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Full Length Research Paper

Study of physical chemistry on biosorption of zinc by using *Chlorella pyrenoidosa*

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Discharge of heavy metals from metal processing industries is known to have adverse effects on the environment. Biosorption of heavy metals by metabolically inactive biomass of microbial organisms is an innovative and alternative technology for removal of these pollutants from aqueous solution. Presence of heavy metals in the aquatic system is posing serious problems and zinc has been used in many industrials and removal of Zn ions from waste waters is significant. Biosorption is one of the economic methods that is used for removal of heavy metals. In the present study, the biomass generated from the dried *Chlorella pyronoidsa* was used for evaluating the biosorption characteristics of Zn ions in aqueous solutions. Batch adsorption experiments were performed on these leaves and it was found that the amount of metal ions adsorbed increased with the increase in the initial metal ion concentration. In this study effect of agitation time, initial metal ion concentration, temperature, pH and biomass dosage were studied. Maximum metal uptake was observed at pH = 5. Maximum metal uptake (q_{max}) was 101.11 mg/g. The biosorption followed both Langmuir and Freundlich isotherm model. The adsorption equilibrium was reached in about 1 h. The kinetic of biosorption followed the second – order rate. The biomass could be regenerated using 0.1 M HNO_3. A positive value of $\Delta H°$ indicated the endothermic nature of the process. A negative value of the free energy ($\Delta G^{°}$) indicated the spontaneous nature of the adsorption process. A positive value of $\Delta S°$ showed increased randomness at solid–liquid interface during the adsorption of heavy metals, it also suggests some structural changes in the adsorbate and the adsorbent. Fourier transform infrared (FTIR) spectrums of *C. pyrenoidosa* revealed the presence of hydroxyl, amino, carboxylic and carbonyl groups. The scanning electron micrograph (SEM) clearly revealed the surface texture and morphology of the biosorbent.

Key words: Biosorption, *Chlorella pyronoidosa*, Zn, isotherm models, kinetic.

INTRODUCTION

Heavy metal pollution has posed a serious threat to the aquatic environment. A thigh concentrations, metals are toxic to animals and plants alike, as they could be dispersed in water and consequently in human beings through food chain biomagnifications that could cause serious health hazards. In view of the human health impacts, each metal imparts different effects and symptoms. For instance, in the case of minor zinc exposure, irritability, muscular stiffness, loss of appetite and nausea are common (Bhattacharya et al., 2006). Zinc is an essential element as enzyme activators in humans, but it is equally toxic at levels of 100.500 mg/day and it is a known carcinogen (Volesky and Holan, 1995). However, these methods were found to be either inefficient or expensive when metal ions exist in low concentrations (<100 mg/L) and may also be associated with the generation of secondary environmental problems from waste disposal (Ahalya et al., 2003). Biosorption is the binding and concentration of heavy metals from aqueous solutions (even very dilute ones) by certain types of inactive, dead, microbial biomass (Macaskie et al., 1992). Some of the advantages of biosorption include competitive performance, heavy metal selectivity, cost-effectiveness, regenerative and no sludge generation. Sources of biomass include seaweeds, microorganisms

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Abbreviations: FTIR, Fourier transform infrared; **SEM,** scanning electron micrograph; **BOD,** biochemical oxygen demand; **COD,** chemical oxygen demand; **NCIM,** national collection of industrial microorganisms; **EDTA,** ethylene diamine tetraacetic acid; **HCL**, hydrogen chloride.

(bacteria, fungi, yeast and molds), activated sludge and fermentation waste. Studies using biosorbents have shown that both living and dead microbial cell are able to uptake metal ions and offer potential inexpensive alternative to conventional absorbents (Khoo and Ting, 2001; Knorr, 1991).

However, living cell is subject to toxic effect of the heavy metals, resulting in cell death. Moreover, living cell often require the addition of nutrients and hence increase the Biochemical oxygen demand (BOD) and Chemical Oxygen Demand (COD) in the effluent. For these reasons, the use of non-living biomaterials or dead cells as metal binding compounds has been gaining advantage because toxic ions do not affect them. In addition, dead require less care and maintenance and cheaper (Mofa, 1995). Furthermore, dead biomass could be easily regenerated and reused. The capability of some living microorganisms to accumulate metallic elements has been observed at first from toxicological point of view (Volesky, 1990a,b,c) However, further researches have revealed that inactive/dead microbial biomass can passively bind metal ions via various physicochemical mechanisms. Therefore researches on biosorption have become an active field for the removal of metal ions or organic compounds. Biosorbent behavior for metallic ions is a function of the chemical make-up of the microbial cells of which it consists (Volesky and Holan, 1995). Mechanisms responsible for biosorption, although understood to a limited extent, may be one or combination of ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation and micro precipitation (Veglio and Beolchini, 1997; Vijayaraghavan and Yun, 2008; Wang and Chen, 2006).

MATERIALS AND METHODS

Biomass and culture medium

In this study *Chlorella pyrenoidosa* (2738) obtained from National Collection of Industrial Microorganisms (NCIM) from PUNE - India, which was isolated and thoroughly pure. The *C. pyrenoidosa* was maintained in modified Fog's Media at 28°C with using 3000 lx light intensity. After 21 days cultivation period cells were harvested by centrifugation and were washed several times with deionised water in order to remove culture media and was kept on a filter paper to reduce the water content. The biomass was dried at 60°C in an oven for 24 h and milled to a gritty consistency. The biomass was sieve for particle size smaller than 1 mm and stored in dark bottle and keeps in a dry cabinet for experiments. All of the media are sterilized by autoclaving at 121°C for 20 min.

Preparation of synthetic sample

A stock solution of 1000 mg/l of Zn was obtained by dissolving Zinc chloride (Merck Company) in distilled water. The test solutions of various concentrations range from 10 mg/L to 100 mg/L were prepared from the stock solution. The solution pH was adjust using 0.1 M HNO_3 and 0.1 M NaOH at the beginning of the experiment and not controlled afterwards. The conical flasks (250 ml) were shaken at 120 rpm in a temperature controlled rotatory shaker.

Analysis of zinc ions

Zinc ions were determined spectrophotometrically by atomic adsorption spectrophotometer (UNICAM, model 929, United Kingdom).

Batch biosorption studies

Batch mode adsorption studies for individual metal compounds were carried out to investigate the effect of different parameters such as adsorbate concentration, adsorbent dose, agitation time and pH. Solution containing adsorbate and adsorbent was taken in 250 ml capacity conical flask and agitated at 120 rpm in a shaker at predetermined time intervals. The adsorbate was decanted and separated from the adsorbent using whatman No. 41 filter paper.

