J. Microbiol. Biotechnol. (1998), **8**(1), 53 – 60

JOURNAL OF
MICROBIOLOGY AND
BIOTECHNOLOGY

Evaluation of Metal Biosorption Efficiency of Laboratory-grown *Microcystis* under Various Environmental Conditions

PRADHAN, SUBHASHREE, SARITA SINGH, LAL CHAND RAI*, AND DOROTHY L. PARKER[1]

Deparment of Botany, Banaras Hindu University, Varanasi 221 005, India
[1]Department of Biology and Microbiology, University of Wisconsin, Oshkosh, WI 54901, U.S.A.

Received: December 11, 1997

Abstract This study examines the effect of pH, temperature, metal ion concentration and culture density on metal biosorption by the nuisance cyanobacterium *Microcystis aeruginosa*. Ni biosorption was higher at pH 9.2 than at neutral and acidic pH. In contrast the biosorption of Cu and Zn was maximum at pH 7.0. However, biosorption of Zn was difficult to measure at pH values 9.2 and 10.5, owing to the formation of insoluble complexes. All the test metals (Cu, Zn, and Ni) showed maximum biosorption rate at low culture densities of 40 mg dry wt l^{-1}. The biosorption of Cu, Zn, and Ni was maximum at 40°C. However, no worthwhile difference in Zn and Ni sorption was noticed at 4 and 29°C as compared to 40°C. Of these three metals used *Microcystis* showed a greater binding capacity (K_f value=0.84, Freundlich adsorbent capacity) and accelerated biosorption rate for Cu under various environmental conditions. Fitness of mathematical models on metal biosorption by *Microcystis* confirmed that the biological materials behave in the same way as physical materials. These results suggest that before using a biosorbent for metal recovery, the environmental requirements of the biosorbent must be ascertained.

Key words: *Microcystis*, metal biosorption, pH, temperature, culture density, adsorption isotherms

The study of heavy metal contamination of aquatics has attracted the attention of many research teams all over the world. These elements persist over long period of time in the sediments of water bodies [31]. For this reason restoration of metal degraded waters is one of the prime issues before environmental biotechnologists worldwide. A number of techniques have been used for removing metals from wastewater. The advantages of biological method are many fold. Biological materials are cheaply and abundantly available, nontoxic, biodegradable and highly selective for certain ion species. Biosorption is one of the promising alternative technologies for metal recovery and removal [26]. The mechanisms associated with metal sorption by microorganisms are complex and may be placed into two categories: (i) intracellular uptake and entrapment of particles associated with live cells, and (ii) cell surface adsorption, which includes ion-exchange and complexation of metals by stoichiometric interaction of metal ions with chemically reactive groups present in the cell surface [17].

Successful attempts have been made to use algae, [10, 20] bacteria, [5, 6] fungi, [13, 25] yeast, [7, 24] mosses, [21] macrophytes [22] and a few higher plants [8] for the metal recovery from water systems. Dead and living microorganisms that are immobilized in a polymer matrix seem to offer several advantages for waste water purification and nutrient recycling [9]. It is well established that several freshwater [2, 14] and marine [3] algae are able to take up various heavy metals selectively from aqueous media and accumulate them within their cells. Several researchers are of the opinion that separation of metal saturated algae from the medium is an economic method for removing heavy metals from wastewater.

The cell surfaces of microorganisms, whether living or nonliving, possess abundant functional groups that bind metal ions. Many microorganisms secrete polymeric materials outside of the cell which play an important role in metal binding [5, 11, 12]. External polysaccharides of gram-negative bacteria are similar to those of cyanobacteria, and offer many functional groups such as carboxylate, hydroxyl, sulphate, phosphate and amino that can interact coordinatively with heavy metals ions [12, 36]. Dense blooms of the cyanobacterium *Microcystis* constitute approximately 85% of total population in certain Indian ponds and assume special

*Corresponding author
Phone: 91-542-317074; Fax: 91-542-310620/311555;
E-mail:

significance in metal removal due to the presence of various functional groups which interact strongly with cations [29, 30].

Various factors known to affect microbe-mediated metal removal from aqueous solution include chemistry of metal ions, specific surface properties of the organism, chemical modification and particle size, cell metabolism, pH, temperature and culture density. pH is known to affect metal speciation, binding sites on algal surfaces and precipitation of metal compounds inside the cells [11, 15, 31]. Concomitant with increase in temperature, the initial biosorption rates and equilibrium biosorption for Cr (VI) and Fe (III) were increased, and these metals were efficiently adsorbed by *Rhizopus arrhizus* [33] at low pH. The abundant growth of otherwise useless *Microcystis* present in Indian ponds and the lack of information about the metal biosorption strategies of *Microcystis* under different environmental conditions were the two pressing considerations for taking up the present investigation. Attempts have been made to (i) find out the most effective temperature, pH, and culture density for maximum biosorption of Cu, Zn, and Ni, and (ii) evaluate whether the metal biosorption properties of *Microcystis* fit with mathematical models used for physico-chemical adsorption.

