UNIVERSITI SAINS MALAYSIA

Laporan Akhir Projek Penyelidikan Jangka Pendek

Biosorption of Heavy Metals by Macrofungi

by

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LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA PENDEK

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Progress Report

Biosorption of heavy metals by *Pycnoporous sanguineus*

1.0 Introduction

Heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration. Heavy metals include lead (Pb), cadmium (Cd), zinc (Zn), mercury (Hg), arsenic (As), silver (Ag) chromium (Cr), copper (Cu) iron (Fe), and the platinum group elements. Heavy metals can be emitted into the environment by both natural and anthropogenic causes. Untreated industrial effluent which consist metal ions can pose a harmful effects to aquatic plant including humans. Conventional methods used to remove metal ions such as ion exchange, activated carbon adsorption; membrane technology and electrodialysis were inapplicable as it is expensive and inefficient.

Biosorption process becoming an alternative method used for heavy metals removal from industrial waste as it offer high efficiency and low cost. Biosorption is a process that utilized microorganisms such as algae, bacteria, fungal and yeast as a biosorbent. These microorganisms are cheap and abundant which make biosorption process has been selected as a promising method used for heavy metals removal. In this work, a low cost biosorbent from white rot fungi, *Pycnoporus sanguineus* (*P. sanguineus*) is developed to remove metal ions from an aqueous solutions.

2.0 Biosorption equilibrium procedure

2.1 Microorganism and production medium

P. sanguineus capable of adsorbing heavy metals was obtained from the Forest Research Institute of Malaysia (FRIM), Kepong, Selangor (Mashitah et al., 1999). It was maintained by weekly transfer on malt extract agar slants incubated at 30°C for 6 days, after which the slants were stored at 4°C until required. The composition of the production medium was (g/L): glucose 20, yeast extract 10 and malt extract 10. The pH of the medium was adjusted to 9.0 prior autoclaving at 121°C (1.5 bar) for 15 minutes.

2.2 Immobilized cell preparation

A cell suspension was prepared by inoculating a stock culture of *P. sanguineus* onto malt extract agar plates and incubating them at 30°C for 6 days. The mycelial mat formed was scraped off by using a sterile blade and mixed with 10 ml sterile Tween 20 solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was vortexed for 3 minutes so that the mycelium was evenly distributed in the liquid.

Fifteen ml of the cell suspension was inoculated into an Erlenmeyer flask containing 135 ml of the production medium. The flask was incubated on a rotary shaker at 30°C, 150 rpm for 66 hr. The harvested sample was centrifuged at 3500 rpm for 4 minutes at 25°C and known as free cells of *P. sanguineus*. Immobilized cells of *P. sanguineus* beads were prepared by dropping a mixture of 1.5 % (w/v) sodium alginate solution and *P. sanguineus* mycelium into a 2% (w/v) $CaCl_2$ solution stirring slowly at room temperature, 25$\pm$ 3.0°C. The beads were stirred slowly for 30 minutes, then collected by throughout filtration, washed three times with sterile deionized water and stored in Tris-HCl buffer (pH 7) at 4°C until used.

2.3 *Preparation of metal ions*

Metal solutions were prepared by diluting 1000 mg/L of Pb $(NO_3)_2$ (Mallinckrodt), CdCl (Sigma) and Cu $(NO_3)_2$. $3H_2O$ (Merck) solutions with deionized water to a desired range of 50 to 300 mg/L. For each solution, the initial metal concentrations and the concentrations in the samples after biosorption treatment were determined by using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

2.4 *Batch equilibrium and kinetics biosorption*

The biosorption of Cd (II), Cu (II) and Pb (II) ions using free and immobilized cells of *P. sanguineus* from aqueous solution was evaluated in batch biosorption equilibrium experiments. Effects of initial metals concentration, pH, biomass loading and temperature on the biosorption rate and capacity were examined.