Effect of agitation time and initial concentration

For the determination of rate of metal biosorption by biomasses from 100 ml (at 10, 20, 50 and 100 mg/L) on conical flask 250 mL, the supernatant was analyzed for residual metal at different time intervals. The pH and the adsorbent dosage was kept constant at pH = 5±0.01, which varied according to the adsorbent and adsorbate under consideration. Amount of biomass dosage was 0.1±0.001 g for biomass (*C. pyronoidosa*) and temperature was 25±1°C and agitation speed of shaker was 120 rpm.

Effect of adsorbent dosage and initial concentration

The effect of adsorbent dosage that is, the amount of the biomasses on the adsorption of metals was studied at different dosages ranging from 0.1 to 3 g with varied metal concentrations of 10, 20 and 50 mg/L. The equilibrium time and the pH were kept constant depending on the metal under consideration. The pH and the adsorbent dosage was kept constant at pH = 5±0.01, which varied according to the adsorbent and adsorbate under consideration. Agitation time was 120 min for biomass (*C. Pyronoidosa*) and temperature was 25±1°C and agitation speed of shaker was 120 rpm.

Effect of pH and initial concentration

To determine the effect of pH on the adsorption of metal solutions (100 ml) of different concentration ranges (10, 20 and 50 mg/L) at conical flask 250 ml were adjusted to desired pH values and mixed with constant amount of adsorbent and agitated at preset equilibrium time. The equilibrium time and adsorbent dosage varied with the metal and adsorbent under consideration. Amount of biomass dosage was 0.1±0.001 g for e biomass (*C. pyronoidosa*) and temperature was 25±1°C and agitation speed of shaker was 120 rpm and contact time was 120 min.

Effect of temperature

Optimum biomass concentration with optimum pH was used to monitor the temperature effect on biosorption. Experiments were carried out at different temperatures from 10 to 40°C for each culture and kept on rotary shaker at 120 rpm. The samples were allowed to attain equilibrium. To determine the effect of temperature on the adsorption of metal solutions (100 ml) of concentration 50 mg/L at conical flask 250 ml were adjusted to desired pH values and mixed with constant amount of adsorbent and agitated at preset equilibrium time. The equilibrium time and adsorbent dosage

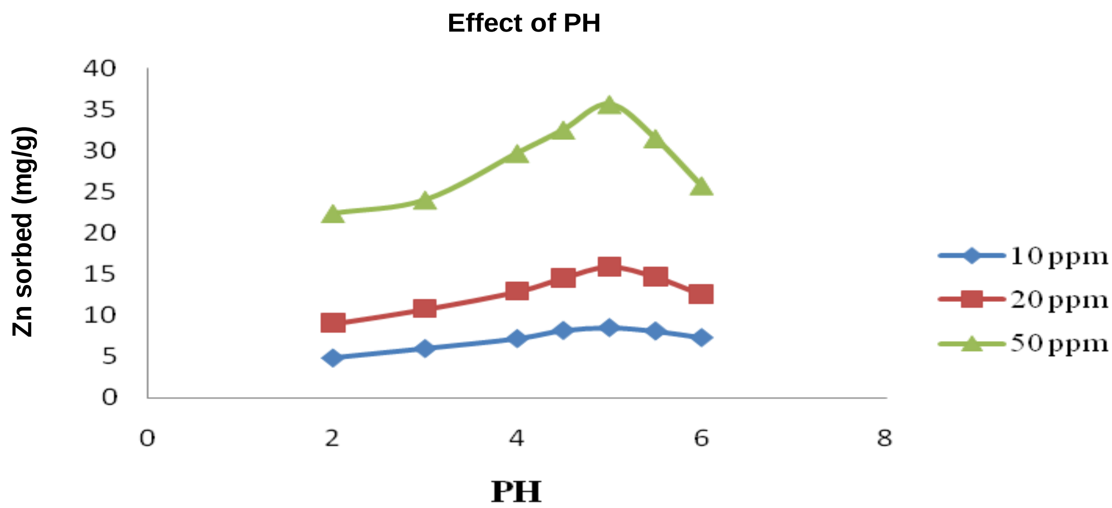

Figure 1. Effect of pH on biosorption of Zinc by *C. pyrenoidosa* (biomass dose = 0.1 g, initial Zinc ion concentration = 10, 20 and 50 mg L^{-1}; temperature = 25°C; agitation speed = 120 rpm; contact time = 120 min).

varied with the metal and adsorbent under consideration. Amount of biomass dosage was 0.1±0.001 g for biomass (*Chlorella*) and pH was 5±0.001 and agitation speed of shaker was 120 rpm and contact time was 120 min.

Desorption studies

After adsorption, the adsorbates loaded adsorbent were separated from the solution by centrifugation and the supernatant was drained out. The adsorbent was gently washed with water to remove any unadsorbed adsorbate. Regeneration of adsorbate from the adsorbate laden adsorbent was carried out using the desorbing media distilled water at pH ranges using dilute solutions of ethylene diamine tetraacetic acid (EDTA), Hydrogen chloride (HCl) and HNO_3 (Stirred at 120 rpm for 120 min at 25°C). Then they were agitated for the equilibrium time of respective adsorbate. The desorbed adsorbate in the solution was separated and analyzed for the residual heavy metals.

FT-IR spectroscopy (Fourier transform infrared)

In order to determine the functional groups responsible for Zn biosorption, infrared (IR) spectroscopy was used that about 0.1 g biomass was mixed with KBr for FT-IR spectra analysis (Shimadzu model 8400).

SEM (scanning electron microscopy)

The SEM was used to investigate the morphology of the biosorbent. We used samples with pH=5 and C_0 = 10 mg.L^{-1}. SEM (SEM, JEOL, JSM-6360A) used for this study.