MATERIALS AND METHODS

Microorganism and Growth Conditions

Naturally immobilised *Microcystis* was colleced from Laxmi Kund pond in Varanasi (25° 20'N, 83° 0'E) and grown in DP medium [28] at pH 9.2 under continuous illumination of 72 μ mole photon m^{-2} s^{-1} PAR light intensity and 29±2°C temperature. Cultures from the logarithmic phase were used for biosorption studies. Stock solutions of $CuCl_2 \cdot 2H_2O$, $ZnCl_2$ and $NiCl_2 \cdot 6H_2O$ were sterilized by passing through Sartorius membrane filters (0.22 μm) before adding to the culture medium.

Metal Biosorption Study

Exponentially grown *Microcystis* cells were harvested, washed twice in Mili Q grade water (>18 MΩ) and known quantities of homogeneous *Microcystis* cell suspensions were added to flasks containing known doses of test metals in 10 ml Mili Q water adjusted to pH 9.2 by 1 N NaOH/1 N HCl. To avoid any alteration in pH the Mili Q water was buffered with 5 mM MES buffer for pH 4.0 and 5.5, 5 mM HEPES buffer for pH 7.0 and 5 mM Tris base for pH 9.2 and 10.5. The flasks were agitated at 300 rpm in the environmental shaker (model 3597-ICOGMPR, U.S.A.) having above mentioned illumination and temperature. Samples were withdrawn at known time intervals. Residual concentrations of Cu, Zn and Ni in the aqueous solution were measured at 324.8, 213.9 and 232 nm, respectively, by Perkin-Elmer atomic absorption spectrophotometer model-2380. Biosorption was measured by recording the difference in metal contents between supernatants from control and test flasks containing biomass. Sorbents were separated from solution by vaccum filtration using 0.45 μm pore size cellulose acetate filters (Sartorius). To study the effect of pH (4.0~10.5), temperature (4, 29, and 40°C) and culture density (40~240 mg dry wt l^{-1}) on metal biosorption the test organism was incubated in freshly prepared metal solution having different initial concentrations (1~35 μg ml^{-1}) of test metals. Algal biomass was filtered through preweighed Sartorius membrane filters (0.45 μm pore size) dried at 80°C for 1 h, and weighed to compute dry weight.

Theoretical Aspects

Mathematical models developed by Freundlich and Langmuir were used to understand the surface behaviour of biosorbent, mechanisms of biosorption, and distribution of metal ions between the liquid and solid phases.

Freundlich isotherm. This isotherm suggests a concentration-dependent increase in metal biosorption onto the adsorbent. It is not only based on a heterogeneous energetic distribution of active sites but also the interaction between sorbed metals. There may be multilayer reversible binding. Biosorption can be explained by the following empirical equation:

$$x/m = K_f C^{1/n}$$

The linear form of Freundlich equation can be written as:

$$\log x/m = \log K_f + 1/n \log C$$

where C : equilibrium concentration (μg ml^{-1}) of unbound metal
x : amount of solute adsorbed (μg) at equilibrium
m : mass of adsorbent (mg dry wt)
K_f: Freundlich constant known as adsorbent capacity (μg solute mg^{-1} dry wt adsorbent)
n : Freundlich exponent known as adsorption intensity.

Langmuir isotherm. This isotherm assumes that the adsorption is a monolayer because the surface contains a finite number of identical sites. The atoms of adsorbent on the surface have residual force due to unshared electrons. Thus, bonds between the adsorbate and adsorbent may be physical or chemical. In this case adsorption is localized; hence no transmigration of sorbate in the plane of the surface occurs. The binding may be irreversible in nature. Biosorption of metal can be explained by following equation.

$$C/Q = 1/K_1K_2 + C/K_2$$

where C : equilibrium concentration of the free metal ion
Q : amount of metal adsorbed per unit of biomass
K_2: amount of metal adsorbed per unit biomass at saturation
K_1: equilibrium constant

RESULTS AND DISCUSSION

Effect of pH on Metal Biosorption

Figure 1A shows the initial biosorption rate of Cu over a pH range of 4.0 to 10.5. The binding rate of Cu increased with increasing pH from 4 to 7 and reached a maximum value at pH 7.0. The sorption rate of copper at an initial concentration of 30 μg ml^{-1} was 6.3 μg Cu mg^{-1} dry wt min^{-1} at pH 7.0, but was only 1.2 to 1.4 μg Cu mg^{-1} dry wt min^{-1} at pH 4, 5.5, 9.2 and 10.5. Our data do not agree with those of Harris and Ramelow [17] who studied pH-induced variation in Cu sorption by *Chlorella vulgaris* and *Scenedesmus quadricauda* and found it to be maximum at pH 5.0. It is quite likely that the negatively charged groups present on the surface of microbial cell wall may undergo protonation at low pH leading to an increase in the positive charge density on the cell surface thus resulting into a competition between H^+ and cationic ions for the same binding sites. The elecrostatic attraction so developed between the anionic metal complexes and the protonated functional groups results into decreased biosorption at acidic pH [15, 31]. Further, Cu exists in hydroxide form at alkaline pH, hence a decreased metal removal was observed in alkaline pH. [17].

