Biosorption optimization of lead(II), cadmium(II) and copper(II) using response surface methodology and applicability in isotherms and thermodynamics modeling

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ABSTRACT

The present study was carried out to optimize the various environmental conditions for biosorption of Pb(II), Cd(II) and Cu(II) by investigating as a function of the initial metal ion concentration, temperature, biosorbent loading and pH using *Trichoderma viride* as adsorbent. Biosorption of ions from aqueous solution was optimized in a batch system using response surface methodology. The values of R^2 0.9716, 0.9699 and 0.9982 for Pb(II), Cd(II) and Cu(II) ions, respectively, indicated the validity of the model. The thermodynamic properties ΔG°, ΔH°, ΔE° and ΔS° by the metal ions for biosorption were analyzed using the equilibrium constant value obtained from experimental data at different temperatures. The results showed that biosorption of Pb(II) ions by *T. viride* adsorbent is more endothermic and spontaneous. The study was attempted to offer a better understating of representative biosorption isotherms and thermodynamics with special focuses on binding mechanism for biosorption using the FTIR spectroscopy.

1. Introduction

A world wide environmental problem has been invited over the past few decades due to tremendous increase in the metallic contents in the environment. Heavy metals are the main group of inorganic contaminants, and a considerable large area of land is contaminated with them due to use of sludge, pesticides, fertilizers, and emissions from municipal waste incinerators, car exhausts, residues from metalliferous mines, and smelting industries [1,2]. The industrial effluents discharge containing toxics heavy metals drain into the river, a source of drinking water for downstream towns. Wastewater treatment facilities in most of the developing countries are not well equipped to remove traces of heavy metals, thus exposing every consumer to unknown quantities of pollutants in the water they consume. Biosorption is a process that utilizes low cost biosorbents to sequester toxic heavy metals [3]. Biological treatment of wastewater is an innovative technology available for heavy metal remediation. Biosorbents such as algae, fungi and bacteria are examples of biomass tested for biosorption of several metals species with very encouraging results and are known to tolerate and accumulate heavy metals. To overcome the disadvantages toxic effect at elevated toxicant concentrations on living biomass; non-viable or dead biomass is preferred [4]. Among the microorganisms, fungal biomass seems to be a good sorption material, because, it can be produced easily and economically using simple fermentation techniques with a high yield of biomass and economical growth media [5]. Potential of filamentous fungi in bioremediation of heavy metal containing industrial effluents and wastewaters has been increasingly reported from different parts of the world [6]. However, filamentous fungi of heavy metals polluted habitat in India are not largely screened and exploited for their bioremediation potential. The advantages of biosorption over the conventional methods are low operating cost, selectivity for specific metal, short operational time and no chemical sludge [7]. It is necessary to submit a controlled application, either living or nonliving microbial cells under most influencing conditions, such as pH, temperature, sorbent mass and ionic concentration [8]. The response surface methodology successfully applied for the optimization of process variables indicated that it is a decision making tool. The main goal of the study was to investigate metals ions removal efficiency of the enveloping *Trichoderma viride* as a cost effective biosorbent using response surface methodology.

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Table 1
Process variables and their level.

Factors	Name	Units	Low actual	High actual	Low coded	Middle coded	High coded
A	Temperature	°C	20	40	−1	0	1
B	Adsorbent dose	g/l	0.5	2.5	−1	0	1
C	pH		2	6	−1	0	1
D	Initial metal ions concentration	ppm	20	100	−1	0	1

The biosorption data are used to evaluate various thermodynamics parameters, such as $\Delta H°$, $\Delta G°$, $\Delta E°$ and $\Delta S°$.

2. Materials and methods

2.1. Source and genesis of fungal biomass adsorbent

The fungal strain *T. viride* was isolated from electroplating industrial soil. One gram of the soil was inoculated in the nutrient broth media amended with heavy metal ions solutions. The strain was isolated on nutrient agar media containing (g/l): agar 20.0, D-glucose 10.0, Bacteriological Peptone 5.0, KH_2PO_4 1.0, $MgSO_4 \cdot 7H_2O$ 0.5, Streptomycin 0.03, Agar 15.0 and pH 6.0 ± 0.5. The pure colony was preserved on the slants at 4 °C and identified from Microbial Type Culture Collection on the basis of spore's morphology. The fungal biomass was prepared in the nutrient broth by inoculating the spore suspension in the 250 ml flasks containing 100 ml of nutrient broth on the rotary shaker cum incubator at 35 °C and 125 rpm. The fully cultured biomass was harvested, filtered through sieve and washed with double distilled water. The biomass was dried at 50 °C and preserved in the polythene bags for the bisorption study.

2.2. Response surface methodology

Box–Behnken design of four variables and three levels each with three concentric point combination [9] was used to unearth the optimum pH, temperature, initial metal ions concentration and adsorbent dose. The design was taken as it fulfills most of the requirement for optimization of the biosorption study. The main objective of RSM is to determine the optimum operational conditions of the process that satisfies the operating specifications [10]. The Box–Behnken design of quadratic model contained 29 experiments for each metal ion.

2.3. Design variables of biosorption study

The design variables of Box–Behnken for bioremediation include: adsorbent loading (0.5–2.5 g/l), initial metal ions concentration (20–100 ppm), temperature (20–40 °C) and pH was selected (2–6) to avoid the interference due to precipitation at higher pH. The biosorption experiments were carried out at 120 rpm for 1 h of equilibrium period. pH of the synthetic solution was adjust using 1N NaOH/HCl. Three levels for each design variables for Box–Behnken for confined biosorption are listed in Table 1.

Various metals ions solutions were made using 1000 ppm stock solution prepared from $Pb(NO_3)$, $3CdSO_4 \cdot 8H_2O$ and $CuSO_4 \cdot 5H_2O$. The residual heavy metals ions quantification was analyzed by Atomic Absorption Spectrophotometer (Shimadzu AA-6300, Japan). The amount of heavy metal ions removal was obtained by using the following expression:

$$\% \, Removal = \frac{C_{initial} - C_{final}}{C_{initial}} \times 100 \qquad (1)$$

where $C_{initial}$ is the metal ions concentration before equilibrium, C_{final} is the metals ions concentration after 1 h equilibrium period.

2.4. Design of experiments

Optimization is a research area with a long tradition, particularly in the field of operational analysis, which has given rise to a wealth of techniques. In conventional one-factor-at-a-time experimentation, effect of interaction among the factors is ignored as the experimenter varies a single factor, while other factors are held as constant. Response surface methodology is a systematic statistical approach to explore the relationships between design variables and responses that can give a better overall understanding with the minimal number of experiment runs. The Box–Behnken creates designs with desirable statistical properties but, most importantly, with only a fraction of the trials required for a 3-level factorial so the quadratic model is appropriate. The number of experiments required for Box–Behnken design can be calculated as follows:

$$N = k^2 + k + cp \qquad (2)$$

where k is the factor number and cp is the replicate number of the central point [11]. Response surface methodology uses quantitative data in an experimental design to determine, and simultaneously solve multivariate equations, to optimize processes and products [12]. For the better accuracy, the second-order model is used. The general form for the second-order model is expressed as

$$Y = b0 + b1A + b2B + b3C + b12AB + b13AC + \cdots \qquad (3)$$

where Y is the response, bn is the coefficient associated with factor n, and the letters, A, B, C, ... represent the variables in the model.

