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# Biosorption of copper (II) onto immobilized cells of *Pycnoporus sanguineus* from aqueous solution: Equilibrium and kinetic studies

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#### ABSTRACT

The ability of white-rot fungus, *Pycnoporus sanguineus* to adsorb copper (II) ions from aqueous solution is investigated in a batch system. The live fungus cells were immobilized into Ca-alginate gel to study the influence of pH, initial metal ions concentration, biomass loading and temperature on the biosorption capacity. The optimum uptake of Cu (II) ions was observed at pH 5 with a value of 2.76 mg/g. Biosorption equilibrium data were best described by Langmuir isotherm model followed by Redlich–Peterson and Freundlich models, respectively. The biosorption kinetics followed the pseudo-second order and intraparticle diffusion equations. The thermodynamic parameters enthalpy change (10.16 kJ/mol) and entropy change (33.78 J/mol K) were determined from the biosorption equilibrium data. The FTIR analysis showed that –OH, –NH, C–H, C=O, –COOH and C–N groups were involved in the biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus*. The immobilized cells of *P. sanguineus* were capable of removing Cu (II) ions from aqueous solution.

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#### 1. Introduction

Malaysia's rapid industrialization in electroplating, manufacturing, mining and automotive has accelerated the heavy metals pollution to the environment. Copper (II) is one of the toxic compounds commonly found in an electroplating industrial effluents [1]. Disposal of industrial effluents containing copper (II) ions into natural water beyond limits may harm the living organisms including human [1,2]. Conventional methods used to remove metal ions from industrial wastewater include chemical precipitation, electrochemical treatment, ion exchange process, membrane separation and evaporation [2,3]. However some of the conventional technologies are ineffective and unfavorable as they cause sludge disposal problem, expensive and incomplete removal [4,5].

The utilization of biosorption technology for the treatment of heavy metal contaminated wastewaters has become an alternative method to conventional treatments [6–9]. Biosorption utilizes various natural materials including fungi, yeast and bacteria that have been studied to sequester metal ions from aqueous solution [10]. Metal ions uptake by microorganisms involves several chemical processes including adsorption, ion exchange, covalent binding and co-ordination. [11]. Fungi have been known capable to remove metal ions from industrial effluent [12]. Many fungal species such as *Aspergillus niger*, *Mucor* spp., *Phanerochaete crysosporium*, *Rhizopus* spp. and *Saccharomyces* spp., have been extensively studied as a potential biosorbent in metal ions removal as it is inexpensive and abundant [8,13–15]. Fungi are also been recognized as a promising low-cost biosorbent for heavy metals biosorption from aqueous solution [16,8,17–19].

White-rot fungus, Pycnoporus sanguineus have been reported to be capable of decolorizing 98% and 100% of bromophenol blue and malachite green [20]. Though dead cells of P. sanguineus are also reported as a potential biosorbent for Pb (II), Cu (II) and Cd (II), no studies have reported on Cu (II) biosorption by immobilized live cells system of P. sanguineus [1]. In industrial operation, immobilization is one of the methods used to overcome the incorporating free suspended cells in wastewater treatment. It offers several advantages include minimal clogging in continuous systems [10,21,22]. The cells are easy to separate from the reaction system and can be regenerated [10,23]. However, immobilized biosorbents have major disadvantages such as cost, poor mechanical strengths, instability at low pH, cell leakage and diffusional limitations [22,24–29]. Biopolymers such as alginate, agarose, cellulose acetate and gluteraldehyde are widely used as immobilization matrices as they are non-toxic, inexpensive and efficient [30].

