



Biotechnological potential of *Microcystis* sp. in Cu, Zn and Cd biosorption from single and multimetallic systems

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Received 25 October 2000; accepted 13 December 2000

Key words: *Microcystis* sp., biosorption, Cu, Zn, Cd, adsorption isotherm, bimetallic and trimetallic combinations

Abstract

This paper provides information on biosorption of Cu, Zn and Cd by *Microcystis* sp. in single, bi and trimetallic combination. Highest biosorption of Cu followed by Zn and Cd in single as well as in mixtures containing two or three metals was noticed. The order of inhibition of Cu, Zn and Cd biosorption in bi and trimetallic combinations was suggestive of screening or competition for the binding sites on the cell surface. This observation was reconfirmed by Freundlich adsorption isotherm. K_f values were maximum for Cu ($K_f = 45.18$), followed by Zn ($K_f = 16.71$), and Cd ($K_f = 15.63$) in single metallic system. The K_f values for each test metal was reduced in solution containing more than one metal. Further, the reduction in biosorption of each metal ion due to presence of other metal ion was of greater magnitude at relatively higher concentrations of interfering metal ion. The biosorption of Cu at saturation was less affected when secondary metal (Cd or Zn) was added in the medium. Above results suggest that *Microcystis* holds great potential for metal biosorption from mixture.

Introduction

Disastrous effects of heavy metals on biota discharged anthropogenically in the aquatics has aroused considerable attention of environmental biotechnologists to develop suitable strategies for reducing burgeoning burden of metals from the system. A variety of mechanisms exist for the removal of heavy metals from aqueous solution by algae, bacteria, fungi, mosses, macrophytes and higher plants (Cotoras *et al.* 1992; Holan & Volesky 1994; Leusch *et al.* 1995; Knauer *et al.* 1997). Of the different techniques employed for metal removal from multi elemental system, biosorption has been found to be highly selective depending on the typical binding profile of the biosorbents (Knauer *et al.* 1997; Crist *et al.* 1994).

Most studies have been performed by taking one or two metals at a time. A vast literature has been accumulated on sequestering and accumulation of single metallic ions by different organisms. Leusch *et al.* (1995) described biosorption of five metals by two ma-

rine biomass of *Sargassum fluitans* and *Ascophyllum nodosum*. Simmons *et al.* (1995) compared Ag and Cu uptake capacities between different strains of *Saccharomyces cerevisiae* and between *Saccharomyces cerevisiae* and *Candida* sp. The study of bacterial sorption of metal ions has been evoking attention of bio-scientists due mainly to its environmental and technological implications (Cotoras *et al.* 1992). An interesting piece of work on simultaneous binding of Pb, Zn and Cu by *Chlorella vulgaris* has been produced by Pascucci & Sneddon (1993). Zhou *et al.* (1998) demonstrated a strong competition between Cu and Cd for *Sargassum kjellmanianum* and *Laminaria japonica*. A recent study on the biosorption of Cr and Fe in two metal system using algae (Chong & Volesky 1996; Aksu *et al.* 1997) peat moss and lignite (Allen & Brown 1995; McKay & Porter 1997) as biosorbents has been carried out and results were analysed using isotherms. It is worth stating that mathematical formulations (adsorption isotherms) so developed for such studies are extremely useful in expressing the

relationships between the quantity of one component adsorbed and the concentration of all other components, either present in solution or already adsorbed. However, interference and competition produced by metals for adsorption sites have been explained with the help of mathematical formulation of the equilibrium. This is especially relevant when the sorption of one ion influences the binding of another, as is the case when different ions compete for the same binding sites (Schiewer & Volesky 1996).

One of the difficulties in studying the adsorption of metal ions from wastewaters is the presence of a host of metals in such waters. Although lot of work has been conducted on metal biosorption by various researchers using different microorganisms, no attempt seems to have been initiated to investigate the selectivity and capacity of *Microcystis* sp. for biosorption of different metal ions from single and multimetal systems. It deserves mention that the test cyanobacterium *Microcystis* sp. selected for this purpose is easily and abundantly available in eutrophic ponds of India as well as in different parts of the world. Some other characteristics of this organism are: efficient and rapid biosorption of metals, low production cost of the biosorbent, ease in separation of the biosorbent from the solution and high selectivity in metal biosorption. In view of the above characteristics as well as the perceptible gap in our knowledge on metal biosorption by *Microcystis* sp. from metal mixtures, it was decided to employ this organism for the present study. The aim of this study was (i) to evaluate competitive sorption of Cu, Zn and Cd by *Microcystis* sp. in binary and ternary metal mixture and compare with a single metal ion situation in batch stirred system when equal amounts of metals are spiked in biosorption medium, and (ii) selective biosorption potential of test cyanobacterium for Cu, Zn and Cd ions from the solution.