Zinc biosorption registered an increase with increasing pH from 4.0 to 7.0 and reached a maximum level at pH 7.0 (Fig. 1B). Due to formation of some insoluble complexes, the biosorption of Zn at pH 9.2 and 10.5 could not be measured accurately [19, 32]. Although it was difficult to analyse the actual amount of metals adsorbed by *Microcystis* at alkaline pH (data not shown), a decreased biosorption of Zn at acidic pH could be due to its repulsion by the alga as suggested for Cu. Our results further point toward the formation of zincate when the pH of the Zn solution was adjusted by the addition of NaOH [4]. Zn biosorption was, however, found to saturate (Fig. 1B) at 30 μg ml^{-1} metal ion concentration where maximum biosorption (1.518 μg mg^{-1} dry wt min^{-1}) was obtained at pH 7.

In contrast to Cu and Zn, the rate of Ni biosorption was higher at pH 9.2 than at other pH values tested. At 30 μg ml^{-1} initial Ni concentration, the initial biosorption rate was 2.115 μg Ni mg^{-1} dry wt min^{-1} (Fig. 1C). Difference in pH optima for different metals could be due to the difference in isoelectric points of the cells and the nature of chemical interaction between metal species

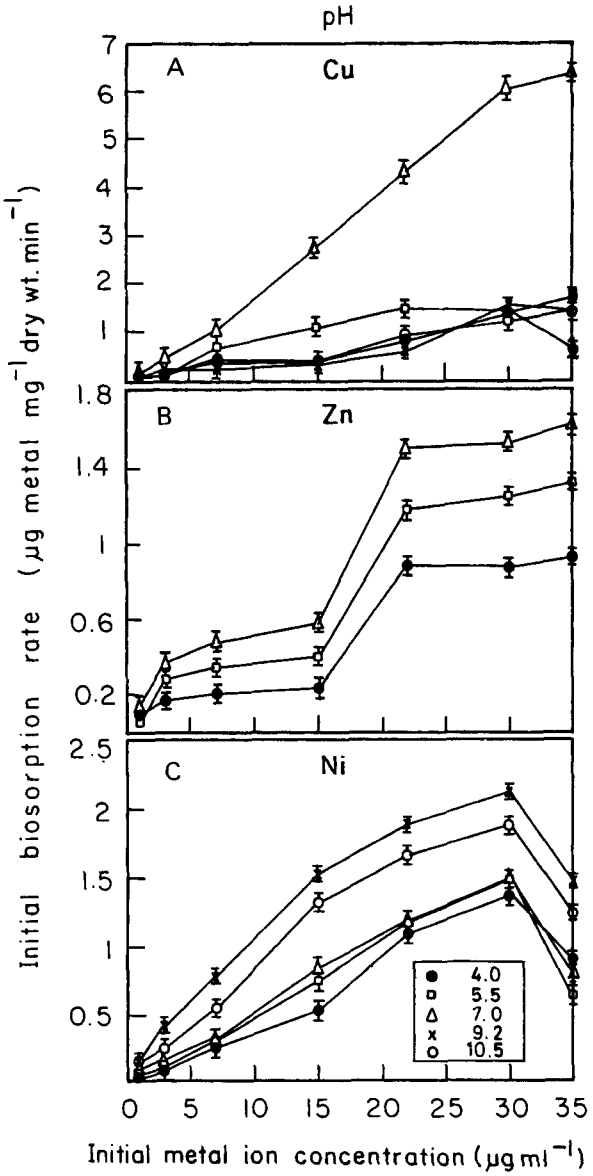

Fig. 1. Effect of pH on Cu, Zn and Ni biosorption by *Microcystis*.

and microbial cells [33] or to differences in the metal solubility. Maximum biosorption of Ni at pH 9.2 also agrees with the findings of Mavros *et al.* [23] who demonstrated high Ni removal at alkaline pH and low at acidic pH. Since nickel is known to exist in different forms viz., $NiOH^+$, Ni $(OH)_2^+$ and Ni $(OH)_4^{4+}$ at pH 9.2, it is possible that these may either interact or precipitate with negative sites on the surface.

Experimental results demonstrated significant differences between the sorption of Cu, Ni and Zn suggesting the differences in their ionic radii, electronegativity, chemical properties, and coordination tendencies at various pH [1]. A higher amount of Cu adsorbed at saturation (166.287 μg

Table 1. Comparison of Freundlich and Langmuir constants at various pH values.

