http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 14 (5): 327-335, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.327.335

Biosorption Kinetics of Cu (II) Ions Removal from Aqueous Solution using Bacteria

P. Sethuraman and M. Dharmendira Kumar
Department of Chemical Engineering, A.C. Tech. Campus, Anna University,
Chennai-600 025, India

Abstract: The present study highlights the effective removal of Cu (II) ions from synthetic solution using bacteria such as *B. subtilis*, *P. aeruginosa* and *E. cloacae*. Batch biosorption studies show that the biosorption of *B. subtilis* is effective when the concentration ranges from 25-200 mg L^{-1}. Biomass dosage, pH and the initial metal ion concentration have a profound effect on the biosorption process and this is reported in this study. In order to understand the nature of the biosorption process, Langmuir and Freundlich isotherm models were applied. Pseudo first and second order models were used to study the biosorption kinetics. The results show that these bacterial strains are very much suitable for the removal of Cu (II) ions. Being cost effective and efficient in toxic metal ion removal, these bacteria can be used on a large scale.

Key words: Bacteria, Cu (II) removal, biosorption, kinetics, isotherms, waste water removal

INTRODUCTION

The presence of toxic heavy metals in industrial effluents has become a matter of environmental concern. The contamination of water resources by toxic heavy metals through the discharge of industrial wastewater is a world wide environmental problem. Metals which have a density greater than or equal to 6.0 gcm^{-3} are classified as heavy metals (Barrera *et al*., 2006). The most familiar metals are cadmium (8.65 g cm^{-3}), chromium (7.19 g cm^{-3}), cobal (8.90 g cm^{-3}), copper (8.95 g cm^{-3}), lead (11.34 g cm^{-3}), mercury (13.53 g cm^{-3}), nickel (8.91 g cm^{-3}) and zinc (7.14 g cm^{-3}) (Anandkumara and Mandal, 2009).

In the human body, Cu is needed to catalyze many enzymatic reactions and is also necessary for the overall growth of connective tissues, nerve coverings and in the growth of bones. However, when a large dosage is ingested, it becomes lethal and carcinogenic. There are several reports on the acute copper poisoning/toxicity and these include abdominal pain, nausea, vomiting, headache, lethargy, diarrhea, tachycardia, respiratory difficulties, hemolytic anemia, gastrointestinal bleeding, liver, kidney failure and death (Uriu-Adams and Keen, 2005; WHO, 1998). Since heavy metal ions are not biodegradable, they are usually removed from the contaminated water by physical or chemical treatment processes. Conventional treatment methods such as precipitation, membrane separation, ion exchange, reverse osmosis and electrolysis are not often feasible because of the high treatment costs involved, the need for continuous input of chemicals and the production of a complicated toxic sludge (Tunali and Akar, 2006; Han *et al*., 2006). Therefore, research studies have been carried on to develop environment friendly technologies like bioremediation of heavy metal pollution. Microorganisms are potent bioremediators, removing heavy metals via biosorption mechanisms (Tiemann *et al*., 1998).

Biosorption by non-living, non-growing biomass or active groups of some molecules is a non-metabolism dependent (passive) uptake process (Vijayaraghavan and Yun, 2008; Hawari and Mulligan, 2006). According to the location from where the metal has been removed, biosorption process may be classified as: extracellular biosorption/precipitation and cell surface sorption /precipitation: Ion exchange, complexation, physical adsorption and precipitation (Vijayaraghavan *et al*., 2005; Preetha and Viruthagiri, 2007). The passive metal uptake may be present even when the cell is metabolically active and conversely, it may be suppressed by active metal exclusion processes. Passive metal uptake is relatively rapid and can be reversible (Cangeevaram *et al*., 2007). As opposed to a simple phenomenon of biosorption, biosorption is based on metabolism dependent (active) metal uptake and it occurs by transport across cell membrane and precipitation. It is an energy-driven process. According to the location, biosorption is an intracellular accumulation realized by transport across cell membrane (Hussein *et al*., 2005). This complex process is still not fully understood.

Corresponding Author: M. Dharmendira Kumar, Department of Chemical Engineering, A.C. Tech. Campus, Anna University Chennai, Chennai-600 025, India

Metabolically active cells from the exponential growth phase probably contain highly active enzymes, some of which may be involved in complexing and binding the metal ions. However, little information is available about the participation of enzymes in the mechanisms involved for the uptake of heavy metals in fungi and yeast (Ito *et al*., 2007). Bacteria alter the digestion of organic maters in aqueous solution (Hudnell *et al*., 2011). Strains of *Citrobacter* sp. produced acid phosphatase during growth and biosorbed heavy metals when resuspended in metal-supplemented media (Macaskie *et al*., 1992). The use of fungus belonging to the genus *Rhizopus* as a source of acid phosphatase has been defined in literature (Tsekova and Galabova, 2003). Metal uptake is mediated via the activity of an over produced acid-type phophatase, that serves also in non-growing cells to release HPO_4^{2-} from appropriate organic phosphate with the precipitation of heavy metals on the cell surface. In addition, the presence of heavy metal ions in the fermentation medium can increase the cellular enzyme activity.