RESULTS

A result on the effect of pH of Zn at different initial metal ion concentrations by *C. pyrenoidosa* is presented in Figure 1. Maximum percentage of biosorption accursed at the initial concentration of 10 mg/L at the time of 120 min at pH= 5 for Zn was 85.55% and metal ion uptake capacity was 8.55 mg/g and when initial concentration of Zn increased to 50 mg/L, percentage of biosortion of Zn was 71.33% and uptake capacity was 35.66 mg/g for *C. pyrenoidosa.* With increase the initial concentration percentage of biosorption decreased and metal ion uptake capacity was increased. A result on the contact time of zinc at different initial metal ion concentrations by *C. pyrenoidosa* is presented in the Figure 2. The time required to reach equilibrium for Zn adsorption by *C. pyrenoidosa* was 60 min for all initial metal ion concentrations. In the initial concentration of 10 mg/L at the time of 60 min percentage of remove of Zn was 82.27% and metal ion uptake capacity was 8.22 mg/g and when initial concentration of Zn increased to 100 mg/L, percentage of remove of Zn was 64.61% and uptake capacity was 64.61 mg/g for *C. pyrenoidosa*. The time taken for Zn adsorption by *C. pyrenoidosa* was dependent on initial metal ion concentration and increased with increase in concentration of Zn. With increase the initial concentration percentage of biosorption decreased and metal ion uptake capacity was increased.

A result on the effect of biomass dosage of Zn at different initial metal ion concentrations by *C. pyrenoidosa* is presented in the Figure 3. percentage of biosorption accursed at the initial concentration of 10 mg/L at the time of 120 min at pH = 5 and 0.1± 0.001 g of biomass for Zn was 85.55% and metal ion uptake capacity was 8.56 mg/g and when initial concentration of Zn increased to 50 mg/L, percentage of biosortion of Zn was 71.33% and uptake capacity was 35.66 mg/g for *C. pyrenoidosa.* When amount of biomass increased from 0.1±0.001 g to 3±0.001 g, percentage of biosorption accursed at the initial concentration of 10 mg/L at the

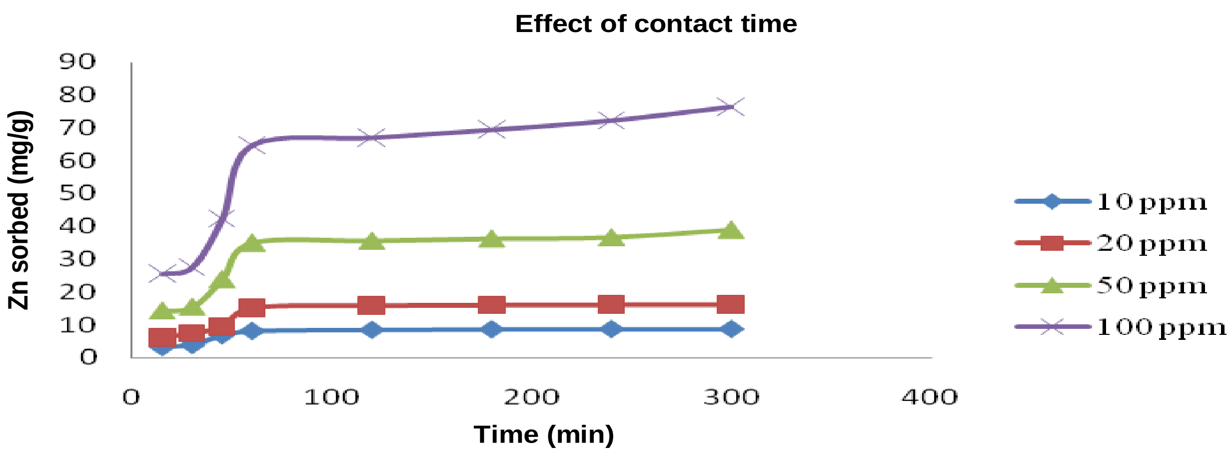

Figure 2. Effect of Contact Time on biosorption of Zinc by *C. pyrenoidosa* (biomass dose=0.1 g, pH=5, initial Zinc ion concentration = 10, 20 50 and 100 mg L^{-1}; temperature = 25°C; agitation speed = 120 rpm).

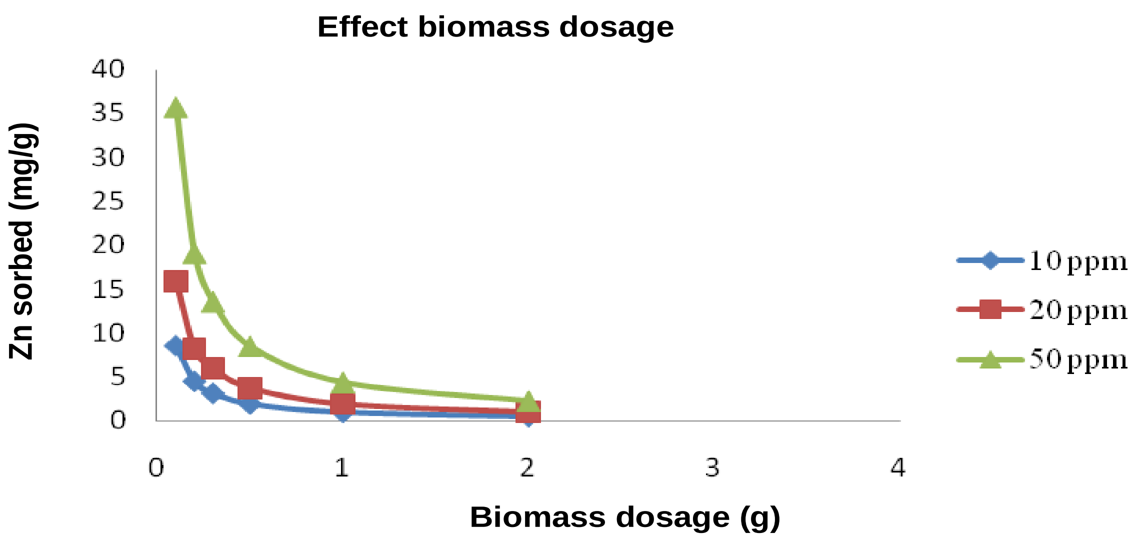

Figure 3. Effect of biomass dosage on biosorption of zinc by *C. pyrenoidosa* (pH = 5, initial Zinc ion concentration = 10, 20 and 50 mg l^{-1}; temperature = 25°C; agitation speed = 120 rpm; contact time = 120 min).

time of 120 min at pH= 5 for Zn was 97.45% and metal ion uptake capacity was 0.325 mg/g and when initial concentration of Zn increased to 50 mg/L, percentage of biosortion of Zn was 91.55% and uptake capacity was 1.52 mg/g for *C. pyrenoidosa.* With increase the initial concentration percentage of biosorption decreased and metal ion uptake capacity was increased. With increased the amount of biomass observed that percentage of biosorption increased and metal ion uptake capacity was decreased. A result on the effect of temperature of Zn at initial metal ion concentration of 50 mg/L by *C. pyrenoidosa* is presented in the Figure 4. Maximum percentage of biosorption accursed at the initial concentration of 50 mg/L at the time of 120 min at pH= 5 for Zn was 75.44% and metal ion uptake capacity was 37.72 mg/g at the temperature of 40°C for *C. pyrenoidosa.* The findings of *C. pyrenoidosa* indicate that the sorption percentage increased with increase in temperature up to 40°C.