Metal	pH	Freundlich			Langmuir		
		K_f	n	R^2	K_1	K_2	R^2
Cu	4.0	0.805	1.562	0.811	0.238	47.619	0.661
	5.5	0.475	0.843	0.873	-0.012	-250.000	0.062
	7.0	2.005	1.051	0.579	0.000	0.000	0.003
	9.2	0.822	1.557	0.806	0.035	125.000	0.162
	10.5	0.997	1.897	0.716	0.003	1000.000	0.008
Zn	4.0	0.770	1.658	0.813	1.250	100.000	0.614
	5.5	0.777	1.282	0.877	0.022	200.000	0.150
	7.0	1.203	7.246	0.897	0.108	111.111	0.615
Ni	4.0	0.211	0.798	0.787	-0.019	-71.428	0.232
	5.5	0.514	1.055	0.886	0.042	100.000	0.307
	7.0	0.862	1.416	0.906	0.086	90.909	0.619
	9.2	1.288	1.766	0.932	0.200	100.000	0.898
	10.5	1.109	1.545	0.909	0.116	100.000	0.757

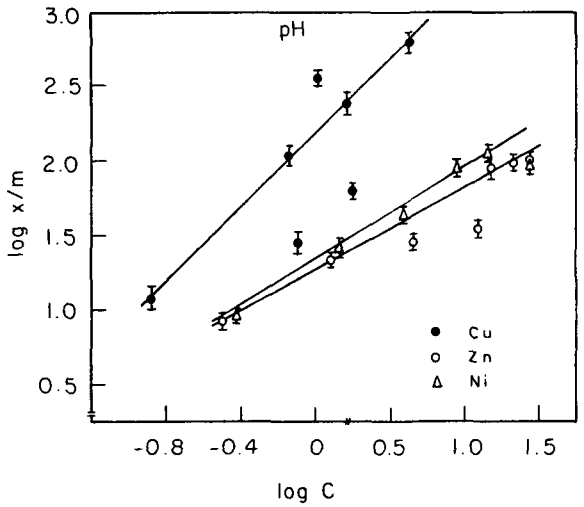

Fig. 2. Freundlich isotherm for Cu, Zn, and Ni biosorption by *Microcystis* at optimum pH.

mg^{-1}) than Zn (91.137 μg mg^{-1}), and Ni (126.95 μg mg^{-1}) could be due to additional coordination binding to uncharged functional groups [11]. Likewise the higher removal efficiency of Cu may be due to its higher electronegativity (1.9 pauling) than Ni (1.8 pauling) and Zn (1.6 pauling). Compared to Cu, *Microcystis* registered approximately 24% and 45% reduction in biosorption of Ni and Zn.

To evaluate the efficiency and to understand the pattern of metal biosorption, the experimental data were fitted into Freundlich and Langmuir isotherms. Data incorporated in Table 1 showed better performance of the Freundlich over the Langmuir isotherm for Cu, Zn and Ni biosorption at various pH values. Figure 2 represents a clearcut relationship of Freundlich isotherm

Table 2. Comparison of Freundlich and Langmuir isotherm constants for metal biosorption by *Microcystis* at various culture densities.

Metal	Culture density (mg dry wt l^{-1})	Freundlich			Langmuir		
		K_f	n	R^2	K_1	K_2	R^2
Cu	40	1.597	1.968	0.747	0.222	250.000	0.966
	80	1.390	2.564	0.693	0.094	100.000	0.467
	160	1.108	1.831	0.734	0.200	71.428	0.841
	240	1.107	2.597	0.334	0.000	-000.000	0.000
Zn	40	1.089	1.197	0.988	0.010	1000.00	0.512
	80	0.821	1.114	0.970	0.028	250.000	0.622
	160	0.455	1.088	0.975	0.014	200.000	0.446
	240	0.351	1.048	0.923	0.019	125.000	0.203
Ni	40	1.347	1.466	0.912	0.097	250.000	0.918
	80	0.141	1.540	0.903	0.133	125.000	0.897
	160	0.898	1.636	0.876	0.149	58.820	0.909
	240	0.921	2.298	0.666	0.186	35.714	0.848

for biosorption of metals at optimum pH. This could be due to a heterogeneous adsorbent surface or to the multilayer reversible binding. The Freundlich analysis was used to determine the maximum loading capacity (K_f) of the cyanobacterial cells for different metals. This capaciy was found to be highest for Cu (K_f=2.005), followed by Ni (K_f=1.288) and Zn (K_f=1.203) at the optimum pH values of each metal (Table 2). The Freundlich constants obtained from the isotherm further support our experimental result of higher binding capacity of *Microcystis* for Cu than for Ni and Zn.

Effect of Culture Density on Metal Biosorption

Figure 3 shows initial biosorption rates for Cu, Zn and Ni at different cell biomass of *Microcystis*. The biosorption rate was maximum at 40 mg dry wt l^{-1} culture and thereafter it registered a decrease with increasing biomass from 40 to 240 mg dry wt l^{-1}. These results are in agreement with the observations of Veglio *et al.* [35] who noticed an impact of biomass concentration on biosorption. The hypothesis of Gadd and White [13] explained that an increase in biomass concenration leads to interference between binding sites leading to decreased biosorption. Electrostatic interactions among the cells are the main factor for cell concentration dependency of metal adsorption. An increase in culture density leads to a decreased electrostatic interaction between metal ions and binding sites [18]. These results are also supported by the Poisson Boltzmann equation which states that large quantity of cations are adsorbed on the cell anions in suspension when the distance between cells is great [27]. Reduction in the effective adsorption area as a result of aggregation of cells in