The present study was conducted to determine the ability of live immobilized cells of *P. sanguineus* in alginate to adsorb Cu (II) ions in a batch system. The biosorption equilibrium and kinetic

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$a_{\rm rp}$ Redlich-Peterson isotherm constant $(L/mg)^{\beta}$ $C_{\rm e}$ equilibrium concentration $(mg/L)$ $C_{\rm f}$ final concentration $(mg/L)$ $C_{\rm i}$ initial concentration $(mg/L)$ $E_{\rm a}$ activation energy $(kJ/mol)$ $\Delta G^{\circ}$ Gibbs free energy change $(J/mol)$ $\Delta H^{\circ}$ standard enthalpy $(kJ/mol)$							
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$\Delta H^{\circ}$ standard enthalpy (kJ/mol)							
15 ( 5/ )							
<i>k</i> <sub>1</sub> rate constant of first-order biosorption (min <sup>-1</sup> )							
<i>k</i> <sub>2</sub> rate constant of second-order biosorption	1						
(g/mg min)							
<i>K</i> <sub>b</sub> Langmuir equilibrium constant (L/mg)							
K <sub>f</sub> Freundlich constant							
$K_{\rm rp}$ Redlich–Peterson isotherm constant (L/mg)							
$K_{\rm s}$ intraparticle diffusion constant (mg/(g min <sup>0.5</sup> ))							
MT metric ton							
<i>n</i> Freundlich constant							
q metal ions biosorbed per gram of biomass (mg/g)							
$q_e$ amount of metal ions uptake at equilibrium (mg/g							
$q_{\text{max}}$ maximum specific uptake corresponding to the sites	saturation (mg/g)						
saturation $(mg/g)$	-						
$q_t$ amounts of adsorbed Cu (II) ions on the biosorben	2						
$\frac{dt time t (iiig/g)}{dt time t (l/mol K)}$							
R gas law constant (J/IIIOLK)							
$\Lambda_{\rm L}$ Separation identity (1/mol K)							
$\Delta S^{*}$ Standard entropy (J/mork)							
W weight of biosorbent (g)							
w weight of biosofbent (g)							
Greek letter							
$\beta$ Redlich–Peterson isotherm constant							

data are fitted using different models and process parameters were evaluated.

#### 2. Material and methods

#### 2.1. Microorganism, medium and growth conditions

*P. sanguineus* capable of adsorbing heavy metals was obtained from Forest Research Institute Malaysia (FRIM), Kepong, Selangor. The culture was maintained by weekly transfers on malt extract agar slant incubated at 30 °C for 6 days, after which they were stored at 4 °C until required.

The composition of the medium was comprised of: glucose; 20 g/L, yeast extract; 10 g/L and malt extract; 10 g/L, respectively. The pH of the medium was adjusted to pH 9 prior autoclaving at  $121 \degree$ C ( $150 \text{ kN/m}^2$ ) for 15 min.

The cell suspension was prepared by inoculating a stock culture of *P. sanguineus* onto malt extract agar plates and incubated at 27 °C for 6 days. The mycelium mat that was formed was scraped by a sterile blade and mixed with 10 mL of sterile Tween 20 solution prior to putting it into a sterile sampling bottle (100 mL). The sampling bottle was then vortexed for 3 min so that the mycelium will be evenly distributed in the liquid.

Fifteen millilitre of cell suspension was inoculated into an Erlenmeyer flask containing 135 mL of the production medium. The flask was incubated in a rotary shaker at  $30 \degree$ C, 150 rpm for 66 h. The sample was harvested and centrifuged at 3500 rpm for 4 min.

#### 2.2. Methods

#### 2.2.1. Preparation of immobilized cell beads

Immobilized cell beads were prepared by dropping a mixture of sodium alginate solution and *P. sanguineus* cells into 2% (w/v) CaCl<sub>2</sub> solution under magnetic stirring (slow) at  $25 \pm 3.0$  °C. The beads of  $50 \pm 0.2$  mm in diameter, were stirred in this solution for 30 min. Successively, these were collected by filtration, washed three times with sterile deionized water and stored in Tris–HCl buffer pH 7 at 4 °C until further use.