Equilibrium isotherms

The isotherm studies were performed in the solution with the initial concentrations ranging from 10 to 100 mg/L at optimum pH values for ions (pH=4.5 or pH = 5). After shaking the flask containing the mixture of biomass (120 rpm, 25°C) and ions for 120 min, the amount of residual ions in the filtrated solution was analyzed. The biosorption equilibrium uptake capacity for each sample

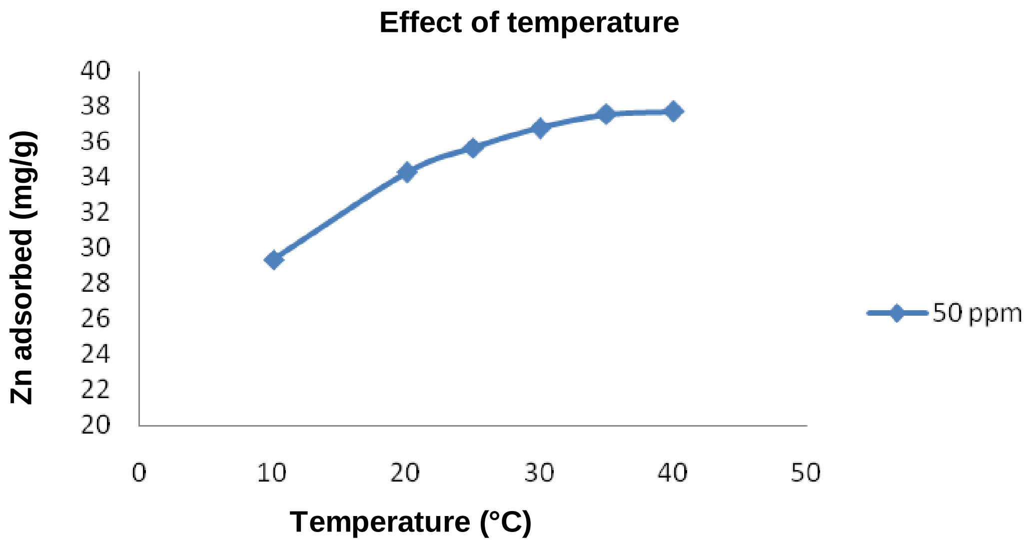

Figure 4. Effect of temperature on biosorption of zinc by *C. pyrenoidosa* (biomass dose = 0.1 g, pH=5, initial Zinc ion concentration = 50 mg l^{-1}; temperature = 25°C; agitation speed = 120 rpm; contact time = 120 min).

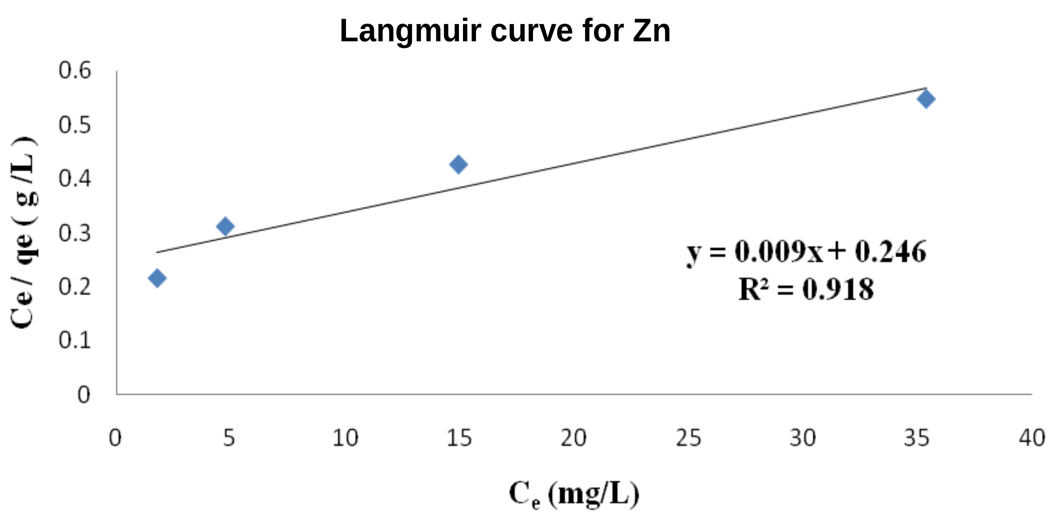

Figure 5. Langmuir adsorption isotherm for Zn by *C. pyrenoidosa* (q_{max} = 111.11 mg/g, b = 0.036 L/mg).

was calculated according to mass balance on the ions expressed in this equation: $q_e = \frac{(C_0 - C_e)}{M} \times V$

where V is the sample volume (L), C_0 is the initial ion concentration (mg/L), C_e is the equilibrium or final ion concentration (mg/L) , M is the biomass dry weight (g), and q_e is the biomass biosorption equilibrium ions uptake capacity (mg/g). Langmuir and Freundlich isotherms, the two classical adsorption models, were used to describe the equilibrium between adsorbed ions on the biomass cell (q_e ,q) and ions in the solution (C_e, q) in this study.