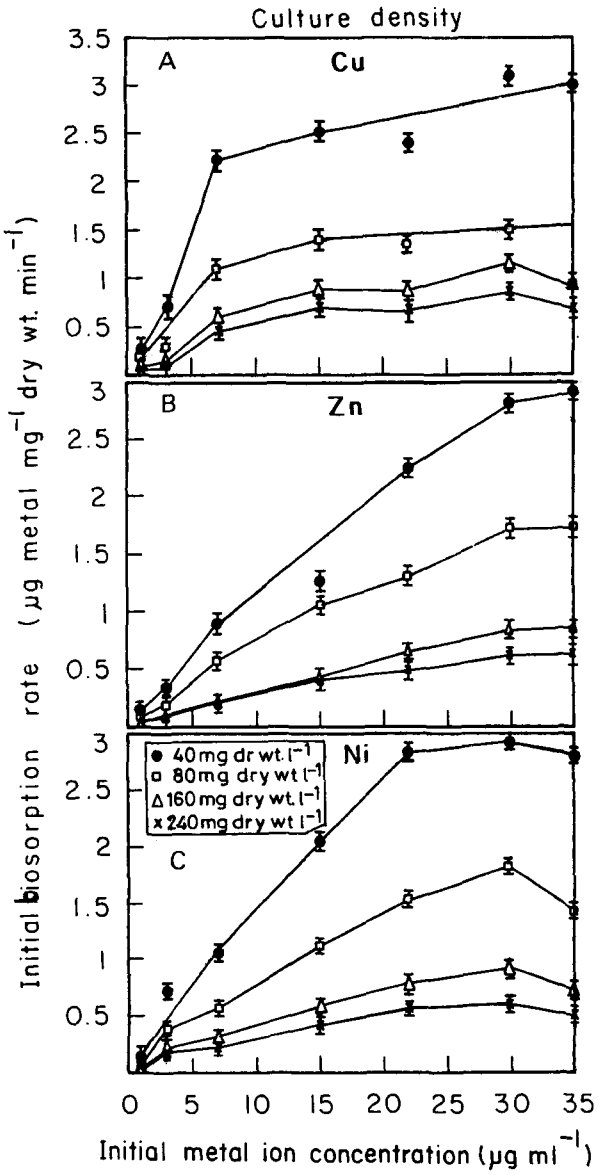

Fig. 3. Effect of culture density on Cu, Zn, and Ni biosorption by *Microcystis*.

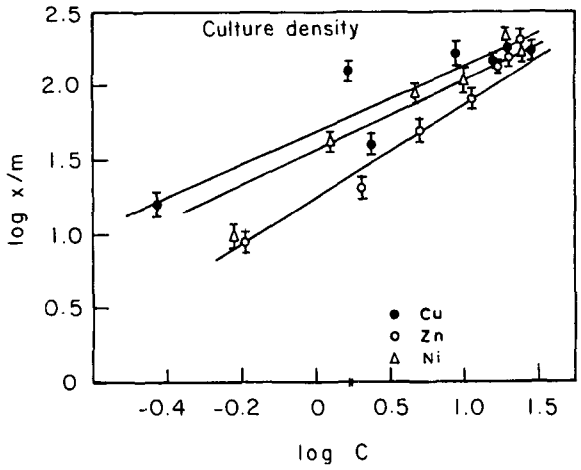

Fig. 4. Freundlich isotherm for Cu, Zn, and Ni biosorption by *Microcystis* at 40 mg dry wt l^{-1}.

biosorption medium [2] and reduced mixing of biomass [24, 34] at high culture density also support our results. These observations are consistent with our data of slow biosorption rate for Cu, Zn, and Ni at high cell density.

Maximum biosorption rates of 3.087 μg Cu mg^{-1}, 2.810 μg Zn mg^{-1} min^{-1} and 2.907 μg Ni mg^{-1} min^{-1} were obtained at 30 μg ml^{-1} initial ion concentration. As compared to 40 mg dry wt l^{-1} culture density, approximately 72, 79, and 82% reduction respectively for Cu, Zn, and Ni biosorption (expressed as μg metal mg^{-1} dry wt) was noticed at 240 mg dry wt l^{-1}. Further compared to Cu about 6 and 8% decrease in Ni and Zn biosorption efficiency of *Microcystis* was observed. The data for Freundlich and Langmuir constants as calculated from culture density isotherms are presented in Table 2. A very effective adsorption at low concentration of algal biomass can be seen from the data of K_f values which were always higher for Cu (1.597) followed by Ni (1.347) and Zn (1.089) at 40 mg dry wt l^{-1} (Fig. 4). A good fit of data on Freundlich isotherm compared to Langmuir isotherm implies that the metal ion load on the adsorbent will increase with increase in the metal ion concentration in the liquid medium. Better performance of Langmuir isotherm and failure of Freundlich isotherm (low R^2 value) for Cu at 40 and 160 mg dry wt l^{-1} and for Ni at 160 and 240 mg dry wt l^{-1} suggest the formation of monolayer binding on *Microcystis*.