Materials and methods

Microcystis cells were harvested by centrifugation, washed and the pellets resuspended in Milli-Q water instead of growth medium as constituents of the growth medium may interfere with sorption of test metals by the test organism. The concentrated and washed algal biomass was diluted with predetermined volume of Milli-Q water for obtaining desired biomass concentration (40 mg l⁻¹). The biomass was dried at 70 °C in a hot air oven till a constant weight was obtained. This exercise was necessary to express re-

sults in term of dry wt. Chemicals used were of Loba grade. Metal solutions were prepared by dissolving CuCl₂, CdCl₂ and ZnSO₄ in Milli-Q water. The pH of biosorption medium was adjusted between 6.5 to 7.0 with 1 N NaOH or 1 N HCl.

Preparation of biosorption media containing single metal ions

The calculated amount of concentrated stock culture was added to 10 ml experimental solution containing varying concentrations (ranging from 1 to 50 µg ml⁻¹) of metals, to give a final biomass concentration of about 40 mg l⁻¹. The flasks were agitated at 30 rpm in continuous light at 29 ± 2 °C in an environmental shaker (model 3597 – ICOGMPR, USA) for 1 h. After incubation, the cells were separated from metal solution by vacuum filtration using 0.45 µm cellulose acetate Sartorius membrane filters. The residual metal concentration in the solution was measured by atomic absorption spectrophotometer (model 2380). Control experiments were also carried out using culture vessels containing metal solution but without alga. Controls and blanks were examined throughout the sorption experiment to check sorption of metals if any by the glassware.

Metal biosorption from bimetallic and trimetallic solutions

Efforts have been made to find out the sorption behaviour of the test cyanobacterium exposed to bi and trimetallic solution. Metal solution contained Cu-Cd; Cu-Zn; and Zn-Cd in bimetallic and Cu, Zn and Cd in trimetallic solution in the concentration range of 1 to 50 µg ml⁻¹ for each metal. Batch equilibrium sorption experiments were carried out using standard batch methodology. Samples were withdrawn at predetermined time intervals and the metal adsorbed was determined by subtracting the metal left in the solution after 1h of biosorption from the initial metal ion concentration.

In order to determine adsorption characteristics of Cu in binary metal mixture, the initial concentration was varied from 1 to 50 µg ml⁻¹ while the Zn concentration in each biosorption medium was held constant at 1, 10, 20 and 40 µg ml⁻¹. The pH of biosorption media was adjusted to the optimum value for the biosorption of Zn with 1 N HCl and 1 N NaOH. To determine sorption characteristics of Zn in binary metal mixture the initial concentration of Zn was varied from

