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EQUILIBRIUM MODELLING AND SPECTROSCOPIC STUDIES FOR THE BIOSORPTION OF ZN^{+2} IONS FROM AQUEOUS SOLUTION USING IMMOBILIZED *SPIRULINA PLATENSIS*

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ABSTRACT

Biosorption equilibrium of zinc ions to *Spirulina platensis* both in free and immobilized forms were studied in batch system with respect to pH, metal ion concentration, algal dosages and time. The maximum adsorption was observed at pH=8, optimum metal ion concentration and algal dose were 100 mg/L and 1g/100mL, respectively. Biosorption equilibrium was established in 90 minutes. The maximum attainable biosorption was found to be 97.1% for *Spirulina platensis*. The equilibrium adsorption capacities of adsorbents used for zinc ions were measured and extrapolated using Langmuir and Freundlich isotherms models. Langmuir model was found to be in better correlation with experimental data. The maximum Langmuir constants $Q_{\circ}$ (mg/g) and b were 92.93 and 0.0012, respectively for *Spirulina platensis* embedded in calcium alginate matrix. The immobilized *Spirulina platensis* in calcium of alginate matrix was the best biosorbent. 0.1 M EDTA was used as an eluant, which allowed the reuse of biomass in three biosorption-desorption cycles without considerable loss in biosorption capacity. 89-95 % zinc ions were desorbed with EDTA. The functional groups involved in zinc biosorption were identified by using Fourier Transform Infra Red spectroscopy. Spectroscopic analysis of algae revealed the presence of carboxyl, hydroxyl, amino, amide and imine groups, which were responsible for biosorption of zinc ions.

Key words: Zinc ions, *Spirulina platensis*, immobilization, functional groups

INTRODUCTION

Main zinc producing industries are galvanizing iron and steel for corrosion protection, alloys, vulcanization of rubber, photocopy paper, paints, T.V. tubes, rayon glass, enamel and plastic industries, fertilizers, medicines and cosmetics industries. Acute zinc toxicity in human includes vomiting, dehydration, drowsiness, lethargy, electrolytic imbalance, abdominal pain, nausea, lack of muscular coordination and renal failure. Workers exposed to zinc fumes from smelting or welding have suffered from short term illness called metal fume fever (Sharma and Aggarwal, 2005).

Sorption of heavy metals by various biological materials has been proposed as an efficient and potentially cost effective tool for metal enriched industrial effluents. Biosorption is a property of certain types of inactive, dead microbial biomass to bind and concentrate heavy metals from very dilute aqueous solutions (Kratochvil and Volesky, 1998). It is particularly the cell wall of certain algae, fungi and bacteria which is found to be responsible for the phenomenon of biosorption. The cell wall of cyanobacteria includes carboxyl, hydroxyl, carbonyl, sulphahydryl, thioether, sulphonate, amine, imine, imidazole and phosphodiester groups (Davis *et al.*, 2003). Immobilized biomass has shown greater potential in terms of control of particulates size, efficient regeneration of biomass and separation from effluents. In addition, easy operation of repeated biosorption-desorption cycles with biosorption beads make the biosorption process potentially more economic and competitive (Rajendran *et al*., 2003).

Small particles size and low mechanical strength

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of biomass creates a problem for effective biosorption. This problem can be solved by immobilization of biomass which provides desirable and better strength to biomass (Rajendran *et al.,* 2003).

The present work reports the spectral study and biosorption equilibrium of Zn^{2+} ions by free and immobilized *Spirulina platensis*. *S. platensis* in biosorption equilibrium, initial metal ion concentrations, algal dosages, time and pH were analyzed. Equilibrium adsorption isotherms (Langmuir and Freundlich) were obtained quantitatively to describe the Zn^{2+} ions uptake in aqueous solution.

MATERIALS AND METHODS

Biomass

The cyanobacterium *S. platensis* was obtained from the national facility from Blue Green Algae Division, Indian Agricultural Research Institute (IARI), New Delhi, India. The axenic culture was grown in the *Zaurrock's* media composition and is shown in Table 1 (Zaurrock's, 1966). The culture was maintained in the culture room illuminated with cool day light 3000 lux under 16 h light/8 h dark cycle at 24 ± 1°C.