2.5. Fourier transforms infrared (FTIR) spectroscopy

FTIR spectrum study was carried out to explain biosorption mechanism for identifying the presence of functionalities of the fungal biomass. The spectra were collected using PerkinElmer spectrum BX FTIR system (Beaconfield Buckinghamshire HP9 1QA) equipped with diffuse reflectance accessory with the range of 400–4000 cm^{-1}. To get the information specific to the group, and also on the interaction of the group with other parts of the molecule and on the spatial properties of the group by FTIR, the biosorption study for the metal ions were carried out holding temperature 30 °C, adsorbent loading 1.5 g/l and initial metal ions concentration 60 ppm at the central design point. pH the fourth most important parameter was adjusted according to the optimum point design by the model. The adsorption equilibrium experiments for FTIR study were carried out for 1 h at 120 rpm. The control pure biomass adsorbent was also run parallel in the distilled water at optimum pH. After equilibrium the metal loaded biomass was filtered through Watmaan filter paper and washed with double distilled water to remove the loosely bind ions or impurities. The metals loaded and pure biomass was dried at 50 °C in a heating oven. The samples were grounded in an agate pestle and mortar with KBr. The background obtained from KBr disc was automatically subtracted from the sample discs spectra prepared with KBr. All spectra were plotted using the same scale on the transmittance axis.

Table 2
Experimental design in term of coded factors and results of the Box–Behnken model.

	Temperature (°C)	Adsorbent dose (g/l)	pH	Initial metals ions (ppm)	Responses (% removal)		
					(Pb)	(Cd)	(Cu)
1	−1	−1	0	0	48.5	38.33	42.96
2	1	−1	0	0	50.9	35.4	31.7
3	−1	1	0	0	76.05	66.58	50.66
4	1	1	0	0	78.16	72.08	53.7
5	0	0	−1	−1	15.58	0.09	11.34
6	0	0	1	−1	54.07	64.75	56.3
7	0	0	−1	1	2.89	1.57	9.2
8	0	0	1	1	70.05	61.25	55.35
9	−1	0	0	−1	66.89	59.73	45.62
10	1	0	0	−1	73.12	60	59.2
11	−1	0	0	1	68.1	58.4	58.4
12	1	0	0	1	68.7	62.25	41.16
13	0	−1	−1	0	1.02	0.9	0.5
14	0	1	−1	0	10.6	17.16	7.3
15	0	−1	1	0	29.1	39.25	38.1
16	0	1	1	0	66.25	64.25	57.5
17	−1	0	−1	0	6.5	0.9	5.23
18	1	0	−1	0	7.98	1.56	11.5
19	−1	0	1	0	49.2	52.33	63
20	1	0	1	0	55.24	69.66	44.23
21	0	−1	0	−1	35.32	28.5	38.68
22	0	1	0	−1	61.36	76	55
23	0	−1	0	1	39.05	41.6	40.9
24	0	1	0	1	75.7	62.2	49.75
25[a]	0	0	0	0	66.28	63.41	44
26[a]	0	0	0	0	66.81	63.22	44.5
27[a]	0	0	0	0	66.19	62.98	43.58
28[a]	0	0	0	0	66.01	6245	44.89
29[a]	0	0	0	0	60.01	63.66	43.6

[a] Experiments carried out model central values.

3. Results and discussion

The results are obtained with the experimental design that was aimed at identifying the best levels of the selected variables, i.e. temperature (20–40 °C), adsorbent loading (0.5–2.5 g/l), pH (2–6) and initial metal ions concentration (20–100 ppm) (Table 2). The effects of temperature, adsorbent loading, pH and initial metal ions on the biosorption were studied. The second-order polynomial equation was used to find out the relationship between variables and response. The regression equation coefficients were calculated

and data was fitted to a second-order polynomial equation. The flat surface on the three-dimensional response indicates an optimum condition for the biosorption. The analysis of variance (ANOVA) for biosorption study of Pb(II), Cd(II) and Cu(II) ions with fungal biomass adsorbent was used in order to ensure a good model. The test for significance of regression model and the results of ANOVA are in Table 3. Prob > F less than 0.05 indicated model terms are significant. Non-significant value lack of fit shows the validity of the quadratic model for bisorption by *T. viride*. The predicted R^2 and adjusted R^2 values 0.8442 and 0.9432, 0.8494 and 0.9397, 0.9900

Table 3
Analysis of variance (ANOVA) for Pb(II), Cd(II) and Cu(II) ions removal.

Source	Sum of squares (Pb)	Sum of squares (Cd)	Sum of squares (Cu)	DF	Prob > F			
					(Pb)	(Cd)	(Cu)	
Model	16922.17	16802.34	9284.295	14	<0.0001	<0.0001	<0.0001	Significant
A	29.64163	50.75853	49.53203	1	0.3752	0.2628	<0.0001	
B	2247.624	2531.417	547.6954	1	<0.0001	<0.0001	<0.0001	
C	6502.57	9037.09	6048.479	1	<0.0001	<0.0001	<0.0001	
D	27.45187	0.27	10.79203	1	0.3929	0.9334	0.0101	
A2	36.54933	15.25708	48.21336	1	0.3264	0.5327	<0.0001	
B2	344.955	226.1711	35.81908	1	0.0075	0.0274	<0.0001	
C2	6950.26	4720.742	1609.037	1	<0.0001	<0.0001	<0.0001	
D2	0.023351	17.52415	126.6557	1	0.9799	0.5042	<0.0001	
AB	0.021025	17.76622	51.1225	1	0.9809	0.5013	<0.0001	
AC	5.1984	69.47222	156.7504	1	0.707	0.1938	<0.0001	
AD	7.924225	3.2041	237.4681	1	0.6431	0.7737	<0.0001	
BC	190.0262	19.0969	39.69	1	0.036	0.486	<0.0001	
BD	28.14303	180.9025	13.95023	1	0.3872	0.0449	0.0045	
CD	205.4922	6.2001	0.354025	1	0.0302	0.6896	0.599	
Residual	494.6207	521.9816	17.11925	14				
Lack of fit	462.3879	427.2502	15.80573	10	0.0534	0.2992	0.0717	Not significant
Pure error	32.2328	94.73132	1.31352	4				
Cor total	17416.79	17324.32	9301.414	28				

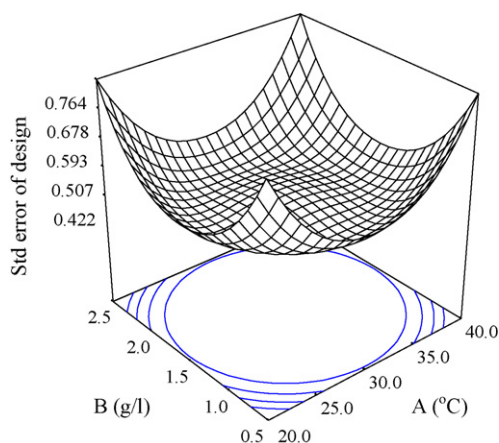$R^2 = 0.9716$ (Pb), $R^2 = 0.9699$ (Cd), $R^2 = 0.9982$ (Cu).