Effect of Temperature on Metal Biosorption

Figure 5 demonstrates a temperature dependence of Cu, Zn, and Ni biosorption by the *Microcystis* biomass. This figure demonstrated a decrease in Cu biosorption at 29°C and increase at 4 and 40°C. At 15 μg ml^{-1} initial Cu ion concentration, maximum biosorption rate of Cu was 2.183 μg mg^{-1} min^{-1} at 40°C. The rate was 2.029 μg mg^{-1} min^{-1} and 2.047 μg mg^{-1} min^{-1} at 30 μg ml^{-1} initial Zn and Ni ion concentration, respectively. Furthermore, the biosorption rate of the test organism at 4°C was either equal or slightly higher than at 29°C for all the test metals.

The inhibition of metabolism dependent slow active uptake is quite plausible at high temperature but the surface and cell wall binding of metals to negatively charged functional groups may be quite appreciable. Many metabolic processes of the cells would be arrested at 4°C [33], but the adsorption will continue to operate. Gourdon *et al.* [16], on the basis of a similar kind of study suggested that adsorption of solutes at the liquid-solid interface is generally an exothermic process and

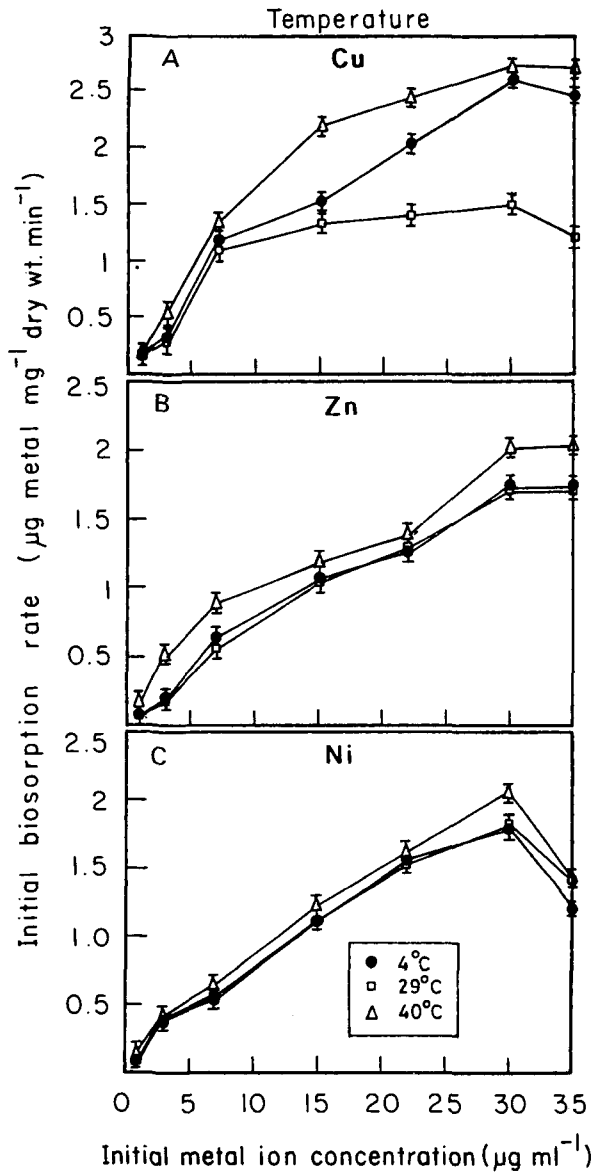

Fig. 5. Effect of temperature on Cu, Zn, and Ni biosorption by *Microcystis*.

therefore the metal uptake might increase at lowered temperature if adsorption were exclusively passive. Further an increase in biosorption by increasing the temperature from 29° to 40°C could be due to simultaneous operation partly of metabolic processes and partly the physical processes. Therefore, due to the operation conceivably of two processes the biosorption was maximum at 40°C. The high temperature might expand the cell surface thus exposing more sites for increased metal biosorption. Another alternative could be the increased lysis of the cell in response to a comparatively greater toxicity of Cu than other metals at 29°C.

Table 3. Comparison of the Freundlich and Langmuir adsorption constants for metal biosorption at different temperatures.

Metal	Temperature (°C)	Freundlich			Langmuir		
		K_f	n	R^2	K_1	K_2	R^2
Cu	4.0	1.454	1.683	0.876	0.876	250.000	0.789
	29.0	1.390	2.540	0.693	0.094	100.000	0.467
	40.0	1.689	2.049	0.814	0.946	113.036	0.860
Zn	4.0	0.837	1.107	0.949	0.021	333.333	0.547
	29.0	0.821	1.114	0.970	0.028	250.000	0.622
	40.0	1.451	2.164	0.912	0.241	90.900	0.941
Ni	4.0	1.132	1.584	0.896	0.120	140.845	0.381
	29.0	1.141	1.540	0.903	0.133	125.000	0.897
	40.0	1.482	2.380	0.854	0.909	100.000	0.941

