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EXPERIMENTAL DESIGN FOR THE OPTIMIZATION OF COPPER BIOSORPTION BY IMMOBILIZED NANOSCALE *Lentinus edodes*

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Abstract

An experimental design methodology was applied to study the copper removal from aqueous solutions by immobilized nanoscale *Lentinus edodes*. The effect of the various variables (biomass loading, initial metal ions concentration, PH, contact time, agitation speed and temperature) were studied in the batch experiment. The results showed that pH, contact time and agitation speed were the most significant variables on biosorption of copper ions determined by Minimum Run Equireplicated Res V Design. Then Central Composite Design was employed to optimize the process conditions for the maximum removal of copper. A maximum copper removal (97.57%) was found to occur under pH of 6 and contact time of 540 min and agitation speed of 125 rpm. The kinetic data agreed well with the pseudo-second-order model and the equilibrium sorption data followed the Freundlich model. Scanning electron microscope (SEM) and energy dispersive spectrometry (EDS) analysis confirmed the apparent change of the surface morphology and the existence of copper ions on the biosorbent after metal binding. The biosorbent could be regenerated by 1M HNO_3 with only 8.66% decrease in adsorption capacity and the desorption of the biosorbent was of high percentage after three adsorption-desorption cycles. The present work suggests that immobilized nanoscale *Lentinus edodes*, an abundant low-cost biomaterial, was an efficient biosorbent for copper removal from wastewater.

Keywords: biosorption, copper, *Lentinus edodes*, optimization, response surface methodology

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The conventional techniques for removing Cu^{2+} from wastewaters, include chemical precipitation, flocculation, ion exchange, chelating (Bulai et al., 2009), reverse osmosis, membrane filtration, solvent extraction etc. Due to the disadvantages like expensive cost, low efficiency, less applicable to a wide range of pollutants and possible production of secondary toxic compounds, to find out a more feasible technique is needed. Compared to the conventional techniques, adsorption especially biosorption is considered to be an attractive technology because of its low cost, high efficiency, easy operation and regeneration of adsorbent (Anis et al., 2013; Goksungur et al., 2005; Hasan and Srivastava, 2009; Hasan et al., 2009). Adsorbents like drying bed activated sludge (Benaissa and Elouchdi, 2011), bacteria (Isik, 2008), fungi (Xiaoming et al., 2008) and nut hulls (Tajar et al., 2009) have already been researched, showing that biomaterials can be used for the removal of heavy metals from wastewater. The application of dead biomass is preferred since it is not affected by toxic heavy metals, does not require nutrients, and may be regenerated and reused in several number of adsorption-desorption cycles (Kumar et al., 2008; Wang et al., 2004). Edible mushroom biomass such as *Agaricus bisporus* (Ertugay and Bayhan, 2008; Ertugay and Bayhan, 2010), *Amanita rubescens* (SarI and Tuzen, 2009), *Agaricus macrospores* (Melgar et al., 2007) and *Pleurotus platypus* (Vimala and Das, 2009) have successfully been applied to the removal of heavy metals from wastewater recently, owing to its high ability to bind heavy metals as well as its high availability to industrial residues (Pakshirajan and Swaminathan, 2009; Preetha and Viruthagiri, 2007).

In this study, nanoscale *Lentinus edodes* was applied to the biosorption of copper ions in artificial aqueous solution since no report available until now. The immobilization of the biomass is carried out because of minimal clogging in continuous systems (Vilar et al., 2008), facility to reuse and especially convenient to separate the solid biomass from the bulk liquid (Bayramoğlu et al., 2006). Natural polymers like alginate, chitosan and chitin were widely used as immobilization matrices since they are non-toxic, efficient and cheap (Bayramoglu et al., 2003). Alginate is selected in this study as it is common in laboratory. In order to reduce the number of experiments, two experimental design methodologies namely factorial design and response surface design (RSM) were used to investigate the adsorption characteristics of copper(II) onto immobilized nanoscale *L. edodes*.

2. Material and methods

2.1. Preparation of the biosorbent

Fresh *L. edodes*, an adsorbent, was purchased from a mushroom production site located in the suburbs of Chengdu, China. It was washed with distilled water several times, dried in an oven at 50℃ for three days followed by grinding with a pulverizing mill (Joyoung, JYL-CO12) to obtain raw biomass. Finally, the biomass was conducted by a High-energy nano-impact mill to obtain nanoscale *L.edodes*. In order to confirm that the obtained materials was nanoscale, scanning electron microcopy (SEM) was employed. The SEM image is shown in Fig. 1.

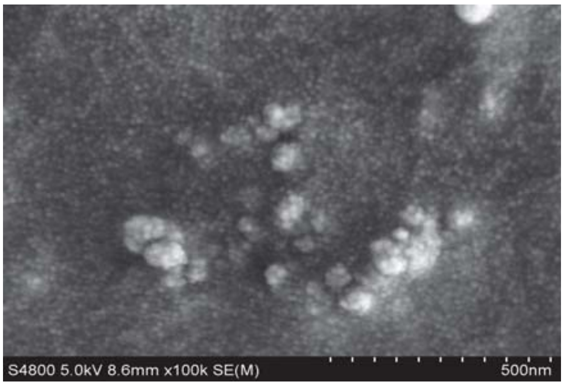


Fig. 1. SEM micrograph of nanoscale *L.edodes*

2.2. Immobilization of nanoscale L.edodes into sodium alginate

To immobilize the nanscale *L.edodes* into sodium alginate, a 2% sodium alginate solution was mixed with the same weight of the nanscale *L.edodes*. The mixture was then introduced drop-wise from 8-10cm away into a cooled 0.5M $CaCl_2$ using 2.5mL syringe with the solution stirred continuously. The beads harden immediately once dropped into the $CaCl_2$ solution and were left in the solution overnight at 4ºC. Then the resulting beads were washed with distilled water three times and were collected by filtration and dried at room temperature. Beads of irregular shape as well as inappropriate size were discarded. Finally, the beads of approximately 2mm size were obtained for the following experiment.