Fig. 1. Standard error design of the model adsorbent loading verses temperature holding pH and initial metals ions concentration at central value.

and 0.9963 are in reasonable agreement for Pb(II), Cd(II) and Cu(II) respectively, which are closer to 1.0, indicates the better fitness of model in the experimental data.

The minimum value of standard error design 0.4422 around the centroid and maximum prediction variance 0.583 at the design points also indicate that present model can be used to navigate the design space for the present study (Fig. 1). The residuals are used to check the homogeneous variance assumption by plotting the (studentized) residuals against the predicted probability values. Homogeneously spread data about either side of zero (line) indicated the suitability of the model for the present study (Fig. 2). The final responses for the removal of metal ions in terms of coded factors are in Eqs. (4)–(6):

$$
\begin{aligned}
\% \, \text{Removal (Pb)} = {} & +65.06 + 1.57 \times A + 13.6 \times B + 23.28 \times C \\
& + 1.51 \times D + 2.37 \times A^2 - 7.29 \times B^2 - 32.73 \times C^2 \\
& + 0.060 \times D^2 - 0.072 \times A \times B + 1.14 \times A \times C \\
& - 1.41 \times A \times D + 6.89 \times B \times C + 2.65 \times B \times D \\
& + 7.17 \times C \times D
\end{aligned} \tag{4}
$$

$$
\begin{aligned}
\% \, \text{Removal (Cd)} = {} & +61.14 + 2.06 \times A + 14.52 \times B + 27.44 \times C - 0.15 \\
& \times D - 1.53 \times A^2 - 5.90 \times B^2 - 26.98 \times C^2 - 1.64 \\
& \times D^2 + 2.11 \times A \times B + 4.17 \times A \times C + 0.89 \times A \\
& \times D + 2.18 \times B \times C - 6.72 \times B \times D - 1.24 \times C \times D
\end{aligned} \tag{5}
$$

$$
\begin{aligned}
\% \, \text{Removal (Cu)} = {} & +44.11 - 2.03 \times A + 6.76 \times B + 22.45 \times C - 0.95 \\
& \times D + 2.73 \times A^2 - 2.35 \times B^2 - 15.75 \times C^2 + 4.42 \\
& \times D^2 + 3.58 \times A \times B - 6.26 \times A \times C - 7.71 \times A \\
& \times D + 3.15 \times B \times C - 1.87 \times B \times D + 0.30 \times C \times D
\end{aligned} \tag{6}
$$

where A, B, C and D are the coded terms for temperature, adsorbent loading dose, pH and initial metal ions concentration.
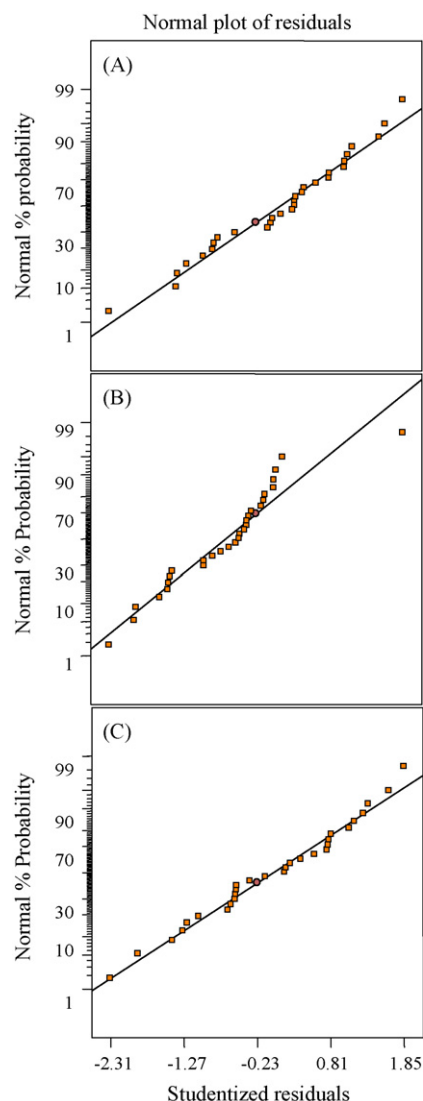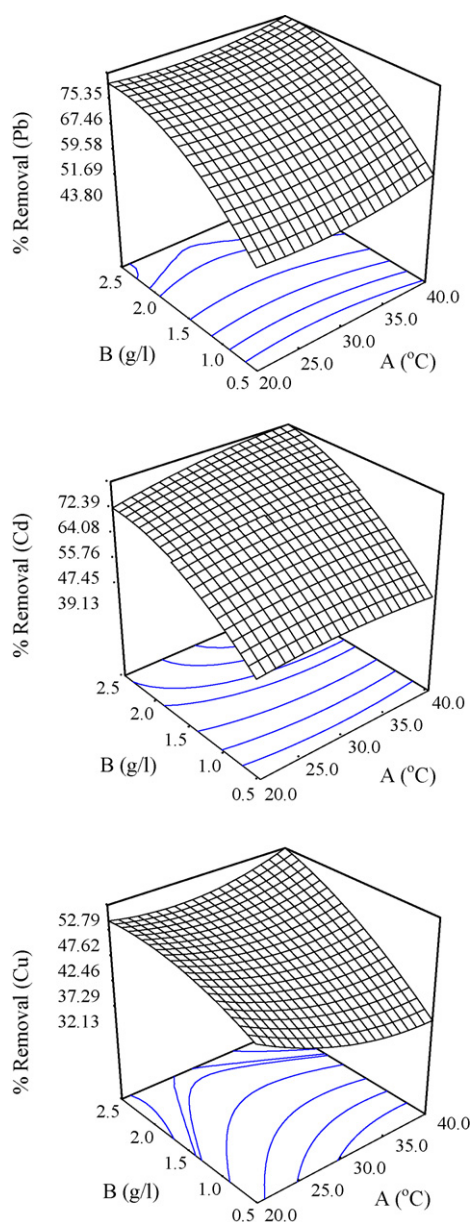

Fig. 2. Normal plot of studentized residuals verses normal % probability for bearing the experiment for Pb (A) ions, Cd(II) ions (B) and Cu(II) ions (C).

