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Exploring single and multi-metal biosorption by immobilized spent *Tricholoma lobayense* using multi-step response surface methodology

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ABSTRACT

A multi-step response surface methodology was successfully applied to interpret the adsorption characteristics of lead, cadmium and copper ions onto immobilized spent *Tricholoma lobayense* (ISTL) in single and ternary systems in this study. Firstly, the most significant medium factors, which were pH, biomass loading and contact time, on biosorption of three metals, were determined by Minimum Run Res V Design. Then central composite design followed by mixture design was utilized to identify the affinity and predict the adsorption capacities of three metals onto the biosorbent. The results showed that the preference of ISTL for three metals was in the order of Pb(II) > Cu(II) > Cd(II) and lead ions could still be effectively removed from aqueous solution in the presence of both cadmium and copper while removal of the cadmium and copper ions would be suppressed by lead. Sorption isotherm data were better represented by Freundlich model. The both adsorption and desorption process of three metals followed the pseudo-second-order kinetics model. Scanning electron microscope (SEM) and energy dispersive spectrometry (EDS) analysis testified the obvious change of the surface morphology and the presence of three metals on the biosorbent after metal binding. The ISTL could be regenerated with 1 M HNO_3, which allowed the reuse of the biomass in three biosorption–desorption cycles without considerable loss of adsorption capacity. The present work suggests that ISTL as an abundant low-cost biomaterial was an efficient biosorbent for heavy metals removal from wastewater.

1. Introduction

Heavy metals, discharged into the environment through a variety of natural and anthropogenic pathways, are of toxicity, non-biodegradability and bioaccumulation. Especially for aquatic environments, with the rapid development of various industries such as metal plating, electroplating, mining, ceramic, batteries, pigment manufacturing, leather, photography, automobile, refrigeration industries, the industrial effluents containing heavy metals drain into the river leading to various disorders and diseases in the ecosystem [1,2]. Usually lead, cadmium and copper are the important metals found in industrial wastewater and they are toxic even at low concentration. World Health Organization (WHO) recommended maximum 0.01, 0.003 and 2 mg/L as acceptable concentrations of lead, cadmium and copper, respectively, in drinking water [3].

The technologies for the removal of heavy metals from wastewaters include chemical precipitation, flocculation, membrane filtration, solvent extraction, ion exchange and adsorption. In these methods, biosorption with low-cost materials (industrial or agricultural residues) has been found to be superior to other techniques in virtue of the low-priced cost, high efficiency, easiness of operation, regeneration of biosorbent and possibility of metal recovery [4–6]. Therefore, the wastes from industry and agriculture such as papaya wood [7], grape stalk [8], rice husks [9] and some other mushrooms such as *Agaricus bisporus* [10,11], *Agaricus macrosporus* [12], *Lentinus edodes* [13], *Amanita rubescens* [14] have been successfully employed as biosorbents for removing toxic metals recently. In this study, spent *Tricholoma lobayense*, by-product of mushroom, was first used to remove heavy metals from artificial wastewater. Moreover, mushrooms are widely grown in Sichuan, China and the spent mushroom has no commercial value and will exert environmental burden as a result of decay. Therefore, it is a low-cost, eco-friendly and readily available biosorbent. The immobilization of biomass may also provide several advantages such as minimal clogging in continuous systems [15,16], facility to reuse and separation of solid biomass from the bulk liquid for a few cycles [17,18]. Natural polymers such as alginate, chitosan, chitin and cellulose derivatives are usually used as immobilization matrices as they are non-toxic, inexpensive and efficient [17–19]. In these polymers, alginate has been extensively used for immobilization to removing heavy metals from aqueous solutions, developing

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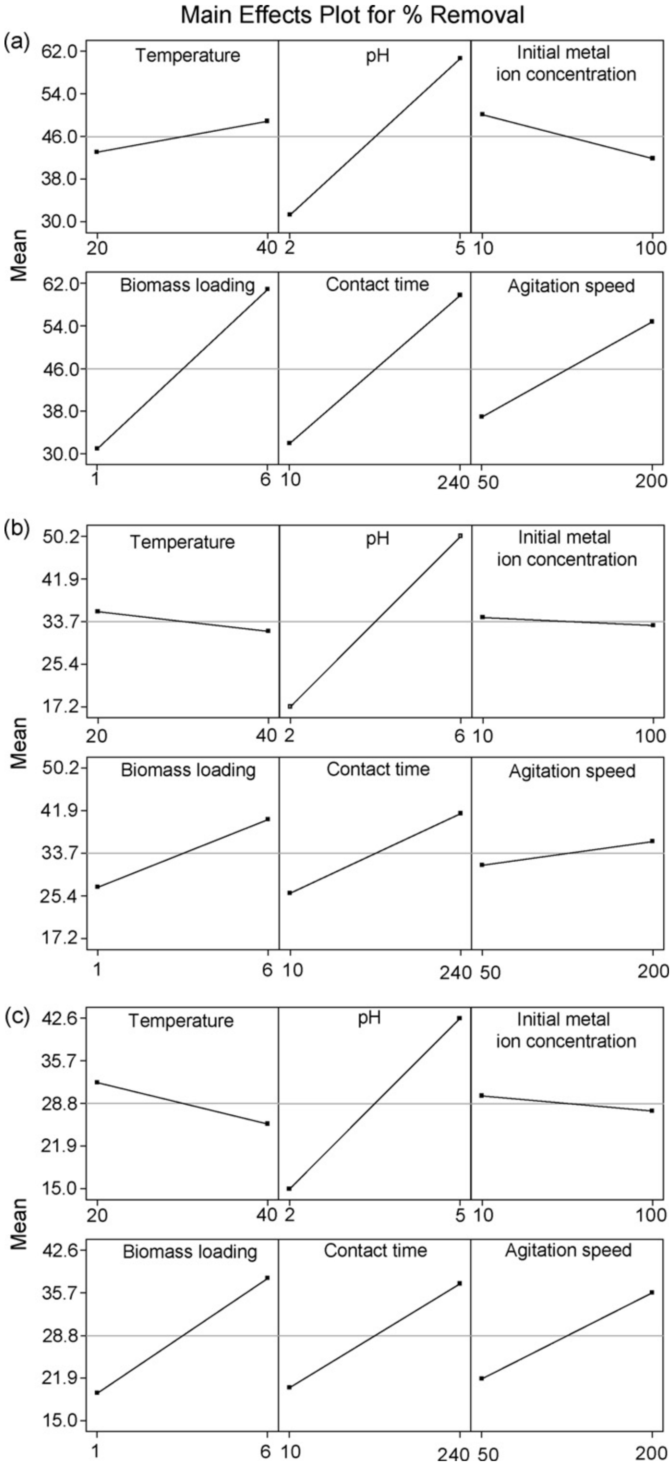

Fig. 1. Main effects plot for (a) removal of Pb(II), (b) removal of Cd(II) and (c) removal of Cu(II) by immobilized spent *T. lobayense*.

a new approach to physicochemical technologies for wastewater treatment [15,18].

In recent years, biosorption studies have more focused on single metal system than multi-metal system despite the fact that industrial effluents often contain several metal ions concomitantly [20–23]. When more than one metal is present, the studies become very complicated since the interaction of one metal sorption from other metal ions may be synergistic, antagonistic or non-interactive. The traditional one-factor-at-a-time experiments cannot successfully predict possible interactions between the metal ions in aquatic systems. Thus, it is necessary to develop a new method for exploring multi-metal sorption.

Three experimental design methodologies, factorial design, response surface design (RSM) and mixture design were used to interpret the characteristics of three metals removal from single and multi-metal aqueous solution by immobilized spent *T. lobayense* (ISTL). Factorial design was useful for screening many factors to find the significant few and estimating main effect and interactions [24,25]. RSM was used to predict the biosorption results under different metal compositions [26], instead of finding the optimal combination of different variables which impacted heavy metals removal from wastewater in previous studies [6,27,28]. Two mixture designs, standing for high and low overall metal concentrations with the same metal components, were employed to evaluate the effect of total metal concentration on biosorption capacity. Furthermore, through these designs the number of experiments can be markedly reduced.

