubation time: 60 min was found to be sufficient for optimal and reproducible biosorption. It was confirmed that the metal cations were not accumulated by the polyurethane foam cubes. Mycelia of *Rhizopus delemar* (free- and immobilized cells) were examined with respect to their ability to remove cobalt ions from aqueous solutions. The relationship between mycelial density, residual metal ions concentration and cobalt biosorption is studied. Control experiments, either lacking biomass or cobalt ions, were included. The immobilized mycelium showed considerable higher specific biosorbent capacities than free cells in all tested cases of mycelial density. With carefully optimized biomass concentrations, the cobalt ions could be removed completely from the aqueous phase by immobilized biosorbent. Maximal value for specific biosorbent capacity of the immobilized mycelium was calculated to be 19.6 mg/g. At the experimental conditions used, the biomass concentration was found to have a significant effect on biosorption. In general, the amount of heavy metal adsorbed per unit weight was higher at lower biomass concentrations and decreased with increasing amounts of biomass. Reduction in biomass concentration in the suspension at a given metal concentration enhances the metal/biosorbent ratio, and thus increases metal uptake per g of biosorbent, as long as the latter is not saturated. Thus, maximal uptake 8.8 mg/g and 20.1 mg/g was achieved for both cases at initial concentration of 58.93 mg/L cobalt ions with an initial cobalt/biosorbent ratio, of 58.3 mg/g and 62.6 mg/g free and immobilized biosorbent, resp. All these data, obtained with simple experiments, can be of great interest in scale-up processes to optimize industrial effluent purification. The results obtained in these experiments showed

that using immobilized batch system of filamentous fungus enhanced the biosorption levels, as compared with native mycelia. It is quite possible that the immobilization of mycelia on this carrier generates more accessible potential ligands within the cell wall, hence allowing more cobalt ions to precipitate at these surfaces. Increased uptake of metal ions can be expected at equal weight of mycelia as result of a larger distance between cells, resp. smaller electrostatic interaction between function groups of the cell surface (Rome and Gadd, 1987).

Effect of pH of the solution on Biosorption

The initial pH of the metal solution is an important parameter affecting the biosorption of metal ions. The effect of pH on metal biosorption has been studied, and the results indicated that the pH value of solution could significantly influence the biosorption. The effect of initial pH [(]3 - 7) on the removal of Mn, Fe, Cr and Se using immobilized cells of *Spirulina platensis* were represented in Figure 4. A trend of increasing metal ion binding with increasing pH could be observed for all the metals. The optimal removal efficiencies of all the metals were observed at the pH 6. The maximum uptake at pH 6 and initial metal concentration of 5 mg/L, for Mn, Fe, Cr and Se using immobilized cells of *Spirulina platensis* observed as 4.97 mg/L, 4.99 mg/L, 4.99 mg/L and 4.98 mg/L. The effect of pH on metal biosorption has been studied, and the results indicated that the pH value of solution could significantly influence biosorption. The influence of initial pH on the adsorption characteristics of 134Cs using Ca-alginate (CA) and immobilized algal biomass (IAB) beads is studied. A trend of increasing metal ion binding with increasing pH could be observed for all sorbents at pH values above 2.5. The optimal removal efficiencies of 134Cs were observed for the pH range 6.5-7.5. The cell wall of algae contains a large number of surface functional groups, in which carboxyl is generally the most abundant acidic functional group according to (Figuera, 2000). At low pH, cell wall ligands are closely associated with the excess of hydronium ions (H_3O^+) and hence the sorption of positive Cs ions to the ligands will be restricted. With increasing pH , (H_3O^+) decreased and carboxyl groups will be exposed to attraction with the positive charges Cs ions and hence an increases in biosorption onto the cell surface was observed in agreement with (Matheickal, 1999). Alteration in pH over a wide range does not measurably change the average intracellular pH (Pothstein, 1956), suggesting that the binding sites must be located peripherally, under the influence of extra cellular pH rather than sites exposed to the constancy of intracellular pH. This view does not take into account that a hydrogen ion gradient across the cell membrane must be present to allow for cation uptake (Wang, 2002). It is apparent that when the initial pH <5.0, the adsorption capacity decreases dramatically, which may be due to the fact that the hydrogen ion as a cation, can compete with binding sites of the biomass surface for cadmium ions. PH of the adsorption medium is a key parameter that shows a substantial effect on bio-adsorption capacity of removing the cadmium ion from aqueous solution. The percent adsorption increased with increase in pH; at near neutral pH 6 of the solution were found to favor adsorption very strongly. At lower pH the percentage adsorption was very low; it may be due to preferential adsorption of hydrogen ions. These observations were in agreement with those reported in

earlier investigations (Senthilkumar *et al*., 2006; Bayramoglu *et al*., 2006; Marques *et al*., 1999; Say *et al*., 2001; Yan and Viraraghavan, 2003; Vasudevan *et al*., 2003; Zafar *et al*., 2007; Bishnoi *et al*., 2007; Sari and Tuzen, 2008; Murthy *et al*., 2007; Fagundes-Klen *et al*., 2007) for different metals using various biosorption materials.
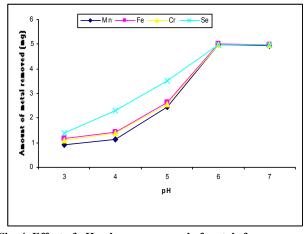

Fig. 4. Effect of pH values on removal of metals from aqueous solution (initial metal concentration – 5 mg/L, algal biomass dose – 0.025 gm).