2.3. Preparation of Cu^{2+} stock solution

All reagents used in this study were of analytical grade and were purchased from KeLong Chemical Reagent Factory, Chengdu, China. 1000mg/L of Cu^{2+} stock solution was prepared by dissolving 3.805g of $Cu(NO_3)_2 \cdot 3H_2O$ in 1 L of deionized distilled water. All the working solutions in the followed experiments were obtained by successive dilution.

2.4. Experimental design

A software named Design-Expert 7.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA) was used for experimental design and data analysis. Two experimental design methodologies namely factorial design methodology and response surface methodology contained in this software were applied to this study.

2.4.1. Factorial design methodology

Factorial design is generally used for screening important variables that all variables chosen in the experiment were used in selecting main effects under a wide range of conditions with the minimum of resources.

A Minimum Run Equireplicated Res V Design, which is a new class of design that contains the minimum number of runs to estimate all main effects and all two-factor interactions was employed to evaluate the influence of factors on the removal rate (%) of copper(II). Six factors namely biomass loading, initial metal ions concentration, pH, contact time, agitation speed and temperature were selected in order to study the removal rate (%) of copper(II) by the biosorbent of immobilized nanoscale *L.edodes*. Each factor was indicated by two levels (coded as -1 or low, and +1 or high), regardless of its relative magnitude. The factors and their respective level are showed in Table 1.

2.4.2. Response surface methodology (RSM)

Response surface methodology is widely applied to find out the optimization of experimental conditions. In this study, the Central Composite Design (CCD) was chosen to investigate the effects of variables on the removal rate (%) of copper(II) by the biosorbent of immobilized nanoscale *L.edodes*. The variables used in the CCD are the significant variables screened by the Factorial design methodology.

2.5. Batch experiments

All the experiments were carried out in 150 mL Erlenmeyer flasks with required concentration and pH of 50mL copper(II) solution in a shaker incubator (SUKUN, SKY-211B). The solution pH is adjusted by adding HNO_3 (0.1M, 1M) and NaOH (0.1M, 1M) solutions and the pH value is measured by a pH meter (Model phs-25, calibrated with buffers of ph 4.00, 6.86 and 9.18). Each experiment was carried out with three replications and the final result was obtained as the average value.

The concentration of copper(II) in solution was measured using flame atomic adsorption spectrometry (AAS) (VARIAN, spectraa-220Fs).

The removal rate (%) of copper(II) and adsorption capacity (q_e) were calculated by following Eqs. (1-2):

$$\text{Removal rate (\%)} = \frac{C_o - C_e}{C_o} \cdot 100 \qquad (1)$$

$$q_e (mg/g) = (C_o - C_e) \qquad (2)$$

where: C_0 and C_e are the initial and the equilibrium copper(II) concentration (mg/L), respectively; q_e is the amount biosorbed at equilibrium; V is the volume of the copper(II) solutions (mL); M is the weight of immobilized beads (g).

2.6. pH at the point of zero charge (pHpzc)

The pH value at the point of zero charge (pHpzc) was measured by a mass titration method. Three solutions with different initial pH values namely pH=3, 6 and 11 were prepared via adding 0.05 M HNO_3 or NaOH, while $NaNO_3$ was used as the background electrolyte. For each initial pH, Erlenmeyer flasks were filled with 30 mL of the solution and different amounts of adsorbent were added to make the solid fraction 0.05%, 0.1%, 0.5%, 1%, 5% and 10%. The mixture was then shaken for 24 h and the equilibrium pH was measured. A plot of the equilibrium pH versus mass fraction yielded a curve, showing a plateau and the pHpzc was identified as the point at which the change of pH is zero. Then the pHpzc was taken as the average of the three asymptotic pH values. Below this pH the surface is positively charged, while above this pH the surface is negatively charged.

2.7. Kinetics of biosorption

In order to study the kinetics of biosorption, a series of experiments that certain amount of absorbent with certain initial concentrations of copper(II) solution with definite time intervals ranging from 5min to 600min at 25°C were carried out. The kinetics data were analyzed by two models namely the pseudo-first-order rate Lagergren model and the pseudo-second-order kinetic model.

The pseudo-first-order kinetics equation is proposed when Lagergren studied the phenomenon of solute adsorbed with charcoal from solution in 19th century. This equation based on the solid-liquid adsorption system, which is often used to verify the kinetic adsorption process of biosorbents.

Table 1. Factors and levels used in the Minimum Run Res V Design

Factors	***Name***	***Units***	***Levels and range***	
			-1	+1
A	Biomass loading	g/L	1	6
B	Initial metal ion concentration	mg/L	10	100
C	pH		2	6
D	Contact time	min	10	300
E	Agitation speed	rpm	50	200
F	temperature	°C	20	40

The pseudo-first-order kinetics equation reflects that the slewing rate of the adsorption capacity per unit mass to the time and the D-value of the adsorption capacity in maximum and in time t is in direct ratio. The pseudo-first-order model is described by Eq. (3).

$$Ln(q_e - q_t) = \ln(q_e) - k_1 t \qquad (3)$$

where: q_e and q_t are the amounts of Cu^{2+} ions adsorbed on the biosorbent at equilibrium and at time t (min) respectively and k_1 is the first-order biosorption rate constant (min^{-1}).

The pseudo-second-order kinetic model is established based on the assumption that the detecting ion concentration is equal to the ion concentration of the cell surface. The pseudo-first-order model is described by Eq. (4):

$$\frac{1}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e} t \qquad (4)$$

where q_e and q_t are the biosorption capacity at the equilibrium and at the time t (mg/g), respectively; k_2 is the second-order biosorption rate constant ($gmg^{-1}min^{-1}$).

2.8. Equilibrium isotherms of biosorption

In order to study the equilibrium isotherms of biosorption, a series of experiments that certain amount of absorbent with various initial copper(II) solution concentrations ranging from 10 to 100 mg/L at 25°C were carried out. The adsorption equilibrium data were analyzed by two common models namely Langmuir and Freundlich.