The effect of solution pH between 2 and 6 on the biosorption of Cd (II), Cu (II) and Pb (II) by free and immobilized *P. sanguineus* preparation was evaluated in cultures kept at 150 rpm, 30°C for 5 hr for Cd (II) and Cu (II) and 2 hr for Pb (II). For initial metals concentration, the biosorption studies were conducted at optimum pH with concentration of metal ions varied from 58 to 300 mg/L. The effect of biomass loading was evaluated at 1 to 6 g with other procedures as described previously. For the equilibrium study, the initial Cd (II), Cu (II) and Pb (II) concentration was 100 mg/L and the working pH was 6, 5 and 4. The contact time varied from 15 and 300 min at 30°C (303 K), 35°C (308 K) and 40°C (313 K), respectively.

The amount of metal ions bound by the biosorbent was calculated as:

$$q = \frac{V(C_i - C_f)}{W} \qquad (1)$$

where q is mg of metal biosorbed per g of biomass (mg/g), C_i (mg/L) is the initial concentration, C_f (mg/L) is the final or equilibrium concentration, V (L) is the volume of metal solution in the flask and W (g) is the weight of the biosorbent. Each experiment was repeated three times and the results given are average values. Samples taken after the desired incubation period were measured with an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

Kinetics experiments were carried out at known Cd (II), Cu (II) and Pb (II) concentrations with various biosorbent loadings and shaking at 150 rpm. At pre-determined time interval, samples were withdrawn, filtered and the metal ions concentration measured with an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

2.5 Desorption procedures

Desorption of metal ions was performed only on immobilized cells of *Pycnoporus sanguineus* since it is more practical to be done compare to free cells. The desorption experimental procedure were carried out using different concentration of acidic solution in order to determine the reusability of the biosorbents. The desorption of Cd (II), Cu (II) and Pb (II) ions were carried out using (a) 0.01, 0.05 and 0.1 M nitric acid and (b) 0.01, 0.05 and 0.1 M hydrochloric acid. The loaded cells with Pb (II) ions were placed in the desorption medium and stirred at 150 rpm for 60 min at 25°C. The final concentration of

Pb (II) ion in the aqueous solution was determined by Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

2.6 Column studies

The experiment was performed in a packed bed column with inner diameter 4 cm and 60 cm height (Figure 1). Glass wool was introduced at the bottom and top of the column to prevent the biosorbent from floating. The Cd (II), Cu (II) and Pb (II) were pumped into the column downstream using a peristaltic pump to study the effect of the following parameter such as (a). Bed height (5-13 cm) (b). Flow rate (4-12 ml/min) (c). Initial metals concentration (50-300 mg/L) (d). Regeneration of biosorbent

Sample are collected at different intervals of time and analysed using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650). Metals solution fed into the column kept at optimum pH obtained from preliminary experiments. All the column experiments were performed at constant temperature (25 ± 3^0C) and repeated two times and only average values are discussed in this study.

Desorption column was performed by pumping 0.1 M HCl at 5 ml/min into the column downstream using a diaphragm pump when effluent metals concentration exceeded 99.5 mg/L. After elution, this regenerated column was reused for next cycles. These cycles were repeated two times to determine the biomass biosorption capacity.