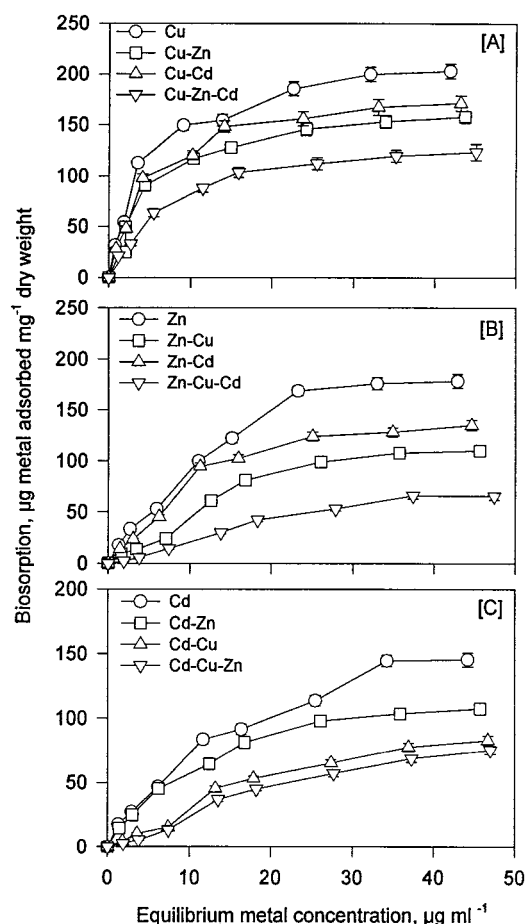


Fig. 1. Metal biosorption characteristics of *Microcystis* sp. from single, bimetallic and multimetallic system. (A) Cu biosorption in single, bimetallic (Cu-Zn, Cu-Cd) and multimetallic (Cu-Zn-Cd) system. (B) Zn biosorption in single, bimetallic (Zn-Cu, Zn-Cd) and multimetallic (Zn-Cu-Cd) system. (C) Cd biosorption in single, bimetallic (Cd-Cu, Cd-Zn) and multimetallic (Cd-Cu-Zn) system. Values are mean of three replicates.

1 to 50 $\mu\text{g ml}^{-1}$ while Cu concentration was held constant at 1, 10, 20 and 40 $\mu\text{g ml}^{-1}$. Similar procedure was applied for the study of biosorption characteristics of Cu in presence of fixed concentration of Cd and vice versa as well as biosorption characteristics of Zn in presence of fixed concentration of Cd and vice versa. In order to study the effect of secondary metal on percent biosorption of primary metal by *Microcystis* from a solution containing a mixture of two metals, primary metal ion concentration was held constant at 1, 10 and 20 $\mu\text{g ml}^{-1}$, respectively, while the initial secondary metal ion concentration was varied from 1 to 50 $\mu\text{g ml}^{-1}$.

Results

Figure 1 (A, B, C) shows biosorption of Cu, Zn and Cd by *Microcystis* sp. in single, bi and trimetallic combination. In single metallic system, Cu was more readily biosorbed by *Microcystis* sp. than from a mixed solution containing Cu and Zn; Cu and Cd; and Cu, Zn and Cd, respectively (Figure 1A). In Cu-Zn combination, Zn efficiently lessened Cu biosorption but in Cu-Cd combination, Cd was not as effective as Zn in decreasing the biosorption of Cu by test alga. In trimetallic system, the biosorption of Cu was further decreased as compared to the bimetallic system. Further, when the initial Cu concentration was low, the biosorption of Cu in bimetallic combination was not affected by the second metal. However, an increase in the concentration of both primary and secondary metals from 10 to 50 $\mu\text{g ml}^{-1}$, Cu biosorption was substantially inhibited. Biosorption of Zn was low in presence of Cu in bi and trimetallic condition respectively. Zn sorption was appreciably decreased when equal amount of Cu was added in the solution. But when equal amounts of both Zn and Cd were added in the sorption medium, Zn biosorption was not as strongly inhibited by Cd at low concentration of both the metals. However, on increasing the concentration of both the metals, a decline in Zn biosorption was clearly noticed. As compared to the biosorption of Cu and Zn in single metallic condition, biosorption of Cd by *Microcystis* was low (Figure 1C). In Cd-Cu and Cd-Zn combinations, both Cu and Zn antagonistically affected the Cd biosorption.

It is *sine-qua-non* that Langmuir and Freundlich adsorption isotherm models have been used over time to explain the efficiency and pattern of binding. Figure 2 compiles the data of solid-phase equilibrium metal concentration, expressed as $\mu\text{g metal per mg dry weight sorbent}$, versus the liquid phase equilibrium metal ion concentration ($\mu\text{g ml}^{-1}$). The linear biosorption plots ($\log Q_{eq}$ against $\log C_{eq}$) drawn for *Microcystis* subjected to different concentrations of test metals in single, bi and trimetallic combination followed the Freundlich equation (Figure 2). The series of straight lines in Figure 2 vividly reflected the best fitness of the data. Freundlich constants i.e. adsorbent capacity (K_f) and intensity (n) determined for all the systems studied are compiled in Table 1. This shows that the K_f values were maximum for Cu ($K_f = 45.18$), followed by Zn ($K_f = 16.710$), and Cd ($K_f = 15.63$). These values not only support the experimental data but depict a much stronger bind-

Table 1. Freundlich constants and r^2 values obtained from Freundlich isotherm in single and multimetallic combination