The aim of this study is to find out the biosorption properties of *B. subtilis*, *P. aeruginosa* and *E. cloacae* for the removal of Cu (II) form aqueous solution. Biosorption conditions for biomass were screened by varying the pH, bioadsorbent dosage, contact time and initial metal ion concentration. Experimental results were analysed using the Langmuir and Freundlich adsorption isotherms. The experimental data were fitted to pseudo second order kinetic models. The mechanism of biosorption and the surface structure of the biomass were examined by Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscope (SEM) analysis, respectively.

MATERIALS AND METHODS

Microorganism growth and preparation for biosorption: *B. subtilis* (MTCC-121), *P. aeruginosa* (MTCC-424) and *E. cloacae* (MTCC-509) are the required bacterial cultures which were received from Microbial Type Culture Collection (MTCC) Chandigarh, India. The nutrient broth was prepared using the prescribed growth medium containing beef extract 1.0 g, yeast extract 0.1 g, peptone 5.0 g sodium chloride 5.0 g and distilled water 1.0 L. The bacterial culture was sterilized in an autoclave maintained at 15 lbs for a time period of 15 min as per the guidelines of (MTCC).

Preparation of samples: Synthetic Cu (II) solution were prepared using potassium dichromate salt of Cu (II). All chemicals used in the study were of analytical grade and were obtained from Ranbaxy Fine Chemicals Ltd., India. The Copper sulfate ($CuSO_4$. $5H_2O$) solutions were prepared using double distilled water. Cu (II) solutions of varying concentrations were acquired by diluting the stock solution. 1 N Sodium Hydroxide (NaOH) and 1 N Hydrochloric Acid (HCl) solutions were used to adjust the solution pH. Characterization of the bioadsorbent was done using Scanning electron microscope (SEM) and FT-IR analysis following which the results were studied. Optical Scanning electron microscopic (S-3400N-HITACHI, Japan) study was conducted to observe the surface texture and porosity of bioadsorbent. Fourier transform infrared spectroscopy (FTIR; Model Tensor 27, Bruker Optic GmbH, Germany) was used to determine the type of functional groups present in the bacteria and that which is responsible for Cu (II) metal biosorption. Atomic absorption spectrophotometer (AAnalyst 800; PerkinElmer, USA) was used for the determination of Cu (II) content in standard and treated solutions, respectively. The pH of the solution was measured using a pH Meter (L1 120; Elico-India) containing standard buffer solutions. Centrifuge (R-24, Research Centrifuge. REMI-India) was used for the bioadsorbent centrifugation. Incubated shaker (Scingenic Biotech/ ORBITEK-with temperature mode) at a constant speed of 150 rpm was used for the different bioadsorbents.

Batch biosorption of Cu (II): Batch experiments were conducted with solutions prepared from the stock solution. A known quantity of metal concentrated solution was taken into several flasks and biomass was added. The flasks were stirred at a constant speed of 150 rpm at room temperature for about 6 h. Test samples were collected at regular intervals of time, centrifuged and filtrated for the estimation of Cu (II) concentration. Experiments were carried out over a wide range of operating conditions and the percentage of Cu (II) removal, i.e., R (%) was calculated using the following equation:

$$R(\%) = \frac{(C_0 - C_e)}{C_0} \times 100 \quad (1)$$

where, C_0 and C_e represent initial and final Cu (II) concentration. The biosorption capacity can be estimated as:

$$q_e = \frac{(C_0 - C_e)}{M} \times V \quad (2)$$

where, q_e is the amount of adsorbed metal ion onto the biomass at equilibrium (mg L^{-1}), M represent is the amount of biomass in the suspension (g) and V is the volume of the suspension (L).

RESULTS AND DISCUSSION

Effect of pH: The important parameter in adsorption studies which controls metal ion sorption process is the effect of acidity in the medium (pH). Different adsorbents exhibit diverse uptake rates which will have different ranges of pH for optimum adsorption. In synthetic solutions the effect of pH on biosorption of Cu (II) ion onto *B. subtilis*, *P. aeruginosa* and *E. cloacae* was studied by varying the pH ranges from 2 to 9 as shown in Fig. 1. The Cu (II) uptake was observed to be high in *B. subtilis* at a pH of 2 while in *P. aeruginosa* and *E. cloacae* at a pH of 3 and 6, respectively. The effect of pH on the adsorption of Cu (II) is attributed to interactions between ions in solution and complexes formed at the adsorbent surface. On the Cu (II) ion removed Fig. 1, very clearly shows that *E. cloacae* has a higher removal percentage (92.86%) compared to *B. subtilis* (54.7%) and *P. aeruginosa* (26.6%). At a pH value lower than 3, the adsorption capacities were found to below due to the competitive adsorption of HO_3^+ ions and metal ions at the same active adsorption site. As the pH increases, the adsorption surface becomes less positive and therefore, electrostatic attraction between the metal ions and sawdust surface increases. The maximum sorption efficiency is observed at a pH of 6 and this may be due to the interaction of M^+, $M(OH)^+$, $M(OH)_2$ with the surface functional groups present in the sawdust. A slight decrease in adsorption at high pH is due to the formation of soluble hydroxyl complexes. These species are adsorbed at the surface of sawdust by ion exchange mechanism with the functional groups present in sawdust or by hydrogen bonding (Elliott and Huang, 1981).

$$2\,(\text{-ROH}) + M^{2+} \rightarrow 2(\text{RO})\,M + 2H^{+}\text{-ROH} + M(OH)^{+} \rightarrow (\text{-RO})\,M\,(OH) + H^{+}\ \text{ion exchange} \quad (3)$$

$$2\,(\text{- ROH}) + M\,(OH)_2 \rightarrow (\text{-ROH})_2 + M\,(OH)_2 \quad \text{H-bonding} \quad (4)$$

where, M represents the metal ions and R represents the matrix of sawdust, respectively.