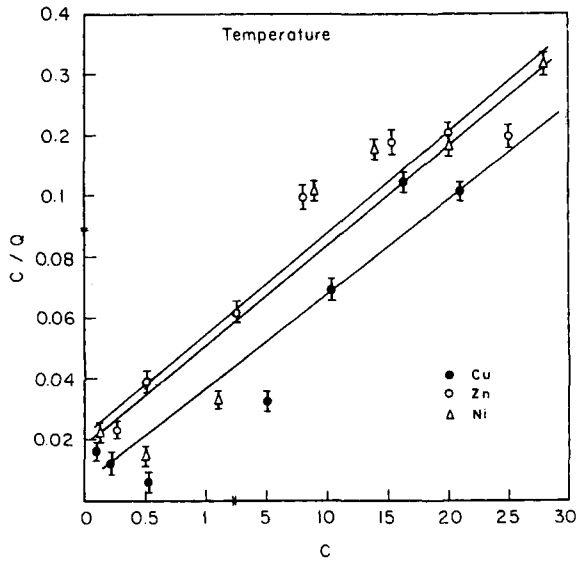

Fig. 6. Langmuir isotherm for Cu, Zn, and Ni biosorption by *Microcystis* at 40°C.

Effect of temperature on isotherm constants and R^2 values obtained from Freundlich and Langmuir isotherms is compiled in Table 3. Freundlich isotherm gave better fitness at 4°C and 29°C but Langmuir model was the best fit at 40°C (Fig. 6). The distribution coefficient (defined as µg metal sorbed/mg dry wt/ equilibrium concentraion of metals) was 29.396, 5.987, and 6.100, respectively, for Cu, Zn, and Ni at 40°C. The large distribution coefficient for Cu suggests that *Microcystis* has the ability to remove more Cu from aquatic environment [21]. A critical analysis of these results showed that (i) biosorption of metals is pH dependent; pH 7 for Zn and Cu and pH 9.2 for Ni are suitable for maximum biosorption by *Microcystis*, (ii) 40°C temperature and 40 mg dry wt l^{-1} culture density may be used to efficiently remove these tes metals, (iii)

Microcystis has greater affinity for Cu followed Ni and Zn, and (iv) *Microcystis* seems to hold great biotechnological potential for use as biosorbent for heavy metal removal from the aquatic system.

Acknowledgments

We are thankful to the Department of Biotechnology, Ministry of Science and Technology, Government of India for financial support in the form of project to L. C. Rai. Thanks are due to Professor H. D. Kumar FNA for reviewing the manuscript and Professor J. S. Singh FNA, Coordinator, CAS in Botany for facilities.