The purpose of this study was to investigate the adsorption characteristics of Pb(II), Cd(II) and Cu(II) onto immobilized spent *T. lobayense* (ISTL) in single and multi-metal systems, respectively. The main objectives were (1) to apply a six factor, two-level factorial design and quadratic equations for finding the most significant effect, (2) to estimate the affinity of three heavy metals and to predict the adsorption capacity onto the biosorbent in multi-metal system with RSM and mixture designs, (3) to provide a quantitative description and prediction of the metal uptake behavior of ISTL using the isotherms and kinetics models, (4) to reveal the changes in morphology and also to verify the presence of heavy metals onto ISTL after biosorption by scanning electron microscope (SEM) and energy dispersive spectrum (EDS) analysis, respectively, and finally (5) to evaluate elution-reuse of the biosorbent in consecutive adsorption–desorption cycles and to represent reaction pathways and rates by desorption kinetics models.

2. Materials and methods

2.1. Preparation of the biosorbent

Fresh spent *T. lobayense* was obtained from a mushroom growing area in the suburbs of Chengdu, Sichuan, China. It was washed with deionized water, dried in an oven at 50 °C, ground with a pulverizing mill, and sieved with a 200-mesh to a size < 0.075 mm.

2.2. Immobilization of spent T. lobayense into Ca-alginate

A 2% sodium alginate solution with spent *T. lobayense* (sodium alginate: spent *T. lobayense* = 1:1) was dropped into a cooled 0.5 M $CaCl_2$ with the help of a syringe and the solution was stirred slowly during this process [15,29,30]. After overnight hardening in the same solution at 4 °C, the resulting beads were collected by filtration and washed with deionized water for removing excess $CaCl_2$. Finally, the beads were dried at room temperature to obtain the xerogels which were approximately 2 mm for the following experiment.

2.3. Preparation of metal stock solution

The heavy-metal adsorbates used in this study were lead ($Pb(NO_3)_2$), copper ($Cu(NO_3)_2 \cdot 3H_2O$) and cadmium ($Cd(NO_3)_2 \cdot 4H_2O$) which were analytical grades and their main characteristics are summarized in Table 1. 1000 mg/L of stock solutions were prepared by dissolving above-mentioned metal salts in one percent HNO_3 solution. The required working solutions of heavy metals for the adsorption experiments were obtained by diluting each stock solution. Heavy-metal ions in solutions were

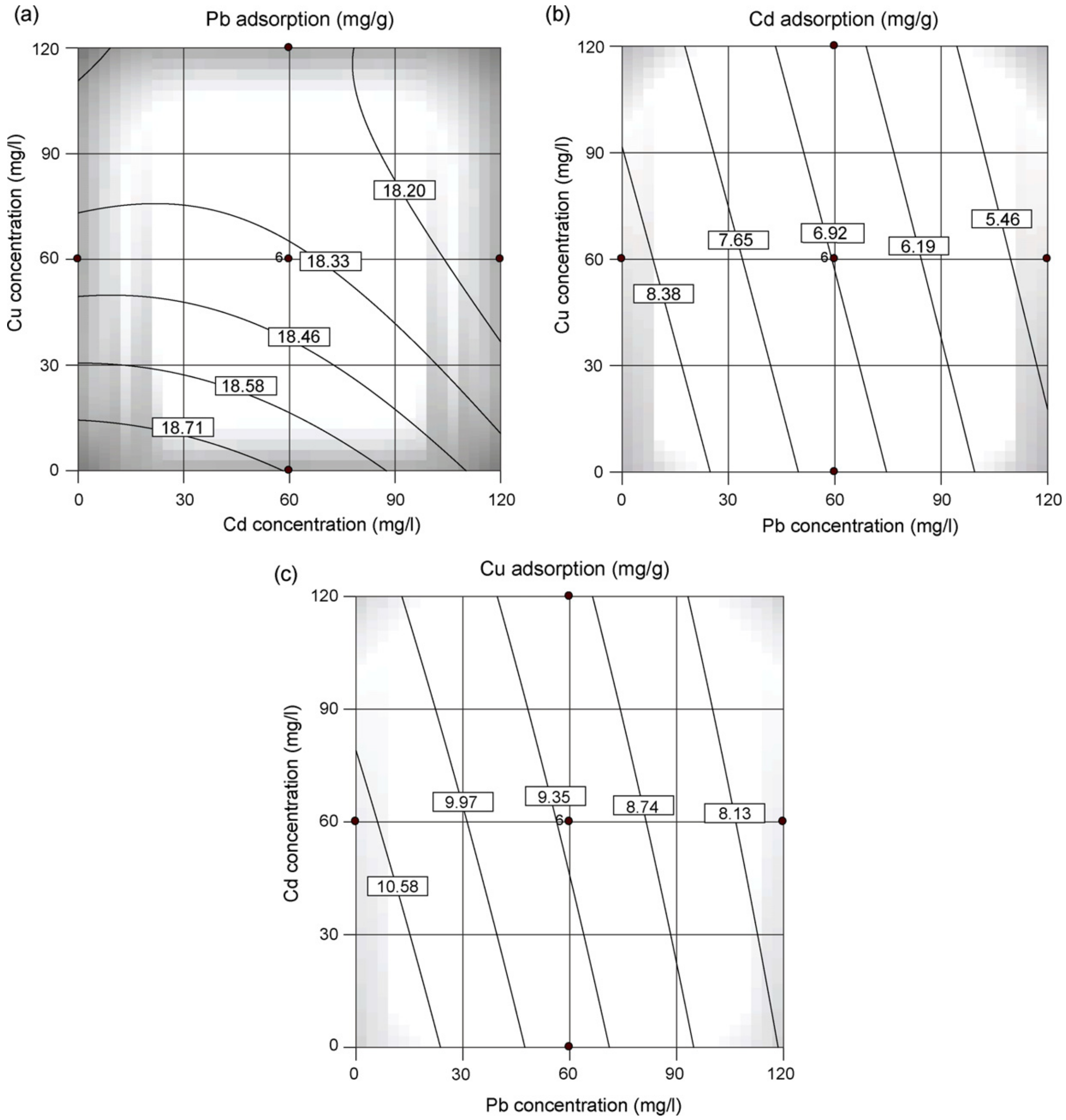

Fig. 2. Response surface contour plots for the competitive biosorption of (a) Pb(II), (b) Cd(II) and (c) Cu(II) onto immobilized spent *T. lobayense* in ternary system (total metal concentration 120 mg/L) holding another variable at central values. Temperature = 30 °C, pH = 5, biomass loading = 2 g/L, agitation speed = 200 rpm, sorption time = 9 h.

Table 1
Physicochemical properties of the metals tested.

Property	Pb	Cd	Cu
Formula	Pb(II) from $Pb(NO_3)_2$	Cd(II) from $Cd(NO_3)_2$	Cu(II) from $Cu(NO_3)_2$
Atomic weight	207.2	112.41	63.54
Charge	2	2	2
Number of coordination	4,6	4	4
Electronic configuration	$[Xe]4f^{14}5d^{10}6s^{2}6p^{2}$	$[Kr]4d^{10}5s^{2}$	$[Ar]3d^{10}4s^{1}$
Ionic radii	1.19 Å	0.97 Å	0.72 Å
Electronegativity	2.33	1.69	1.90

analyzed by flame Atomic Absorption Spectrometer (AAS; VARIAN, SpectrAA-220Fs).

2.4. Batch biosorption experiments

Each experiment was carried out in 150 mL erlenmeyer flask containing 50 mL heavy-metal solution whose concentration was set according to experimental design methodology. The initial pH of the solution was adjusted to the required value by adding HNO_3 (0.1 M, 1 M) and NaOH (0.1 M, 1 M) solutions.