Metal ion	System reduction	K_f	n	r^2	% K_f
Cu	Cu	45.18	2.212	0.917	–
Zn	Zn	16.71	1.464	0.977	–
Cd	Cd	15.63	1.607	0.984	–
Cu	Cu-Zn	30.61	2.061	0.816	32
Zn	Cu-Zn	5.984	1.212	0.958	64
Cu	Cu-Cd	37.23	2.188	0.919	18
Cd	Cu-Cd	2.971	1.079	0.964	81
Zn	Zn-Cd	12.56	1.449	0.948	25
Cd	Zn-Cd	13.64	1.709	0.974	13
Cu	Cu-Zn-Cd	22.49	2.020	0.948	50
Zn	Cu-Zn-Cd	1.70	0.967	0.907	90
Cd	Cu-Zn-Cd	1.34	0.904	0.977	91

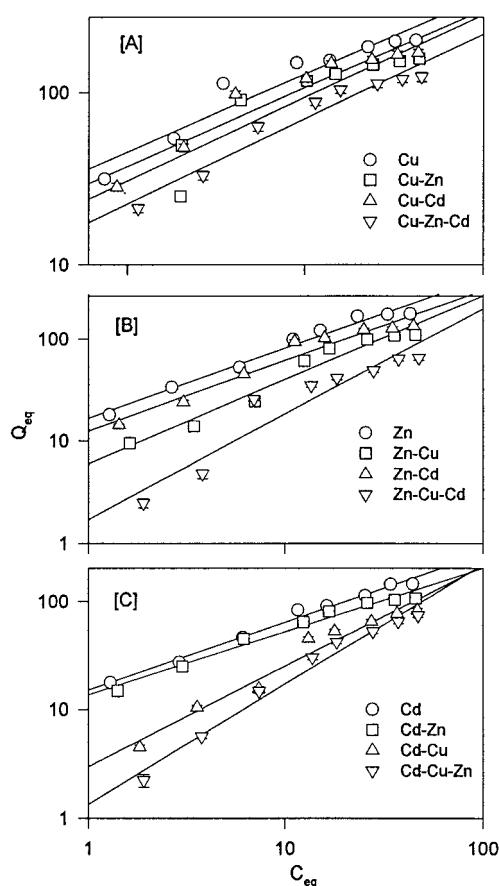


Fig. 2. Freundlich adsorption isotherm for biosorption of Cu [A], Zn [B] and Cd [C] from single, bi and trimetallic systems. Values are mean of three replicates.

ing capacity of *Microcystis* for Cu than Zn and Cd. In Cu – Zn combination, sorption capacity of *Microcystis* (K_f value) for these metals was reduced by 32 and 64% as compared to its biosorption capacity in single metal species. Likewise, reduction in biosorption capacity of Cu and Cd was noticed when both the metals were present in combination. However, Cd biosorption was more inhibited than Cu. No clear cut difference in biosorption of Zn and Cd in bimetallic combination of Zn – Cd was noticed. K_f values of these metals were respectively 12.56 and 13.64 and there was only 25 and 13% reduction in biosorption when both were present in the solution. In trimetallic combination sorption capacity (K_f) of *Microcystis* for Cu, Zn and Cd was respectively 22.49, 1.7 and 1.34, and approximately 50, 90 and 91% reduction in sorption capacity of *Microcystis* for biosorption of these three metals was obtained when equal amounts of these metals were present in metal mixture.

Table 2 compiles the data of effect of secondary metal on biosorption characteristics of primary metal in term of maximum biosorption at saturation (Q_{max}). Cu biosorption at saturation (Q_{max}) when used singly was $185.22 \mu\text{g mg}^{-1}$ dry wt. Q_{max} was decreased to 175.22, 157.52, 140.72 and $125.30 \mu\text{g mg}^{-1}$ dry wt. when 1, 10, 20 and $40 \mu\text{g mg}^{-1}$ Zn was added in the biosorption medium. Likewise, biosorption of Zn as reflected by Q_{max} value, decreased gradually at increasing concentration of Cu ion in biosorption medium. Further, effect of Cu on Zn biosorption was more pronounced as compared to effect of Zn on Cu biosorption in bimetallic system of Cu-Zn (Table 2). Similar trend of reduction in Q_{max} of primary metal ion with increasing concentration of secondary metal ions was noticed in Zn-Cd system. No clear cut decrease in sorption characteristics of Zn and Cd in Zn-Cd system by *Microcystis* sp. in the presence of increasing concentration (from 1 to $40 \mu\text{g ml}^{-1}$) of Cd and Zn was observed in a system containing Zn- Cd. In contrast to this, Cu biosorption at saturation was not much affected when Cd concentration in biosorption medium was held constant. Approximately 2, 15, 18 and 21% reduction in Q_{max} was noticed respectively at 1, 10, 20 and $40 \mu\text{g ml}^{-1}$ Cd and approximately 9, 23, 32 and 46% reduction in biosorption of Cd at saturation (Q_{max}) was obtained respectively at 1, 10, 20 and $40 \mu\text{g ml}^{-1}$ Cu.