The Langmuir isotherm is put forward based on the assumption that the adsorption is taken place on the specific as well as homogeneous adsorption sites, which are on the surface of adsorbent. It is often used to describe the relationship between the adsorption amount of metal ions and the equilibrium concentration of metal ions in the adsorption system. The linear form is given by Eq. (5):

$$\frac{C_e}{q_e} = \frac{1}{k_a q_m} + \frac{C_e}{q_m} \qquad (5)$$

where: C_e is the equilibrium metal concentration (mg/L); q_e and q_m are the amount of metal ions adsorbed at equilibrium and at maximum (mg/g), respectively; k_a is the equilibrium constant (L/mg).

The Freundlich isotherm is established based on the adsorption happened on multiphase surface of adsorbate. It has been widely used in the isothermal adsorption. It describes heterogeneous surface energies in multilayer adsorption. The linear form is given by Eq. (6):

$$Lnq_e = \ln K_F + \frac{1}{N} LnC_e \qquad (6)$$

where: q_e is the amount of metal ions adsorbed at equilibrium (mg/g); C_e is the equilibrium metal concentration (mg/L); K_F is Freundlich isotherm constant related to the adsorption capacity of the adsorbent and *1/N* is Freundlich isotherm constant represents the surface heterogeneity (Crisafully et al., 2008).

2.9. Characterization of biosorbent

The surface morphology features of the biosorbent of immobilized nanoscale *L. edodes* before and after copper adsorption were identified using the scanning electron microscopy (SEM) (JSM-5900LV, Japan). Energy dispersive spectrometry (EDS) (incapentafet-x3, Oxford) was also used to further confirm the existence of metal ions onto the beads. The infrared spectroscopy analysis of the biosorbent before and after copper adsorption was obtained using a Fourier transform infrared (FTIR) spectrometer (NEXUS-650, America).

2.10. Desorption experiments

The beads were collected after adsorption to conduct the desorption experiments so as to explore the reusability of the biosorbent. The desorption experiments were conducted in 150mL conical flasks with 50mL 1M HNO_3 solution in half an hour (Yanru et al., 2010). Then the same beads were used for the adsorption under the same conditions. Three continuous adsorption-desorption cycles were carried out and the copper concentration was measured using flame atomic adsorption spectrometry (AAS) (VARIAN, spectraa-220Fs). The reusability of the biosorbent was described by the desorption ratio, which was calculated by the amount of copper ions adsorbed on the beads as well as the amount of copper ions desorbed in the HNO_3 solution.

3. Results and discussion

3.1. Screening of the important variables using Minimum Run Res V Design

The experimental design matrix derived from Minimum Run Res V Design and the observed response on removal rate (%) of copper were shown in Table 2. The results of removal rate (%) of copper, which were obtained by calculating on average were ranging from 2.11% to 73.7%.

The analysis of variance (ANOVA) was utilized for the study of copper biosorption. The results of the analysis of variance (ANOVA) were shown in Table 3. The Model F-value of 20.07 implies that the model is significant and there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise, indicating that this model is available.

Table 2. Experimental design and results of the Minimum Run Res V Design

Run	***Factors***						***Response***
	A	*B*	*C*	*D*	*E*	*F*	*Removal rate of copper (%)*
1	1	10	2	300	50	20	9.8
2	1	100	2	300	50	40	7.9
3	6	10	2	300	200	20	21.8
4	1	10	6	300	50	40	40.7
5	6	10	6	10	50	20	30.8
6	6	100	6	10	50	40	21
7	1	10	2	10	200	20	5.4
8	1	10	2	10	50	40	4.1
9	1	100	2	300	200	20	18.1
10	6	100	2	300	200	40	18.2
11	6	100	6	300	50	20	53.6
12	6	10	2	10	200	40	7.75
13	6	100	6	10	200	20	40.05
14	1	100	6	10	50	20	24.25
15	1	100	2	10	200	40	2.11
16	6	100	6	10	50	20	24.65
17	6	10	2	300	50	40	13.05
18	6	100	2	10	50	20	2.3
19	6	10	6	300	200	40	73.7
20	1	10	6	10	200	40	31.7
21	1	100	6	300	200	40	33.3
22	1	10	6	300	200	20	67.9

Values of "Prob > F" less than 0.0500 indicate model terms are significant, while values greater than 0.1000 indicate the model terms are not significant. In this case, C, D, E standing for pH, contact time and agitation speed, respectively. The value of the predicted R^2 of 0.7559 is in reasonable agreement with the adjusted R^2 of 0.8449. The adequate precision measures the signal to noise ratio. The adequate precision ratio of 14.344 obtained in this study indicates an adequate signal to noise ratio, because a ratio greater than 4 is desirable. In this case, this model can be used to navigate the design space.

The main effects plot shown in Fig. 2 were used to estimate the main effects of the factors on the removal rate (%) of copper. The main effects plot revealed that the magnitude of positive or negative effect of each variable as well as the interaction on removal rate (%) of copper. The positive value of effect implies that the removal rate (%) of copper increases with the increment of the variable level. On the other hand, a negative value of effect implies that the removal rate (%) of copper decreases with the increase on the variable level. The linear regression model built taking the main factors into account in this study is shown by Eq. (7) in terms of actual factors:

$$\text{Removal rate}(\%) = -18.34 + 1.077A - 0.077B + 8.083C + 0.059D + 0.064E - 0.187F \quad (7)$$

In this case, pH, contact time and agitation speed have a significant effect on the removal rate (%) of copper, according to a greater degree of departure from the mean overall. Biomass loading, pH, contact time and agitation speed represent positive effect, but initial metal ions concentration and temperature show a negative effect. Considering the conclusions mentioned above, 2g/L of biomass loading, 50mg/L of initial metal ions concentration and a temperature of 25°C were chosen for the following experiments.

3.2. Optimal conditions of copper biosorption using response surface methodology (RSM) design

According to the results above, pH, contact time and agitation speed were applied to the response surface methodology in order to select the optimal conditions of copper biosorption. The factors and their respective level are shown in Table 4. The experimental design matrix derived from response surface methodology and the observed response in the name of removal rate (%) of copper are shown in Table 5. The analysis of variance (ANOVA) was used for studying copper biosorption so as to ensure the regression model. The results of the analysis of variance (ANOVA) are shown in Table 6. The Model F-value of 183.38 implies the model is significant and there is only a 0.01% chance that a "Model F-Value" this large could occur due to noise, indicating that this model is available.

