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# Biosorption of copper (II) from aqueous solution by mycelial pellets of *Rhizopus oryzae*

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In the present study, mycelial pellets of *Rhizopus oryzae* (MPRO) were investigated as a potential biosorbent for the removal of copper (II) from aqueous solution. The effects of the pellet diameter, solution pH, contact time, initial copper (II) concentration and temperature were studied. The optimum diameter and pH for biosorption of copper (II) was found to be 1.0 to 1.2 mm and 4.0. Evaluation of the experimental data in terms of biosorption dynamics showed that the biosorption of copper (II) onto MPRO followed the pseudo-second-order kinetic model. The equilibrium data fitted very well to the Langmuir adsorption model in the concentration range of copper (II) and at all the temperatures studied. The calculated thermodynamic parameters ( $\Delta G^\circ$ ,  $\Delta H^\circ$  and  $\Delta S^\circ$ ) showed that the biosorption of copper (II) were feasible, spontaneous and endothermic at the temperature ranges of 298 to 318K.

**Key words:** Biosorption, copper (II), mycelial pellet, *Rhizopus oryzae*.

## INTRODUCTION

Heavy metal pollution is one of the most serious environmental problems today. Various industries, such as mining and smelting of metalliferous, energy and fuel production, metallurgy, iron and steel, production dis-

charge wastes containing different heavy metals into the environment. Thus, metal as a resource is depleting and also brings about serious environmental pollution, threatening human health and ecosystem (Ahluwalia and Goyal, 2007; Wang and Chen, 2009). Copper is a common useful metal but poses serious environmental problems as well. Undesired amounts of copper ions are released by several industries, e.g. dyeing, paper, petroleum, copper and brass plating (Rome and Gadd, 1987). The removal of copper from industrial waste waters is a problem of increasing concern that has been mostly solved by chemical and physical methods of treatment. Nevertheless, these processes are expensive in which some technological problems exist especially when applied to diluted metal solutions (Bhainsa and D'Souza, 2008). Therefore, the search for clean and competitive technologies is strongly recommended.

Biosorption is usually considered as an effective method and can significantly reduce the quantity of heavy metals in aqueous solutions (Ahluwalia and Goyal, 2005; Goyal et al., 2003). It has been proven that there are wide variety of micro-organisms, such as fungi (Fourest et al., 1994), yeasts (Park et al., 2005), bacteria (Mameri et al., 1999) and algae (Davis et al., 2003) that qualify in

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**Abbreviations:** MPRO, mycelial pellets of *Rhizopus oryzae*;  $C_i$ , Initial copper (II) concentration in aqueous solution (mg/L);  $C_q$ , final copper (II) concentration in aqueous solution (mg/L);  $V$ , volume of the solution (L);  $W$ , dry weight of MPRO (g);  $q_e$ , the amounts of copper (II) adsorbed on MPRO at equilibrium (mg/g);  $q_t$ , the amounts of copper (II) adsorbed on MPRO at a given time  $t$  (mg/g);  $k_1$ , the rate constant of Lagergren-first-order kinetic model ( $\text{min}^{-1}$ );  $k_2$ , the rate constant of pseudo-second-order kinetic model ( $\text{g}/(\text{mg}\cdot\text{min})$ );  $t$ , time for biosorption (min);  $C_e$ , the copper (II) concentration at equilibrium in aqueous solution (mg/L);  $K_F$ , the Freundlich constant ( $\text{mg}^{1-1/n}\text{L}^{1/n}/\text{g}$ );  $n$ , the Freundlich constant;  $q_m$ , the Langmuir constant (mg/g);  $K_L$ , the Langmuir constant (L/mg);  $R$ , the universal gas constant (8.314 J/(mol·K));  $T$ , the absolute temperature in Kelvin (K);  $\Delta G^\circ$ , change in Gibbs free energy (kJ/mol);  $\Delta H^\circ$ , change in enthalpy (kJ/mol);  $\Delta S^\circ$ , change in entropy (kJ/(mol·K)).

the uptake of heavy metals from aqueous solution. Mechanisms of biosorption are independent of cell metabolism. They are based on physical or chemical interactions between contaminants and functional groups of the cell wall (Li et al., 2009; Vierira and Volesky, 2000). Fungi is considered to be the superior biosorbent for heavy metals because of the abundant sources of different functional groups such as carboxyl, amine, hydroxyl, phosphate and sulphonate which are responsible for metal sorption in the cell wall of fungi (Tsezos, 1983; Baik et al., 2002; Carlile, 2001). There are several reports involving removal of heavy metals using freely suspended fungal biomass (Fourest and Roux, 1992; Sag and Kutsal, 1998). However, the use of freely suspended biomass for large scale process utilization is not practicable because of its smaller particle size, poor mechanical strength and little rigidity. In order to overcome this problem, some researchers try to use immobilized fungal biomass for copper uptake (Zhou and Kiff, 1991). The immobilized biomass, however, have major disadvantages, such as cost, cell leakage strengths, instability at low pH, poor mechanical and rate-limitation in diffusion, which make it hardly applied in industrial operation (Ganguly et al., 2007; Grimm, et al., 2005).