Langmuir isotherm model: $q_e = \frac{q_{\max} C_e b}{1 + C_e b}$

That after arrange we have; $\frac{C_e}{q_e} = \frac{1}{q_{\max} b} + \frac{C_e}{q_{\max}}$

These values q_{max} and b (where b, is the adsorption equilibrium constant) can be obtained from the slopes and the intercepts of the linear plots respectively, where experimental data of Ce/qe as the function of C_e. as shown in Figure 5. The empirical Freundlich equation

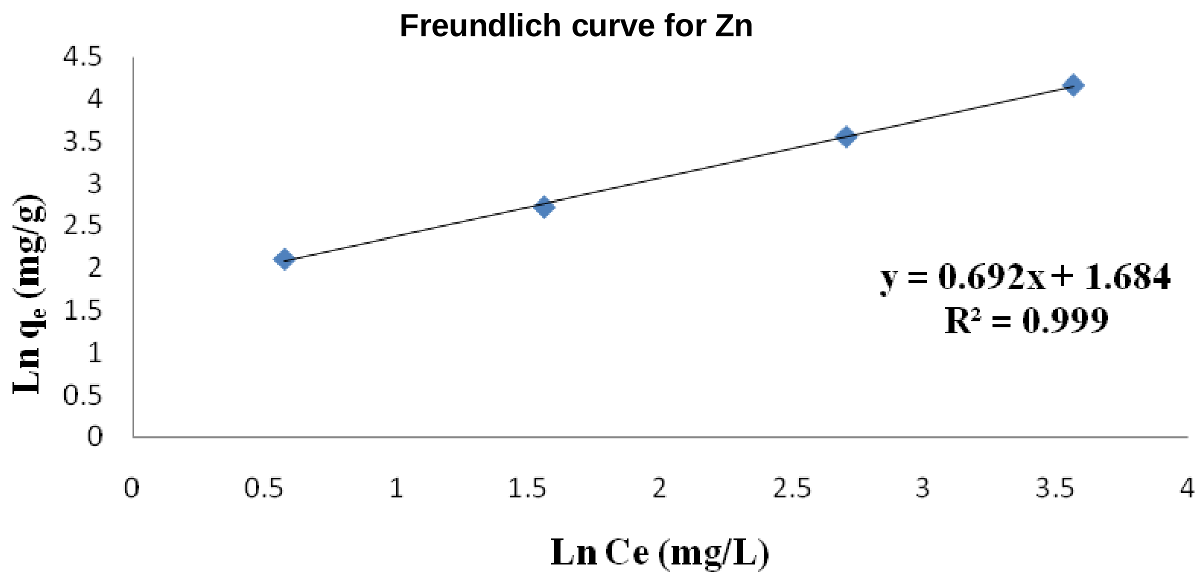

Figure 6. Freundlich adsorption isotherm for Zn by *C. pyrenoidosa* (n = 1.44, K = 5.38).

Table 1. Parameters of isotherm models for chromium.

Biomass	Langmuir Parameters			Freundlich parameters		
	q_{max} (mg/g)	b(L/mg)	R^2	K_f	n	R^2
Chlorella pyrenoidosa	101.11	0.036	0.918	5.38	1.44	0.999

based on sorption on a heterogeneous surface, on the other hand, is as follows:

$$q_e = K_f (C_e)^n$$

K and n: An experimental constant, K is an indication of the adsorption capacity of the adsorbent; n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. The equation can be linearized in the following logarithmic form:

$$\ln q_e = \ln k_f + \frac{1}{n} \ln C_e$$

These values n and K_f can be obtained from the slopes and the intercepts of the linear plots, respectively, where experimental data of Ln q_e as the function of Ln $C_{e.}$ .The equilibrium experimental results of Zinc ions have been fitted in the Langmuir and Freundlich models. For biosorption of Zinc using *C. pyrenoidosa* the coefficient of determination (R^2) of both models was mostly close to 1 as shown in Figure 6. This indicates that both models adequately describe the experimental data of the biosorption of Zinc. In the biosorption of Zinc by *Chlorella*, most of the metal ions were sequestered very fast from the solutions in the first phase of contact time 60 min and almost no increase in the level of bound metal have been occurred after this time interval. Biosorption equilibrium isotherms were plotted for metal uptake q against the residual metal concentration in the solution. The q verses C_f sorption isotherm relationship was mathematically expressed by Langmuir and Freundlich models. The higher the values of k and n; lower the value of b, the higher the affinity of the biomass. Table 1 describes summaries of linear regression data for Langmuir and Freundlich isotherms for Zinc biosorption using *C. pyrenoidosa* biomass. Langmuir and Freundlich constant k were obtained from the linear equations of both models. As indicated in Table 1, the coefficients of determination (R^2) of both models are close to 1. In the Table 1 the values of K_f, n, q_{max} and b were given.

Kinetic modeling

Figure 7 shows the experimental break through curves for the effects of contact time on a bound rate of Zn. It can be observed that the adsorption of Zinc ions quickly increased at the beginning of biosorption, but after 15 min, the adsorption slowed down. The result indicated that the maximum adsorbed amount of the Zinc ions was achieved within 60 min and then followed by a longer equilibrium period. After this equilibrium period, the

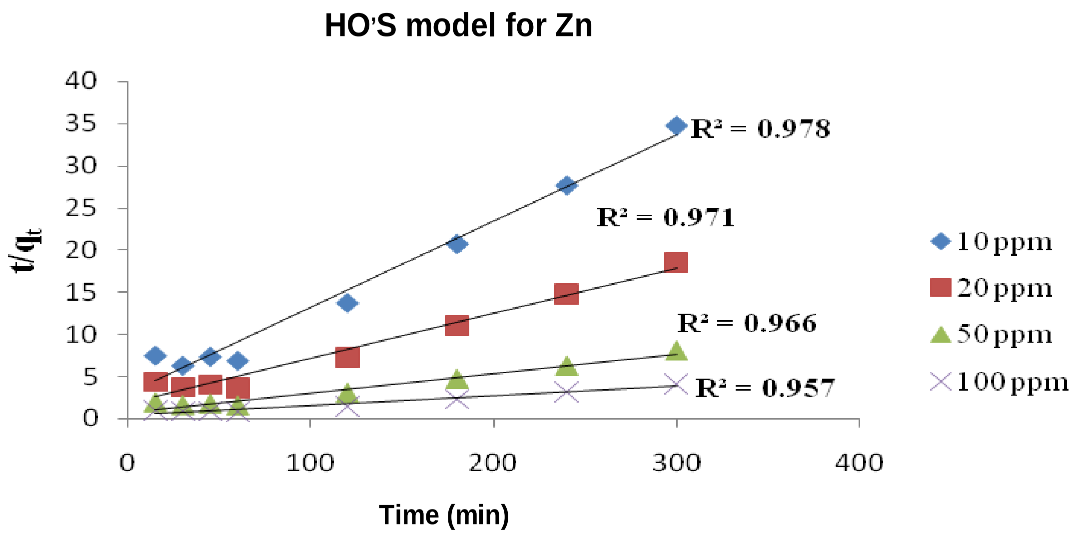

Figure 7. Plot of HO'S Model (pseudo second order rate) for Zinc by *C. pyrenoidosa*.