Table 1: Standardized media composition for mass culturing the *Spirulina platenesis* (Zaurrock', 1966)

Chemicals	Concentration (g/L)
NaCl	1.00
$NaNO_3$	2.50
Na_2EDTA	0.08
$CaCl_2$	0.04
$FeSO_4$. $7H_2O$	0.01
K_2SO_4	1.00
$MgSO_4$	0.2
K_2HPO_4	0.50
$NaHCO_3$	16.80
A_5 (Micronutrient solution)	1mL
Water	1L(pH=9)
H_3BO_3	2.86
$MnCl_2$.$2H_2O$	1.81
$ZnSO_4$.$7H_2O$	0.22
Na_2MoO_4.$2H_2O$	0.39
$CuSO_4$.$5H_2O$	0.07

For biosorbent preparation, exponentially grown cells (9 days) were harvested by centrifuging at 4000 rpm for 10 minutes. The biomass was then washed twice with deionized water and dried for 24 h at 60°C in oven. The dried biomass was ground and sieved through 200 µm screen.

Chemicals and reagents

All the chemicals used in the study were of analytical grade and supplied by Qualligenes fine chemicals, Bombay, India. The stock metal solution of Zn^{+2} ions were prepared by dissolving appropriate quantities of pure analytical grade metal salt in deionized water. The stock solution was further diluted with deionized water to obtain working solution of desirable concentrations.

Immobilization of biomass

Immobilization of *S. platensis* was done according to Srinath *et al.*,(2003). Calcium alginate and agar beads were prepared with cyanobacterial biomass and were used for the evaluation of biosorption of Zn^{+2} ions at room temperature. For each immobilization process, 0.02 g of biomass was entrapped in 1 g of matrix at room temperature. The preparations were obtain as follows:

1.Calcium alginate beads: 2% (w/v) sodium alginate was dissolved in hot distilled water with constant stirring. At room temperature biosorbent was added under stirring condition for even dispersal. The slurry solution was dripped through the nozzle drop wise into 0.05 M $CaCl_2$. As a result, spherical beads were formed immediately due to phase inversion process as the alginate was cross linked with Ca^{+2} ions. The beads (3.2 ± 0.1 mm) were moderately agitated in deionized water for 24 hrs at 4°C. Finally, the beads were stored at 4°C in ultra pure double distilled water until for further use (Srinath *et al.*, 2003).

2. Agar beads: the agar beads were prepared by dissolving it in distilled water at 90°C. The biomass was added and dispersed by stirring. Spherical beads were obtained on drop wise addition of slurry into a hydrophobic liquid phase (sun flower oil) over distilled water. The beads were collected and then washed with 0.001% Triton X 100 to eliminate the residual oil phase (Srinath *et al.,* 2003).

Infrared spectroscopy

The Fourier Transform Infra Red(FTIR) spectroscopy of biosorbent was obtained from analytical instrumentation facility, Punjab University, Chandigarh, India. IR spectra of free biosorbent before and after biosorption of zinc ions were recorded on Nicolt Model 6000. FTIR spectrometer was equipped with a liquid nitrogen cool detector. The spectrum was recorded in the range of 400 to 4000 cm^{-1}.

Zn^{+2} ions sorption by free and immobilized cyanobacterial biosorbent

Standard stock solution of Zn^{+2} ions (1000 ± 2 mg/l) was diluted to the concentration to be investigated for metal biosorption. The pH of the metal solution was adjusted with the help of 0.01M HCl or 0.01M NaOH as desired in the experiments. Biosorption capacity of cyanobacterial biosorbent, both free and immobilized in calcium alginate and agar matrix, was determined by 100 mL metal solution of known concentration (20-400 mg/L) to cyanobacterial biosorbent (0.2-2 g) in 250 mL shaking flask. The metal ion solution incubated with biosorbent was shaken at 100 rpm at 25°C in tightly stoppered flask. Free biosorbent was removed from metal solution by centrifugation at 3500 rpm for 5 minutes; where as immobilized biosorbent was separated by simple decantination. The filtrate was analysed for residual Zn^{+2} ions with the help of atomic absorption spectrophotometer, Perkin Elemer 280.