The removal rate and amount of adsorbed metal ions (Q_e) per gram of ISTL were calculated as follows:

$$\text{removal rate } (\%) = 100 \times \frac{(C_i - C_e)}{C_i} \tag{1}$$

$$\text{adsorption amount (mg/g)}: \quad Q_e = \frac{(C_i - C_e)V}{M} \tag{2}$$

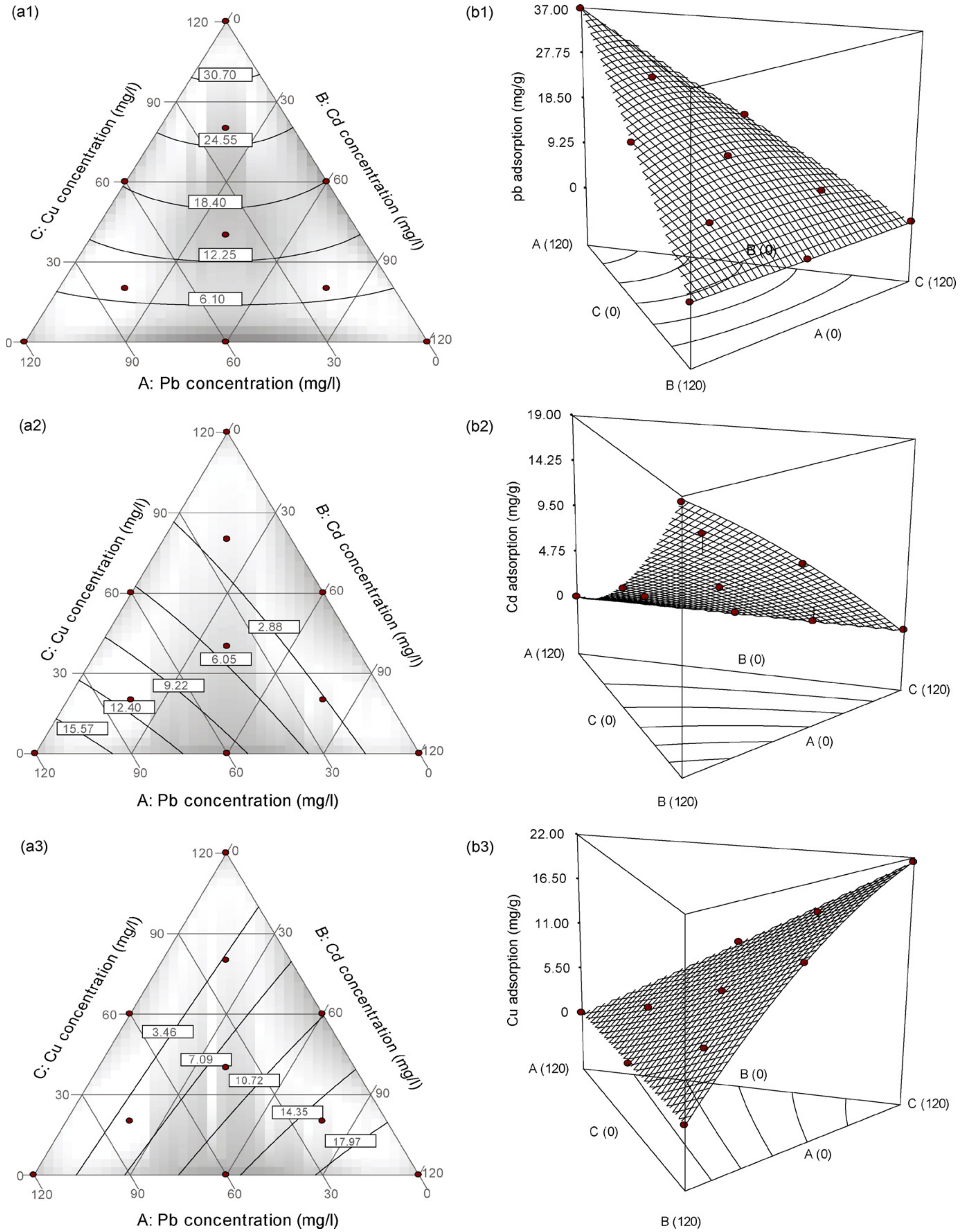

Fig. 3. Graphical representation for the biosorption of Pb(II), Cd(II) and Cu(II) onto immobilized spent *T. lobayense* in ternary system. (a) Triangular contour diagrams and (b) triangular three-dimensional biosorption surfaces (the total metal concentration was 120 mg/L; the values indicated in (a1), (a2) and (a3) were biosorption capacities in a unit of mg/g).

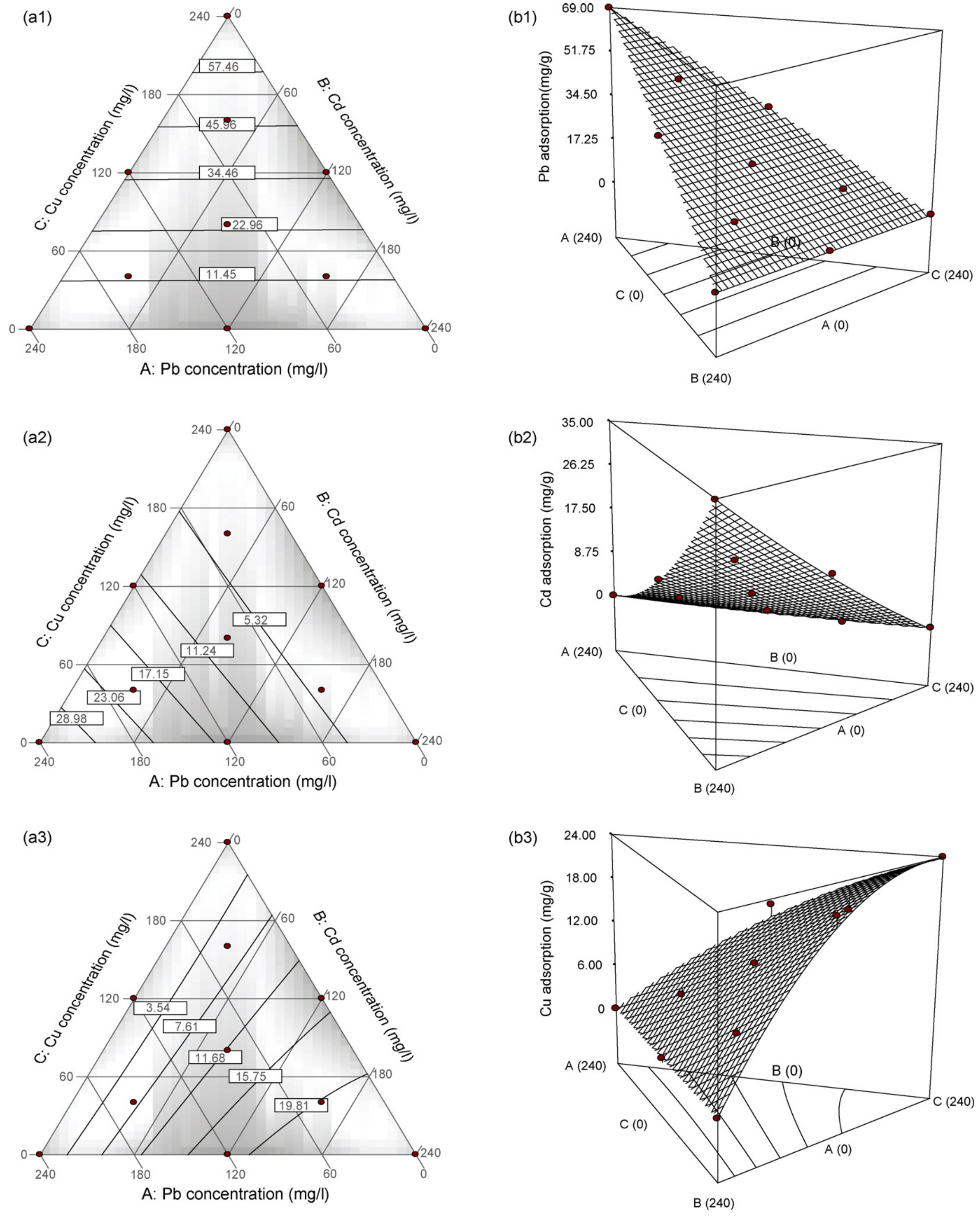

Fig. 4. Graphical representation for the biosorption of Pb(II), Cd(II) and Cu(II) onto immobilized spent *T. lobayense* in ternary system. (a) Triangular contour diagrams and (b) triangular three-dimensional biosorption surfaces (the total metal concentration was 240 mg/L; the values indicated in (a1), (a2) and (a3) were biosorption capacities in a unit of mg/g).