Table 2. Type of isotherm for various R_L.

R_L	$R_L>1$	$R_L=1$	$0< R_L<1$	$R_L = 0$
Type of isotherm	Un favorable	Linear	Favorable	Irreversible

Table 3. Parameter of kinetic model for chromium of *C. pyrenoidosa*.

Concentration of Cr	Equation	R^2	K_2
10 mg/L	y = 0.103x + 2.468	0.991	4.30×10^{-3}
20 mg/L	y = 0.055x + 1.577	0.989	1.92×10^{-3}
50 mg/L	y = 0.023x + 0.762	0.988	9.94×10^{-4}
100 mg/L	y = 0.011x + 0.479	0.986	2.53×10^{-4}

amount of adsorbed ions did not significantly change with the adsorption time. Therefore, for the following experiments, the contact time was maintained for 60 min to ensure that equilibrium was fully achieved. The pseudo-second-order equation is also based on the sorption capacity, which is expressed as:

$$\frac{t}{q_t} = \frac{1}{(K_2 q^2{}_e)} + \frac{t}{q_e}$$

Where K_2 is the rate constant of pseudo-second-order sorption (g· mg^{-1}·min^{-1}). K_2qe^2 is the initial rate constant (represented by h, mg·g^{-1}·min^{-1}). Plotting t/q_t versus t will give a straight line. The values of qe and K_2 can be determined from the slope and intercept of the plot, respectively. The results showed that the pseudo-second-order model fitted the simulation curve much better than the pseudo-first-order model for Zn. The results of pseudo-second-order model are shown on Table 2. The coefficient of determination (R^2) and K_2 HO'S Model for the different metal ion concentration under study has been established as:10 > 20 > 50 > and 100 ppm. With increase the initial concentration coefficient of determination (R^2) and K_2 decreased.

Equilibrium parameter R_L

The essential characteristics of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter R_L, which is defined by the equation:

$R_L = 1/1+bC_o$

Where C_o is the initial adsorbate concentration (mg/L) and b is the Langmuir constant (L/mg). The parameter indicates the shape of the isotherm as follows (Table 3). The R_L values at different initial adsorbate

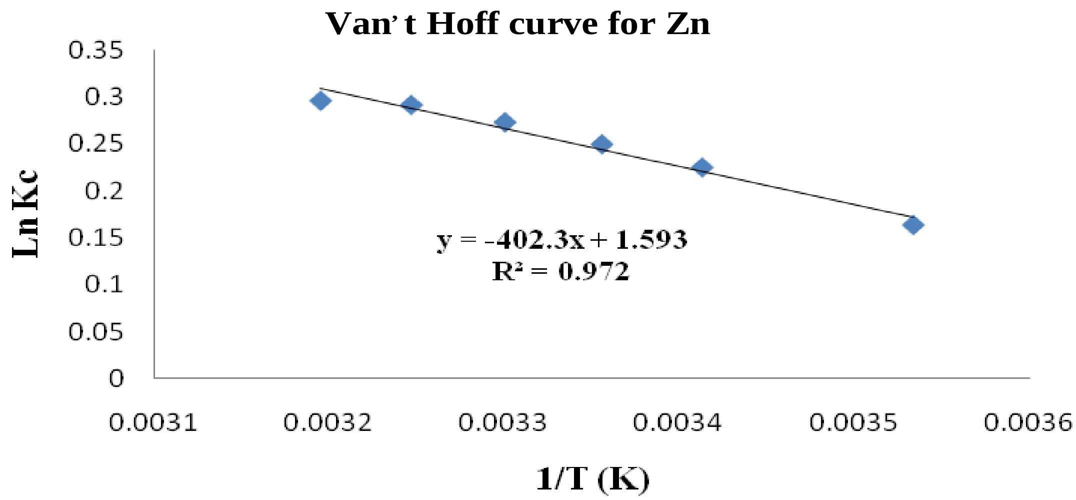

Figure 8. Plot of Van' t Hoff equation for the estimation of thermodynamic parameters for biosorption of Zinc by *C. pyrenoidosa*.

Table 4. Thermodynamic parameters for the adsorption of heavy metals by *C. pyrenoidosa*.

Heavy metal	ΔH° (J $mol^{-1}K^{-1}$)	ΔS° (J $K^{-1}mol^{-1}$)	-Δ°G (J.$mol^{-1}K^{-1}$)					
			283 K	293 K	298 K	303 K	308 K	313 K
Zn	3344.72	13.24	13.61	37.39	51.84	68.01	84.74	98.31

concentrations indicate favorable adsorption for all the adsorbents and adsorbates studied.

Thermodynamic studies

Adsorption, studies in the temperature range 283 to 313 K were conducted to determine thermodynamics constants such as Gibbs free energy change (ΔG°), enthalpy change (ΔH°) and entropy change (ΔS°) for the system and to ascertain the sorption mechanism. For this study, adsorbent dosage selected was 0.1 gr. and Zinc concentration was 50 mg L^{-1} with pH = 5 in a conical flask and allowed to equilibrate for 1 h at the different temperatures ranging from 283 to 313K. In order to determine thermodynamic parameters, experiments were carried out at different temperatures in the range of 283 to 313K for heavy metals adsorption. The thermodynamic parameters such as standard Gibbs free energy change (ΔG°), enthalpy change (ΔH°) and entropy change (ΔS°) were estimated to evaluate the feasibility and nature of the adsorption process. The Gibbs free energy change, of the process is related to equilibrium constant by the equation:

$$\Delta G^{\circ} = -RT \ln K_C$$

Where, T is temperature in K, R is ideal gas constant having value as 8.314, J mol^{-1} K^{-1} and K_C is thermodynamic equilibrium constant. The thermodynamic equilibrium constant, was determined as:

$$K_C = \frac{C_a}{C_e}$$

Where, C_a is mg of adsorbent adsorbed per liter and C_e is the equilibrium concentration of solution, mg/L. According to thermodynamics, the Gibbs free energy change is also related to the enthalpy change (ΔH°) and entropy change (ΔS°) at constant temperature by the following Van' t Hoff equation:

$$\ln K_C = \frac{-\Delta H^{\circ}}{R}\frac{1}{T} + \frac{\Delta S^{\circ}}{R}$$