#### 2.2.2. Preparation of metal ions

Metal solutions were prepared by diluting 1000 mg/L of  $Cu(NO_3)_2 \cdot 3H_2O$  (Merck) solutions with deionized water to a desired concentration range between 50 and 300 mg/L. The initial concentration of the metal in the solution and samples after biosorption treatment were determined using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

#### 2.2.3. Biosorption procedures

Biosorption of copper (II) ions on the immobilized cells of P. sanguineus was investigated in a batch system. The effect of pH, initial metal concentration, biomass loading and temperature were studied. The effect of solution pH on the biosorption rate of the immobilized P. sanguineus preparation with Cu (II) ions (100 mL) was investigated in the range of 2-5 (which was adjusted with 0.1 M HCl or 0.1 M NaOH at the beginning of the experiment and not controlled during experimentation). Experiments were conducted in 250 mL Erlenmeyer flasks at constant temperature where metal ions with solutions of different pH (100 mL) were placed in these flasks. 3.0 g of immobilized cells were added into metals solution and shaken at 150 rpm, 30 °C for 5 h. The effect of initial metal concentration on the biosorption capacity of immobilized cells of *P. sanguineus* was investigated in the range of 50–300 mg/L carried out at pH 5 and similar procedures were repeated. The effect of biomass loading was varied between 1.0 and 6.0 g and the effect of temperature was studied at different temperatures of 30 °C (303 K), 35 °C (308 K) and 40 °C (313 K), respectively. The Cu (II) ions biosorption using free cell of P. sanguineus was also been carried out in this study with a solution pH in the range of 2-5.

The amount of metal bound by the biosorbent was calculated using the following Eq. (1):

$$q = \frac{V(C_{\rm i} - C_{\rm f})}{W} \tag{1}$$

where *q* is milligram of metal ion biosorbed per gram of biomass (mg/g),  $C_i$  (mg/L) is the initial concentration,  $C_f$  (mg/L) is the final concentration, *V* (L) is the volume of metal solution in the flask and *W* (g) is the weight of biosorbent. Each experiment was repeated three times and the average values are used in this study. Samples taken after the desired incubation period were analyzed for concentration of Cu (II) ions using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

Kinetic experiments were carried out at a known biosorbent loading with varied temperature and agitation speed at 150 rpm. At different time intervals, samples were withdrawn, filtered and the Cu (II) concentration was measured using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

#### 2.3. Analysis methods

The infrared (IR) spectra of immobilized cells of *P. sanguineus* before and after biosorption treatment were obtained using Fourier Transform Infrared Spectrometer (PelkinElmer FTIR 2000, USA). The biosorbent was encapsulated in a KBr disk and by pressing the ground material with 8 MT pressure bench press, the translucent disks were obtained.

#### 2.4. Equilibrium and kinetics studies

#### 2.4.1. Equilibrium isotherm models

The biosorption isotherm data of Cu (II) ions onto immobilized cells of *P. sanguineus* at different temperatures were analyzed using Langmuir, Freundlich and Redlich–Peterson equations.

The Langmuir model assumes a monolayer sorption of a solute from a liquid solution [31,32]. The Langmuir equation is given by Eq. (2)

$$q_{\rm e} = \frac{q_{\rm max}K_{\rm c}C_{\rm e}}{1 + K_{\rm c}C_{\rm e}} \tag{2}$$

where  $q_{\text{max}}$  is the maximum specific uptake of metal ions corresponding to the sites saturation (mg/g) and  $K_c$  is an equilibrium constant (L/mg). The parameters can be determined from a linearised form of Eq. (2)

$$\frac{C_{\rm e}}{q_{\rm e}} = \frac{1}{q_{\rm max}K_{\rm c}} + \frac{C_{\rm e}}{q_{\rm max}} \tag{3}$$

where  $C_e$  is the equilibrium concentration (mg/L),  $q_e$  is the amount of metal ions uptake at equilibrium (mg/g).