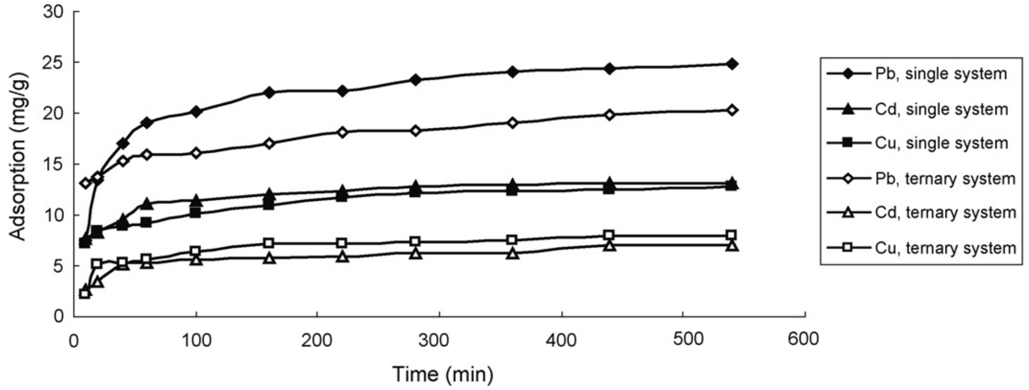

Fig. 5. Time-course adsorption of Pb(II), Cd(II) and Cu(II) by immobilized spent *T. lobayense* in single and ternary systems. Initial metal ions concentration = 60 mg/L, biomass loading = 2 g/L, pH = 5, temperature = 30 °C, agitation speed = 200 rpm.

where C_i and C_e are the metal ions concentrations (mg/L) initially and at a given time t, respectively; V is the volume of the heavy-metal solutions (mL); M is the weight of immobilized beads (g). Experiments were conducted in triplicate and the average values were taken as result. The residual metal ions concentrations in solutions were determined with flame Atomic Absorption Spectrometer (AAS; VARIAN, SpectrAA-220Fs).

2.5. *Experimental design*

The experimental design methodologies used in this study are described in detail as follows. The software used for experimental design and obtained data analysis was Design-Expert 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA).

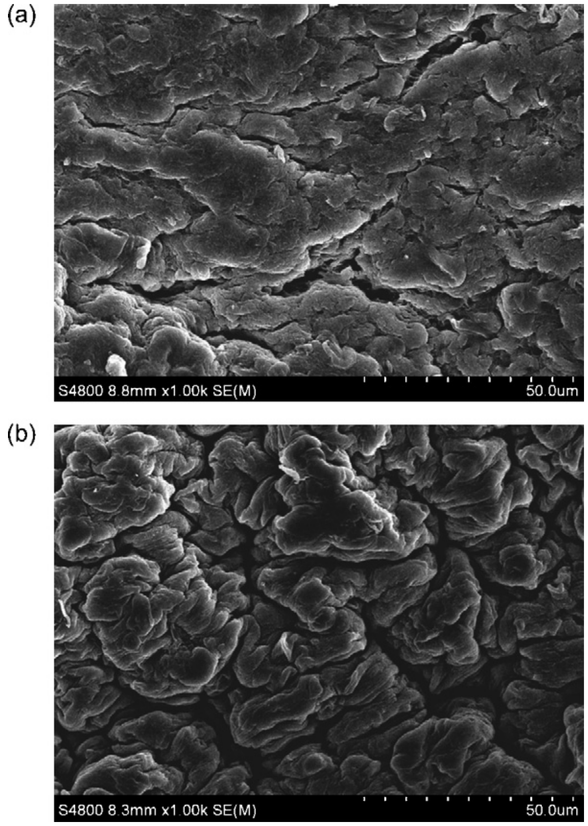

Fig. 6. SEM micrograph of immobilized spent *T. lobayense*: (a) control (surface image before biosorption) and (b) surface image after Pb(II), Cd(II) and Cu(II) biosorption.

2.5.1. *Factorial design methodology*

Factorial design is usually used for screening important variables and has successfully been applied by researchers [24,25]. The advantages of this method are: (1) all experimental variables are used in sifting main effects, leading to the most efficient use of resources, (2) the effects are evaluated by a wider range of conditions with the minimum of resources, (3) a series of factorial treatments are optimized for estimating main effects and interactions.

In order to evaluate the factors that affected the removal rate (%) of heavy metals (Pb(II), Cd(II) and Cu(II)), a Minimum Run Equireplicated Res V Design was applied. Six factors, temperature, pH, initial metal ions concentration, biomass loading, contact time and agitation speed, were chosen to study the response as removal rate (%) of heavy metals by biosorption onto ISTL. Each factor was represented at two levels—high and low, denoted by (+1) and (−1) signs, respectively. The factors and their respective level are summarized in Table 2.

2.5.2. *Response surface methodology (RSM)*

In recent years, RSM has been generally applied to optimize experimental conditions. However, in this study, RSM was used

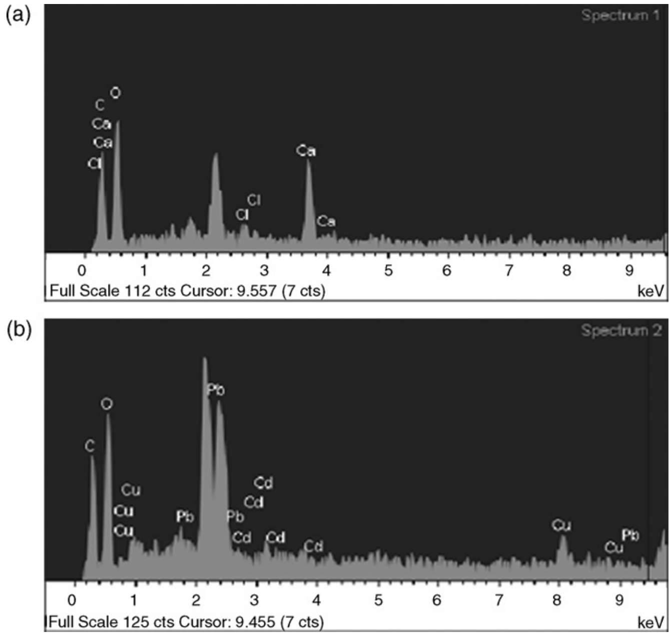

Fig. 7. EDS analysis of immobilized spent *T. lobayense*: (a) control (before biosorption) and (b) after Pb(II), Cd(II) and Cu(II) biosorption.