In the pH range studied (5 to 9) all the heavy metal resistant bacterial isolates growth were increased gradually at initial pH 7 and decreased at increased pH. The biosorption of the cell was sensitive to pH (Simie *et al*., 1998). The cell surface metal binding sites and availability of metal in solution are affected by pH. At low pH, the cell surface sites are closely linked to the H^+ ions, thereby making these unavailable for other cations. However, with an increase in pH, there is an increase in ligand with negative charges which results in increased binding of cations (Abuja *et al*., 1999). Charge on the surface of the cell which favored electrochemical attraction and adsorption of metal (Gourdon *et al*., 1990). *Bacillus* sp., *Pseudomonas* sp. and *Micrococcus* sp. has the ability to adsorb maximum Cu, Cd and Pb at pH 7, 6 and 6 respectively which are similar to the results of (Wang and Chen, 2006 and Blackwell *et al*. 1995). In their result, the highest adsorption occurs at pH ranges from 4 - 8. This pH range is widely accepted as being optimal for metal uptake of almost all types of biomass.

Conclusion

Experimental investigations have been carried out to determine the potential of immobilized cells of *Sprulina platensis* to remove some metals from the aqueous solution. The algae *Sprulina platensis* has important potential for the removal of Cr, Fe, Mn and Se ions from aqueous solution. The immobilized algal biomass (IAB) enhances the adsorption capacity of Ca-alginate beads especially in case of low weight (0.025g/10ml gel). The pH of the adsorbate (Cr, Fe, Mn and Se) solution and the weight of the immobilized algal biomass are controlling factors in the biosorption process. In the present investigation the biosorption process is highly dependent upon the initial metal concentration, pH and algal biomass dose.

REFERENCES

Abdel-Hameed, M. S. (1994). Continuous removal and recovery of lead by alginate beads and free biomasses (*Rhizopus arrhizus, Mucor miehei* and *Penicillium chrysogenum*): pH control and cationic activation. Fems Microbiol Rev, 14, 325–332.

Ahuja, P., Gupta, R., Saxena, R. K. (1999). Sorption and desorption of cobalt by *Oscillatoria anguistissima*. Curr. Microbiol, 39, 49-52.

Aksu, Z. (2002). Equilibrium and kinetic modeling of cadmium (II) biosorption by *C. vulgaris* in a batch system: effect of temperature. Separation and Purification Technology, 21, 285-294.

Albergoni, V., Piceinni, E., Coppellotti, O. (1980). Response to heavy metals in organisms, excretion and accumulation of phsiological and non physiological metals in *Euglena gracilis*. *Comp*. Biochem. Physiol, 67, 121 – 127.

Bai, S.R., Abraham, T.E. (2001). Biosorption of Chromium(VI) from aqueous solution and electroplating wastewater using fungal biomass. Chemical Engineering Journal, 135, 202-208.

Bai, S.R., Abraham, T.E. (2003). Studies on chromium(VI) adsorption desorption using immobilized fungal biomass. Bioresource Technology, 87, 17-26.

Bayramoglu, G., Tuzun, I., Celik, G., Yilmaz, M., Arica, M.Y. (2006). Biosorption of mercury (II), cadmium (II) and lead (II) ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads. Int J Miner Process. 81, 35-43.

Bishnoi, N.R., Kumar, R., Kumar, S., Rani, S. (2007). Biosorption of Cr (III) from aqueous solution using algal biomass *spirogyra sp.* J Hazard Mat, 145, 142- 147.

Blackwell, J. K., Singleton, I., Tobin, M. J. (1995). Metal cation uptake by yeast: a review. Appl. Microbiol. Biotechnol, 43, 579-584.

Dhabab, J. M. (2011). Removal of Fe(II), Cu(II), Zn(II), and Pb(II) ions from aqueous solutions by duckweed. Journal of OceanograpHy and Marine Science, 2(1), 17 -22.

Dend, L., Su, Y., Su, H. (2006). Biosorption of copper (II) and lead (II) from aqueous solutions by nonliving green algae *Cladophora fascicularis*: equilibrium, kinetics and environmental effects. *Chemistry and Materials Sci*, 12, 267–277.