3.1. Interactive effects of two variables

The sensitivity of the response to the two interacting variables can be included by the three-dimensional graphs by holding the other variable at the central values. On the basis of quadratic polynomial Eqs. (4)–(6) of the response surface methodology, the effect of interacting variables: temperature (20–40 °C), adsorbent loading (0.5–2.5 g/l), pH (2–6) and initial metal ions concentration (20–100 ppm) on the biosorption of Pb(II), Cd(II) and Cu(II) were analyzed. The large value for pH in the linear coefficient terms (Eqs. (4)–(6)) illustrates the significant, positive effect of the variable on the biosorption. The positive linear coefficient indicates that biosorption increased with increasing the variable. Conversely, the negative quadratic coefficient for pH was evaluated since it affects the number of cellular surface sites available to bind cations, as well as metal speciation. The optimal settings of adsorbent loading weight–temperature surface could examine from Fig. 3. The differences in the initial biosorption rate of metal ions may be due to the nature and distribution of active groups on the adsorbent and the affinity between the metal ions and the adsorbents [13–15]. The removal of the metals ions increases with increase in the adsorbent loading for all the metals ions. The effect of adsorbent loading weight on the removal of metals ions by the adsorbent loading was

Fig. 3. Three-dimensional plots showing effects of adsorbent loading and temperature the independent variables on the % removal of metals ions holding another variable at central values.

This is due to the greater availability of the exchangeable sites or surface area at higher loading dose of the adsorbent. These results are in agreement with previous studies on many other adsorbents [16,17]. The amount of protons exchange between the adsorbent and the metal solutions increased with increasing biomass concentration [17]. Whereas the uptake capacity of the adsorbent was decreased from 5820, 4599.6 and 5155.2 mg/g to 1825.2, 1597.92 and 1215.84 mg/g for Pb(II), Cd(II) and Cu(II), respectively. It is interesting to compare the sorption uptake of metal ions towards the same adsorbent. The increasing temperature clearly showed the increased in the adsorption of Pb(II) and Cd(II), whereas decreasing trends for Cu(II) was observed. The non-significant value of interaction coefficient for Pb(II) and Cd(II) clearly indicates the increasing effects of temperature on the removal of metal ions (Fig. 3). The significant value $P > F$ for Cu(II) indicates that it is the most affected metals ions during bisorption with change in temperature (Table 3). At very low pH values (with a high proton concentration) cell wall ligands would be closely associated to H_3O^+, and access of metal ions to ligands would be restricted as a result of repulsive forces [18]. As pH levels increase, more ligands with negative charge would be exposed with the subsequent increase in attraction sites to positively charged metal ions [19]. Increased temperatures of metal solutions may alter the physical/chemical structure of the biosorbents, which could either increase or decrease the number of metal adsorption sites [20]. The decrease of the biosorption at increased temperature indicated that the biosorption of copper(II) ions is exothermic in nature. The adsorption isotherms kinetics also indicated that the Cu(II) biosorption at higher temperature has negative ΔH° value (Table 4). The decrease in adsorption with the rise of temperature may be due to the weakening of adsorptive forces between the active sites of the adsorbents and adsorbate species and also between the adjacent molecules of the adsorbed phase [21]. The exothermic nature of metal ion adsorption/biosorption has also been reported previously for adsorptive removal of copper(II) ions by sugar beet pulp [22] and activated rubber wood sawdust [23].
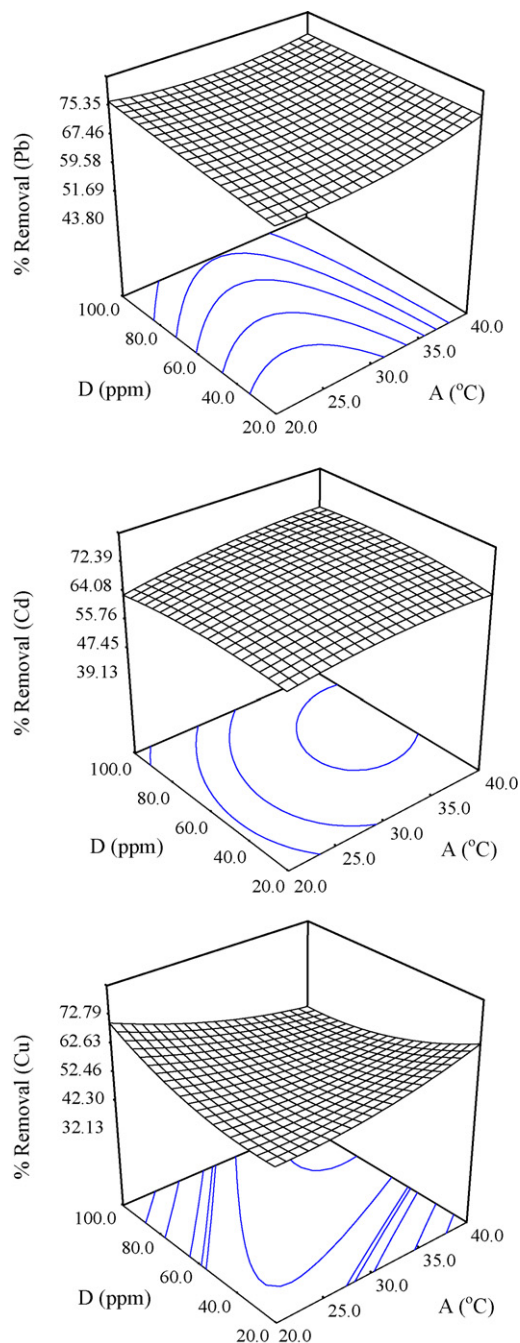
The interactive effect of initial pH and temperature are the most important environmental parameters influencing the biosorption of the heavy metal ions. Influence of pH the most dominant parameter on the biosorption capacity for the different metals is shown in Fig. 4. pH affects the isoelectric point of the biosorbent, at isoelectric points electrostatic repulsion between adsorbed molecules is at a minimum and molecules have a higher structural stability, and therefore a smaller tendency to spread at the interface resulting maximum adsorption at isoelectric point [24]. In this study, metals cations biosorption is maximum around initial pH 5.0, would be expected to interact more strongly with negatively charged binding sites on the biosorbent. The metals ions biosorption capacity of biosorbent increased with increasing initial pH upto 5.0 and then decreased with the increase in initial pH at 20 °C temperature. The linear significant value of pH also indicates that it is the most important parameter. The significant value for the quadratic term of all the metal ions indicates the existence of the maximum

varied from 0.5 to 2.5 g/l of metal ions solution containing 60 mg/l and 4 pH of each of the investigated metals. It can easily be inferred that the percent removal of metal ions increases 48.5%, 38.33% and 42.96% to 76.05%, 66.58% and 50.66% with increasing weight of adsorbent from 0.5 to 2.5 g/l for Pb(II), Cd(II) and Cu(II), respectively.

Table 4
The thermodynamic constants obtained for the metal ions during adsorption process.