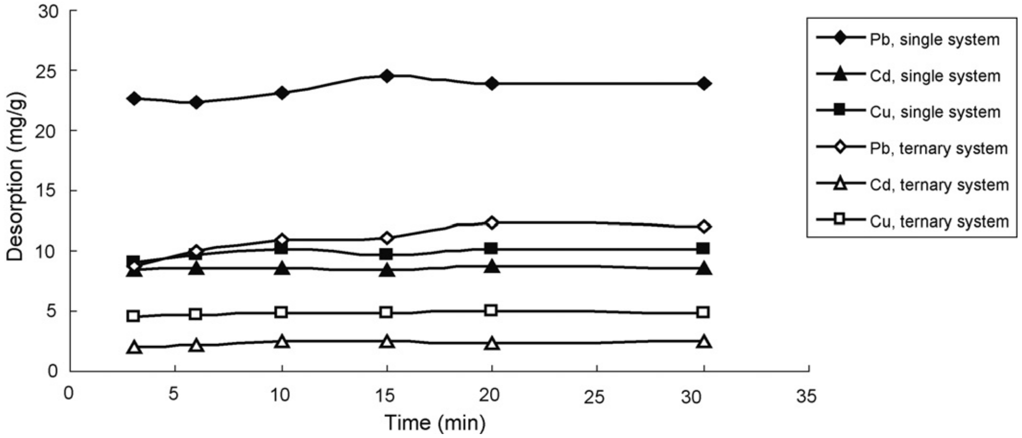

Fig. 8. Desorption kinetics of Pb(II), Cd(II) and Cu(II) onto immobilized spent *T. lobayense* in single and ternary systems.

Table 2
Factors and levels used in the Minimum Run Equireplicated Res V Design.

Factors	Name	Units	Levels and range (coded)	
			−1	+1
A	Temperature	°C	20	40
B	pH		2	5
C	Initial metal ions concentration	mg/L	10	100
D	Biomass loading	g/L	1	6
E	Contact time	min	10	240
F	Agitation speed	rpm	50	200

Table 3
The list of experimental designs and results according to response surface methodology, mixture designs and combinative design (no. 1–31) for multi-metal biosorption using immobilized *Tricholoma lobayense* as the biosorbent.

No.	RSM (face-centered) (mg/L)			Mixture design (120 mg/L)			Mixture design (240 mg/L)			Exp. uptake (mg/g)		
	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu
1	0	0	0							0	0	0
2	120	0	0	120	0	0				36.8	0	0
3	0	120	0	0	120	0				0	18.5	0
4	120	120	0				120	120	0	35.9	12.5	0
5	0	0	120	0	0	120				0	0	21.5
6	120	0	120				120	0	120	35.7	0	16.3
7	0	120	120				0	120	120	0	16.7	20.1
8	120	120	120							35.4	10.9	15.6
9	0	60	60	0	60	60				0	10.0	13.5
10	120	60	60							35.8	4.5	8.5
11	60	0	60	60	0	60				18.3	0	10.7
12	60	120	60							18.2	13.0	9.0
13	60	60	0	60	60	0				18.8	6.0	0
14	60	60	120							18.0	5.0	16.5
15	60	60	60							18.2	6.2	9.6
16	60	60	60							18.5	8.7	10.3
17	60	60	60							18.4	6.2	8.6
18	60	60	60							18.1	6.5	8.8
19	60	60	60							18.5	7.8	8.2
20	60	60	60							18.6	5.8	8.4
21				80	20	20				26.5	2.3	3.3
22				20	80	20				8.1	13.3	4.7
23				20	20	80				7.3	1.5	16.3
24				40	40	40				15.2	5.5	7.9
25							240	0	0	68.9	0	0
26							0	240	0	0	34.9	0
27							0	0	240	0	0	24.0
28							160	40	40	47.9	3.7	4.8
29							40	160	40	12.9	20.1	6.4
30							40	40	160	12.0	2.3	17.9
31							80	80	80	23.5	8.8	11.7

to interpret the interaction of three co-existing metals during sorption and predict biosorption results of ternary system. The Face-Centered Composite (FCCD) design which was a modified Center Composite design was chosen to explore the behavior of multi-metal biosorption onto ISTL [31]. The design contained a total of 20 experiments, being made up of six points on the six cubic surfaces and eight points on the eight vertices, as well as six replicates at the central points. The composition of three metals in this design is given in Table 3.

2.5.3. Mixture design methodology

In RSM, the concentration of each metal was independent on that of other two metals. However, in mixture design, each heavy metal was dependent and the summation of three metals was definite [26]. The factors in this design were the percentage of three metals. The simplex-lattice design which had enough points to estimate a full cubic model was selected to study the effects of mixture components. In order to cover the points in the interior of the simplex-lattice design and detect the lack of fit, the center point and check blends were added to the design. Therefore, the design developing from simplex-lattice called augmented simplex-lattice design was applied in this study at last. Moreover, in real wastewater, the concentrations of heavy metals may be high or low. Two groups of mixture designs including the total heavy-metal concentrations of 120 and 240 mg/L were conducted, respectively. The each of two designs contained a total of nine experiments and the composition of three metals is also given in Table 3. The experimental points from 1 to 20 and from 21 to 31 were, respectively, the mutual and different experiments in three designs as mentioned above (Table 3).

2.6. Equilibrium isotherms and kinetics studies

2.6.1. Equilibrium isotherm models

In order to explore the biosorption behavior and predict the biosorption capacity of ISTL, Langmuir and Freundlich models which could calculate the amount of sorbate that a sorbent retained and remained in the solution at equilibrium were chosen in this study [32–34].

The Langmuir model assumes that uptake takes place on a homogeneous surface by monolayer sorption without any interaction between the sorbed molecules [35]. The linear equation of Langmuir model is given by Eq. (3)

$$\frac{C_e}{q_e} = \frac{1}{q_{max}K_c} + \frac{C_e}{q_{max}} \tag{3}$$

where q_e and q_{max} are the observed and maximum uptake capacities (mg/g), respectively; C_e is the equilibrium metal concentration (mg/L); K_c is the equilibrium constant (L/mg).

The Freundlich model proposes a multilayer sorption based on a heterogeneous surface, and with interaction between the sorbed molecules [35]. The linear equation of Freundlich model is described by Eq. (4)

$$\ln\ q_e = \frac{1}{n}\ \ln C_e + \ln K_f \tag{4}$$

where K_f (L/g) and n are Freundlich isotherm constants related to adsorption capacity and adsorption intensity, respectively; q_e is the observed uptake capacity (mg/g); C_e is the equilibrium metal concentration (mg/L).

2.6.2. Kinetics modeling

The kinetics represents reaction pathways and reaction rates that the isotherms cannot give any information about. Meanwhile, adsorption kinetics depends mainly on the physical and/or chemical characteristics of the biosorbent which also affect the adsorption mechanism. In order to investigate the mechanism, pseudo-first-order and pseudo-second-order models have been used to test the experimental data.

The pseudo-first-order equation is represented as [35,36]

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t \tag{5}$$

where q_e and q_t are the amounts of adsorbed metal ions on the biosorbent at equilibrium and at time t (respectively, mg/g); k_1 is the first-order biosorption rate constant (min^{-1}).

The pseudo-second-order equation is also based on the sorption capacity of the solid phase and is expressed as [36]

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \tag{6}$$

where k_2 is the second-order biosorption rate constant (g/mg min); q_e is the biosorption capacity at the equilibrium (mg/g).

2.7. Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) analysis

In order to directly observe the surface morphology and analyze the elemental constitute of metal-free and metal-loaded ISTL, as well as to further confirm the identity of metal ions onto the beads, SEM (Inspect F, FET) and EDS (INCAPentaFET-x3, Oxford) were employed in this study. The above-mentioned samples were coated under vacuum with a thin layer of gold and then examined by SEM and EDS.

2.8. Desorption and kinetics modeling

In order to determine the reusability of the ISTL, consecutive adsorption–desorption cycles were repeated three times using the same beads. Desorption of Pb(II), Cd(II) and Cu(II) was performed with HNO_3 solution (50 mL, 1 M). The ISTL loaded with Pb(II), Cd(II) and Cu(II) ions were immersed in this desorption medium and stirred 200 rpm for 20 min at 30 °C. The final metal ions concentration in the aqueous phase was determined using an AAS as described above. Desorption ratio was calculated from the amount of metal ions adsorbed on the immobilized beads and the final metal ions concentration in desorption medium, as the following equation:

$$\text{desorption rate} = \frac{\text{amount of metal ions desorbed to the elution medium}}{\text{amount of metal ions adsorbed on the immobilized beads}} \times 100 \tag{7}$$

Furthermore, to describe the desorption mechanism, the experimental data from desorption experiments also were fitted to pseudo-first-order and pseudo-second-order models.