Values of "Prob > F" less than 0.0500 indicate model terms are significant, while values greater than 0.1000 indicate the model terms are not significant. In this case, A, B, C, AB, AC, BC, A^2, B^2, C^2 are significant model terms, where A, B, C are standing for pH, contact time and agitation speed, respectively. The “Lack of Fit F-value” of 1.15 implies the Lack of Fit is not significant relative to the pure error. There is a 44.00% chance that a "Lack of Fit F-value" this large could occur due to noise.

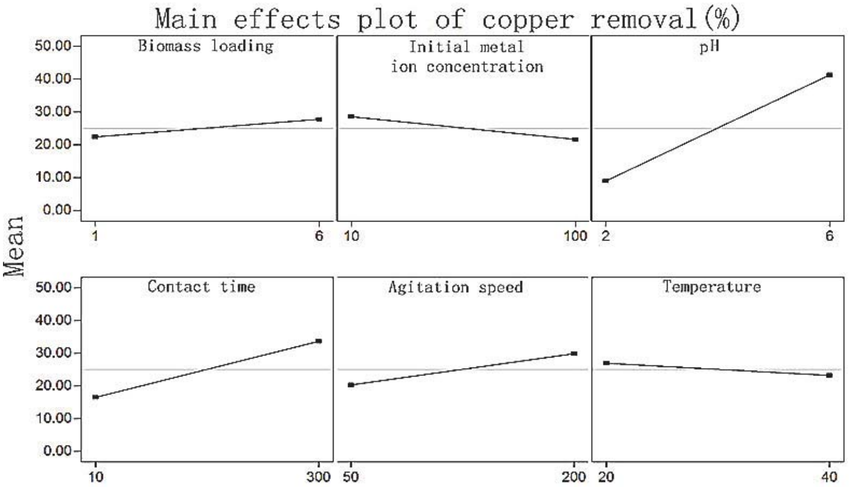


Fig. 2. Main effects plot of copper removal by immobilized nanoscale *L.edodes*

Table 3. Analysis of variance (ANOVA) for copper removal using Minimum Run Res V Design

Source	***Sum of***	***df***	***Mean***	***F Value***	***p-value***	
	Squares		*Square*		*Prob >F*	
Model	7643.11	6	1273.85	20.07	< 0.0001	significant
A	154.57	1	154.57	2.44	0.1395	
B	254.25	1	254.25	4.01	0.0638	
C	5575.76	1	5575.76	87.86	< 0.0001	
D	1573.14	1	1573.14	24.79	0.0002	
E	488.10	1	488.10	7.69	0.0142	
F	74.34	1	74.34	1.17	0.2962	
Residual	951.95	15	63.46			
Cor Total	8595.06	21				

R^2 =0.8892, Adj R^2=0.8449, Pred R^2=0.7559

Table 4. Factors and levels used in the Central Composite Design

Factors	***Name***	***Units***	***-1 Level***	***+1 Level***	***-alpha***	***+alpha***
A	pH		2	6	2	6
B	Contact time	min	300	540	300	540
C	Agitation speed	rpm	50	200	50	200

alpha = 1

Table 5. Experimental design and results of the Central Composite Design

Run factors				***Response***
	A	*B*	*C*	*Removal rate of copper(%)*
1	6	300	50	80.1
2	6	540	50	91.55
3	4	540	125	90.2
4	6	300	200	85.3
5	2	300	50	71.95
6	6	540	200	89.3
7	4	420	125	90.6
8	2	300	200	84.25
9	2	540	50	81.2
10	2	420	125	90.3
11	4	420	125	90.7
12	4	420	125	89.5
13	4	300	125	84.45
14	4	420	50	80.1
15	6	420	125	95.6
16	4	420	125	90.75
17	4	420	125	91.1
18	2	540	200	85.55
19	4	420	125	90.95
20	4	420	200	85.45

Table 6. Analysis of variance (ANOVA) for Response Surface Quadratic Model

Source	***Sum of***	***df***	***Mean***	***F***	***p-value***	
	Squares		***Square***	***Value***	***Prob > F***	
Model	573.74	9	63.75	183.38	< 0.0001	significant
A	81.80	1	81.80	235.29	< 0.0001	
B	100.81	1	100.81	289.98	< 0.0001	
C	62.25	1	62.25	179.07	< 0.0001	
AB	3.00	1	3.00	8.63	0.0148	
AC	23.46	1	23.46	67.49	< 0.0001	
BC	29.65	1	29.65	85.28	< 0.0001	
A^2	24.83	1	24.83	71.41	< 0.0001	
B^2	18.88	1	18.88	54.32	< 0.0001	
C^2	141.39	1	141.39	406.73	< 0.0001	
Residual	3.48	10	0.35			
Lack of Fit	1.86	5	0.37	1.15	0.4400	not significant
Pure Error	1.62	5	0.32			
Cor Total	577.22	19				

Non-significant lack of fit is well indicating the model is fit. The value of the predicted R^2 of 0.9793 is in reasonable agreement with the adjusted R^2 of 0.9886. The adequate precision measures the signal to noise ratio. The adequate precision ratio of 58.060 obtained in this study indicates an adequate signal to noise ratio for a ratio greater than 4 is desirable. In this case, this model can be used to navigate the design space.

The 3D surface plot was used to estimate the interaction of the factors. The combined effect of pH and contact time while the agitation speed was at a constant value (125rpm) was shown in Fig. 3a.

It was obvious that the pH exert stronger influence than contact time because of the removal rate showed a wider range when pH varied from 2 to 6 than contact time varied from 300min to 540min. In general, no matter the contact time was at a high or low level, the removal rate (%) of copper increased as pH increased from 2 to 6.

This behavior may be explained by the electrostatic attraction between the biosorbent and copper as well as the competition for the active sites of the biosorbent between copper ions and hydrogen ions. In this study, the pHpzc value of the biosorbent was 6.67. The removal rate increased at higher pH value due to the electrostatic attraction force between the biosorbent and copper increased, because the augmentation of pH leads to the surface of biosorbent more negative.