The values of enthalpy change (ΔH°) and entropy change (ΔS°) were calculated from the slope and intercept of the plot lnKc versus, 1/T. Results on the plots of van' t Hoff equation for the estimation of thermodynamic parameters by *C. pyrenoidosa* is presented in Figure 8. The calculated values of thermodynamic parameters are reported in Table 4. A positive value of ΔH° indicated the endothermic nature of the process. A negative value of the free energy (ΔG°) indicated the spontaneous nature of the adsorption process. It was also noted that the

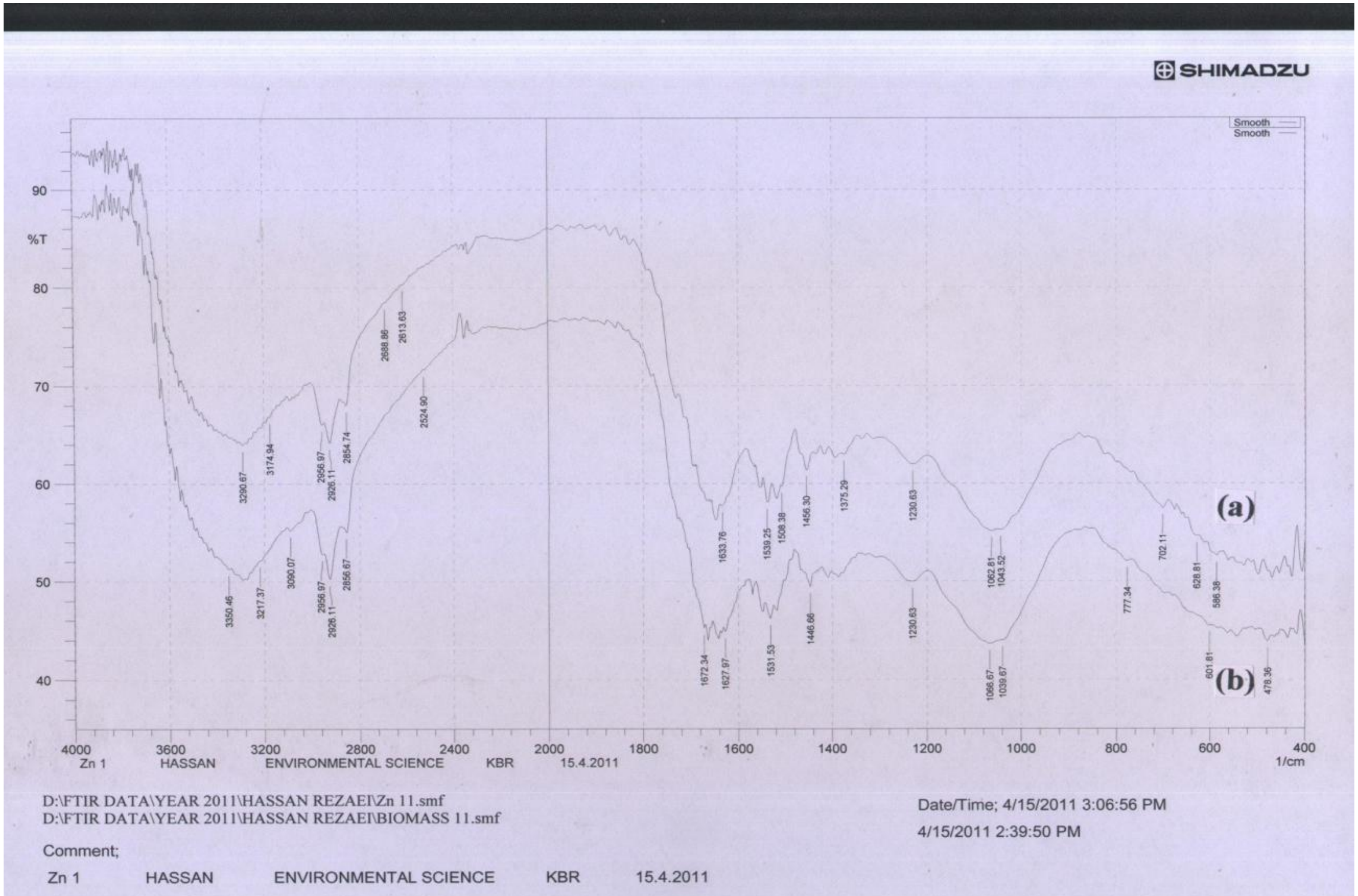

Figure 9. FTIR Spectrum of the *C. pyrenoidosa* (before (a) and after (b) biosorption of Zinc).

change in free energy, increases with increase in temperature, which exhibits an increase in adsorption with rise in temperature. This could be possibly because of activation of more sites on the surface of biomasses with increase in temperature. A positive value of $\Delta S°$ showed increased randomness at solid–liquid interface during the adsorption of heavy metals, it also suggests some structural changes in the adsorbate and the adsorbent.

FTIR spectroscopy of *C. pyrenoidosa*

Spectrums of *C. pyrenoidosa* are presented in Figure 9 that revealed the presence of hydroxyl, amino, carboxylic and carbonyl groups. The

Table 5. Assignment of bands to functional group on the surface of *C. pyrenoidosa* as observed from FTIR spectroscopy.

Wave numbers (Cm^{-1})		Assignment
Before	**After**	
3433.41	3373.61	O-H stretching /
3325.39	3174.94	N-H stretching
3255.95	3091.99	
3213.51		
3174.94		
2924.18	2924.18	C-H stretching aliphatic
2854.74	2854.74	C-H stretching
2746.73	2744.80	
1737.92	1732.13	C=O stretching vibration
1639.55	1645.33	C=O Stretching vibration /
	1618.33	N-H Stretching vibration
1450.52	1454.38	CH_2Bonding vibration
1317.43	1315.50	CH_3 Bonding vibration
1033.88	1031.95	C-O Stretching
756.12		C-X (X = F, Cl, Br and I)

presence of OH group along with carbonyl group confirmed the presence of carboxylic acid groups in the biosorbent. The presence of NH group and OH group along with carbonyl group might be attributed the presence of amino acid groups in the biosorbent. All of them are summarized in Table 5.

Scanning electron microscopy (SEM)

SEM of *C. pyrenoidosa* at before and after biosorption of Zinc is presented in Figures 10 and 11, respectively. The SEM clearly revealed the surface texture and morphology of the biosorbent at different magnifications. The SEM analysis revealed important information on surface morphology. In these micrographs structures with large surface area were evident.