3. Results and discussion

3.1. Screening of the parameters by Minimum Run Res V Design

The design matrix of uncoded values for the tested factors and the observed responses in terms of the removal rate (%) of Pb(II), Cd(II) and Cu(II) for all experimental runs including replicates, are shown in Table 4. The analysis of variance (ANOVA) for biosorption study of Pb(II), Cd(II) and Cu(II) ions with ISTL was used in

Table 4
Minimum Run Equireplicated Res V Design for determination of the most important variables affecting the metal ions removal.

Std.	Temperature (°C)	pH	Initial metal ions (mg/L)	Biomass loading (mg/L)	Contact time (min)	Agitation speed (rpm)	Responses (% removal)		
							Pb(II)	Cd(II)	Cu(II)
1	20	2	100	6	10	200	36.2	24.7	26.7
2	40	5	10	1	10	50	25.0	24.3	12.4
3	20	2	10	1	240	50	26.5	11.5	7.5
4	40	5	100	6	10	50	49.3	42.0	27.5
5	20	5	10	6	10	200	79.4	48.5	48.6
6	40	5	100	1	10	200	39.3	23.1	22.9
7	40	2	10	6	10	200	48.6	14.3	15.6
8	20	5	10	6	240	50	75.6	68.3	66.3
9	40	2	100	1	10	50	11.4	20.4	9.1
10	40	2	100	6	240	200	68.7	24.9	32.7
11	40	2	10	6	240	50	77.2	22.9	11.3
12	40	5	10	6	240	200	97.9	84.1	91.9
13	20	5	10	1	240	200	91.9	68.4	68.4
14	40	5	100	1	10	50	22.1	37.2	20.7
15	20	2	100	6	240	50	42.1	29.0	28.9
16	40	2	10	1	240	200	32.8	10.1	6.2
17	20	5	100	1	10	50	15.0	27.6	22.5
18	20	2	10	1	10	200	6.4	12.5	6.0
19	20	2	10	5	10	50	9.8	16.4	7.9
20	20	5	100	5	240	200	94.7	64.2	65.5
21	40	5	100	1	240	50	38.0	45.6	20.2
22	20	2	100	1	240	200	23.4	21.6	15.1

order to ensure a good model. The test for significance of regression model and the results of ANOVA are in Table 5. Prob > F less than 0.05 indicated model terms were significant. The predicted R^2 and adjusted R^2 values 0.7473 and 0.8384, 0.6305 and 0.7562, 0.6199 and 0.7512 were in reasonable agreement for Pb(II), Cd(II) and Cu(II), respectively. The adequate precision value was the "signal to noise ratio". A ratio greater than 4 was desirable. A ratio of 17.039 for lead, 11.916 for cadmium and 13.083 for copper obtained indicated an adequate signal. Therefore, this model can be used to navigate the design space. The regression models obtained from Minimum Run Res V Design represented the effect of the tested factors on the responses. The models proposed are as follows:

$$\text{Pb(II) removal (\%)} = +0.460 + 0.029A + 0.150B - 0.041C + 0.150D + 0.140E + 0.089F \quad (8)$$

$$\text{Cd(II) removal (\%)} = +0.340 - 0.019A + 0.170B - 7.681 \times 10^{-3}C + 0.066D + 0.077E + 0.023F \quad (9)$$

$$\text{Cu(II) removal (\%)} = +0.290 - 0.033A + 0.160B - 0.012C + 0.093D + 0.084E + 0.070F \quad (10)$$

where, A, B, C, D, E and F are medium temperature, pH, initial metal ions concentration, biomass loading, contact time and agitation speed, respectively. As it is clear from Eqs. (8)–(10), medium pH, biomass loading and contact time have a main effect on the response.

The main effects were also analyzed using main effects plot for removal of Pb(II), Cd(II) and Cu(II) as shown in Fig. 1. It indicates the relative importance of effects of various factors. For three metal ions removal, the effect of pH, biomass loading and contact time were characterized by a greater degree of departure from the overall mean. This means that pH, biomass loading and contact time had a significant effect on metal ions removal as Eqs. (8)–(10) previously identified. For Pb(II), medium temperature, pH, biomass loading, contact time and agitation speed had a positive effect while initial Pb(II) ion concentration was found negative. For Cd(II) and Cu(II), medium pH, biomass loading, contact time and agitation speed showed a positive effect while temperature and initial metal ions concentration were negative. In view of above-mentioned conclusions, medium temperature for 30 °C, pH for 5, biomass loading for 2 g/L, agitation speed for 200 rpm were used for exploring the behavior of multi-metal biosorption in following RSM and mixture designs.

3.2. *Data analysis from response surface methodology (RSM) design*

Results obtained from multi-metal biosorption experiments are listed in Table 3. The statistical significance of the response surface

Table 5
Analysis of variance (ANOVA) for Pb(II), Cd(II) and Cu(II) ions removal using Minimum Run Equireplicated Res V Design.

Source	Sum of squares			*F*-Value			Prob > *F*			
	Pb	Cd	Cu	Pb	Cd	Cu	Pb	Cd	Cu	
Model	1.59	0.77	1.00	19.15	11.85	11.57	<0.0001	<0.0001	<0.0001	Significant
A	0.018	7.775 E−003	0.024	1.29	0.72	1.66	0.2737	0.4107	0.2172	
B	0.46	0.58	0.57	33.20	53.67	39.25	<0.0001	<0.0001	<0.0001	
C	0.036	1.259 E−003	3.172 E−003	2.61	0.12	0.22	0.1273	0.7382	0.6459	
D	0.48	0.092	0.18	34.43	8.49	12.79	<0.0001	0.0107	0.0028	
E	0.41	0.13	0.15	29.68	11.70	10.46	<0.0001	0.0038	0.0056	
F	0.17	0.011	0.10	12.29	1.03	7.18	0.0032	0.3253	0.0171	
Residual	0.21	0.16	0.22							
Cor. total	1.80	0.94	1.22							

R^2 = 0.8845 (Pb), 0.8258 (Cd), 0.8223 (Cu).

models was evaluated by the analysis of variance (ANOVA). The *F*-value (10017.41 for lead, 75.69 for cadmium and 106.80 for copper) with a low probability value ($P < 0.0001$) demonstrates a high significance for three regression models. The determination coefficient obtained at 95% of confidence level was 0.9999 for lead, 0.9722 for cadmium and 0.9801 for copper, revealing that these regressions were statistically significant. Besides, the non-significance lack of fit (0.5995 for lead, 0.5383 for cadmium and 0.1364 for copper, more than 0.05) explained that three models were valid for the present work.

The response surface contour plots were created using the design-expert 7.0.0 software for predicting ternary metal biosorption by ISTL. The predicted profiles for the selective biosorption of Pb(II), Cd(II) and Cu(II) are illustrated in Fig. 2(a)–(c), respectively. Each contour plot represented a number of combinations of two test variables holding the other variable at middle levels. Fig. 2(a) shows the simultaneous effect of initial cadmium and copper ions concentration on the lead uptake in ternary system. The lead uptake decreased slightly with increase of initial cadmium ion concentration as well as with initial copper ion concentration ranging from 0 to 120 mg/L, respectively. That is to say, the present of cadmium and copper had a little suppression of lead uptake in multi-metal system. In addition, copper concentration showed a remarkable effect while a little effect of cadmium concentration was observed. Fig. 2(b) depicts the cadmium adsorption as a function of the initial lead ion concentration and the initial concentration of copper in the aqueous solution. The biosorption capacity of cadmium onto ISTL decreased sharply when the initial lead ion concentration increased from 0 to 120 mg/L compared with that copper ranged from 0 to 120 mg/L. This means that the suppression effect of lead ions on cadmium ions adsorption was much greater than copper ions. Fig. 2(c) presents the interactive effect of the initial lead and cadmium ions concentration on the copper uptake in multi-metal solution. It was observed that there was a similar trend for the effect of lead and cadmium ions on the adsorption of copper ions as well as that about cadmium uptake.