Mycelial pellets are often the preferable morphological forms of filamentous fungi in industrial fermentation processes (Schügerl et al., 1983), and the mycelial pellets of *Rhizopus* sp. are more and more applied in organic acid industrial production (Liao et al., 2007). Mycelial pellets have a compact central core with a fluffy (hairy), loosely packed filamentous, outer zone and many free mycelia flocculated at the outer zone (Fu et al., 2009). There has been no report on heavy metal biosorption by mycelial pellets of fungi until now. In this work, the potential of the pellet biomass of *Rhizopus oryzae* TZ-F46, a fumaric acid producing strain, for copper (II) removal from the aqueous solution was investigated. The biosorption process was studied with regard to the effect of pellet diameter, initial pH, contact time, initial copper (II) concentration and temperature. All the results obtained in this paper would provide a sound basis for further exploration.

## MATERIALS AND METHODS

### Preparation of the metal ion stock

A stock solution of copper (II) was prepared by dissolving required amount of analytical grade  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) in double distilled water to obtain a concentration of 500 mg/L. Further dilutions were made during the course of the experiment to obtain different concentrations as desired.

### Preparation of biomass

*R. oryzae* TZ-F46, a fumaric acid producing strain, was grown

aerobically in batch cultures at 35°C, as described previously (Fu et al., 2009). The different diameters of mycelial pellets were harvested under different culture conditions and collected by using stainless steel sieve, followed by boiling for 15 min in 0.2 M NaOH solution (1:10 w/v). The resulting biomass was washed extensively with distilled water, and then it was pressed against filter paper to remove the bound water.

### Batch biosorption studies

Copper (II) uptake was investigated in 250 ml Erlenmeyer flask. All experiments were conducted by mixing 100 ml of aqueous copper (II) solutions with 1.0 g (wet weight) of mycelial pellets of *R. oryzae* (MPRO). The mixtures of MPRO and copper (II) solution were shaken in an environmental shaker at 150 rpm and desired temperature for an appropriate time. The pH values of solutions were adjusted with dilute NaOH or  $\text{H}_2\text{SO}_4$  solution before introducing MPRO into the solution. At given time intervals, 1.0 ml of the suspensions were collected and centrifuged at 10,000 g for 5 min. The concentration of the residual copper (II) was analyzed by an atomic absorption spectrophotometer (PerkinElmer SIMAA 6000) (Chen et al., 2010). In all the studies involving wet biomass, an equal amount of the same biomass (triplicate) was dried at 333K over night to obtain the dry weight and the mean dry weight was used to calculate the copper (II) uptake (mg/g dry weight) given as:

$$q = (C_i - C_q) * V / W \quad (1)$$

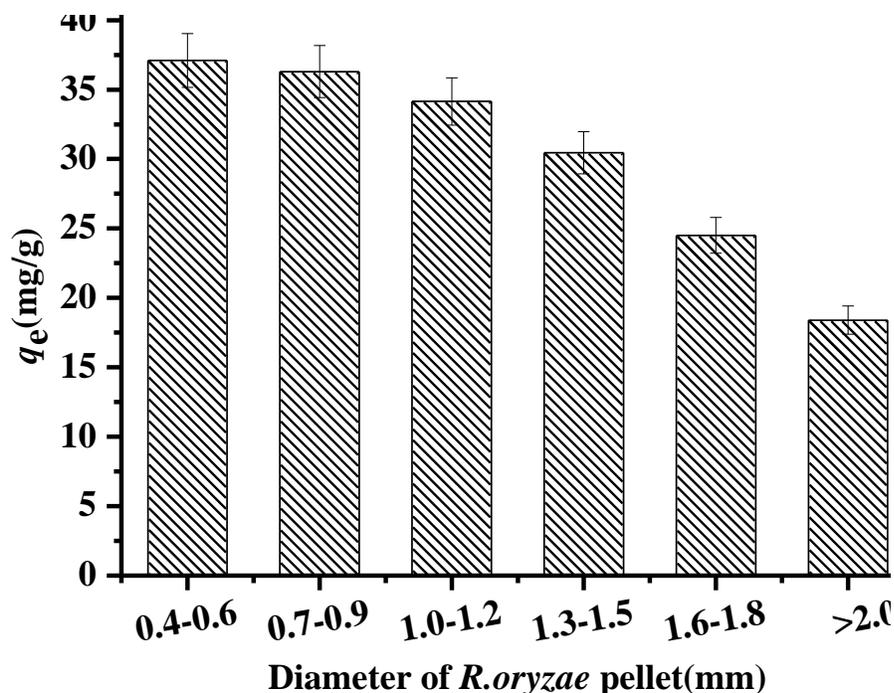
where  $C_i$  and  $C_q$  refer to the initial and final copper (II) concentration (mg/L), respectively,  $V$  represents volume (L) of the solution and  $W$ , the dry weight (g) of the biosorbent used in the study.