A similar theory was proposed by several earlier works for metal sorption on different adsorbents. At higher pH values above 6, metal precipitation appears and the bioadsorbent gets deteriorated with the biosorption of metal ions (Acar and Eren, 2006). Therefore, a pH of 6 was selected to be the optimum pH for further studies.

Effect of biosorbent dosage: The studies indicate that the biosorbent dosage for removal of Cu (II) ions from aqueous solution was carried-out at different biosorbent doses (0.2-1.0 g) using 25-200 mg L^{-1} of Cu (II) (Fig. 2). it was observed that quantitative removal of Cu (II) ion was attained for adsorbent dosage of 0.2-0.4 g. The Cu (II) ion metal removed almost constant for a biosorbent dosage greater than 0.2 g for *B. subtilis* and *E. cloacae* and when it was greater than 0.4 g for *P. aeruginosa*. The biosorption for Cu (II) on *B. subtilis* decreased from 96 to 94% while in *P. aeruginosa* it decreased from 87 to 83% and in *E. cloacae* it decreased from 94 to 91%. Increase in the percentage of Cu (II) ion removal with biosorbent dosages could be attributed to the increase in biosorbent surface areas, resulting in the augmentation of the number of adsorption sites available for adsorption. On the other hand, the increase in the biosorbent dosage causes a decrease in the amount of Cr (II) ion adsorbed per gram of

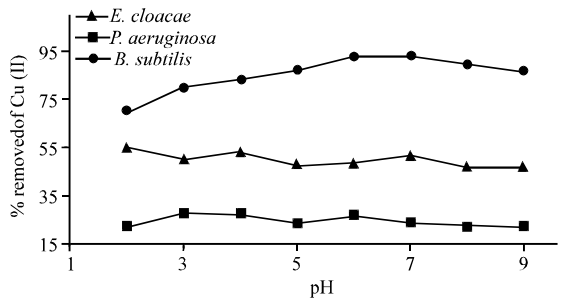

Fig. 1: Effect of initial pH on the biosorption of Cu (II) ion (conditions: in *B. subtilis*; biomass dosage = 0.2 g, contact time = 6 h, temperature = 27°C, concentration = 100 mg L^{-1}, agitation rate = 150 rpm, the same parameter condition were kept as a constant for *P. aeruginosa* and *E. cloacae*

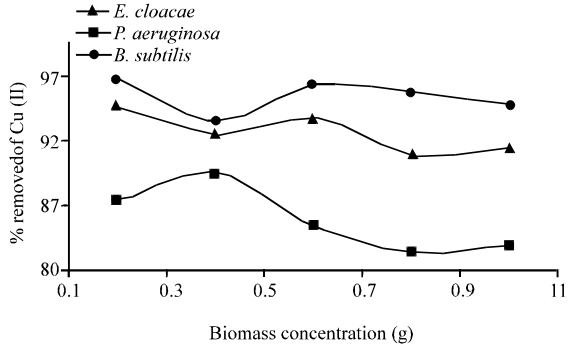

Fig. 2: Effect of Biomass dosage on biosorption of Cu (II) ion (conditions: in *P. aeruginosa* and *E. cloacae*; pH = 3 and 6, time = 6 h, concentration = 100 mg L^{-1}, temperature = 27°C, agitation rate = 150 rpm; for *B. subtilis*; (pH-2 and other parameter constants are same as the other biomass)

biosorbent (q_e) Fig. 2. The decrease in q_e value may be due to the splitting effect of flux (concentration gradient) between adsorbate and biosorbent with increasing biomass concentration causing a decrease in amount of Cu (II) ion adsorbed per gram of biomass. This effect may be attributed to the reduction in the overall surface area of the biosorbent probably because of aggregation during the adsorption process (Kumar and Porkodi, 2007).

Effect of contact time: Figure 3 shows the effect of contact time for the biosorption of Cu (II) ions by biosorbents. In order to determine the effect of contact time on the biosorption of Cu (II) ions, the contact time was varied from 0.5 to 6.0 h. It is apparent from the figure that significant removal of Cu (II) ion took 3.5 h. It was observed that in Cu (II) biosorption the initial rapid phase is probably due to the abundant availability of active metal binding sites on the biosorbent surface and the gradual occupancy of those sites. The sorption becomes less efficient in the slower stage (Saeed *et al*., 2005) as a result of the decrease in competition for available active binding sites on the biosorbent surface and so the metal ions remain in the solution. The rate of metal-sorption is of greater significance in developing a microbial origin sorbent-based water treatment technology and putting it to practical usage (Akar and Tunali, 2005).