These results indicated that in all cases, there was an inhibitory effect of one metal on binding of other metals, with lead showing the greatest effect. In a word, the adsorption order in ternary system was found to be Pb(II) > Cu(II) > Cd(II). The findings which were in agreement with previous studies [26,37] can be explained by the physicochemical properties of metal ions (listed in Table 1). Cadmium and copper ions have the same coordination number, charge, similar diamagnetism and similar electronegativity, which imply that the two ions would compete similarly with the lead ions. Moreover, lead has a greater affinity to biomass, which may be due to the fact that the metal has the largest atomic weight, the highest standard reduction potential, the most electronegativity, two possible coordination numbers (4, 6) and is paramagnetic compared to cadmium and copper ions. Generally, the metals with the highest uptake capacity in the mono-component system showed greater inhibitory effect on the biosorption of other metal ions in the multiple-component system [23]. As depicted in Fig. 2(b) and (c), when the lead ions exist in aqueous solution, the uptake of cadmium and copper decreased markedly.

3.3. Data analysis from mixture designs

The uncoded values of the test variables and the experimental results of uptake of three metals in each case are also presented in Table 3. The experiments were carried out with the same conditions as RSM. The result of ANOVA showed that the larger *F*-values (8326.51 for lead, 152.57 for cadmium and 1245.33 for copper) and the smaller *P*-values (<0.0001) indicated the more significant model term. The square of correlation coefficient for each response was computed as the *R*-square (R^2) that is a measure of the amount of variation around the mean explained by the model. The values of R^2 (0.9999 for lead, 0.9896 for cadmium and 0.9987 for copper) and correspondingly adjusted R^2 (0.9997 for lead, 0.9831 for cadmium and 0.9979 for copper) were close to 1.0 that indicated a high correlation between the observed values and the predicted values. Besides, the predicted R^2 (0.9996 for lead, 0.9798 for cadmium and 0.9976 for copper) and the adjusted R^2 were also in reasonable agreement with each other. The adequate precision of 242.362 for lead, 33.082 for cadmium and 88.785 for copper indicated good reliability of the experiments which were carried out.

Fig. 3 describes the biosorption of lead, cadmium and copper ions onto ISTL from ternary system with a total metal concentration of 120 mg/L. The triangular contour plots (Fig. 3(a1)–(a3)) show that the preference of ISTL on biosorption of a target metal from multi-metal system was in the order of Cu(II) > Cd(II), Pb(II) > Cu(II) and Pb(II) > Cd(II). Therefore, the affinity of ISTL for three metals followed as: Pb(II) > Cu(II) > Cd(II). The experimental results were in accord with that predicted in RSM. At the same time, as seen from the triangular three-dimensional diagrams (Fig. 3(b1)–(b3)), the design points mainly matched the predicted surfaces, suggesting that the augmented simplex-lattice design could predict accurately the behavior of multi-metal biosorption.

However, in real wastewater, the metal ions concentration could be higher. To heighten the practicality of this study, a total metal concentration of 240 mg/L was investigated for multi-metal biosorption with the augmented simplex-lattice design. The experimental data also corresponded to the predicted surface perfectly as shown in Fig. 4. The trend of competitive sorption (Pb(II) > Cu(II) > Cd(II)) was uniform with that observed in previous experiments with a total metal concentration of 120 mg/L. Meanwhile, comparing the results given in Fig. 3 with those in Fig. 4, the triangular 3D surfaces were very similar for the two mixture designs with different total metal concentrations (120 and 240 mg/L, respectively). These indicate that the interaction of the target metal with its co-existing metal ions in multi-metal biosorption was greatly similar no matter what the total metal concentration was. But, for a higher total metal concentration, the uptake of target metal was usually more than the relatively low one. This suggests that a total metal concentration of 120 mg/L did not completely saturate the adsorption sites of ISTL as well as the preference of the biosorbent for heavy metals would not change with the total metal concentration. Besides, when the total metal concentration was known in wastewater, the individual adsorption capacity of three metals can be predicted from the 3D surface plots (Figs. 3 and 4).

In this study, according to the results of RSM and two mixture designs (Table 3), maximum metal uptake capacities of ISTL were found as 68.9, 34.9 and 24.0 mg/g for lead, cadmium and copper, respectively. Say et al. [38] found the maximum metal uptake by dried fungal biomass as 69.77 mg/g for lead, 23.04 for cadmium and 20.23 mg/g for copper. Lu et al. [39] found the maximum lead, cadmium and copper uptake capacities of *Enterobacter* sp. J1 as 50.9, 46.2 and 32.5 mg/g. Mata et al. [29] found the highest value of lead, cadmium and copper uptake as 2.26, 0.579 and 0.617 mmol/g by immobilized *Fucus vesiculosus*. Göksungur et al. [5] found the maximum metal uptake of ethanol treated yeast biomass as 60.24 and 31.75 mg/g for lead and cadmium, respectively. The comparison of the biosorption capacities of biomass used in this study with those reported in the literature shows that ISTL is suitable for biosorption processes.

3.4. Analysis of adsorption isotherms

The tests were carried out at the concentration range 10–120 mg/L for 9 h holding other experimental parameters same as RSM. The adsorption constants of Langmuir and Freundlich

Table 6
Equilibrium constants obtained from Langmuir and Freundlich isotherms for Pb(II), Cd(II) and Cu(II) biosorption onto immobilized *Tricholoma lobayense* in single and ternary systems.

	Metal ions	Langmuir			Freundlich		
		q_{max} (mg/g)	K_c (L/mg)	R^2	K_f	n	R^2
Single metal system	Pb(II)	526.3	6.21×10^{-4}	0.9287	0.338	1.012	1.0000
	Cd(II)	769.2	2.01×10^{-4}	0.6248	0.159	1.011	1.0000
	Cu(II)	92.6	2.41×10^{-3}	0.6945	0.309	1.136	0.9981
Ternary metal systemPb(II) + Cd(II) + Cu(II)	Pb(II)	370.4	8.72×10^{-4}	0.9635	0.356	1.040	1.0000
	Cd(II)	22.1	7.27×10^{-3}	0.9851	0.260	1.275	0.9896
	Cu(II)	91.7	2.33×10^{-3}	0.6642	0.296	1.134	0.9978

Table 7
Kinetic parameters obtained from pseudo-first-order and pseudo-second-order for Pb(II), Cd(II) and Cu(II) biosorption onto immobilized *Tricholoma lobayense* in single and ternary systems.