Fagundes-Klen, M. R., Ferri, P., Martins, T.D., Tavares, C. R. G., Silva, E. A. (2007). Equilibrium study of the binary mixture of cadmium–zinc ions biosorption by the *Sargassum filipendula* species using adsorption isotherms models and neural network. Biochemical Engineering Journal, 34, 136-146.

Favero, N., cattalini, F., Bertaggia, D., Albergoni, V. (1996). Metal accumulation in biological indicator (*Ulva rigida*) from lagoon of Venice (Italy). Arch. Environ. Contam. Toxical, 31, 9- 18.

Figuera, M. W., Volesky, B., Ciminelli, V. S., Roddick, F. A. (2000). Biosorption of metals in brown seaweed biomass. Water Res, 34 (1),196-204.

Gourdon, R., Bhande, S., Rus, E., Sofer, S. S. (1990). Comparison of Cadmium biosorption by gram positive and gram negative bacteria from activated sludge. Biotechnol. Lett, 12, 839-842.

Handy A. (2000). Removal of Pb^{2+} by biomass of marine algae. Current Microbiology, 41, 232–243.

Marques, P. A., Pinheiro, H. M., Teixeira, J. A., Rosa, M. F. (1999). Removal efficiency of Cu^{2+}, Cd^{2+} and Pb^{2+} by

waste brewery Biomass: pH and cation association effects. Desalination, 124, 137-144.

Matheickal, J. T., Yu, Q. M., Woodburn, G. M. (1999). Biosorption of Cadmium(II) from aqueous solutions by pre-treated biomass of marine alga *Durvillaea potatorum*. Water Res, 33, 335– 342.

Murthy, C. V. R., Kumar, K. K., Jayaraju, K., Silas, S. (2007). Biosorption studies for removal of cadmium from wastewater using immobilized *Saccharomyces cerevisiae*. Asian Journal of Chemistry, 19, 3502- 3510.

Piccinni, E., Gutierrez, J. C. (1995). Protists as a bioindicators in the environment. Symposium 9, Proccedings of the Second European Congress of Prolistology Clermont – Ferrand, 173 – 183.

Pothstein, A., Hayes, A. D. (1956). The relationship of the cell surface to metabolism. XIII. The cation-binding properties of the yeast cell surface. *Arch. Biochem. BiopHys*, 63, 87-99.

Rachlin, J. W., Jensen, T. E., Warkentine, B. (1984). The toxicological response of the Alga *Anabaena flos-aquae* (Cyanophyceae) to cadmium. Arch. Environ. Contam. Toxicol, 13, 143 – 151.

Rome, L., Gadd, D. M. (1987). Copper adsorption by *Rhi*and *zopus arrhizus, Cladosporium resinae* and *Penicillium italicum*. Appl. Microbiol. Biotechnol, 26, 84-90.

Saini Randeep Singh. (2006). A Handbook on Air, Water and Land Pollution. B.R. Publishing Corporation, Delhi – 110 052.

Sari, A., Tuzen, M. (2008). Biosorption of Pb(II) and Cd(II) from aqueous solution using green alga (*Ulva lactuca*) biomass. J Hazard Mat, 152, 302- 308.

Sastri, M.N. (1995). Water (Ist Ed^n). Himalaya publishing house, Girgaon, Mumbai –400 004.

Say, R., Denizli, A., Arica, M. Y. (2001). Biosorption of cadmium (II), Lead (II) and Copper (II) with the filamentous fungus *PHanerochaete chrysosporium*. Bioresour Technol, 76, 67-70.

Sekhar, K. C., Kamala ,C.T., Chary ,N.S., Anjaneyulu, Y. (2003). Removal of heavy metals using a plant biomass with reference to environmental control, Int. J. Miner. Process, 68 , 37– 45.

Senthilkumar, R., Vijayaraghavan, K., Thilakavathi, M., Iyer, P. V. R., Velan, M. (2006). Seaweeds for the remediation of wastewaters contaminated with zinc (II) ions. J Hazard Mat, 136, 791-799.

Simie, D. D., Finoli, C., Vecchio, A., Ancheoni, V. (1998). Metal ion accumulation by immobilized cells of *Brevibacterium* sp. J. Indust. Microbiol. Biotechnol, 20, 116-120.

Vasudevan, P., Padmavathy, V., Dhingra, S. C. (2003). Kinetics of biosorption of cadmium on Baker's yeast. Bioresour Technol, 89, 281-287.

Wang, J., Chen, C. (2006). Biosorption of heavy metals by *Saccharomyces cerevisiae*: Rev. Biotechnol. Adv, 24, 427-451.

Wang, J. L. (2002). Biosorption of Copper (II) by Chemically Modified Biomass of *Saccharomyces cerevisiae*. Process Biochemistry, 37(8), 847-850.

Yan, G., Viraraghavan, T. (2003). Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*. Water Res, 37, 4486–4496.

Zafar, S., Aqil, F., Ahmad, I. (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil. Bioresour Technol, 98, 2557-2561.

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