Though the surface charge of the biosorbent become more negative at pH > 6 and the removal rate of copper may be increased, it is not indicated that copper could be precipitated instead of being adsorbed at pH > 6. On the other hand, whether pH was at a high or low level, the removal rate of copper increased along with the increase of contact time. Possible explanations may lie in the equilibrium mechanism of adsorption. The function of pH and agitation speed while the contact time was maintained at the middle value of 420min was shown in Fig. 3b.

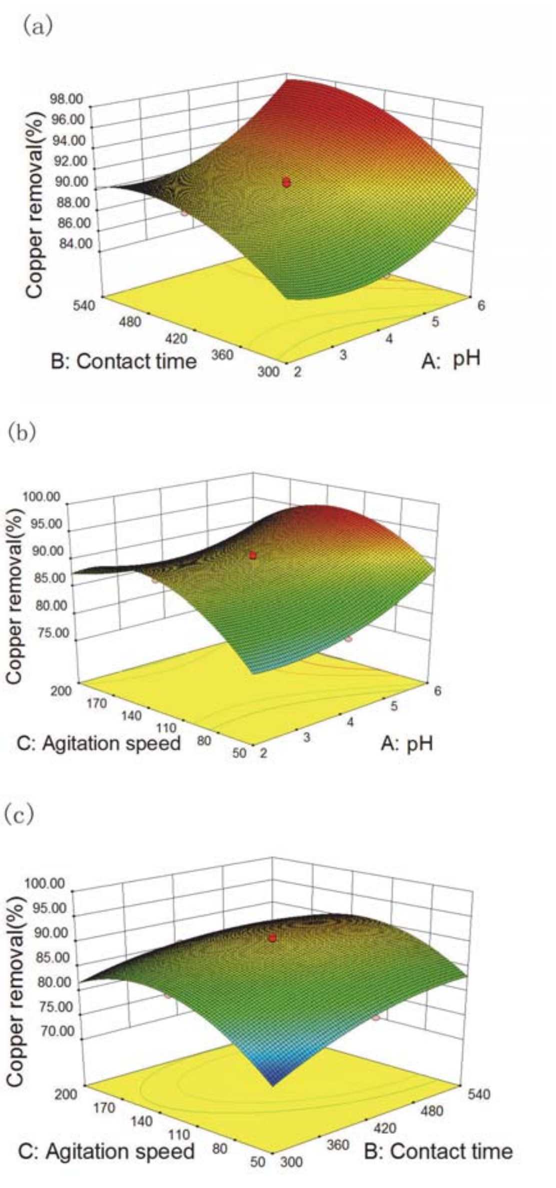


Fig. 3. 3D plot of copper removal by immobilized nanoscale *L.edodes*: (a) the effect of pH and contact time, (b) the effect of pH and agitation speed,(c) the effect of contact time and agitation speed

On the basis of the Figure, no matter the agitation speed was at high or low level, the removal rate (%) of copper increased as pH increased from 2 to 6. the explanation is mentioned above.

On the other hand, when the pH value was invariable, the removal rate of copper increased as agitation speed increased at first until the agitation speed reached the middle value of 125rpm, then the removal rate of copper decreased as agitation speed kept on increasing. This behavior may be explained by the mechanical biomass damage, which was caused by shear stress.

The interaction of contact time with agitation speed while the pH value is stationary at 4 was shown in Fig. 3c. It was apparently that whether the agitation speed was at high or low level, the removal rate (%) of copper increased as contact time increased from 300min to 540min. As stated earlier, possible explanations may lie in the equilibrium mechanism of adsorption. Meanwhile, no matter the contact time was at high or low level, the removal rate of copper increased first and followed by decreasing as the agitate on speed varied from 50 to 200rpm and the maximum value at 125rpm.

According to the results above, the optimal conditions for copper biosorption from aqueous solution using immobilized nanoscale *L.edodes* as a biosorbent are pH of 6 and contact time of 540min as well as agitation speed of 125rpm. Under above conditions, the maximum removal rate of copper could reach to 97.57%, which is much higher than *Agaricus bisporus* of 73.3% (Ertugay and Bayhan, 2008) and immobilized biomass of *Arthrobacter sp.* of 89.56% (Hasan and Srivastava, 2009), but a little lower than *Rhizopus arrhizus* of 98% (Preetha and Viruthagiri, 2007).

3.3. Adsorption kinetics

In order to study the kinetics of biosorption, a series of experiments that 2 g/L of absorbent with 50mg/L of initial copper(II) solution with definite time intervals ranging from 5min to 600min at a PH of 6, agitation speed of 125rpm and the temperature of 25°C were carried out. The experimental data is linear fitted by the pseudo-first-order and pseudo-second-order adsorption kinetic models and their respective parameters were calculated and shown in Table 7.

According to the value of linear correlation coefficient (R^2), it indicated that the adsorption of copper by immobilized nanoscale *L.edodes* could not be well described by the pseudo-first-order model, but could be well described by the pseudo-second-order model. According to the mechanism established by the pseudo-second-order equation, deducing that the chemical sorption process is the rate-limiting step, which may be related to the covalent forces of sharing or exchange of electron between biosorbent and metal ion. Moreover, the calculated q_e was so close to the experimental q_e, which also implied a well agreement. Therefore, the kinetic parameters of the pseudo-second-order model can be used for calculating the equilibrium adsorption capacity as well as removal rate.

Table 7. The related parameters for adsorption kinetics

Adsoption kinetic model	***k(min^{-1})***	***q_e(cal) (mg/g)***	***R^2***	***q_e(exp) (mg/g)***
Pseudo-first-order	0.0430	317.98	0.563	
Pseudo-second-order	0.0028	11.63	0.997	11.05