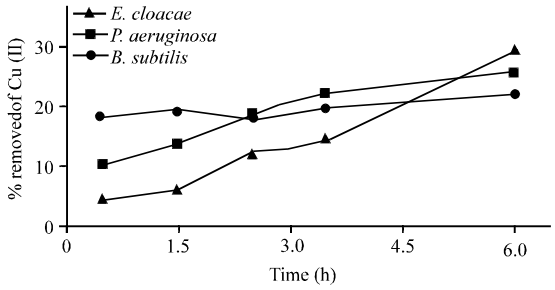

Fig. 3: Effect of contact time on biosorption of Cu (II) ion (conditions: in *P. aeruginosa* and *E. cloacae*; biomass dosage for *P. aeruginosa* = 0.2 g and *E. cloacae* = 0.4 g, pH = 3 and 6, concentration = 100 mg L^{-1}, temperature = 27°C, agitation rate = 150 rpm; for *B.subtilis*; (pH-2, biomass dosage = 0.2 g and other parameter constants are same as the other biomass

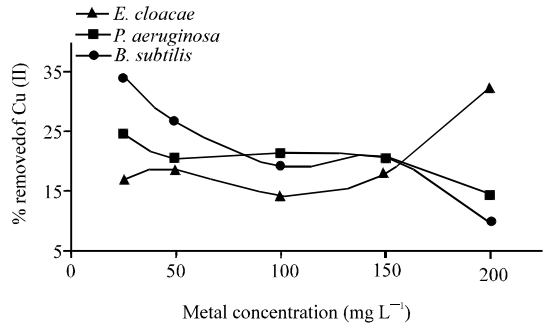

Fig. 4: Effect of metal concentration on biosorption of Cu (II) ion (conditions: in *P. aeruginosa* and *E. cloacae*; biomass dosage for *P. aeruginosa* = 0.2 g and *E. cloacae* = 0.4 g, pH = 3 and 6, contact time = 3.5 h, temperature = 27 °C, agitation rate = 150 rpm; for *B. subtilis*; (pH-2, biomass dosage = 0.2 g and other parameter constants are same as the other biomass)

Effect of initial concentration with time: Effect of initial concentration on Cu (II) removal by biosorbent was studied by carrying out the experiments at different initial concentrations varying from 25-200 mg L^{-1} of Cu (II) and by keeping pH, adsorbent dose by varying the contact time. The removal of Cu (II) by biosorbent percentage chromium removal decreased with increase in maximum concentration Fig. 4. In the given time duration of 3.5 h the maximum Cu (II) removal percentage that occurred in *B. subtilis* was (35% in 25 mg L^{-1}) while in *P. aeruginosa* it was (25% in 25 mg L^{-1}) and in *E. cloacae* it was (34% in 200 mg L^{-1}). The removal of Cu (II) is dependent on pH, initial Cu (II) concentration and adsorbent dosage, so when the initial concentration of Cu (II) is low, adsorbent dosage is higher and pH is optimum, the permissible levels of Cu (II) can be achieved in the solution. At low concentration, the ratio of available surface to the initial Cu (II) concentration is larger, so the removal is higher. However, in case of higher concentrations this ratio is low; hence the percentage removal is also lesser. Higher adsorption capacity at higher metal ion concentration can be attributed to increase in the rate of mass transfer due to increase in the driving force due to concentration difference (Garg *et al*., 2008).

Adsorption behavior of biomass-isotherm studies: The biosorption isotherms reveal the specific relation between the concentration of the adsorbate and its degree of biosorption onto the biomass surface at a constant metal ion concentration. To measure the biosorption capacity of *B. subtilis*, *P. aeruginosa* and *E. cloacae* on the removal of Cu (II) ion from aqueous solution, the Langmuir, Freundlich isotherm models were used.

Langmuir model: This model shows that the biosorption occurs at specific homogeneous sites on the biomass and it is successfully used in many monolayer biosorption

Table 1: Langmuir and Freundlich biosorption isotherm for Cu (II) on *B. subtilis, P. aeruginosa* and *E. cloacae*

	Langmuir constant			Freundlich constant		
Bacteria	K_L	b	R^2	K_F	b_F	R^2
B. subtilis	100	0.095	0.617	11.623	0.934	0.994
P. aeruginosa	17.54	0.354	0.896	2.65	1.451	0.968
E. cloacae	2.174	4.692	0.931	38.02	0.691	0.994

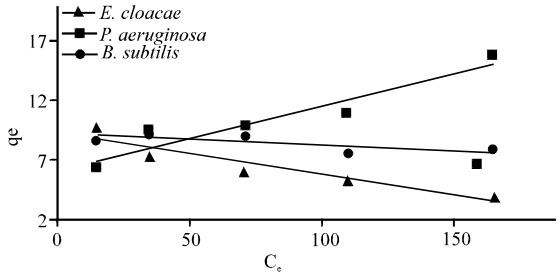

Fig. 5: Langmuir isotherm models for *B.subtilis*, *P.aeruginosa* and *E.cloacae* on Cu (II) ion

processes. The data collected from the equilibrium studies for biosorption of Cu (II) ion onto *B. subtilis*, *P. aeruginosa* and *E. cloacae* may follow the Langmuir model:

$$q_e = \frac{K_L b C_e}{1 + b C_e} \quad (5)$$

The binding constant (K_L) and the sorbent capacity (b) are estimated by plotting C_e/q_e against C_e. The model simulations along with experimental observations for Cu (II)with the experimental values of K_L and b along with the linear regression co-efficient (R^2) are given in Table 1 and Fig. 5, respectively.