Metal ions	Temperature (°C)	$-\Delta G^\circ$ (kJ/mol)	ΔH° (kJ/mol)	ΔE° (kJ/mol)	ΔS° (kJ/mol)
Pb(II)	20	9.43	+29.46	2.28	+38.89
	30	9.84		2.45	
	40	10.11		2.45	
Cd(II)	20	9.25	+90.61	2.26	+99.88
	30	9.76		2.42	
	40	10.41		2.55	
Cu(II)	20	0.442	+122.78	2.03	+123.23
	30	4.92		2.25	
	40	1.68		2.29	

Fig. 4. Three-dimensional plots showing effects of temperature and pH variables on the % removal of metal ions holding another variable at central values.

within the assay range and this maximum was in the vicinity of pH 5–6. Cu(II) uptake increased with pH up to 5–6; beyond these pH values no improvement in the sorption capacity was observed (Fig. 4).

Fig. 5 shows the effect of initial metal ions and temperature on Pb(II), Cd(II) and Cu(II) biosorption capacity of biosorbent holding other variables at their central values. The biosorption capacity of biosorbent increased with the increase in initial metals ions concentration at the initial low temperature. At higher metal ions concentration uptake of the ions did not further increases with increase initial metal ions concentration resulting the saturation of biosorbent surface. The uptake of copper(II) ions increases with increase in the temperature at the low Cu(II) ions concentration. This is perhaps due to the availability of large number of active site available for the biosorption and not much affected by the exothermic nature of the process. The removal decreases at higher metals ions due to the competing of the ions for the available binding site [25]. Very less uptake of Cu(II) ions at high temperature and high metals ions concentration is due the dominance of both exothermic nature as well as competing ions.
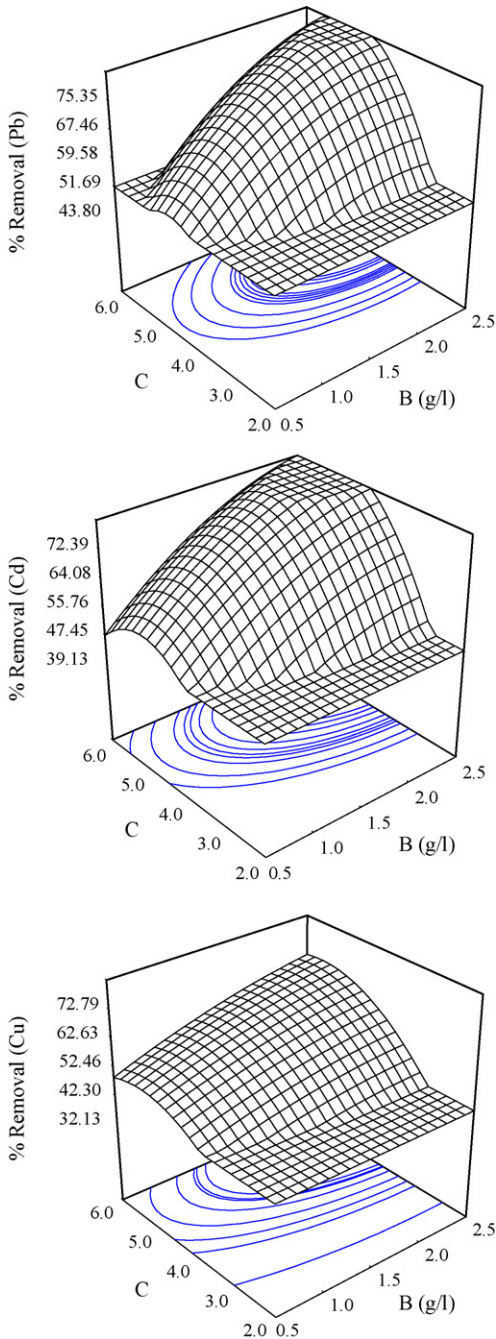

Fig. 5. Three-dimensional plots showing effects of initial metal ions concentration and temperature on the % removal of metal ions keeping pH 4 and adsorbent loading 1.5 g/l.

Three-dimensional response plots show the interactive effect of pH and the adsorbent loading dose (Fig. 6). The biosorption capacity was decreased from 5568, 2765.2 and 1907.76 mg/g for the Pb(II) ions, 5606.4, 2712 and 1872.48 mg/g for Cd(II) ions and 4848.0, 2056.0 and 1378.8 mg/g for the Cu(II) ions with increasing the biosorbent concentration at 0.5, 1.5 and 2.5, respectively. The uptake capacity was calculated at pH 5.0 and initial metal ions concentration 60 ppm. At higher adsorbent loading dose the uptake capacity is low, perhaps due to the unsaturation of biosorption sites through the biosorption reaction and due to the particle interaction, such as aggregation, resulted from high biosorbent concentration [24]. Such aggregation would be lead to decrease in total surface
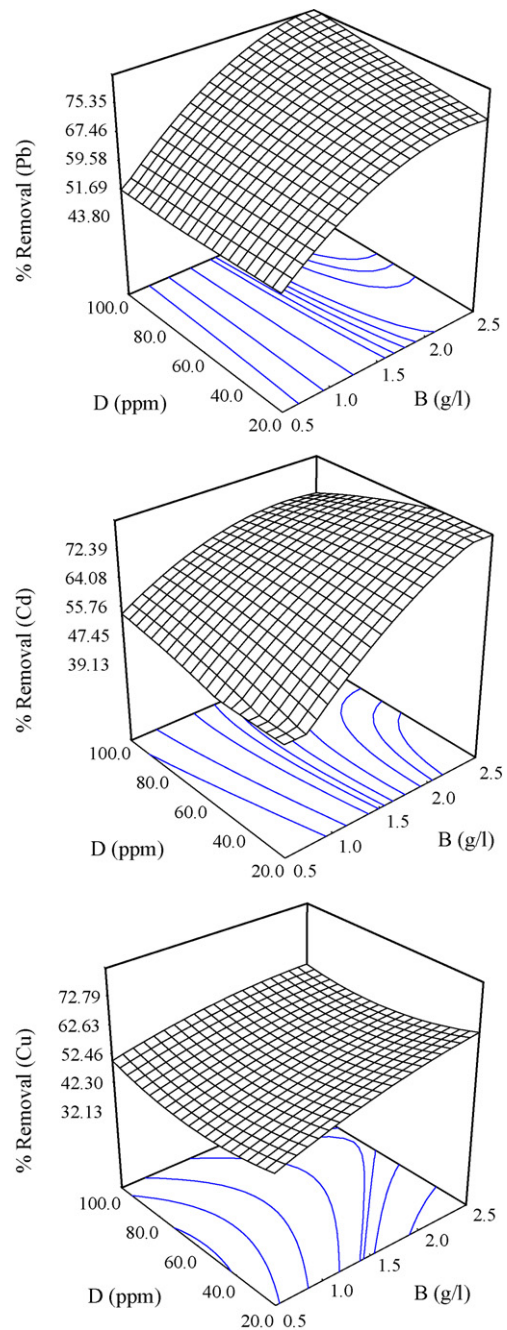
area of the biosorbent and an increase in diffusional path length [26].