## RESULTS AND DISCUSSION

### Effect of pellet diameter

The diameter of pellet determines the surface area of the biosorbent and therefore, is a key factor in all biosorption processes. It also determines the number of metal binding functional groups which are readily exposed to the metal ions in solution. In this work, the effects of different pellet diameter (0.4 to 0.6, 0.7 to 0.9, 1.0 to 1.2, 1.3 to 1.5, 1.6 to 1.8 and >2.0 mm) on copper uptake were investigated at pH 4.0 at an initial copper(II) concentration of 100 mg/L (Figure 1).

It can be clearly observed that the copper (II) adsorption capacity of MPRO decreased from 37.1 to 18.4 mg/g when the pellet diameter increased from 0.4 mm to above 2.0 mm, which corresponded to a 1.02-fold decrease in copper (II) uptake. This was possibly due to an increased surface area, which was responsible for a greater availability of exposed binding sites in biomass of smaller diameter (Sudha and Abraham, 2001). However, the copper (II) uptake by MPRO with diameter in the range of 1.0 to 1.2 mm also reached 34.15 mg/g, which was only 8.0% decrease compared to that in the range of 0.4 to 0.6 mm. It may be the smaller diameter of pellet (< 1.0 mm) that formed the culture in lower pH and lower pH could inhibit the growth of the cell. Meanwhile, the mycelial pellets had a compact central core with a fluffy (hairy),



**Figure 1.** Effect of pellet diameter on copper (II) uptake by mycelial pellets of *Rhizopus oryzae* (Initial copper (II) concentration = 100.0 mg/L; pH = 4.0;  $T = 308\text{K}$ ).

loosely packed filamentous, outer zone and many free mycelia flocculated at the outer zone. So, although the smaller mycelial pellet had an increased surface area, it would supply less metal binding functional group at per unit volume than bigger pellet; but, when the diameter (>1.2 mm) continued to increase, the pellets would be autolysed, which could decrease the metal binding functional groups for copper (II) uptake. The most important was the best pellet diameter for fumaric acid fermentation which was in the range of 1.0 to 1.2 mm (Fu et al., 2009). Thus, the diameter in the range of 1.0 to 1.2 mm was selected as the optimum diameter of MPRO for the following adsorption experiment.

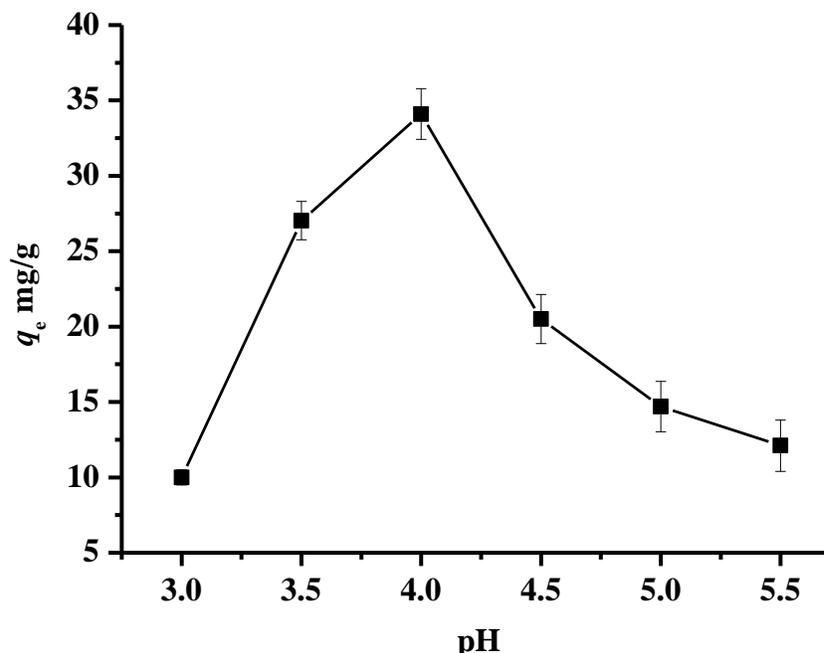
### Effects of pH

It has been consistently shown that pH is the dominant solution parameter controlling adsorption (Bhainsa and D'Souza, 2008; Fourest and Roux, 1992). Figure 2 shows the effect of pH on the adsorption of copper (II) by MPRO. Adsorption capacity of copper (II) by MPRO was analyzed over a pH range 3.0 to 5.5. It could be seen from Figure 2 that the adsorption capacity increased as the pH increased, and reached the maximum (34.1 mg/g) at pH 4.0, then decreased as the pH continued increasing. It was observed that the adsorption capacity was relatively less at low pH value (pH 3.0). The less metal uptake at this pH condition may be explained on