Freundlich model: The Freundlich model is applied for biosorption on heterogeneous surfaces and for multilayer biosorption. The mathematical expression of the Freundlich model can be given as

$$\ln q = \ln K_F + b_F \ln C_e \quad (6)$$

A plot of $\ln q_e$ versus $\ln C_e$ gives a straight line with slope K_F and intercept b_F. The values of K_F and $b_F$along with the linear regression co-efficient (R^2) for the present experimental conditions have been obtained and are given in Table 1 and Fig. 6 shows that the correlation coefficient (R^2) observed from Freundlich isotherm model for *B. subtilis*, *P. aeruginosa* and *E. cloacae* match satisfactorily with the experimental observation.

Kinetic studies: This study describes the solute uptake rate and residence time of biomass uptake at the

Table 2: Biosorption kinetics for Cu (II) on *B.subtilis*

	Pseudo first order			Pseudo second order		
B. subtilis Concentration (mg L^{-1})	q_e	K_1	R^2	q_e	K_2	R^2
25	1.035	0.331	0.846	1.329	1.641	0.978
50	1.713	0.519	0.859	3.688	1.85	0.997
100	4.001	0.517	0.891	7.776	0.725	0.996
150	9.468	0.616	0.972	11.751	0.218	0.984
200	13.53	0.597	0.978	18.52	0.153	0.989

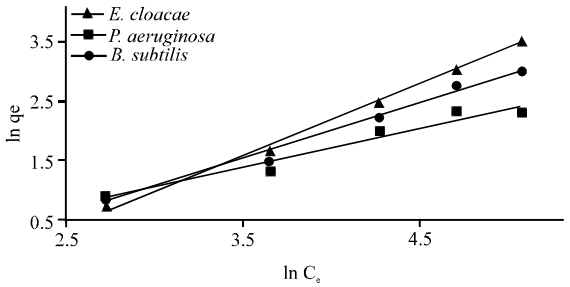

Fig. 6: Freundlich isotherm models for *B. subtilis*, *P. aeruginosa* and *E. cloacae* on Cu (II) ion

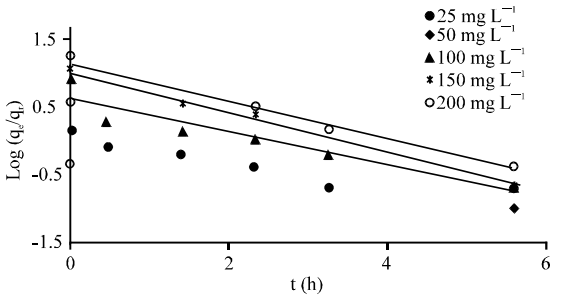

Fig. 7: Pseudo first order plot of time versus t/ log (q_e/q_t) for the Cu (II) on *B. subtilis* at various metal concentration

solid-liquid interface including the diffusion process. The mechanism of biosorption depends on the physical and chemical characteristics of the biomass. The rate of kinetics on *B. subtilis, P. aeruginosa* and *E. cloacae* was analyzed using pseudo first order and pseudo second order kinetic models. The conformity between experimental data and the model predicted values were expressed by correlation coefficient (R^2). The linear form of pseudo first and second order kinetic equations are given as:

$$\log (q_e - q_t) = \log (q_e) - \frac{k_1 t}{2.303} \quad (7)$$

and

$$\frac{t}{q} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (8)$$

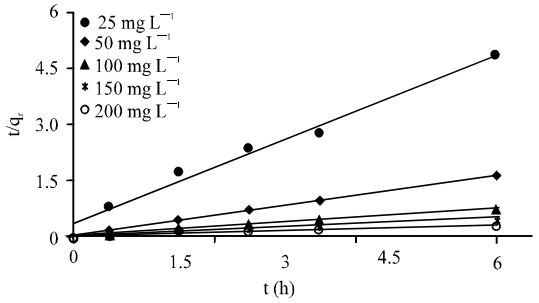

Fig. 8: Pseudo second order plot of time versus t/q_t for the Cu (II) on *B. subtilis* at various metal concentration

Table 3: Biosorption kinetics for Cu (II) on *P. aeruginosa*

P.aeruginosa concentration	Pseudo first order			Pseudo second order		
(mg L^{-1})	q_e	K_1	R^2	q_e	K_2	R^2
25	1.563	0.476	0.961	1.891	0.856	0.962
50	2.461	0.541	0.981	3.058	0.672	0.979
100	3.573	0.640	0.932	5.940	0.649	0.991
150	5.164	0.548	0.962	7.092	0.382	0.984
200	4.207	0.442	0.891	6.802	0.540	0.993