The interactive effects of the adsorbent and initial metal ions concentration can be inferred from the response plot Fig. 7, holding the temperature at 30 °C and pH 4. Difference in biosorption of metal ions Pb(II), Cd(II) and Cu(II) ions towards same biosorbent may be due to the properties of metal sorbets (e.g. atomic weight, ionic size or reduction potential of metal) and due to different functional groups [27].

The three-dimensional response surface plots indicated the interactive effects of the initial metals ions concentration and pH (Fig. 8). The widely spaced isoline (Contour) along the pH indicated the dominance over the initial metals ions concentration.

Fig. 7. Three-dimensional plots showing effect of initial metal concentration and adsorbent loading rate on the % removal of metals keeping pH 4 and temperature 30 °C.

The response plots show that the % removal of the metal ions firstly increased with increase the metal ions concentration and then decreased with increased in the metal ions concentration. The decrease in the percentage removal of metals ions can be attributed with the fact that all the adsorbents had a limited number of active sites, which would have become saturated above a certain initial metal ions concentration.

3.2. Interactive effects of four variables

To study the interactive effects of the four factors at a time the perturbation graphs were used (Fig. 9). The perturbation plot of ions binding ability against all four investigated variables implies

Fig. 6. Three-dimensional plots showing effect of pH and adsorbent loading on the % removal of metal ions keeping initial metal ions concentration 60 ppm and temperature 30 °C.
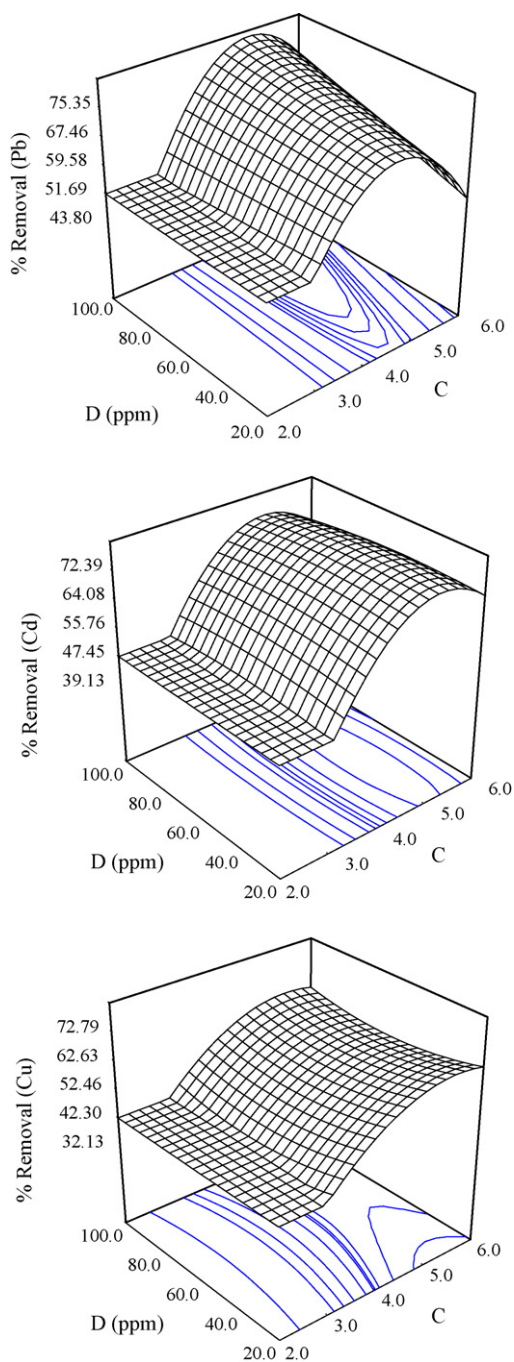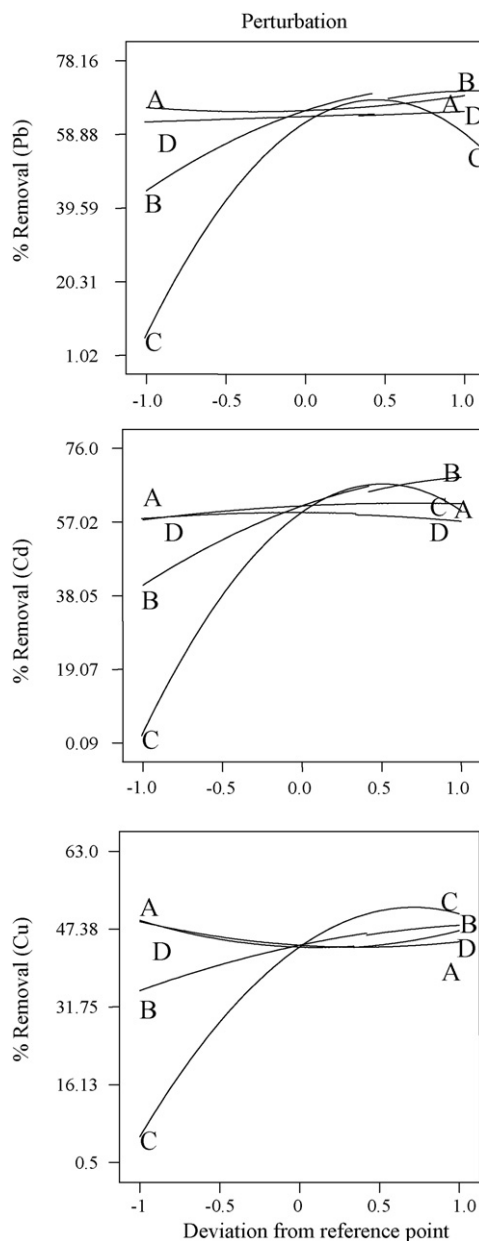
Fig. 8. Three-dimensional plots showing effect of initial metal ions concentration and pH on the % removal metals ions keeping the pH 4 and adsorbent loading rate 1.5 g/l.

Fig. 9. Overlay plot of perturbation of all the variables.

the contribution of each factor to the biosorption process. The perturbation plot illustrates percent metals ions removal as each variable moves from the preferred reference with all other factors held constant at the middle of the design space (the coded zero level). The perturbation plots show the deviation of the factorial level from the adjusted reference point of all the variables. The flat curves for temperature (*A*) and initial metal ions (*D*) indicate that the biosorption changes less due to these variables than adsorbent dose (*B*) and pH (*C*) (Fig. 9). Metal ions levels removal showed the order of Pb > Cd > Cu between pH 2 and 6. The similar trends for metals ions Pb > Cd > Cu biosorption was also obtained by [28].