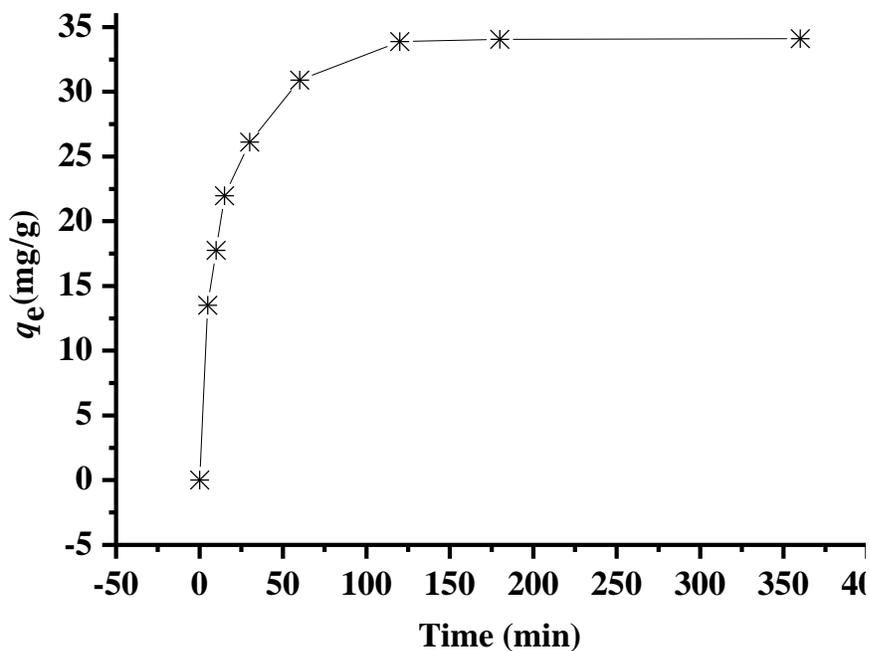
the basis of binding sites being protonated, resulting in a competition between  $\text{H}^+$  and copper (II) ions for occupancy of binding sites (Rome and Gadd, 1987; Sag and Kutsal, 1998). As the pH increased, the cell surface took more negative charges, thus attracting greater metal ions (Volesky and May-Phillips, 1995), but with further increase in pH level, the adsorption capacity decreased remarkably with increasing pH. It may be  $\text{OH}^-$  itself had a tendency to combine with copper (II), and it competed with ligand on cytoderm for metal ions, leading to the decrease of adsorption capacity when the pH was higher than a certain value (Vasconcelos et al., 2008). At the same time, the solution of copper (II) for sorption studies showed that the pH value could not exceed pH 6.0, because the insoluble copper hydroxide started precipitating from the solution at higher pH values, making true sorption studies impossible (Bhainsa and D'Souza, 2008).

### Adsorption kinetics

Equilibrium time is another important parameter to heavy metals waste water treatment process. The effect of contact time on the equilibrium uptake of copper (II) on MPRO for an initial copper (II) concentration of 100 mg/L is shown in Figure 3. Obviously, MPRO showed a good performance in adsorption during the first 30 min and 77% of copper was removed by MPRO in this stage. The