The essential characteristics of a Langmuir isotherm can be represented in terms of dimensionless constant separation factors,  $R_L$  is given as [55]:

$$R_{\rm L} = \frac{1}{1 + K_{\rm C}C_{\rm o}}\tag{4}$$

where  $C_0$  is the initial Cu (II) concentration (mg/L) and  $K_c$  is the equilibrium constant (L/mg). The isotherm is linear when  $R_L = 1$ , the isotherm is favorable when  $0 < R_L < 1$ , the isotherm is unfavorable when  $R_L > 1$  and the isotherm is irreversible when  $R_L = 0$ .

The Freundlich model is based on sorption on a heterogeneous surface [2,33]. Freundlich isotherm model is represented as Eq. (5):

$$q_{\rm e} = K_{\rm f} C_{\rm e}^n \tag{5}$$

The equation may be linearised and represented by Eq. (6)

$$\log q_{\rm e} = \frac{1}{n} \log C_{\rm e} + \log K_{\rm f} \tag{6}$$

where  $q_e$  is the amount of metal ions uptake  $(mgg^{-1})$  at equilibrium,  $C_e$  is the equilibrium concentration (mg/L),  $K_f$  and n are the Freundlich constants indicative of adsorption capacity and adsorption intensity, respectively.

The Redlich–Peterson isotherm incorporates the features of the Langmuir and the Freundlich isotherms and has three parameters  $K_{\rm rp}$ ,  $a_{\rm r}$  and  $\beta$  [2]. It can be described by Eq. (7):

$$q_{\rm e} = \frac{K_{\rm rp}C_{\rm e}}{1 + a_{\rm r}C_{\rm e}^{\beta}} \tag{7}$$

where  $K_{\rm rp}$ ,  $a_{\rm r}$  and  $\beta$  (0 <  $\beta$  < 1) are the Redlich–Peterson isotherm constants. For  $\beta$  = 1, Eq. (7) converts to the Langmuir form. The isotherms constant were obtained by non-linear regression method using Polymath Software [34].

#### 2.4.2. Kinetic modeling

Various kinetic models were proposed to test the experimental data including pseudo-first, pseudo second order and intraparticle diffusion to examine the controlling mechanism involved in the biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus* such as mass transfer and chemical reactions [10].

The pseudo first order equation is given as [10,35–37]:

$$\log(q_{\rm e} - q_t) = \log(q_{\rm e}) - \frac{k_1}{2.303}t$$
(8)

where  $q_e$  and  $q_t$  are the amounts of adsorbed Cu (II) ions on the biosorbent at equilibrium and at time *t* (respectively mg/g) and  $k_1$ , is the first-order biosorption rate constant (min<sup>-1</sup>).

The pseudo second-order equation is also based on the sorption capacity of the solid phase and is given as [10]:

$$\frac{t}{q_{\rm t}} = \frac{1}{k_2 q_{\rm e}^2} + \frac{1}{q_{\rm e}} t \tag{9}$$

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

Intraparticle diffusion equation was introduced to indicate the behaviour of intraparticle diffusion as the rate-limiting step in the biosorption. The intraparticle equation can be described as [38,62]:

$$q_t = K_{\rm S} t^{0.5} \tag{10}$$

where  $q_t$  is the amount of adsorbed Cu (II) ions at time t, t is the contact time (min) and  $K_s$  is the intraparticle diffusion constant.

#### 2.4.3. Thermodynamic parameters

The thermodynamic parameters such as Gibbs free energy change ( $\Delta G^{\circ}$ ), enthalpy ( $\Delta H^{\circ}$ ) and entropy change ( $\Delta S^{\circ}$ ) must be considered in order to determine what processes will occur spontaneously [32,39]. In order to determine thermodynamic parameters, experiments were carried out at different temperatures of 303–313 K for Cu (II) biosorption. The Gibbs free energy change ( $\Delta G^{\circ}$ ) of the sorption reaction is given as:

$$\Delta G^{\circ} = -RT \ln K_{\rm c} \tag{11}$$

$$\ln K_{\rm c} = \frac{\Delta S^{\circ}}{R} - \frac{\Delta H^{\circ}}{RT} \tag{12}$$

where *T* is the temperature (K), *R* is the gas constant (8.314 J/mol K),  $K_c$  is the equilibrium constant obtained from Langmuir isotherm. Enthalpy and entropy change can be obtained from a slope and intercept of a graph of ln  $K_c$  versus 1/T.