3.3. Adsorption isotherm model analysis

In order to optimize the biosorption process parameters, both Langmuir and Freundlich isotherms models were tested. The Langmuir model is described in equation [29,30]:

$$\frac{Ce}{qe} = \frac{1}{bQm} + \frac{Ce}{Qm} \tag{7}$$

where qe adsorbent metal ion quantity per gram of biomass at equilibrium (mg/g), Qm is the maximum amount of metal ion per unit weight of biomass to form a complete monolayer on the surface bound (mg/g) and b a constant related to the affinity of the binding sites (l/mg). Qm and b are calculated from the slope and intercept of the straight lines of plot Ce/qe versus Ce. Freundlich model equation is of the form [29,30]:

$$qe = kC^{1/n} \tag{8}$$

Table 5
Adsorption isotherm parameters for Pb(II), Cd(II) and Cu(II) biosorption on to biomass of *Trichoderma viride*.

Metal ions	Temperature (°C)	Langmuir constant			Frendlinch constant		
		Qm (mg/g)	b (l/g)	R^2	k	n	R^2
Pb(II)	20	0.103	0.236	0.968	48.23	5.263	0.996
	30	0.101	0.242	0.956	49.74	4.878	0.995
	40	0.101	0.256	0.950	48.61	4.347	0.990
Cd(II)	20	0.139	0.180	0.890	44.67	7.407	0.964
	30	0.106	0.234	0.975	48.23	5.263	0.996
	40	0.094	0.258	0.965	54.53	4.167	0.999
Cu(II)	20	0.360	0.091	0.918	1.51	0.103	0.968
	30	0.196	0.126	0.973	7.09	0.101	0.956
	40	0.141	0.155	0.986	1.91	0.102	0.950

where k and n are the Freundlich's constants related to the adsorption capacity and adsorption intensity of the adsorbent characteristics of the system [31]. k and n can be determined from the linear plot of log qe versus log Ce.

The results of the adsorption isotherms at different temperature for the metal ions are presented in Tables 4 and 5. The correlation coefficients values R^2 shows that adsorption isotherms model well fitted to the experimental data. The data fitted indicated the homogeneous distribution of active site on to the adsorbent surface, since Langmuir equation assumes that the surface is homogeneous. The constant b is related to the affinity of the binding sites, which allows us to make a comparison of the affinity of the biomass towards the metal ions. The increasing values of b for the metal ions indicated the increase in the adsorption with increasing temperature. The favorable sorption is indicating by the higher than 1 values of Freundlich sorption constant n for biosorbent. The value of n falling in the range of 1–10 indicates favourable biosorption Aksu and Kutsal [32]. The small K values for Cu(II) indicate a lower extent sorption, while more sorption was observed for Pb(II) ions followed by Cd(II) because of their larger K values. The correlation coefficients obtained from the linear plots, Freundlich equation ($R^2 = 0.996$) fitted better than Langmuir equation ($R^2 = 0.968$) at temperature 20 °C (Table 5). The better fit of equilibrium data to the Freundlich equation refers the multilayer sorption of all the metals ions on the biosorbent [33]. The values of n obtained greater than one for Pb(II) and Cd(II) indicated that physical adsorption, multilayer adsorption and S type isotherms takes place for both the ions. However the value of the n for less than 1 was observed for Cu(II) ions. Optimum temperature observed was 30 °C for Pb(II) and Cu(II), which can be inferred from the more value of k from the Frendlinch equation. The favourable observed for Cd(II) was 40 °C as indicated from the more value of k (54.53).

In order to determine the mean adsorption energy (E) that may provide useful information with regard to whether or not biosorption is subject to a chemical or physical process, Dubinin and Radushkevich [34] isotherm was used in accounting for the effect of the porous structure of an adsorbent:

$$qe = Q_{DR} \exp(-K_{DR} \varepsilon^2) \tag{9}$$

where Q_{DR} is the Dubinin–Radushkevich constant representing the theoretical monolayer saturation capacity [35], K_{DR} is the constant of adsorption energy which is related to mean adsorption energy (E) through $E = 1/\sqrt{2K_{DR}}$, and ε is the Polany potential. The value of the K_{DR} can be found from plot of $\ln Qe$ versus $\ln^2 (1 + (1/Ce))$ and its slope is given by

$$Slope = -K_{DR} R^2 T^2 \tag{10}$$

The mean adsorption energy (E) can be calculated as follows:

$$E = \frac{1}{\sqrt{2K_{DR}}} = \frac{RT}{\sqrt{-2 \times Slope}} \tag{11}$$

In general, the Dubinin–Radushkevich isotherm equation has been widely used to determine the mean adsorption energy (E) in micrporous material that may provide useful information with regard to weather or not biosorption is subjected to chemical or physical process. The results of thermodynamic parameters are presented in Table 4. The calculated E values were smaller than 8 kJ/mol for all the metals ions in the assay ranged indicated the physical adsorption. These results are confirming with the previous result which claims physical adsorption as type of adsorption. The values of $\Delta G°$ become more negative with increasing temperature for Pb(II) and Cd(II) ions, which indicates that the adsorption process is more favorable at high temperature. The value $\Delta G°$ become more negative by Cu(II) ions at the intermediate temperature the adsorption process is more favourable at the intermediate temperature (Table 4). This suggests that sorption process is not spontaneous and as the temperature increases $\Delta G°$ values get lower. The positive values $\Delta H°$ indicates the endothermic nature of the adsorption process. In addition, the small values of $\Delta H°$ are not compatible with the formation of strong chemical bonds hence indicated the physiosorption adsorption. The low value of $\Delta S°$ implied that there was no remarkable change in the entropy as result of biosorption for Pb(II). More positive value of entropy change by the Cu(II) ions implies increased randomness at the solid–solution interface during the sorption compared to the other metals ions. The distribution of Cu(II) ions in solution was obviously more disordered compared to lead ions bound to the biosorbent surface and this resulted in net increase in entropy.