Table 4: Biosorption kinetics for Cu (II) on *E. cloacae*

E.cloacae concentration	Pseudo first order			Pseudo second order		
(mg L^{-1})	q_e	K_1	R_1^2	q_e	K_2	R_2^2
25	1.595	0.673	0.962	1.520	0.936	0.956
50	7.079	1.460	0.904	4.902	1.015	0.996
100	7.762	0.628	0.963	11.236	0.293	0.99
150	8.912	0.617	0.975	11.627	0.238	0.986
200	13.09	0.523	0.967	18.416	0.154	0.987

where, q and q_e are the amount of metal adsorbed per unit weight of biomass (mg L^{-1}) at time t and at equilibrium respectively and k_1 and k_2 are the biosorption rate constants. The pseudo first order and pseudo second order rate constant k_1, k_2 and q_e were estimated from the model for *B. subtilis*, *P. aeruginosa* and *E. cloacae* with corresponding correlation coefficients are presented in Table 2-4, respectively. The Fig. 7 shows *P. aeruginosa* with metal concentration shows time versus log (q_e/q_t) and in Fig. 8 shows that *P. aeruginosa* with time versus t/q_t for various initial concentrations were studied in the present investigations in *B. subtilis*, *P. aeruginosa* and *E. cloacae*. In this investigation it was noticed that the pseudo second order kinetics match satisfactorily with the experimental observation.

Fourier transform infrared spectroscopy (FTIR): The FTIR spectra of metal and metal-loaded biosorbents were used to determine the vibrational frequency changes in the functional groups of the adsorbents. The spectra of the adsorbents were measured within the range of 400-4000 cm^{-1}, confirmed the presence of functional groups that are usually responsible for the biosorption

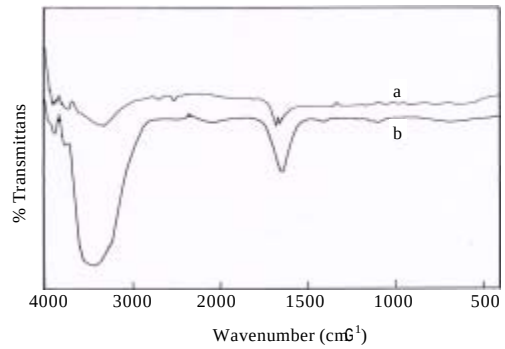

Fig. 9: FTIR Spectra of *B.subtilis* in Cu (II), (a) with out metal (b) with metal loaded

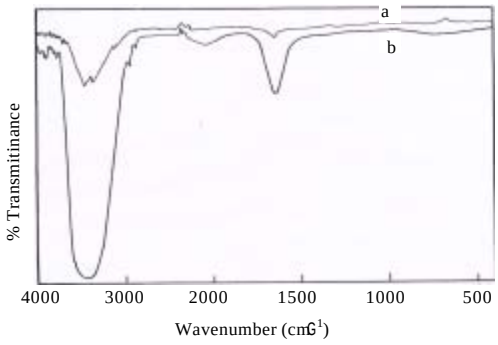

Fig. 10: FTIR Spectra of *P.aeruginosa* in Cu (II), (a) with out metal (b) with metal loaded

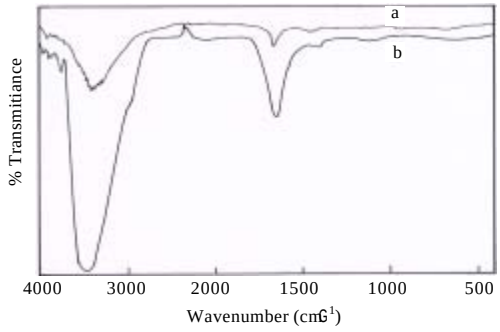

Fig. 11: FTIR Spectra of *E.cloacae* in Cu (II), (a) with out metal (b) with metal loaded

process (Fig. 9-11). The metal ion displays a number of absorption peaks, reflecting the complex nature of the biomass. A peak in the region of 3500-3200 cm^{-1} is due to the stretching of the N-H bond of amino groups and indicates bonded hydroxyl groups (Park *et al*., 2005). The absorption peak at 2352 cm^{-1} is due to C-H bond stretching. bonds. The peaks observed the region 2900-2070 cm^{-1} can be assigned to the stretching and asymmetric vibration of the C-H group. The peaks in the

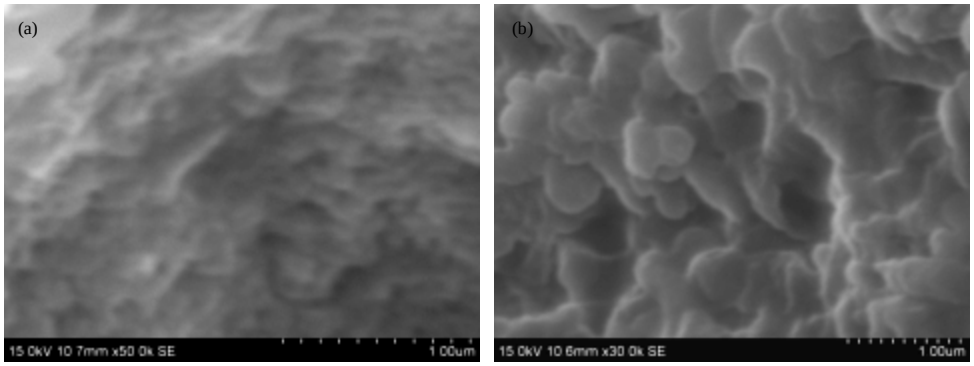

Fig. 12: SEM micrograph of (a) before and (b) after Cu (II) treatment on *B. subtilis*