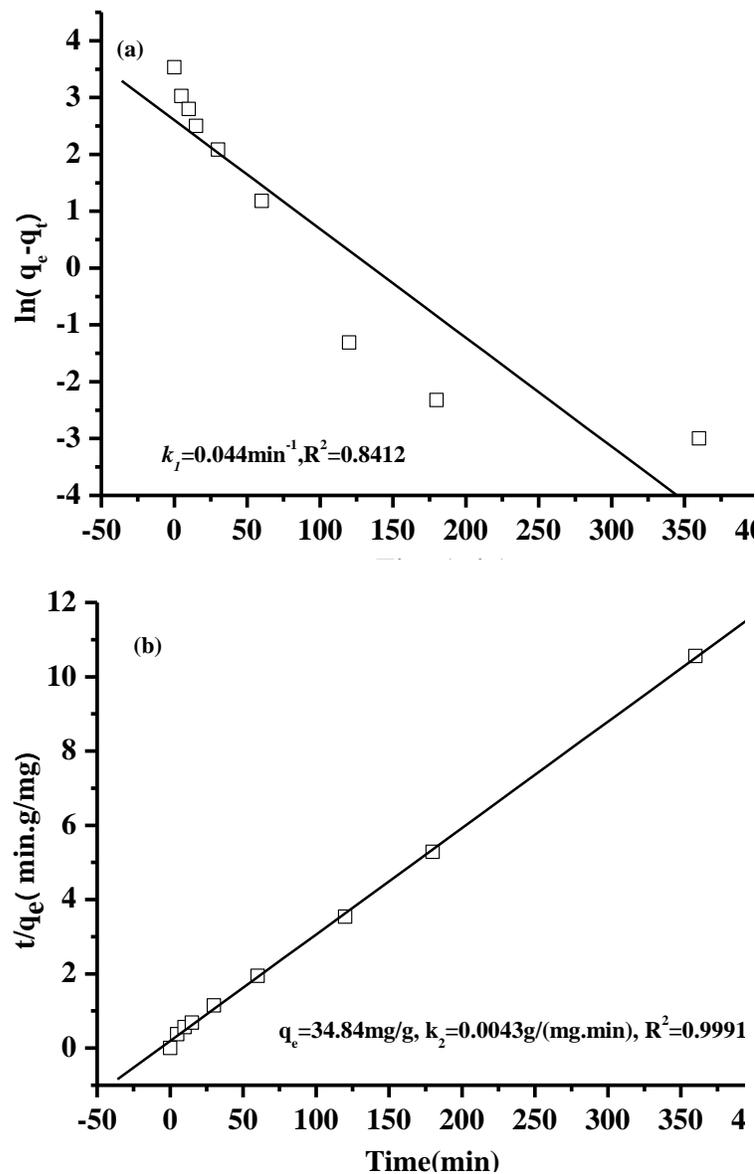
**Figure 2.** Effect of pH on copper (II) uptake by mycelial pellets of *Rhizopus oryzae* (Initial copper (II) concentration = 100.0 mg/L; pellet diameter = 1.0 to 1.2 mm;  $T = 308\text{K}$ ).



**Figure 3.** Effect of contact time on copper (II) uptake by mycelial pellets of *Rhizopus oryzae* (Initial copper (II) concentration=100.0 mg/L, pellet diameter = 1.0 to 1.2 mm, pH = 4.0,  $T = 308\text{K}$ ).

time required to achieve the adsorption equilibrium was only 120 min, and there was no significant change from 120 to 360 min.

In order to investigate the controlling mechanism of adsorption processes such as transfer and chemical reaction, two different kinetic models, Lagergren-first-



**Figure 4.** Linear plot of the kinetic models for copper (II) biosorption by mycelial pellets of *Rhizopus oryzae* (a) Lagergren-first-order kinetic model; (b) pseudo-second-order kinetic model.

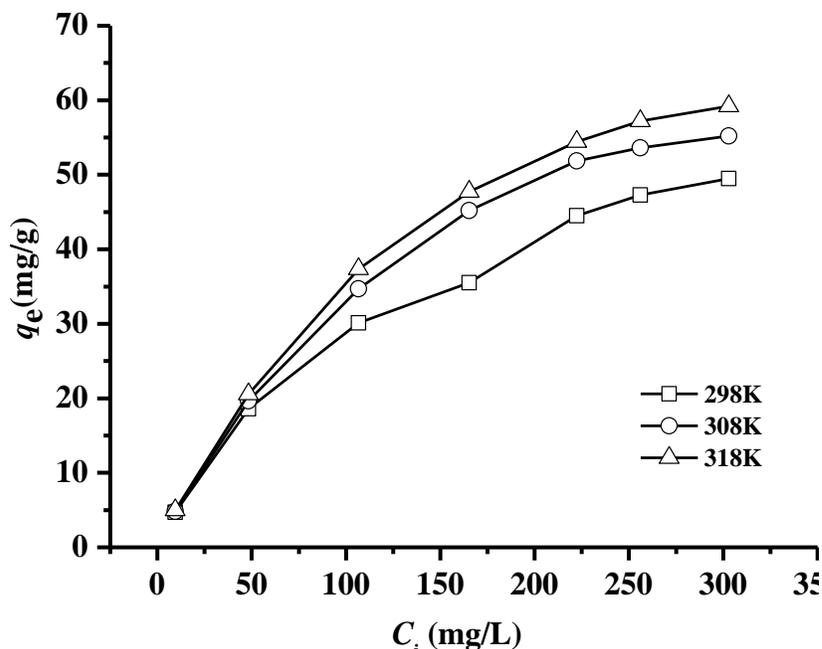
order model (Equation 2) and pseudo-second-order model (Equation 3) have been used to fit experimental data obtained from batch copper (II) removal experiments.