The batch experiments were carried out as function of pH, biosorbent dosage, time and initial ion concentration of metal ions. All experiments were conducted in triplicate and mean values were used.

The quantitative estimation was done by Freundlich and Langmuir isotherms as equations 1 and 2:

$$\log q_e = \log K_f + 1/n \log C_e \quad (1)$$

In which q_e is the metal uptake in (mg/g), K_f is the Freundlich constant for adsorption capacity, n is the Freundlich constant for adsorption intensity and C_e is the equilibrium concentration (mg/L)

The lineraized form of Langmuir equation is

$$1/q_e = 1/ (Q_o b C_{eq}) + 1/Q_o \quad (2)$$

In which Q_o is the Langmuir constant which is measure of adsorption capacity in (mg/g) and b is the Langmuir constant which is measure of adsorption energy in (L/mg).

Zn^{+2} ions adsorption and reusability of free and immobilized S. platensis in repeated batch cycles

The *S. platensis* biosorbent was reused in three biosorption-desorption cycles to determine reusability. For this purpose, immobilized biomass was contacted with 100 mL of 100 mg/L of Zn^{+2} ions solution for biosorption. 0.1 M ethylenediamine tetracarboxylic acid(EDTA) was used as elutant in desorption cycle in 250 mL flask shaken on orbital shaker for 30 minutes at 100 rpm for achieving sorption-desorption equilibrium. The initial and final concentration of the solution was recorded for each cycle, on completion of each. The cyanobacterial biosorbent was washed with deionised water and transferred for the next biosorption cycle.

RESULTS

Fig. 1 shows the effect of pH on biosorption process by *S. platensis*, *S. platensis* embedded in calcium alginate and *S. platensis* embedded in agar matrix. Fig. 2 shows the effect of initial metal ion concentration ranging form 20- 400mg/L. In all three cases, metal uptake was increased rapidly up to 90 minutes and remained constant after that and it was taken as an optimum time. Optimum dose for removal of Zn^{+2} ions was found to be the same i.e. 1g/100mL for all the biosorbents but removal efficiency was maximum for 92.9% for *S. platensis* embedded in calcium alginate matrix. Fig. 3 presents the desorption study using 0.1 M EDTA as elutant. The zinc ions uptake of free and immobilized *S. platensis* was evaluated using Langmuir and Freundlich adsorption isotherms. Table 2 shows the maximum adsorption capacity and the regression correlation coefficient for the three biosorbents. Langmuir model was found to be in better correlation with experimental data. But

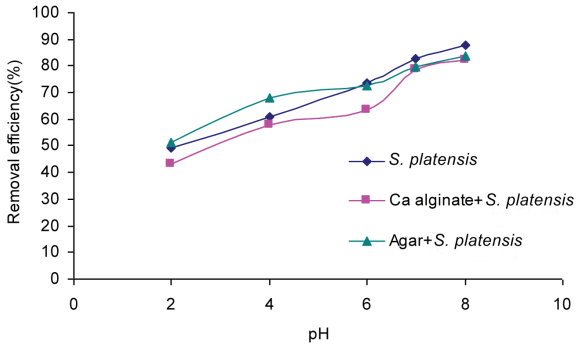

Fig 1. Effect of pH on removal efficiency of Zn^{+2} ions by *Spirulina platensis* in different matrix

in case of *S. platensis* embedded in agar matrix, Freundlich isotherm was better than Langmuir. The maximum Langmuir constant Q_o (mg/g) was 92.93 for *Spirulina platensis* embedded in calcium alginate matrix as shown in Table 2. The native biomass exhibited characteristic absorption at 3500-3000 cm^{-1} and 1200-900 cm^{-1}. The first peak was observed in spectrum occupied by a strong band (3600-3000 cm^{-1}). The sharp peaks at 3000-2800, 2361, 1653, 1542, 1403 cm^{-1} were also seen in the spectrum.