R^2=0.9940, Adj R^2=0.9886, Pred R^2=0.9793

3.4. Adsorption isotherms

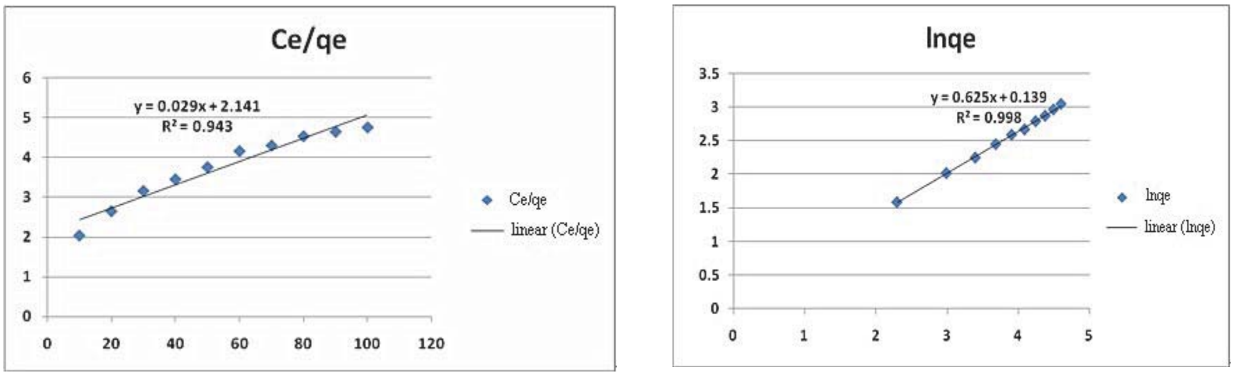
In order to study the equilibrium isotherms of biosorption, a series of experiments that 2g/L of absorbent with various concentrations of initial copper(II) solution ranging from 10 to 100 mg/L at a pH of 6, agitation speed of 125rpm and the temperature 25°C for 9h were carried out. The experimental data is linear fitted by the Langmuir and Freundlich isotherm models and their linear fitting figures were shown in Fig.4 and their respective parameters were calculated and shown in Table 8. As can be seen by the linear correlation coefficient (R^2), a higher value of linear correlation coefficient (R^2) of freundlich model indicated that Freundlich model was better fitted to data than the Langmuir model. This implied that the biosorption of copper by immobilized nanoscale *L.edodes* were more likely heterogeneous surfaced adsorption than momolayer adsorption. K_F is Freundlich isotherm constant related to the adsorption capacity of the adsorbent.

The value 1.149 of K_F indicates that immobilized nanoscale *L.edodes* was of high adsorption capacity of copper. Meanwhile, n is Freundlich isotherm constant represents the surface heterogeneity. The value 1.6 of n, which was greater than 1 demonstrated that the adsorption of copper by immobilized nanoscale *L.edodes* was favorable at studied conditions.

3.5. Charactererization of biosorbent

The Scanning electron microscopy (SEM) images of the biosorbent of immobilized nanoscale *L.edodes* before and after copper adsorption are shown in Fig. 5. It is obvious that the surface morphology of the beads before and after copper adsorption were different from each other. The surface of the bead before copper adsorption was relatively smooth and intensive, but the surface of the bead after copper adsorption was rugged and distorted, which may owe to the damage caused by the copper ion.

Furthermore, the energy dispersive spectrometry (EDS) images shown in Fig. 6 further confirmed the existence of copper ion onto the beads, which gave a direct detection of copper ion onto the beads.



(a) **(b)**

Fig. 4. The linear fitting figures of (a) Langmuir isotherm models and (b) Freundlich isotherm models

Table 8. The related parameters for adsorption isotherns

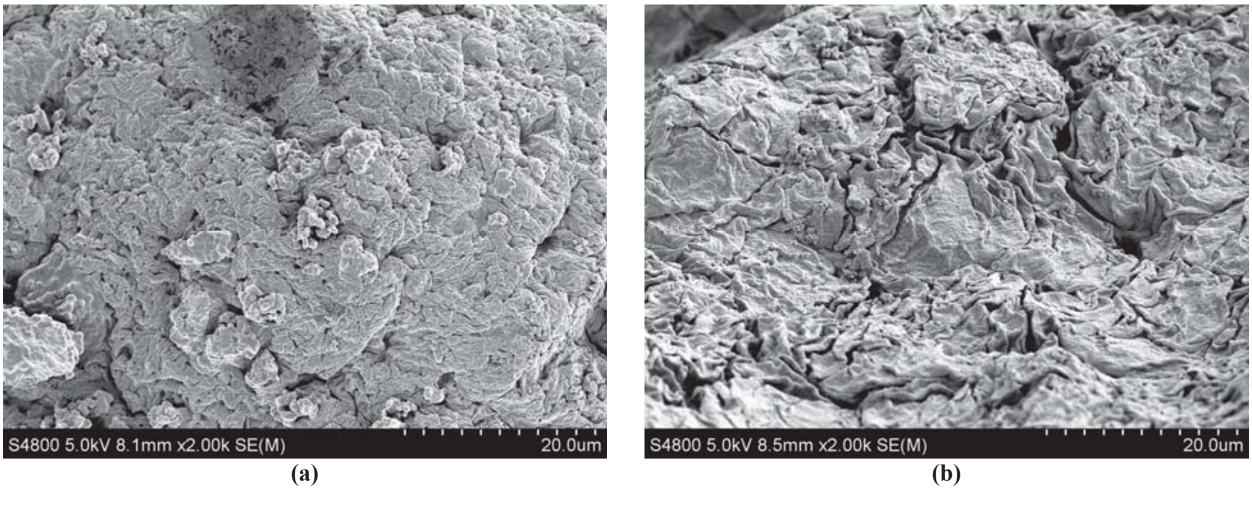
Metal ion	*langmuir*			*freundlich*		
	q_m(mg/g)	*k_a(L/mg)*	*R^2*	*K_F (mg/g)*	*n*	*R^2*
Cu(II)	34.48	0.0135	0.943	1.149	1.6	0.998

The infrared spectroscopy analysis of the biosorbent before and after copper adsorption presenting the evidence of the possible presence of carboxyl, hydroxyl and amino groups and the presence of corresponding characteristic peaks were shown in Fig. 7. The adsorption peak ranged from 3407 to 3420 cm^{-1} representing –OH stretching of carboxylic groups and stretching of –NH groups. alkyl CH_2 peaks at wavenumber 2817 cm^{-1} to 2847 cm^{-1}. The bending of N–H groups appeared at around 1646 cm^{-1} to 1653cm^{-1}. The peaks at 1418 to 1424 cm^{-1}, 1314 to 1317 cm^{-1} and 1035–1038 cm^{-1}represented CH_2 bending vibrations, C-N stretching and C-O stretching, respectively. These shifts may be attributed to the changes in counter ions associated with carboxylate and hydyroxylate anions, suggesting that acidic groups, carboxyl and hydroxyl, are predominant contributors in metal ion uptake.