	Pseudo-first-order			Pseudo-second-order			Experimental
	k_1 (1/min)	$q_{e1,cal}$ (mg/g)	R^2	k_2 (g/mg min)	$q_{e2,cal}$ (mg/g)	R^2	$q_{e,exp}$ (mg/g)
Single system							
Pb	7.2×10^{-3}	11.64	0.9587	1.62×10^{-3}	25.64	0.9992	24.85
Cd	10.5×10^{-3}	5.71	0.9820	5.02×10^{-3}	13.50	0.9996	13.16
Cu	6.7×10^{-3}	5.34	0.9876	3.63×10^{-3}	13.14	0.9982	12.86
Ternary system							
Pb	5.5×10^{-3}	7.14	0.9523	2.60×10^{-3}	20.41	0.9966	20.28
Cd	8.8×10^{-3}	4.43	0.6989	5.06×10^{-3}	7.20	0.9930	7.08
Cu	8.3×10^{-3}	4.42	0.8419	4.57×10^{-3}	8.30	0.9976	8.04

isotherms and their correlation coefficients (R^2) were calculated and represented in Table 6. As seen, mono-component isotherm modeling at different concentrations of three metal ions seemed to be a better option for metal sorption from ternary metal solution [22]. The higher correlation coefficient (R^2) demonstrates that Freundlich model was well fitted to data than the Langmuir isotherm for single and multi-metal biosorption. This suggests that biosorption of three metals by ISTL were more likely heterogeneous surfaced adsorption, instead of monolayer sorption. K_f (L/g) and n are Freundlich constants which indicate the adsorption capacity of the biosorbent and adsorption intensity, respectively. From Table 6, the n values were greater than 1, implying that the biosorption of three metals onto ISTL was favorable at studied conditions [40]. According to the magnitudes of the K_f, the consistent conclusions about the order of adsorption was obtained to be Pb(II) > Cu(II) > Cd(II) in mono-component and ternary systems. This indicates that Pb(II) ions were easily adsorbed from aqueous solution onto the prepared beads with a high adsorption capacity. The preference of biosorbent for different kinds of adsorbates may be related to the solution chemistry, the physicochemical properties of the adsorbates and the characteristics of the binding sites [23]. It is difficult to explain how these physicochemical properties affect the selectivity of the sorption process for the adsorbent. However, it can be speculated that the three metal ions partially share the binding sites on the surface of ISTL, with the presence of Pb(II) introducing competitive interaction with either Cd(II) and Cu(II) individually or combined. In short, there are possible interaction effects between different species in solution and in particular potential interactions on the surface depending on the adsorption mechanism [41].

3.5. Biosorption kinetics studies

Time-course adsorption of Pb(II), Cd(II) and Cu(II) by ISTL in single and ternary systems is shown in Fig. 5. It was observed that uptake of metal ions occurred in two stage i.e. an initial rapid uptake within 10–60 min followed by subsequent slow uptake from 60 to 540 min and reached equilibrium at 540 min in this study. The corresponding parameters obtained from linear regressive equations (Eqs. (5) and (6)) and the experimental adsorption capacities are summarized in Table 7. It is clear that the R^2 values of pseudo-second-order model were higher for the Pb(II), Cd(II) and Cu(II) biosorption than pseudo-first-order model. In addition, the theoretical q_{eq} values for all the studied metal ions were very close to the experimental q_{eq} values in the case of second-order kinetic model. In view of these results, it can be concluded that the sorption process followed the pseudo-second-order kinetics, which agreed with chemical sorption as the rate-limiting mechanism through shar-

Table 8
Kinetic parameters obtained from pseudo-first-order and pseudo-second-order for Pb(II), Cd(II) and Cu(II) desorption from immobilized *Tricholoma lobayense* loaded heavy metals in single and ternary systems.

	Pseudo-first-order			Pseudo-second- order			Experimental
	k_1 (1/min)	$q_{e1,cal}$ (mg/g)	R^2	k_2 (g/mg min)	$q_{e2,cal}$ (mg/g)	R^2	$q_{e,exp}$ (mg/g)
Single system							
Pb	4.32×10^{-2}	2.34	0.5243	0.16	24.15	0.9992	24.60
Cd	4.19×10^{-2}	0.16	0.0606	1.29	8.67	0.9999	8.69
Cu	1.21×10^{-1}	1.27	0.2653	0.28	10.25	0.9994	10.19
Ternary system							
Pb	8.15×10^{-2}	3.93	0.7046	0.05	12.71	0.9972	12.35
Cd	4.16×10^{-2}	0.31	0.2078	1.30	2.46	0.9984	2.53
Cu	3.04×10^{-2}	0.13	0.0638	2.86	4.88	0.9996	4.93

ing or exchange of electron between sorbent and sorbate [42,43]. The q_{eq} of Pb(II), Cd(II) and Cu(II) calculated from the pseudo-second-order model were in the order of Pb(II) > Cd(II) > Cu(II) in mono-component system and Pb(II) > Cu(II) > Cd(II) in ternary system, which were consistent with the results in Fig. 5. Moreover, The differences in the initial biosorption rate of three metals may be due to the nature and distribution of active groups on the adsorbent and the affinity between the metal ions and the adsorbent [44].

3.6. SEM/EDS analysis

The SEM images of heavy-metal-free and metal-loaded ISTL are shown in Fig. 6. It is observed that the surface morphology of the beads changed obviously after metal biosorption. Fig. 6(b) shows that the surface of metal-loaded ISTL looked distorted and rugged and may be damaged by the heavy-metal ions. Another explanation could be the substitution of calcium ions by lead, cadmium and copper onto the ISTL. Besides, most biosorption studies determined metal sorption by measuring the residual metal concentrations in the solution while few directly proved the presence of the metals on the biomass [45]. In this work, the EDS analysis (Fig. 7) confirmed the presence of three metal ions on the biosorbent, giving a direct detection of metals on the ISTL.

3.7. Desorption and kinetics studies

The desorption of the adsorbed Pb(II), Cd(II) and Cu(II) ions from the tested biosorbents was studied in single and ternary systems. The metal ions adsorbed onto ISTL were eluted with 1 M HNO_3. Fig. 8 shows the effects of desorption time on desorption amount of lead, cadmium and copper ions in single and ternary metal systems. Lead, cadmium and copper ions were released from the immobilized beads into eluent within 3–20 min, and desorption of cadmium and copper ions took place more easily than lead, regardless of single and ternary systems. May be, the preference of ISTL for lead ions resulted in the ions eluted more slowly. The kinetic constants calculated from linear regressive equations (Eqs. (5) and (6)) and the experimental desorption amount are summarized in Table 8. The pseudo-second-order model fitted better the desorption kinetics of heavy-metal ions onto ISTL than pseudo-first-order model. In order to explore the reusability of the biosorbent, the adsorption–desorption cycle of lead, cadmium and copper ions in mono-component solution was repeated three times using the same preparations. The adsorption capacities did not markedly change (only a maximum 3% for lead and 5% for copper was observed with the tested beads) for lead and copper while the adsorption capacity decreased by 10% for cadmium during the repeated adsorption–desorption operations. The reduction in lead, cadmium and copper adsorption of ISTL could be attributed to the adverse effect of the desorbing agents on the functional groups or active sites of the biomass [17,46]. These results showed that the ISTL can be used repeatedly for heavy-metal ions removal from wastewater without detectable losses in their initial adsorption capacities, especially for lead and copper.

4. Conclusions

Biosorption of Pb(II), Cd(II) and Cu(II) onto immobilized spent *T. lobayense* (ISTL) was investigated in single and ternary systems. The following conclusions can be drawn from this study:

(1) Medium pH, biomass loading and contact time were found to have a main effect on the removal of the three metals by screening the six independent variables with the Minimum Run Equireplicated Res V Design.
(2) According to Face-Centered Composite (FCCD) design and augmented simplex-lattice design, the biosorption preference of ISTL was following the order of Pb(II) > Cu(II) > Cd(II) for ternary system. Lead ions could still be effectively removed from aqueous solution in the presence of both cadmium and copper ions while removal of the cadmium and copper ions would be suppressed by lead.
(3) The adsorption isotherm followed the Freundlich model, suggesting the multilayer sorption of all the metals ions on the biosorbent. The kinetic data were also well described by the pseudo-second-order model, which agreed with chemisorption as the rate-limiting mechanism, and the equilibrium was established in about 540 min.
(4) Scanning electron microscope (SEM) showed that the surface morphology of ISTL changed obviously after metal binding. The presence of three metal ions on the biosorbent was also verified by energy dispersive spectrometry (EDS) analysis.
(5) The adsorbed metals were effectively eluted with 1 M HNO_3. The biosorbent could be reused for three biosorption–desorption cycles with slight decrease in adsorption capacity. The kinetic process followed well pseudo-second-order model and the equilibrium time was 3–20 min.

Based on the above-mentioned results, it can be concluded that the immobilized spent *T. lobayense* was a suitable biosorbent for removal of heavy-metal ions from wastewater in terms of low-cost, natural and abundant availability.

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