$$\ln(q_e - q_t) = \ln q_e - \frac{k_1 t}{2.3} \quad (2)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (3)$$

where  $q_e$  and  $q_t$  were the amounts of copper (II) (mg/g) adsorbed on the adsorbent at equilibrium and at a given time  $t$ , respectively;  $k_1$  was the rate constant ( $\text{min}^{-1}$ ) of Lagergren-first-order kinetic model,  $k_2$  was the rate constant ( $\text{g}/(\text{mg}\cdot\text{min})$ ) of pseudo-second-order kinetic model.

For Lagergren-first-order plot (Rakhshae et al., 2006), correlation coefficients were found to be 0.8412 (Figure 4A), which suggested that the model was unsuitable to fit the experimental data for the biosorption of copper (II) onto MPRO. Li et al. (2009) indicates that, in most cases, this model was not applicable for all experimental data



**Figure 5.** The effect of initial copper (II) concentration and temperature on copper (II) uptake by mycelial pellets of *Rhizopus oryzae* (pellet diameter = 1.0 to 1.2 mm, pH = 4.0).

throughout the whole biosorption process.

The value of the correlation coefficient ( $R^2$ ) for the pseudo-second-order model (Figure 4B) was  $\geq 0.999$ , and the adsorption capacity calculated by the model (34.84 mg/g) was also close to that determined by experiment (34.2 mg/g). The result indicates that it was feasible for the applicability of pseudo-second-order kinetic model to describe the adsorption process of copper (II) onto MPRO. The pseudo-second-order model is based on the assumption that the rate-determining step may be a chemical sorption involving valence forces through sharing or exchange of electrons between adsorbent and sorbate (Ho and McKay, 1999). In fact, the cell wall of *R. oryzae* contains 70 to 80% carbohydrates, that is, chitin, chitosan,  $\beta$ -1,3-D-glucans,  $\beta$ -1,6-D-glucans and small amount of mannoproteins and lipids. These are abundant sources of different functional groups like carboxyl, amine, hydroxyl, phosphate and sulphonate for metal adsorption (Chatterjee et al., 2010). Therefore, the copper (II) could be adsorbed by interaction between the copper (II) ions and functional groups in MPRO.

#### Effect of initial copper (II) concentration and temperature

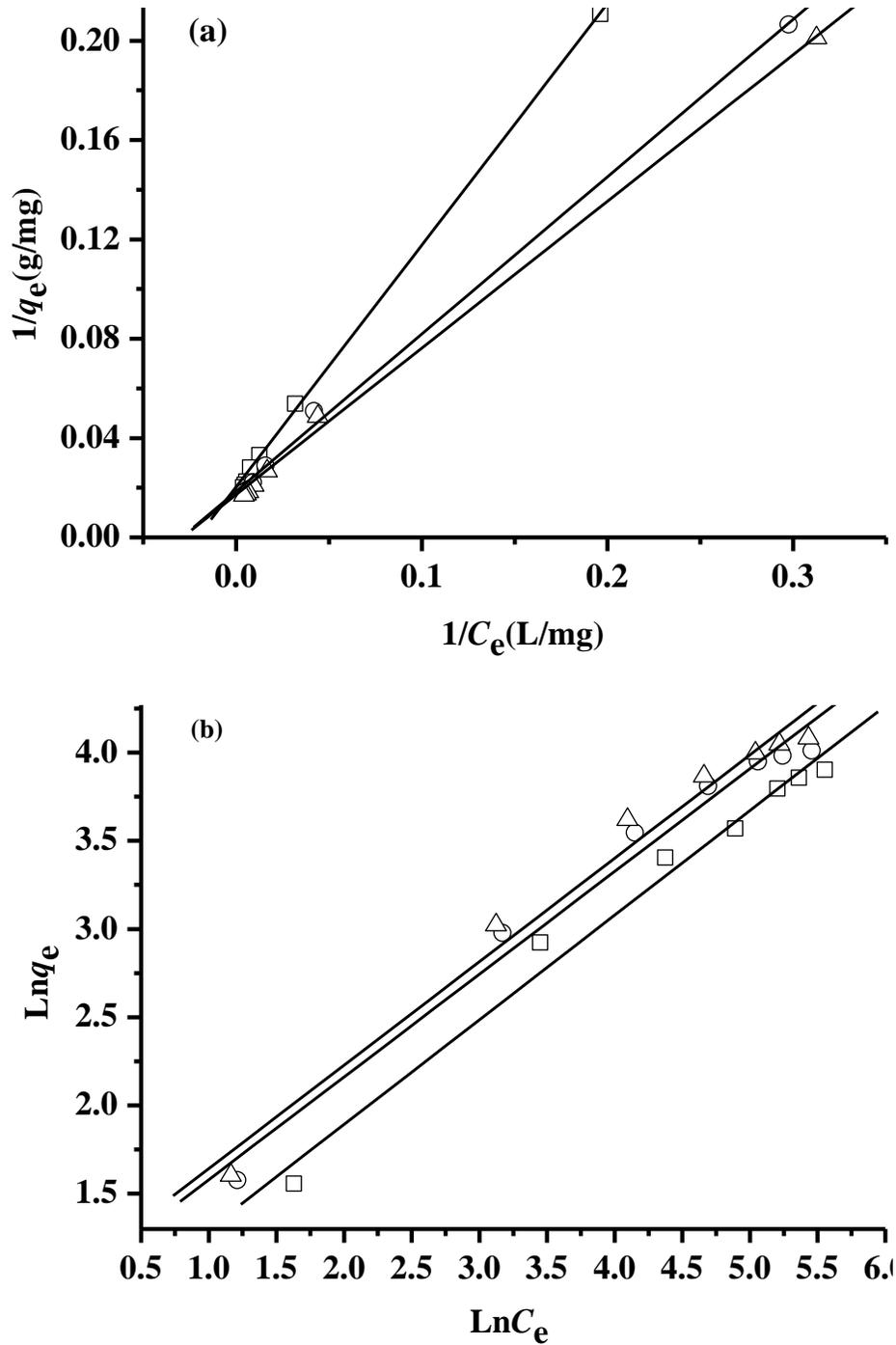
The effect of initial copper (II) concentration on the copper (II) biosorption by MPRO was investigated between 10.0 and 300.0 mg/L at three different temperatures (298, 308 and 318K) and the results are presented in Figure 5. As shown in Figure 5, the