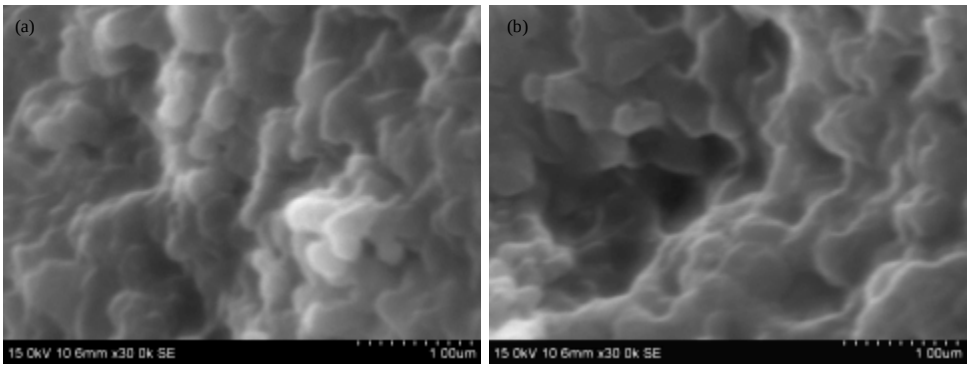

Fig. 13: SEM micrograph of (a) before and (b) after Cu (II) treatment on *P. aeruginosa*

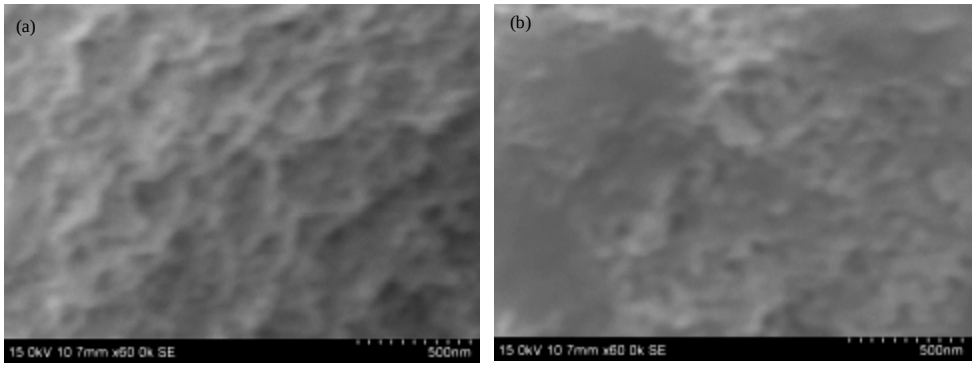

Fig. 14: SEM micrograph of (a) before and (b) after Cu (II) treatment on *E. cloacae*

range of 1645-1630 cm^{-1} are caused by the vibration of C = O and C = C, respectively. The peak at 724 cm^{-1} is due to the presence of aromatic-CH stretching (Tunali *et al*., 2006).

Scanning electron microscopy (SEM): In order to understand the morphology of biosorbent, SEM analyses was done on bacterial samples before and after the adsorption was carried out and are shown in Fig. 12-14,

respectively. The comparison of SEM pictures between the natural and metal loaded biosorbent shows that the particle has undergone remarkable physical disintegration after adsorption in all the three biosorbent.

CONCLUSION

The present study has demonstrated that bacterial biomass possesses the adsorption capacity to remove heavy metal ions and organic compounds. The following are the conclusions arrived from this study.

The investigation shows that *B. subtilis*, *P. aeruginosa* and *E. cloacae* are abundant and hence cost effective bacteria and therefore, can be used as bioadsorbent for the removal of Cu (II) metal ion from aqueous solutions. The biosorption performance is affected by various parameters, i.e., pH, contact time, bioadsorbent dosage and initial metal ion concentrations. The optimum dose of biomass for heavy metal removal is found to be 0.2 g for *B. subtilis* and *E. cloacae* while 0.4 g for *P. aeruginosa*. The kinetic and equilibrium data fitted well with the pseudo second order kinetic model and the Freundlich isotherm model, respectively. The biosorption of Cu (II) onto *B. subtilis*, *P. aeruginosa* and *E. cloacae* was found to follow the process of biosorption on the homogeneous surface via chemisorption process. Of these three bacteria, the maximum biosorption capacity with good rate constant and correlation coefficient was obtained in *B. subtilis*. The proposed biosorption system was successfully applied to real effluent spiked with Cu (II) ions.