Figure 3 summarises the effect of different concentrations of secondary metal on the percent biosorption of primary metal at different selected concentrations. The biosorption of the primary metal $Q_{eq}(M_1)$ ex-

Table 2. Effect of secondary metal on biosorption characteristics of primary metal [maximum biosorption at saturation (Q_{\max})]

Conditions	Secondary metal concentration ($\mu\text{g ml}^{-1}$)	Biosorption of primary metal ion at saturation ($\mu\text{g ml}^{-1}$)	
		Cu	Zn
A. Cu-Zn	0	185.225 \pm 8.59	175.25 \pm 9.50
	1	175.225 \pm 10.50 (5)	170.00 \pm 8.75 (3)
	10	157.525 \pm 8.50 (15)	137.50 \pm 7.93 (21.625)
	20	140.725 \pm 7.50 (24)	120.25 \pm 7.73 (31)
	40	125.30 \pm 8.25 (32)	107.75 \pm 8.50 (38)
B. Zn-Cd	0	175.25 \pm 9.50	144.35 \pm 7.50
	1	168.05 \pm 8.75 (4)	139.35 \pm 5.90 (5)
	10	143.00 \pm 7.37 (18)	125.775 \pm 5.32 (13)
	20	128.125 \pm 6.93 (27)	110.00 \pm 5.20 (24)
	40	113.00 \pm 5.58 (35)	103.375 \pm 4.53 (28)
C. Cu-Cd	0	185.225 \pm 6.90	144.35 \pm 7.50
	1	181.225 \pm 7.53 (2)	131.62 \pm 4.99 (9)
	10	158.25 \pm 6.89 (15)	111.25 \pm 3.59 (23)
	20	153.00 \pm 6.20 (18)	97.75 \pm 3.51 (32)
	40	146.25 \pm 5.20 (21)	77.50 \pm 2.89 (46)

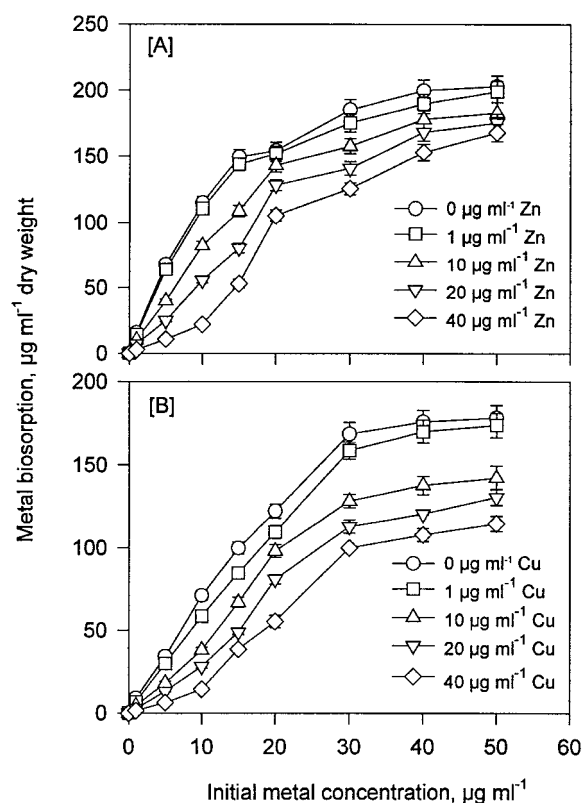


Fig. 3. Effect of Cu and Zn on biosorption of Zn[A] and Cu[B] respectively by *Microcystis* sp. from Cu-Zn system. Values are mean of three replicates.