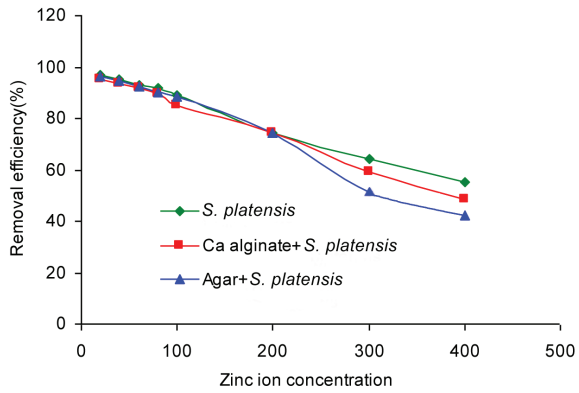

Fig 2. Effect of metal ion concentration on removal efficiency of Zn^{+2} ions by *Spirulina platensis* in different matrix

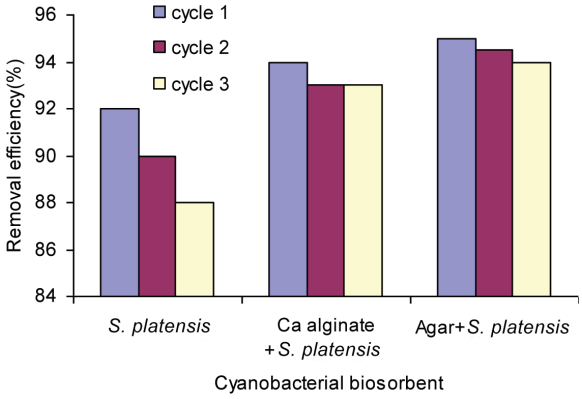

Figure 3. Sorption desorption cycles of *Spirulina platensis*

DISCUSSION

The Zn^{+2} ions uptake pattern by free and immobilized forms of *S. platensis* biosorbent showed similar patterns and maximum percentage removal was observed at pH = 8 (Srivastava *et al.,* 1994). An increase in pH means a lower quantity of protons, which causes a decrease in the competition between proton and heavy metal ion. Increased pH results in more ligands being available for metal Ion binding and hence biosorption is enhanced (Kaewsarn, 2002).

90 minutes was the optimum time for the adsorption process. Adsorption was fast on the first 60 minutes; then the rate started to slow down in later stages because initially a large number of vacant surface sites may be difficult to be occupied due to repulsive forces between the solute molecules of the solid and bulk phase (Ghani *et al.,* 2007). 1g/100 mL was found the optimum adsorbent dose in the experiment. The phenomenon showed increase in Zn^{+2} ions adsorption with increase in adsorbent dose, may be due to more adsorbent surface become available for the solutes to be adsorbed and increasing the rate of adsorption (Mehrotra *et al.,* 1999). In Fig. 2 it is shown that 100 mg/L is the optimum metal ion concentration. Percent adsorption decreased with increasing the metal concentration, due to saturation of all the binding sites with metal ions and establishment of equilibrium between adsorbate and biosorbent (Bai and Abraham, 2001). Desorption study showed that 89-95 % Zn^{+2} ions can be desorbed from the biosorbent using 0.1 M EDTA for three subsequent cycles. EDTA is a hexa-dentate compound containing two nitrogen and four oxygen donor atoms and the metal ions chelated with it. Organic molecules containing more than one functional group with donor electron pairs can simultaneously donate these to a metal atom and this results in the formation of a ring

Table 2: Isotherm parameters for biosorption of Zn^{+2} ions

Species	Freundlich			Langmuir		
	K_f	n	r^2	Q_o	b	r^2
S. platensis	34.77	2.477	0.5098	58.92	0.0019	0.9184
Ca-alginate+*S. platensis*	10.63	1.044	0.8877	92.93	0.0012	0.8953
Agar+*S. platensis*	7.32	0.930	0.929	79.20	0.0018	0.8716