adsorption capacity of copper (II) by MPRO enhanced notably with increasing the initial copper (II) concentration tending to saturation at higher copper (II) concentration. This is possible because a higher initial copper (II) concentration could provide an important driving force to overcome all mass transfer resistances of the copper between the aqueous and solid phases, thus increased the uptake.

The temperature also remarkably influenced the equilibrium copper (II) uptake. As shown in Figure 5, the adsorption capacity of copper (II) by MPRO is enhanced with raising the temperature up to 318K for both concentrations studied. The data also showed that the effect of temperature was significant at higher copper (II) concentrations. At 10.0 mg/L initial copper (II) concentration, the increase in equilibrium uptake capacity of MPRO with increasing temperature from 298 to 318K were only 0.32 mg/g; while the increment raised to 9.7 mg/g, with increasing initial copper (II) concentration up to 300.0 mg/L. The enhancement in adsorption with temperature may be attributed to increase in the number of active surface sites available for adsorption on MPRO.

#### Biosorption isotherms

In order to discover the sorption capacity of MPRO for copper (II), the equilibrium data at different temperatures were fitted by the empirical Langmuir and Freundlich adsorption model (Li et al., 2009; Bhainsa and D'Souza, 2008) (Figures 6a and b) which may be expressed as:



**Figure 6.** Copper (II) biosorption isotherms by mycelial pellets of *Rhizopus oryzae* at different temperature; (a) Langmuir model; (b) Freundlich model.

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{K_L q_m} \quad (4)$$

$$\text{Ln} q_e = \text{Ln} K_F + \frac{1}{n} \text{Ln} C_e \quad (5)$$

where,  $q_e$  is the amount of copper (II) (mg/g) adsorbed on the adsorbent at equilibrium;  $q_m$  (mg/g) and  $K_L$  (L/mg), Langmuir constants related to theoretical maximum adsorption amount of copper (II) and bonding energy of adsorption, respectively;  $C_e$  (mg/L), the copper (II) concentration at equilibrium,  $K_F$  ( $\text{mg}^{1-1/n} \text{L}^{1/n}/\text{g}$ ) and  $n$ , the Freundlich adsorption constants. Linear regression analy-

**Table 1.** Langmuir and Freundlich parameters for copper (II) sorption by MPRO.

T (K)	Langmuir			Freundlich		
	$q_m$ (mg/g)	$K_L$ (L/mg)	$R^2$	$K_F$ (mg <sup>1-1/n</sup> L <sup>1/n</sup> /g)	n	$R^2$
298	52.91	0.019	0.9987	2.03	1.68	0.9844
308	57.47	0.025	0.9978	2.71	1.70	0.9810
318	61.73	0.028	0.9981	2.87	1.72	0.9793

T, the absolute temperature in Kelvin;  $q_m$ , the Langmuir constant;  $K_L$ , the Langmuir constant; R, the universal gas constant;  $K_F$ , the Freundlich constant; n, the Freundlich constant.