REFERENCES

Acar, F.N. and Z. Eren, 2006. Removal of Cu (II) ions by activated poplar sawdust (*Samsun clone*) from aqueous solutions. J. Hazard. Mater., 137: 909-914.

Akar, T. and S. Tunali, 2005. Biosorption performance of *Botrytis cinerea* fungal by-products for removal of Cd(II) and Cu (II) ions from aqueous solutions. Miner. Eng., 18: 1099-1109.

Anandkumara, J. and B. Mandal, 2009. Removal of Cr(VI) from aqueous solution using Bael fruit (*Aegle marmelos correa*) shell as an adsorbent. J. Hazard. Mater., 168: 633-640.

Barrera, H., F. Urena-Nunez, B. Bilyeu and C.B. Diaz, 2006. Removal of chromium and toxic ions presents in mine drainage by *Ectodermis* of *Opuntia*. J. Hazard. Mater., 136: 846-853.

Cangeevaram, S., S. Dhanarani, J. Park, M. Dexilin and K. Thamaraiselvi, 2007. Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J. Hazard. Mater., 146: 270-277.

Elliott, H.A. and C.P. Huang, 1981. Adsorption characteristics of some Cu (II) complexes on aluminosilicates. Water Resour., 15: 849-849.

Garg, U.K., M.P. Kaur, V.K. Garg and D. Sud, 2008. Removal of nickel(II) from aqueous solution by adsorption on agricultural waste biomass using a response surface methodological approach. Bioresour. Technol., 99: 1325-1331.

Han, R., J. Zhang, W. Zou, H. Xiao, J. Shi and H. Liu, 2006. Biosorption of copper(II) and lead(II) from aqueous solution by chaff in a fixed-bed column. J. Hazard. Mater., 133: 262-268.

Hawari, A.H. and C.N. Mulligan, 2006. Biosorption of lead (II), cadmium (II), Copper (II) and nickel (II) by anaerobic granular biomass. Bioresour. Technol., 97: 692-700.

Hudnell, H.K., D. Green, R. Vien, S. Butler, G. Rahe, B.A. Richards and J. Bleth, 2011. Improving wastewater mixing and oxygenation efficiency with solar-powered circulation. Clean Technol. Environ. Policy, 10.1007/s10098-010-0345-x

Hussein, H., S. Farag, K. Kandil and H. Moawad, 2005. Tolerance and uptake of heavy metals by *Pseudomonads*. Process Biochem., 40: 955-961.

Ito, H., M. Inouhe, H. Tohoyama and M. Joho, 2007. Effect of copper on acid phosphatase activity in yeast *Yarrowia lipolytica*. Z. Naturforsch., 62: 70-76.

Kumar, K.V. and K. Porkodi, 2007. Mass transfer, kinetics and equilibrium studies for the biosorption of methylene blue using *Paspalum notatum*. J. Hazard. Mater., 146: 214-226.

Macaskie, L.E., R.M. Empson, A.K. Cheethman, C.P. Grey and A.J. Skarnulis, 1992. Uranium bioaccumulation by a *Citrobacter* sp. as a result of enzymatically mediated growth of polycrystalline HUO2PO4. Science, 257: 782-874.

Park, D., Y.S. Yun and J.M. Park, 2005. Studies on hexavalent chromium biosorption by chemically-treated biomass of *Ecklonia* sp. Chemosphere, 60: 1356-1364.

Preetha, B. and T. Viruthagiri, 2007. Bioaccumulation of chromium(VI), copper(II) and nickel(II) ions by growing *Rhizopus arrhizus*. Biochem. Eng. J., 34: 131-135.

Saeed, A., M.W. Akhter and M. Iqbal, 2005. Removal and recovery of heavy metals from aqueous solution using papaya wood as a new biosorbent. Separation Purif. Technol., 45: 25-31.

Tiemann, K.J., J.L. Gardea-Torresdey, G. Gomez and K. Dokken, 1998. Interferences studies for multi-metal binding by *Medicago sativa* (alfalfa). Proceedings of the Conference on Hazardous Waste Research, May 18-21, Snowbird, pp: 63-74.

Tsekova, K. and D. Galabova, 2003. Phosphatase production and activity in copper(II) accumulating *Rhizopus delemar*. Enzyme Microb. Technol., 33: 926-931.

Tunali, S. and T. Akar, 2006. Zn(II) biosorption properties of *Botrytis cinerea* biomass. J. Hazard. Mater., 131: 137-145.

Tunali, S., A. Cabuk and T. Akar, 2006. Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. Chem. Eng. J., 115: 203-211.

Uriu-Adams, J.Y. and C.L. Keen, 2005. Copper, oxidative stress and human health. Mol. Aspects Med., 26: 268-298.

Vijayaraghavan, K. and Y.S. Yun, 2008. Bacterial biosorbents and biosorption. Biotechnol. Adv., 26: 266-291.

Vijayaraghavan, K., J. Jegan, K. Palanivelu and M. Velan, 2005. Biosorption of copper, cobalt and nickel by marine green alga *Ulva reticulate* in a packed column. Chemosphere, 60: 419-426.

WHO, 1998. Copper. Environmental Health Criteria 200, International Programme on Chemical Safety. World Health Organization, Geneva, Switzerland.