3.6. Desorption studies

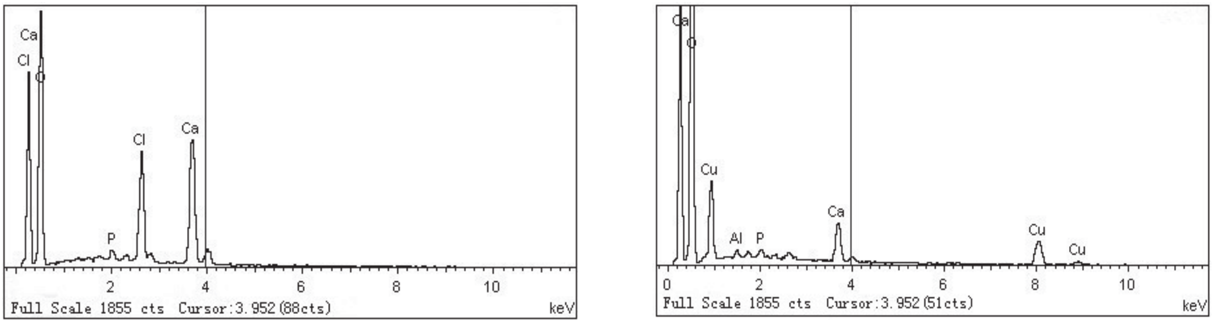
The desorption of the biosorbent is carried out to evaluate the reusability of the biosorbent. In this study, the desorption tests were carried out in 150mL conical flasks with 50mL 1M HNO_3 solution in half an hour. The results of three adsorption-desorption cycle tests were shown in Table 9. It can be perceived that the adsorption capacity of the biosorbent reduced inapparently with only 8.66% after three adsorption-desorption cycles.

Meanwhile, the desorption of the biosorbent was of high percentage, which indicated that the physisorption and chemisorptions were the main mechanism of adsorption (Doyurum and Celik, 2011). Therefore, immobilized nanoscale *L.edodes* could be an excellent biosorbent for its high reusability.



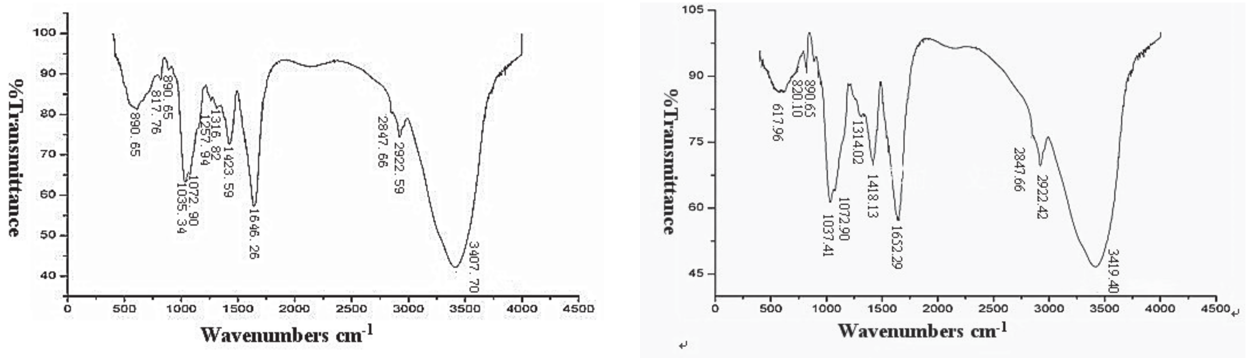
(a) **(b)**

Fig. 5. SEM micrograph of immobilized nanoscale *L. edodes*: (a) surface image before copper adsorption, (b) surface image after copper adsorption



(a) **(b)**

Fig. 6. EDS analysis of immobilized nanoscale *L.edodes*: (a) before copper adsorption; (b) after copper adsorption



(a) **(b)**

Fig. 7. FTIR spectra of immobilized nanoscale *L.edodes*: (a) before copper adsorption; (b) after copper adsorption

Table 9. Three adsorption/desorption cycles of immobilized nanoscale *L.edodes* (biomass loading = 2g/L, pH=6, initial ion concentration=50mg/L, contact time =540 min, agitation speed=125rpm, temperature=25°C)

Cycle one	Adsorption capacity (mg/g)	13.31
	Desorption (%)	96.50
Cycle two	Adsorption Capacity (mg/g)	13.25
	Desorption (%)	96.08
Cycle three	Adsorption Capacity (mg/g)	12.16
	Desorption (%)	94.75

4. Conclusions

This study has demonstrated that the usefulness of a factorial experimental design to model the biosorption of copper by immobilized nanoscale *L.edodes*.

Using the experimental design, the variables namely biomass loading, initial metal ions concentration, pH, contact time, agitation speed and temperature were studied effectively and optimized with a lesser number of experiments.

The optimal conditions for copper biosorption from aqueous solution using immobilized nanoscale *L.edodes* as a biosorbent are pH of 6 and contact time of 540min as well as agitation speed of 125rpm. The maximum removal rate of copper could reach to 97.57%.

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References

Ahmet O., Dursun O., Ayla O., (2004), The adsorption of copper(II) ions on to dehydrated wheat bran (DWB): determination of the equilibrium and thermodynamic parameters, *Process Biochemistry*, **39**, 2183-2191.

Anis M., Haydar S., Bari A.J., (2013), Adsorption of lead and copper from aqueous solution using unmodified wheat straw, *Environmental Engineering and Management Journal*, **12**, 2117-2124.

Bansal M., Mudhoo A., Garg V.K., Singh D., (2016), Sequestration of copper (II) from simulated wastewater using pre-treated rice husk waste biomass, *Environmental Engineering and Management Journal*, **15**, 1689-1703.

Bayramoglu G., Bektas S., Arlca M.Y., (2003), Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*, *Journal of Hazardous Materials*, **101**, 285-300.