REFERENCES

1. Allen, S. J. and P. A. Brown. 1995. Isotherm analyses of single component and multicomponent metal sorption onto lignite. *J. Chem. Tech. Biotechnol.* **62**: 17–24.
2. Aksu, Z. and T. Kutsal. 1990. A comparative study for biosorption characteristics of heavy metal ions with *C. vulgaris*. *Environ. Technol.* **11**: 979–987.
3. Andreas, L., Z. R. Holan, and B. Volesky. 1995. Biosorption of heavy metals (Cd, Cu, Ni, Pb and Zn) by chemically reinforced biomass of marine algae. *J. Chem. Tech. Biotechnol.* **62**: 279–288.
4. Bencheikh-Lehocine, M. 1989. Zinc removal using peat adsorption. *Environ. Technol. Letters* **10**: 101–108.
5. Beveridge, T. J. 1989. Role of cellular design in bacterial metal accumulation and mineralization. *Ann. Rev. Microbiol.* **43**: 147–171.
6. Beveridge, T. J. 1986. The immobilization of soluble metals by bacterial wall. *Biotechnol. Bioeng. Sympo.* **16**: 127–139.
7. Brady, D., A. Stoll, and J. R. Duncon. 1994. Biosorption of heavy metal cations by nonviable yeast biomass. *Environ. Technol.* **15**: 429–438.
8. Breckle, S. W. 1989. Growth under stress: Heavy metals. *In* Y. Waisely, U. Katkaji, and A. Eshel (ed.) *The root system: The hidden half.* Marcel Dekker Inc., New York.
9. Chevalier, P. and J. de la Noue. 1985. Wastewater nutrient removal with microalgae immobilized in carrageenan. *Enzyme Microb. Technol.* 7: 621–624.
10. Corder, S. L. and M. Reeves. 1994. Biosorption of nickel in complex aqueous waste streams by cyanobacteria. *Appl. Biochem. Biotechnol.* **45/46**: 847–859.
11. Crist, R. H., K. Oberholser, D. Schwartz, J. Marzoff, and D. Ryder. 1988. Interactions of metals and protons with algae. *Environ. Sci. and Technol.* **22**: 755–760.
12. Fiore, M. F. and J. T. Trevors. 1994. Cell composition and metal tolerance in cyanobacteria. *Biometals* **7**: 83–103.
13. Gadd, G. M. and C. White. 1989. Removal of thorium from simulated acid process streams by fungal biomass. *Biotechnol. Bioeng.* **33**: 592.
14. Greene, B. and D. W. Darnal. 1990. Microbial oxygenic photoautotrophs (cyanobacteria and algae) for metal ion binding, pp. 277–302. *In* H. L. Ehrlich and C. Brierley (ed.), *Microbial mineral recovery.* Mc Graw Hill, New York.
15. Greene, B., R. McPherson and D. Darnall. 1987. Algal sorbents for selective metal ion recovery, pp. 315–338. *In* J. W. Patterson and R. Parssion (ed.), *Metal speciation, separation and recovery.* Lewis Publishers, Chelsea, MI.
16. Gourdon, R., E. Rus., S. Bhende, and S. S. Sofer. 1990. Mechanism of cadmium uptake by activated sludge. *Appl. Microbiol. Biotechnol.* **34**: 274–278.
17. Harris, P. O. and G. J. Ramelow. 1990. Binding of metal ions by particulate biomass derived from *Chlorella vulgaris* and *Scenedesmus quadricauda. Environ. Sci. Technol.* **24**: 220–228.
18. Itoh, M., M. Yuasa, and T. Kobayashi. 1975. Adsorption of metal ions on yeast cells at varied cell concentrations. *Plant Cell Physiol.* **16**: 1167–1169.
19. Lee, J. D. 1994. *Concise inorganic chemistry: the d-block elements.* pp. 651–854. 4th ed. ELBS with Chapman and Hall, London.
20. Les, A. and R. W. Walker. 1984. Toxicity and binding of Cu, Zn and Cd by blue green alga *Chroococcus paris. Water Air Soil Pollut.* **23**: 129–139.
21. Low, K. S., C. K. Lee, and S. G. Tan. 1997. Sorption of trivalent chromium from tannery waste by moss. *Environ. Technol.* **18**: 449–454.
22. Mallick, N., Shardendu, and L. C. Rai. 1996. Removal of heavy metals by two free floating aquatic macrophytes. *Biomed. Environ. Sc.* **9**: 400–408.
23. Mavros, P., A. I. Zouboulis, and N. K. Lazridis. 1993. Removal of metal ions from wastewaters. The case of nickel. *Environ. Technol.* **14**: 83–91.
24. Meikle, A. J., G. M. Gadd, and R. H. Reed, 1990. Manipulation of yeast for transport studies: critical assessment of cultural and experimental procedures. *Enzyme Microb. Technol.* **12**: 865–872.
25. Mueler, M. D., D. C. Wolf, T. J. Beveridge, and G. W. Bailey. 1992. Sorption of heavy metals by the soil fungi *Aspergillus niger* and *Mucor rouxii. Soil Biol. Biochem.* **24**: 129–135.
26. Muraleedharan, T. R., L. Iyengar, and C. Venkobachar. 1991. Biosorption: An attractive alternative for metal removal and recovery. *Current Sci.* **61**: 379–385.
27. Ohshima, H. 1974. Diffuse double layer interaction between two parallel plates with constant surface charge density in an electrolyte solution I. The interactions between similar plates. *Colloid Polymer Sci.* **252**: 158–164.
28. Parker, D. L. 1982. Improved procedures for the cloning and purification of *Microcystis* cultures (Cyanophyta). *J. Phycol.* **18**: 471–477.
29. Parker, D. L, B. R. Schram, J. L. Plude, and R. E. Moore. 1996. Effect of metal cations on the viscosity of a pectin like capsular polysaccharide from the cyanobacterium *Microcystis flos-aquae* C3-40. *Appl. Environ. Microbiol.* **62**: 1208–1213.

30. Plude, J. L., D. L. Parker, O. J. Schommer, R. J. Timmerman, S. A. Hagstrom, J. M. Joers, and R. Hnasko. 1991. Chemical characterization of polysaccharide from the slime layer of the cyanobacterium *Microcystis flos-aquae* C3-40. *Appl. Environ. Microbiol.* **57**: 1696–1700.
31. Rai, L. C., J. P. Gaur, and H. D. Kumar. 1981. Phycology and heavy metal pollution. *Biol. Rev.* **56**: 98–151.
32. Reid, R. J., J. D. Brookes, M. A. Tester, and F. A. Smith. 1996. The mechanism of Zn uptake in plants: characterisation of the low-affinity system. *Planta* **198**: 39–45.
33. Sag, Y. and T. Kutsal. 1996. The selective biosorption of chromium (VI) and copper (II) ions from binary metal mixtures by *R. arrhizus*. *Process Biochem.* **31**: 561–572.
34. Singleton, I. and P. Simmons. 1990. Factors affecting silver biosorption by and industrial strain of *Saccharomyces cerevisiae*. *J. Chem. Tech. Biotchnol.* **65**: 21–28.
35. Veglio, F., F. Beolchini, and A. Gasbarro. 1997. Biosorption of toxic metals: an equilibrium study using free cells of *Arthrobacter* sps. *Process Biochem.* **32**: 99–105.
36. Volesky, B. and Z. R. Holan. 1995. Biosorption of heavy metals. *Biotechnol. Prog.* **11**: 135–150.