**Table 2.** Thermodynamic parameters for the adsorption of copper (II) ions on MPRO.

T(K)	Initial concentration (mg/L)	Thermodynamic parameters			
		$R^2$ (8.314 J/(mol.K))	$\Delta G^0$ (kJ/mol)	$\Delta H^0$ (kJ/mol)	$\Delta S^0$ (KJ/(mol.K))
298	106.7		-15.210		
308	106.7	0.9933	-15.435	19.505	0.115
318	106.7		-17.010		

T, the absolute temperature in Kelvin; R, the universal gas constant;  $\Delta G^0$ , change in Gibbs free energy;  $\Delta H^0$ , change in enthalpy;  $\Delta S^0$ , change in entropy.

sis may be applied to the data examined by the aforementioned equations. Listed in Table 1 are the corresponding correlation coefficients,  $R^2$ , together with the values of Langmuir constant  $q_m$  and  $K_L$ , the Freundlich constants  $K_F$  and n obtained in the present studies.

All the plots showed a straight line, indicating that the adsorption of copper (II) followed the two models. Comparison of coefficients indicated that the Langmuir model fitted more precisely ( $R^2=0.9987$ , 0.9978, 0.9981) than the Freundlich model ( $R^2=0.9844$ , 0.981, 0.9793) at three different temperature (298, 308 and 318K). The basic assumption of Langmuir adsorption model is based on monolayer coverage of the adsorbate on the surface of adsorbent (Langmuir, 1916), which is an indication of the fact that the adsorption of copper (II) onto MPRO generated monolayer formation. The Langmuir constant  $K_L$  is related to the affinity of the binding sites, which allows us to make a comparison of the affinity of the biomass towards the metal ions. The increasing values of  $K_L$  for the metal ions indicated the increase in the adsorption with increasing temperature. The n values of Freundlich equation can give an indication on the favorability of sorption. It is generally stated that values of n in the range of 1 to 10 indicates favourable biosorption and less than 1 poor sorption characteristics (Aksu and Kutsal, 1991). The result shows that the values of n were all greater than one indicating that the copper (II) were favorably adsorbed by MPRO.

### Thermodynamic parameters

Using the values of thermodynamic parameters, the

process that will occur spontaneously can be determined. The thermodynamic parameters includes change in the Gibbs free energy ( $\Delta G^0$ ), enthalpy ( $\Delta H^0$ ) and entropy ( $\Delta S^0$ ),

$$\Delta G^0 = -RT \ln K_c \quad (6)$$

$$\ln K_c = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \quad (7)$$

Where, R is the universal gas constant (8.314 J/(mol.K)) and T is the absolute temperature in Kelvin;  $\Delta G^0$  (kJ/mol),  $\Delta H^0$  (kJ/mol) and  $\Delta S^0$  (kJ/(mol.K)) are changes of Gibbs free energy, enthalpy and entropy, respectively.  $K_c$  is ratio of copper (II) concentration on MPRO at equilibrium ( $q_e$ ) to the remaining copper (II) concentration in solution at equilibrium ( $C_e$ ).  $\Delta G^0$  were obtained from Equation 6,  $\Delta H^0$  and  $\Delta S^0$  were calculated from the slope and intercept of plot between  $\ln K_c$  versus  $1/T$  for initial copper (II) concentration of 106.7 mg/L. The values of these parameters are given in Table 2.

As shown in Table 2,  $\Delta H^0$  was positive value, suggesting endothermic reaction. The positive value of  $\Delta S^0$  suggested the increased randomness at the solid/solution interface during the adsorption of copper (II) onto MPRO. The negative values of  $\Delta G^0$  implied the spontaneous nature of the adsorption process. Further, the decrease in the values of  $\Delta G^0$  with the increasing temperature indicated the adsorption was more spontaneous at higher temperatures.

## Conclusions

The biosorption characteristics of copper (II) using MPRO were investigated. Experimental parameters affecting the biosorption process such as pellet diameter, pH level, contact time, initial copper (II) concentration and temperature were studied. The adsorption kinetic experiments revealed that the entire adsorption process followed the pseudo-second-order kinetics model. The adsorption of copper (II) onto MPRO followed the Langmuir isotherm model and was spontaneous and endothermic in nature. The overall results indicate that MPRO could be effectively used as a low-cost and alternative biosorbent for the removal of copper (II) from aqueous solution. Further work with the actual copper (II) contaminated wastewater which may contain competing pollutants etc. are needed.

## ACKNOWLEDGEMENTS

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