#### 3. Results and discussion

#### 3.1. Effect of pH solution

The effect of pH solution on Cu (II) ions uptake was carried out in the range of pH 2–5 at 100 mg/L of Cu (II) ions concentration. After shaking for 5 h, the samples were withdrawn and Cu (II) ions concentration was measured using AAS. The maximum Cu (II) uptake was observed at pH 5. At a pH lower than 3.0, little biosorption occurred. This could be due to a competition between copper ions and hydrogen ions thus resulted in lower metals uptake onto immobilized cells of *P. sanguineus* [40]. However, as the pH increased, the metal ion uptake increased. It has been reported in the literature that, pH affects the solubility of metal ions and the ionization of functional groups on the fungal cell wall [10,40,41]. Carboxyl group was identified as the functional groups that were responsible for the metal biosorption [42,43]. As the pH increased, these groups deprotonated and negative charges present resulted in high metals uptake [41]. When the metal solution was adjusted to pH above

## Table 1

Comparison of Cu (II) ions uptake by various biosorbent reported in literature

Biosorbent	Initial concentration (mg/L)	рН	Biomass loading (g)	Cu (II) ions uptake (mg/g)	Reference
Phanerochaete chrysosporium	100	6	0.2	10.72	[15]
Aspergillus niger (pretreated)	250	5	1.0	28.70	[2]
Pycnoporus sanguineus					
Live free cell	100	3	3.0	0.93	Present
Live immobilized	100	5	3.0	2.76	Present
Cladonia rangiformis hoffm					
R. opacus	20	6	2.0	4.79	[49]
A. polytricha	10	3	1.0	0.91	[50]
Spirogyra sp.	150	5	20.0	133.30	[51]

5.0, precipitation occurred and copper (II) biosorption onto biosorbent decreased. Therefore, no experiments were carried out at pH above 5.0. Table 1 shows the comparison of Cu (II) uptake by various biosorbent at different pH value. It is reported that dominant copper species in the solution of pH 3–5 were Cu<sup>2+</sup> and CuOH<sup>+</sup>, while a solution pH of above 6.3, the Cu<sup>2+</sup> ions precipitate and making the biosorption of Cu (II) ions impossible [44–46]. In this study, the Cu (II) uptake by free cells of *P. sanguineus* was not stable and lower compared to immobilized cells of *P. sanguineus*. The highest Cu (II) uptake by free cells of *P. sanguineus* was observed at pH 3. Metal uptake using immobilized cells was more efficient than the free cells [47]. Therefore only Cu (II) biosorption onto immobilized cells of *P. sanguineus* is discussed in the next section.

#### 3.2. Effect of initial Cu (II) concentration

Fig. 1 shows the effect of initial Cu (II) concentration onto immobilized cells of *P. sanguineus*. The biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus* was carried out at different Cu (II) concentrations varying from 50 to 300 mg/L at pH 5. Results showed that as the initial metal concentrations increased, Cu (II) ions uptake increased. Higher Cu (II) ions concentration increased the overall mass transfer driving force and thus the Cu (II) uptake onto the biosorption of Cu (II) onto pretreated *A. niger* [2].

#### 3.3. Effect of biosorbent loading and temperature

Fig. 2 shows the effect of immobilized cells loading on the Cu (II) sorption. The experiments were carried out at different







Fig. 2. Effect of biomass loading on the uptake of Cu (II) ions by immobilized cells of *P. sanguineus* (*Condition*: pH 5.0; 100 mg/L Cu (II) ions; agitation 150 rpm).

loadings of immobilized cells of *P. sanguineus* ranging from 1.0 to 6.0 g. Results showed that when the amount of biosorbent loading increased from 1.0 to 6.0 g, the Cu (II) removal increased from 17.2% to 55.7%. This could be due to the available more binding sites for the biosorption of Cu (II) ions to occur [40]. However, the Cu (II) uptake onto the biosorbents decreased as the loading increased. According to the previous work, higher biosorbent loading could produce a 'screening' effect on the cell wall, protecting the binding sites, thus resulting in lower Cu (II) uptake [1,54].