pressed in term of percent sorption (% Q_{eq}) as affected by different concentrations of secondary metal is compared with the original value when no secondary metal was added [$C_{eq}(M_2)=0$]. When 1, 10 and 20 $\mu\text{g ml}^{-1}$ of Cu was spiked into a fixed biomass, Cu adsorbed per mg dry wt. was 15.73, 114.70 and 154.17 $\mu\text{g mg}^{-1}$ respectively (Figure 3). On changing the initial concentration of secondary metal (Zn) from 1 to 50 $\mu\text{g ml}^{-1}$ and keeping the Cu concentration constant at 1, 10 and 20 $\mu\text{g ml}^{-1}$, a Zn dependent reduction in Cu sorption was observed. Inhibition of Cu was much pronounced at its low (1 $\mu\text{g ml}^{-1}$) initial concentration. However, at 10 and 20 $\mu\text{g ml}^{-1}$ initial Cu concentration and varying Zn concentration (from 1 to 50 $\mu\text{g ml}^{-1}$), no appreciable inhibition of Cu sorption was found (Figure 3). In sharp contrast to this, effect of Cu (secondary metal) on percent Zn biosorption was more pronounced (Figure 3A). Almost similar inhibitory effects of secondary metal on biosorption characteristics of primary metal in Cu-Cd and Zn-Cd combination was observed when metals were used in combination

(data not shown). The biosorption of Cu from the solution containing varying concentration of Cd was found to be always greater and stronger than biosorption of Cd in the presence of fixed concentration of Cu in the solution. These observations led us to conclude that percent sorption of Cd was very much affected by the presence of Cu but sorption of Cu was not so affected by the presence of Cd. The percent biosorption of Cd in the presence of different concentrations of Zn was more affected as compared to Zn biosorption in the presence of various concentrations of Cd.

Discussion

The results obtained from sorption of Cu, Zn and Cd by *Microcystis* in single, bi and trimetallic system revealed a definite sequence, i.e., $\text{Cu} > \text{Zn} > \text{Cd}$, of metal biosorption by test alga. The amount of metal sorbed would also depend on the ionic size, stability of metal ion-*Microcystis* bonding, nature of metal ion-*Microcystis* interaction and the distribution of the reactive groups on the *Microcystis*. High Cu sorption capacity of *Microcystis* is in agreement with Tobin's observations (1984), who established a relationship between sorption efficiency and ionic radii of several metal ions. Tobin's group vividly demonstrated that molecules having smaller ionic radius can be more quickly sorbed onto a fixed surface area of sorbent. The smaller ionic radius of Cu (0.72 Å) than Zn (0.74 Å) and Cd (0.96 Å) may be responsible for its higher biosorption by *Microcystis*. Ionic radius based higher biosorption of Cu followed by Zn and Cd agrees well with the findings of Cho *et al.* (1994) and the hypothesis of Lee (1998). In bimetallic combination of Cu-Zn, Cu-Cd and Zn-Cd, the metals showed antagonistic effects. The order of inhibition of Cu, Zn and Cd biosorption in bimetallic combinations as compared to single metallic species (Figure 1) was suggestive of screening or competition for the binding sites on the cellular surface (Ting & Teo 1994). It is postulated that the effective binding sites available for single metal are reduced in bi and trimetallic combinations depending on the equilibria between adsorption competition from all the cations onto the biomass surface (Sag & Kutsal 1998).

Table 1 depicted that the biosorption capacity of Cu in two binary (Cu-Zn; Cu-Cd) and one ternary (Cu-Zn-Cd) systems was significantly greater than Zn and Cd. High affinity of *Microcystis* for Cu in bi and trimetallic condition is probably due to the existence