Desorption studies

Desorption and regeneration studies of the adsorbates showed that regeneration and recovery of the adsorbates is possible. Chemisorption/ion exchange was the main mechanism by which the adsorbates (metals) were attached to the adsorbents. Physical adsorption played a minimal role in the process .The result of desorption studies of Zn in a batch system showed that HNO_3 (0.1 M) was more efficient in Zn desorption, which remove 95% Zinc ions (Table 6).

Conclusions

The batch experiment conducted with the biosorption demonstrated that biomass of *C. pyrenoidosa* exhibited the potential for Zn removal from aqueous solution. Optimum pH and temperature for biosorption in this study were 5 and 25°C, respectively. The time taken for Zn adsorption by *C. pyrenoidosa* was dependent on initial metal ion concentration and increased with increase in concentration of Zn. With increase the initial concentration percentage of biosorption decreased and metal ion uptake capacity was increased. Which increased the amount of biomass observed that percentage of biosorption increased and metal ion uptake capacity was decreased. The findings of *C. pyrenoidosa* indicate that the sorption percentage increased with increase in temperature up to 30°C and there was a decrease in sorption percentage with further increase in temperature. The removal of Zn increase with increase in biosorbent. The biosorption process was followed both Langmuir and Freundlich isotherm model. The pseudo second-order kinetics described the experimental data well. The equilibrium time was 60 min.

The R_L values at different initial adsorbate concentrations indicate favorable ($0<R_L<1$) adsorption for all the adsorbents and adsorbates studied.HNO_3 (0.1M) had higher efficiency of Zn desorption than EDTA (0.1M) and HCL(0.1M) with 95% efficiency desorption. A positive value of $\Delta H°$ indicated the endothermic nature of the process. A negative value of the free energy (ΔG°) indicated the spontaneous nature of the adsorption process. A positive value of $\Delta S°$ showed increased randomness at solid–liquid interface during the adsorption of heavy metals, it also suggests some structural changes in the adsorbate and the adsorbent. FTIR spectrums of *C. pyrenoidosa* revealed the presence of hydroxyl, amino, carboxylic and carbonyl groups. The

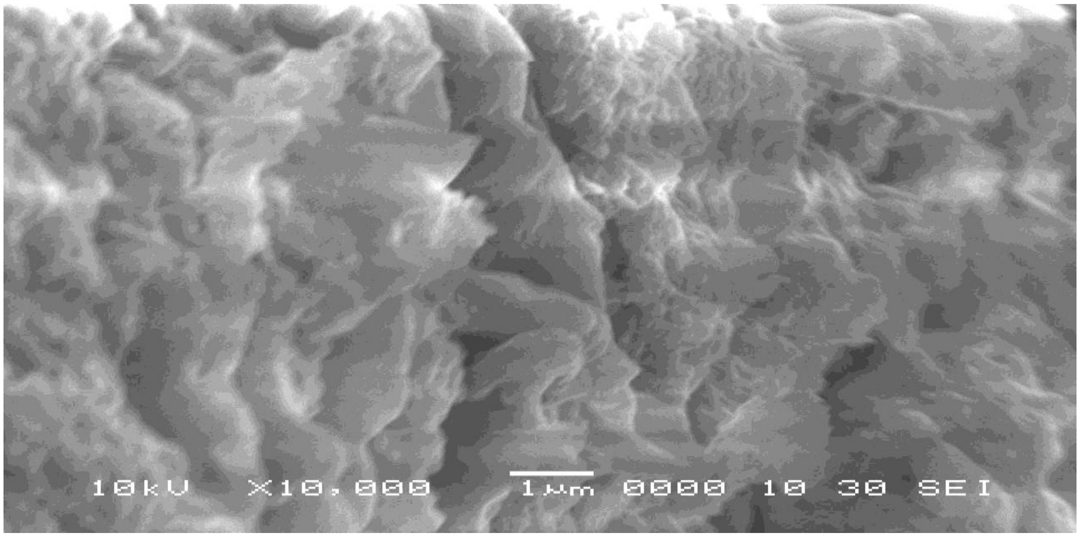

Figure 10. Scanning electron microscopy (SEM) micrographs of the *C. pyrenoidosa* before biosorption.

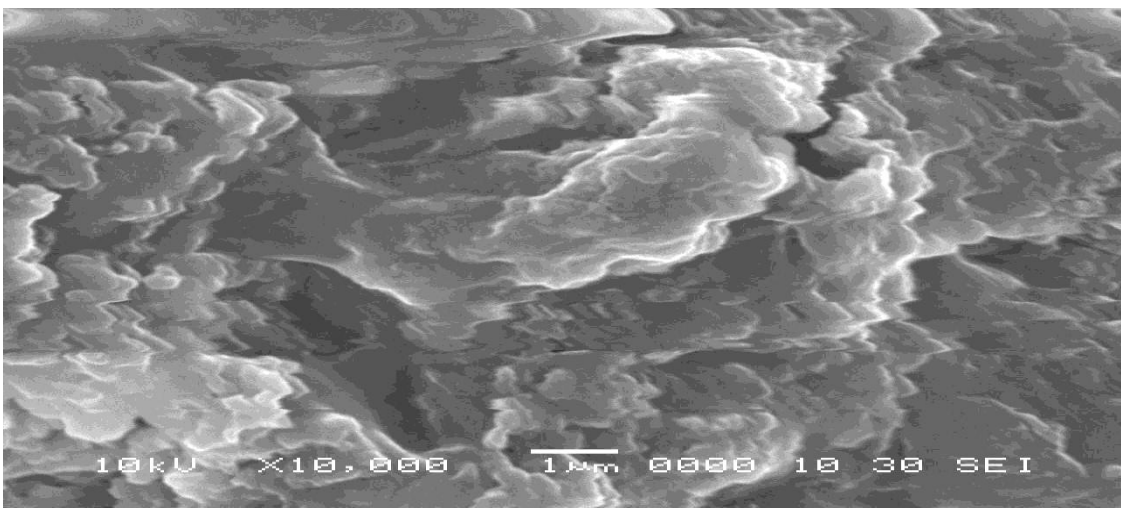

Figure 11. Scanning electron microscopy (SEM) micrographs of the *C. pyrenoidosa* after biosorption of zinc.

Table 6. The desorption efficiency of different desorbent.

Desorbent	EDTA (0.1M)	HCL (0.1M)	HNO_3 (0.1M)
% Desorption of Zn	76.14±4.12	91.36±4.43	94.84±4.01

SEM clearly revealed the surface texture and morphology of the biosorbent.

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