Fig. 3. Effect of temperature on the uptake of Cu (II) ions by immobilized cells of *P. sanguineus* (*Condition*: pH 5.0; 100 mg/L Cu (II) ions; agitation 150 rpm).

T (K)	(K) Langmuir			Freundlich			Redlich–Peterson			
	q <sub>max</sub> (mg/g)	$K_{\rm c}$ (L/mg)	R <sup>2</sup>	K <sub>f</sub>	n	R <sup>2</sup>	K <sub>rp</sub> (L/mg)	$a_{ m r}$ (L/mg) $^{\beta}$	β	$R^2$
303	2.774	1.026	1.000	1.581	7.740	0.796	1.900	0.669	1.000	0.988
308	2.899	1.106	1.000	1.655	7.752	0.836	2.591	0.882	1.000	0.997
313	2.962	1.168	1.000	1.731	8.237	0.840	3.457	1.168	1.000	1.000

Biosorption equilibrium constant obtained from Langmuir, Freundlich and Redlich–Peterson isotherms for the biosorption of Cu (II) ions onto immobilized cells of P. sanguineus

Fig. 3 shows the effect of temperature on the biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus*. The experiments were carried out at different temperatures ranging from 303 to 313 K. As the temperature increased from 303 to 313 K, the Cu (II) uptake increased from 1.44 to 2.18 mg/g. Therefore, it was found that the Cu (II) uptake was favorable at a higher temperature, thus indicating that the biosorption of Cu (II) onto biosorbent was a chemical reaction [55].

#### 3.4. Biosorption equilibrium isotherms

Table 2

Table 2 shows the equilibrium isotherm constants obtained using Langmuir, Freundlich and Redlich-Peterson models. Result shows that Langmuir isotherms model fitted well the experimental data followed by Redlich-Peterson and Freundlich. The values of  $q_{\rm max}$  increase with the increase of temperatures, indicating that the biosorption of Cu (II) ions onto immobilized cells of P. sanguineus is favorable at a higher temperature. R<sub>L</sub> values calculated for biosorption of Cu (II) ions onto immobilized cells at various temperature lies between zero and one indicating favorable biosorption [56]. The values of  $K_{\rm f}$  and n obtained from Freundlich isotherm model also increased with temperature. Even though the correlation coefficient,  $R^2$  is less than 0.9, the *n* values obtained are greater than 1.0 for all temperatures studied. Thus indicating that Cu (II) ions is favorably adsorbed by immobilized cells of *P. sanguineus* as the temperatures increased. For the Redlich–Peterson constant  $K_{rp}$ , the values also increased with the reaction temperatures and the value of  $\beta$  was 1.0 for all temperatures studied.

#### 3.5. Biosorption kinetics modeling

Table 3 lists the results obtained from the experimental data for the biosorption kinetics of Cu (II) ions onto immobilized cells of *P. sanguineus*. Results show that the intraparticle diffusion best described the biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus* followed by a pseudo second order equation. According to Weber and Morris [62], the linear plot of *q* versus  $t^{0.5}$  (Eq. (10)) (figure not shown) obtained indicates that intraparticle diffusion took place in the biosorption process [62]. As reported in literature, a chemical biosorption process took place between Cu (II) ions and the immobilized cells as the experimental data showed a correlation coefficients close to unity by pseudo second order [57].