Bayramoğlu G., Tuzun I., Celik G., Yilmaz M., Arica M.Y., (2006), Biosorption of mercury(II), cadmium(II) and lead(II) ions from aqueous system by microalgae *Chlamydomonas reinhardtii* immobilized in alginate beads, *International Journal of Mineral Processing*, **81**, 35-43.

Benaissa H., Elouchdi M.A., (2011), Biosorption of copper (II) ions from synthetic aqueous solutions by drying bed activated sludge, *Journal of Hazardous Materials*, **194**, 69-78.

Bulai P., Balan C., Bilba D., Macoveanu M., (2009), Study of the Copper (II) removal from aqueous solutions by Chelating Resin Purolite S930, *Environmental Engineering and Management Journal*, **8**, 213-218.

Chunshui Z., Liping W., Wenbin C., (2009), Removal of Cu(II) from aqueous solution by agricultural by-product: peanut hull, *Journal of Hazardous Materials*, **168**, 739-746.

Crisafully R., Milhome M.A.L., Cavalcante R.M., Silveira E.R., Keukeleire D.D., Nascimento R.F., (2008), Removal of some polycyclic aromatic hydrocarbons from petrochemical wastewater using low-cost adsorbents of natural origin, *Bioresource Technology*, **99**, 4515-4519.

Doyurum S., Celik A., (2006), Pb(II) and Cd(II) removal from aqueous solutions by olive cake, *Journal of Hazardous Materials*, **138**, 22-28.

Ertugay N., Bayhan Y.K., (2008), Biosorption of Cr(VI) from aqueous solutions by biomass of A*garicus bisporus*, *Journal of Hazardous Materials*, **154**, 432-439.

Ertugay N., Bayhan Y.K., (2010),The removal of copper(II) ion by using mushroom biomass (*Agaricus bisporus*) and kinetic modelling, *Desalination*, **255**, 137-142.

Goksungur Y., Uren S., Guvenc U., (2005), Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass, *Bioresource Technology*, **96**, 103-109.

Hasan S.H., Srivastava P., (2009), Batch and continuous biosorption of Cu(2+) by immobilized biomass of *Arthrobacter* sp, *Journal of Environmental Management*, **90**, 3313-3321.

Hasan S.H., Srivastava P., Talat M., (2009), Biosorption of Pb(II) from water using biomass of *Aeromonas hydrophila:* central composite design for optimization of process variables, *Journal of Hazardous Materials*, **168**, 1155-1162.

Isik M., (2008), Biosorption of Ni(II) from aqueous solutions by living and non-living ureolytic mixed culture, *Colloids and Surfaces B-Biointerfaces*, **62**, 97-104.

Khalil T.E., Tarek E., Altaher H., Abubeah R., (2016), Adsorptive removal of Cu (II) ions by date pits: kinetic and equilibrium studies, *Environmental Engineering and Management Journal*, **15**, 2719-2732.

Kumar R., Bishnoi N.R., Bishnoi G.K., (2008), Biosorption of chromium(VI) from aqueous solution and electroplating wastewater using fungal biomass, *Chemical Engineering Journal*, **135**, 202-208.

Larous S., Meniai A.H., Lehocine M.B., (2005), Experimental study of the removal of copper from aqueous solutions by adsorption using sawdust, *Desalination*, **185**, 483-490.

Mack C., Wilhelmi B., Duncan J.R., Burgess J.E., (2007), Biosorption of precious metals, *Biotechnology Advances*, **25**, 264-271.

Melgar M.J., Alonso J., Garcia M.A., (2007), Removal of toxic metals from aqueous solutions by fungal biomass of *Agaricus macrosporus*, *Science of the Total Environment*, **385**, 12-19.

Pakshirajan K., Swaminathan T., (2009), Biosorption of copper and cadmium in packed bed columns with live immobilized fungal biomass of *Phanerochaete chrysosporium*, *Applied Biochemistry and Biotechnology*, **157**, 159-173.

Preetha B.,Viruthagiri T., (2007), Application of response surface methodology for the biosorption of copper using *Rhizopus arrhizus*, *Journal of Hazardous Materials*, **143**, 506-510.

SarI A.,Tuzen M., (2009), Kinetic and equilibrium studies of biosorption of Pb(II) and Cd(II) from aqueous solution by macrofungus (*Amanita rubescens*) biomass, *Journal of Hazardous Materials*, **164**, 1004-1011.

Tajar A.F., Kaghazchi T., Soleimani M., (2009), Adsorption of cadmium from aqueous solutions on sulfurized activated carbon prepared from nut shells, *Journal of Hazardous Materials*, **165**, 1159-1164.

Tong K.S., Kassim M.J., Azraa A., (2011), Adsorption of copper ion from its aqueous solution by a novel biosorbent *Uncaria gambir*: Equilibrium, kinetics, and thermodynamic studies, *Chemical Engineering Journal*, **170**, 145-153.

Vilar V.J., Loureiro J.M., Botelho C.M.S., Boaventura R.A.R., (2008), Continuous biosorption of Pb/Cu and Pb/Cd in fixed-bed column using algae *Gelidium* and granulated agar extraction algal waste, *Journal of Hazardous Materials*, **154**, 1173-1182.

Vimala R., Das N., (2009), Biosorption of cadmium (II) and lead (II) from aqueous solutions using mushrooms: a comparative study, *Journal of Hazardous Materials*, **168**, 376-382.

Wang L., Chua H., Sin S.N., Zhou Q., Li. Z.L., (2004), A combined bioprocess for integrated removal of copper and organic pollutant from copper-containing municipal wastewater, *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, **39**, 223-235.

Xiaoming L., Dexiang L., Xueqin X., Qi Y., Guangming Z.,Wei Z., Liang G., (2008), Kinetic studies for the biosorption of lead and copper ions by *Penicillium simplicissimum* immobilized within loofa sponge, *Journal of Hazardous Materials*, **159**, 610-615.

Yanru C., Zhuan L., Guanglei C., Xiao-bing J., Heng X.,(2010) Exploring single and multi-metal biosorption by immobilized spentTricholoma lobayense using multi-step response surface methodology, *Chemical Engineering Journal*, **164**, 183-195.