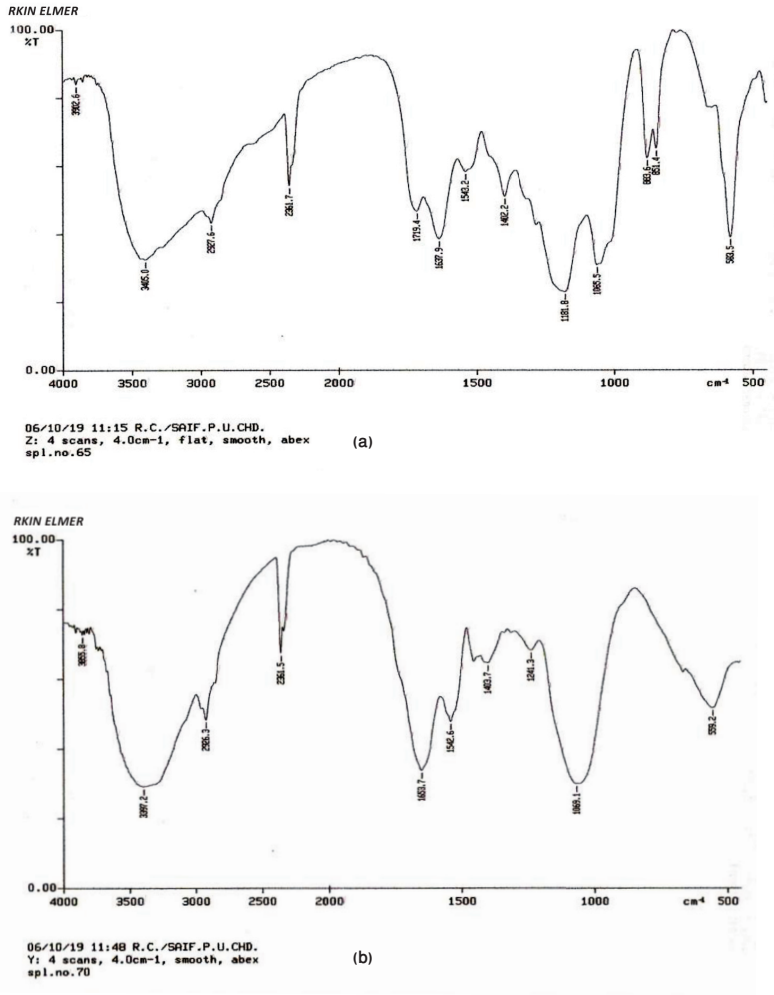

Figure 4 : Infrared spectra of *S. platensis*
(a): Zn untreated; (b): Zn treated

structure involving the metal atom, a process termed 'chelation'. The sharp peak at 3000-2800 cm^{-1} is attributed to the C-H stretching that is characteristic of biological sample. For the native biomass other sharp peaks at 1653 and 1542 cm^{-1} are attributed to the presence of amides I and II bands, respectively (for cellular proteins followed by carboxylic bands at 1653 cm^{-1} and 1403 cm^{-1}). The absorption under 1241.3 cm^{-1} characterises phosphate carrying components, oligo saccharides and poly saccharides of cell wall. These findings are in conformity with earlier studies (Kuyucak and Volesky; 1989, Vannela and Verma, 2006;). The IR spectrum of metal treated cells indicated no such shifts or change in any of characteristics absorbance band exhibited by the native biomass. The only discernible changes observed were in length, width and intensities of the peaks. The changes in above mentioned IR spectrum of metal loaded cells indicate the possible involvement of amide, amino and carboxylic groups in Zn^{+2} ions adsorption. Table 2 shows that the *S. platensis* and *S. platensis* embedded in calcium alginate matrix followed the Langmuir isotherm which indicates the monolayer adsorption. Whereas, the adsorptive behaviour of zinc ion on *S. platensis* embedded in agar satisfied not only Langmuir adsorption assumption but also Freundlich adsorption isotherm, i.e. multilayer formation on surface of the biosorbent with an exponential

distribution of site energy (Meena and Rajgopal, 2003).

The study indicated that *S. platensis* embedded in calcium alginate matrix was the best biosorbent among others for zinc ions removal. Langmuir seems to be suitable for describing zinc ions uptake. The Infrared studies suggests the participation of carboxyl, hydroxyl, amino, amide and imine groups.

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