#### Table 3

Kinetic constants using pseudo first-order, pseudo second-order and intraparticle diffusion models for the biosorption of Cu (II) ions onto immobilized cells of *P. sanguineus* at various temperatures

T(K)	) Pseudo first-order		Pseudo second	-order	Intraparticle diffusion	
	$k_1 ({ m min}^{-1})$	$R^2$	$k_2$ (g/mg min)	R <sup>2</sup>	$K_{\rm s} ({\rm mg}/({\rm gmin^{0.5}}))$	$R^2$
303	0.0058	0.508	0.0069	0.952	0.1534	0.989
308	0.0166	0.749	0.0091	0.961	0.1463	0.966
313	0.0039	0.595	0.0092	0.966	0.1485	0.966



Fig. 4. FTIR spectra of unloaded and Cu (II) loaded onto immobilized cells *P. san-guineus*.

#### 3.6. Thermodynamic studies

The Gibbs free energy change ( $\Delta G^{\circ}$ ) for the biosorption of Cu (II) onto immobilized cells of *P. sanguineus* were -65.84, -258.36 and -403.59 J/mol as the temperature increased from 303 to 313 K. The negative Gibbs free energy change of the process at all temperature indicates the spontaneous nature of the biosorption [2]. The standard enthalpy ( $\Delta H^{\circ}$ ) and entropy ( $\Delta S^{\circ}$ ) calculated were 10.18 kJ/mol and 32.55 J/mol K, respectively. A positive value of  $\Delta H^{\circ}$  indicates that the endothermic nature of the biosorption process and the positive value of  $\Delta S^{\circ}$  suggest increased randomness at the solids/solution interface during the biosorption of metal ions onto the biosorbent [2,58].

The activation energy,  $E_a$  was determined from a linear plot of Arrhenius equation and is given as:

$$\ln k_2 = \ln A_0 - \frac{E_a}{RT} \tag{13}$$

where  $A_o$  is the temperature independent factor called 'frequency factor',  $E_a$  is the Arrhenius activation energy of sorption (kJ/mol), Ris the gas law constant (8.314 J/mol K) and T is the absolute temperature (K). The activation energy evaluated was 20.38 kJ/mol and this high activation energy indicates that the biosorption of Cu (II) uptake onto immobilized cells of *P. sanguineus* is involved of chemisorption process [2].

#### 3.7. FTIR analysis

Fig. 4 shows the FTIR spectra of dried unloaded and loaded immobilized cells of *P. sanguineus* recorded in the range of 400–4000 cm<sup>-1</sup>. The FTIR spectrum at 3434, 2924, 1622, 1434 and 1032 cm<sup>-1</sup> of unloaded biosorbent assigned –OH, –NH groups [59], C–H<sub>2</sub> stretching [55], C=O stretching [60], –COOH [33] and C–N stretching vibrations [33,61]. These peak shifts to 3429, 2919, 1617, 1432 and 1024 cm<sup>-1</sup> after Cu (II) biosorption onto immobilized cells of *P. sanguineus*. This result shows that these functional groups

involved in Cu (II) biosorption onto immobilized cells of *P. san-guineus* [63].

#### 4. Conclusions

- The biosorption of Cu (II) ions has been examined using immobilized *P. sanguineus* cells.
- The results obtained indicate that initial Cu (II) concentration, pH, biomass loading and temperature highly affected the Cu (II) biosorption.
- Cu (II) uptake using immobilized cells of *P. sanguineus* were observed more efficient compared to free cells.
- The maximum biosorption capacity of Cu (II) ions was observed at pH 5. The Langmuir isotherm model fitted well to the experimental data followed by Redlich–Peterson and Freundlich isotherms.
- The kinetics of Cu (II) ions sorption for different loadings of immobilized cell of *P. sanguineus* was best described using pseudo-second order and intraparticle diffusion models. Thus, indicated that both chemical and diffusion reactions involved in the biosorption of Cu (II) ions onto immobilized cell of *P. sanguineus*.
- Thermodynamic constants such as ΔG°, ΔH° and ΔS° calculated indicates that the biosorption of Cu (II) ions onto immobilized cells is a spontaneous process and endothermic in nature.

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