3.4. Characterization of biosorbent using Fourier transforms infrared (FTIR) spectroscopy

The FTIR difference spectra in pure biomass adsorbent were compared to the spectra obtained in metals ions loaded biomass to determine if differences due to interaction of the metals ions with functional groups (Fig. 10). The spectral changes in 412 cm^{-1} spectral region include band that appears in Pb(II) loaded biomass. This change seems logical due to Pb–OH formation and due to increase in the frequency of two O–H vibrations. Mg–OH Infra Red transition modes are found at 412 cm^{-1} equivalent to Raman band [36]. This is perhaps due to Pb(II) soft acid as compared to Cd(II) and Cu(II) and combine with soft base present on the adsorbent (–OH). The two stretching appearing in the 596.6 and 629 in the multi-metals ions are due intra-competition among the ions towards function groups but in case of single metal ions there is very poor stretching. The symmetric stretching at 1043.88 cm^{-1} indicates the presence of C–O–C of ester, there are significant difference in the characteristic absorption bands differ from each others especially at the fingerprint region by the metal ions and Pb(II) causes less stretching compared to other metal ions which shift towards the lower frequency. The bands at 1207.53 cm^{-1} is attributed to the symmet-
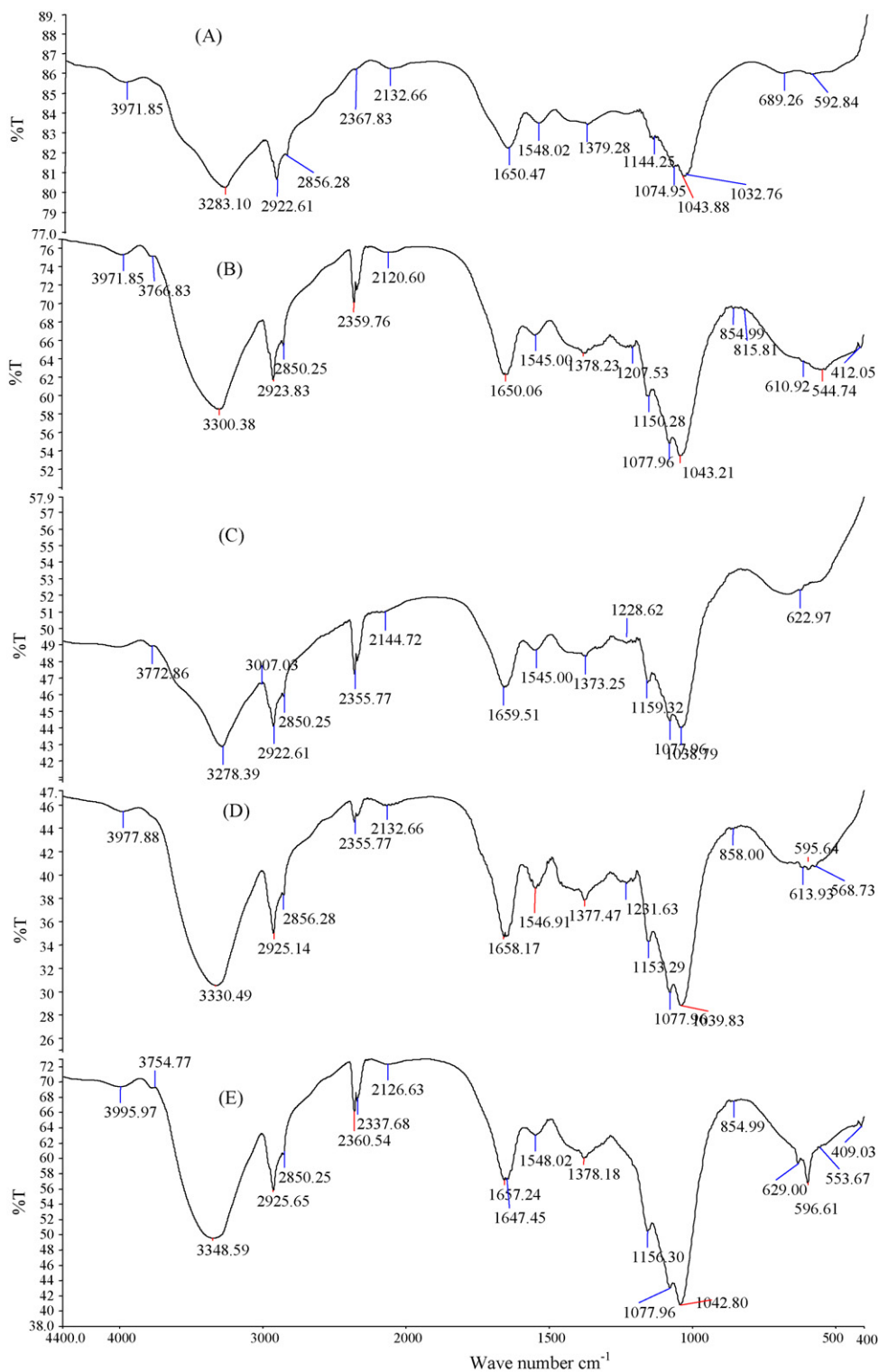
Fig. 10. FTIR Spectra of pure biomass (A) and metals ions loaded biomass Pb(II) (B), Cd(II) (C), Cu(II) (D) and mix metal ions (E) by *T. viride*.

ric stretching of the $-SO_2$ group [37–41]. The appearance of peak at 1377.47 by Cu(II) indicated involvement of $-CH_3$ group towards Cu(II) than other Pb and Cd ions. The small bending vibration at 1548 cm^{-1} in the unloaded biomass due to amide N–H functional group, the unsymmetrical stretching by copper loaded biomass indicated the complexation of the Cu(II) metal ions towards the N–H group in the biomass. The disappearance of the stretching in the multi-metals ions at 1548 indicated the loosely binding

of the copper ions which are easily hindered by the Cd(II) and Pb(II) ions. An interesting phenomenon appearance of stretching related to the vibration 2359.76 by Pb(II), 2355.77 by Cd(II) and Cu(II) indicated the involvement of N=C=O group. A stretching peak of 2367 cm^{-1} assigned to the asymmetric stretching of the isocyanate group (–N=C=O) [42]. There are strong stretching shift to low frequency by Pb(II) and Cd(II) as a result of strong attraction but stretching by Cu(II) is poor indicating less attraction by

the functional group. Simple C–H stretching vibration for saturated aliphatic species was observed at 2856.28 and 2923.83 cm^{-1} by the biomass for all the metals ions indicating the involvement of the methyl and methylene functionalities, respectively, in the adsorption. The stretching at 2108 cm^{-1} frequency for the copper loaded biomass attribute as a function of triple bond (C≡C). The weak umbrella type symmetrical stretching appears for all the metals ions which is at 1378–1377 cm^{-1} due to the –CH$_3$. The stretching due to the copper ions are more pronounced than other metal ions indicating the more attraction to copper ions and stretching is very less in Cd(II) metal ions loaded biomass due to less attraction of this ion. The peaks at 2924.66 and 2856.28 cm^{-1} can be assigned to asymmetric and symmetric CH$_2$ groups and there is small sift in the peaks due to absorption of all the metal ions. The presence of small broad bending at 2120 cm^{-1} is due to C≡C structure.

The broad absorption peaks at around 3200–3500 cm^{-1} indicates the presence of carboxylic acid and amino groups. The stretching vibration band 1658 cm^{-1} is due to carbonyl group (CO). The absorption peak at 1548 cm^{-1} also supported presence of amino group. The band at 1075 cm^{-1} shows C–O stretching vibration. Finally, it should be noted that peaks in the region of lower wave numbers (under 800 cm^{-1}) appeared as a broad peak and this could be attributed to an interaction between lead(II) ions and N containing bioligands [43,44]. The stretching intensities of the metal loaded biomass are significantly higher than then metals unloaded biomass.

4. Conclusions

This study inferred that biosorption has been found to be spontaneous and relatively fast process. The biosorption capacity of the metals ions decreases with increasing adsorbent loading dose. Optimum pH for the biosorption of Pb(II), Cd(II) and Cu(II) are in the vicinity of 5, where maximum biosorption takes place. The response surface methodology proves to be an important tool for process optimization. Langmuir and Frendlich Isotherms, thermodynamics and FTIR analysis of the biosorbent of *T. viride* indicated that biosorbent proves to be economical and cost effective biosorbents to remove metals ions at their initial low concentrations.

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