of a greater number of active sites with high specificity for this metal. Therefore, in presence of Cu these active sites would not be occupied by other cations (Echeverria *et al.* 1998). In the light of Stumm's (1992) explanation on the biosorption behaviour of ions, when attachment of Cu to the sorbent is strong, it will tend to spread out on the surface and inhibit the binding of other ions. In Cu-Zn combination, Cu not only lessened Zn biosorption but registered a higher biosorption as compared to Zn. It can be presumed that when same functional binding sites on the biomass materials are involved, biosorption of Cu may be preferred and it would be less susceptible to Zn interference (de Carvalho *et al.* 1995). Beveridge (1989) put forward a similar proposal on the basis of stability constant of these two metals. The stability constant of Zn compared to Cu is small, hence significantly less Zn than Cu was accumulated by *Microcystis* after 1 h of incubation. The sorption preference for Cu over Zn has also been reported for resin (Chandra & Rempel 1993) or apple residue (Maranon & Sastre 1992). But in Zn-Cd combination, binding capacity of *Microcystis* for Zn and Cd was almost same (i.e., K_f values for Zn = 12.560 and Cd = 13.45). Our results are in agreement with the findings of Martin (1986) which suggest that Zn and Cd ions possibly bind to different surface components of the cell wall having different affinities for these metals. Cd is known to chelate more strongly with sulphur donors, while Zn has a preference for oxygen and nitrogen donors (Martin 1986). Due to difference in the affinities of Zn and Cd to different sites no clear cut difference in biosorption between these metals was noticed. Biosorption of Cu and Cd from Cu-Cd combination indicated a greater affinity of Cu over Cd for binding (Figure 1), leading to a strong inhibition of Cd biosorption by Cu. de Carvalho *et al.* (1995) while working on metal biosorption by *Ascohyllum nodosum* also found a small decrease in the biosorption of Cu in presence of Cd. Gonzalez-Davila *et al.* (1995) like-wise noticed that small decrease in the amount of adsorbed Cu by *Dunaliella tertiolecta* in the mixture containing equal amounts of Cu and Pb in biosorption medium. Difference in the biosorption of Cu and Cd by *Microcystis* may be due to the presence of high and low affinity ligands on surface as observed by Gonzalez-Davila *et al.* (1995) for *Dunaliella tertiolecta*. High affinity sites only include carboxyl groups (-COOH) which are rapidly occupied by Cu. Following this a mild competition between Cu and Cd for these and for low affinity sites occur.

Multicomponent adsorption systems are extremely complicated because of solute-solute competition and solute surface interaction (Zhou *et al.* 1998). When attachment of the sorbate (Cu) to the sorbent is strong, it results into inhibition of Zn and Cd biosorption as was the case with the test alga. After the binding of all the three metals with the functional groups present on *Microcystis*, high concentration of residual Cd and Zn in solution as compared to Cu was found. Notwithstanding above, the sorption of Cu by *Microcystis* was also decreased by the presence of other metal ions in trimetallic combination. In view of the binding sites on the surface of *Microcystis*, any attempt to increase the number of metal cations in the mixture would offer a more intense competition for binding, thus reduced biosorption. A better removal of Cu than Zn and Cd in trimetallic combination is also supported by the data on *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* (Mullen *et al.* 1989). Due to existence of active sites having high specificity for Cu that may not be occupied by other cations (Echeverria *et al.* 1998), Cu realises less competition. Thus Cu was sorbed in higher amounts and with greater binding strength compared to Zn and Cd.

An increase in the inhibition of sorption of one metal at increasing concentration of another metal in solution as shown in Table 2 suggested that metals are competing for binding sites, resulting in the displacement of primary (added first) metal by secondary metal present in excess. It has, however, been found that increasing the secondary concentration of Zn and Cd in the biosorption medium containing varying concentration of primary Cd and Zn, did not severely affect the sorption characteristics of Cd and Zn. Further, it is evident that inhibitory effect of Cd on Cu sorption was not as strong as was the effect of Cu on Cd sorption.

Some metals are responsible for changing the structural integrity of the biomass. Huang *et al.* (1990) and Simmons *et al.* (1995) demonstrated that release of protein from *Saccaromyces cerevisiae* by Cu which bound to the excess Cu in solution and prevented biosorption of competing metal. When high concentration of secondary metal was added into the biosorption medium, it also bound with protein quickly thus inhibition of biosorption of primary metal was clearly obtained at high concentration of secondary metal. It is therefore, concluded that at high concentration of the secondary metal, the inhibition of biosorption of the primary metal might have not been solely due to com-

petitive effect but also change in the structural integrity of biomass.

A critical analysis of these results revealed that sorption from aqueous solution of binary metal mixtures of Cu-Zn, Cu-Cd and Zn-Cd and ternary metal mixture of Cu-Zn-Cd underwent competition for the available sites and Cu was more strongly attracted towards *Microcystis* with higher removal percentage than the other two metals. Thus *Microcystis* can be efficiently employed for removal of Cu from industrial effluents.

Acknowledgements

This study was sponsored by grants from the Department of Biotechnology, Ministry of Science and Technology, Pitamber Pant National Environment Fellowship Award 1996 from the Ministry of Environment and Forests, and Indo-US project from NSF, USA to L.C. Rai.

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