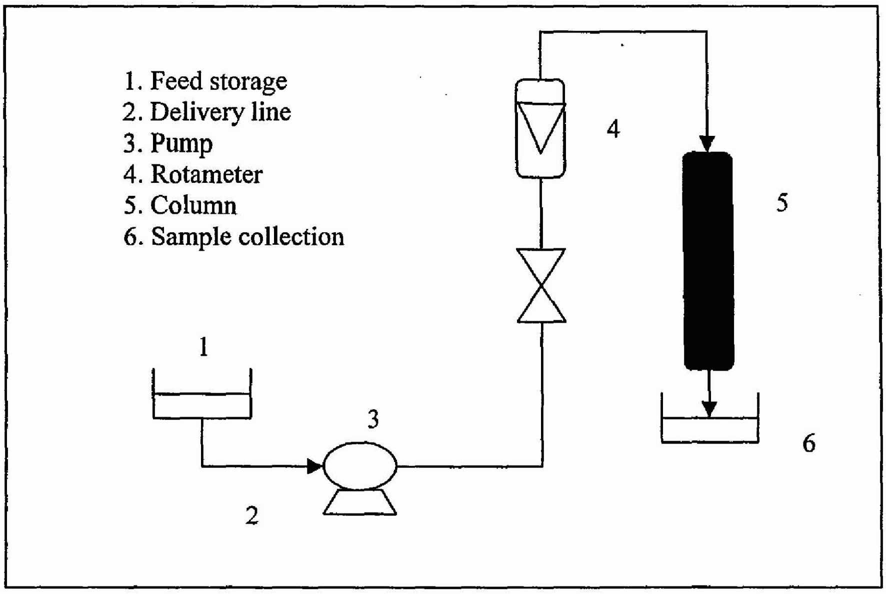


Figure 1: Experimental set up for biosorption studies in fixed bed column

2.7 Characterization of biosorbent before and after biosorption process

2.7.1 SEM studies

Scanning Electron Microscopy was performed using a Leo Supra Scanning Electron Microscope model 50 VP (Germany). Energy Dispersive Spectroscopy (EDAX) analysis was conducted with a linked to SEM apparatus by keeping constant the accelerating voltage at 15 KV. Sample of immobilized beads before and after Pb (II) biosorption was coated with gold under vacuum to increase the electron conduction and improve the quality of the micrographs (Akar et al., 2005).

3.0 Results

3.1 Pb (II) biosorption in batch system by immobilized cells

3.1.1 Effects of pH.

Heavy metals biosorption was found to be significantly dependent on pH (Malkoc and Nuhoglu, 2005). To determine the effect of pH on Pb (II) removal by immobilized cells of *P. sanguineus*, biosorption studies were carried out at pH 2.0, 3.0 and 4.0. Previous studies stated that at lower pH, the surface charge on the cells became positive and that metal cations and protons compete for binding sites on cell wall, which results in lower Pb (II) uptake from the medium (Malkoc and Nuhoglu, 2005). At pH above the isoelectric point, the surface charges are negative and the ionic state of ligands such as amino groups, carboxyls and phosphates promote reactions with the Pb (II) ions, resulting in rapid efficient binding (Arica et al., 2001). In most of Pb (II) ions removal studies, the optimum pH for Pb (II) ions biosorption was in the range pH 3.0 to 7.0 as presented in Table 1. In the present study, the percent removal of Pb (II) by immobilized cells of *P. sanguineus* was more than 90% within 2 h at pH 4. No experiments were conducted at pH above 4 as the Pb (II) ions precipitate.

Table 1 pH for Pb (II) ions biosorption by different biosorbent

Biosorbent material	pH	Reference
Streptomyces noursei	6.1	(Mattuschka and Straube, 1993)
Pennicillium chrysogenum	4.5	(Niu et al., 1993)
Rhizopus arrhizus	5.0-7.0	(Sag and Kutsal, 2000)
Rhizopus arrhizus	4.0-5.0	(Fourest and Roux, 1992) *Zoogloea*
ramigera	4.0-5.0	(Sag and Kutsal, 2000)
Saccharomyces cerevisiae	5.0	(Cabuk et al., 2007) *Phanerochaete*
*chrysosporium*6.0 (		Say et at., 2001)
Cephalosporium aphidicola.	5.0	(Tunali et al., 2006)
Aspergillus niger	4.0	(Dursun, 2005)
Trametes versicolor	4.0-6.0	(Bayramoglu et al., 2003)
Pycnoporus sanguineus	4.0	Present study

3.1.2 Effect of initial Pb (II) ions concentrations

The effect of initial Pb (II) ion concentration on Pb (II) uptake was studied in a range from 58 to 300 mg/L (Figure 2). Increasing the initial Pb (II) concentration increased the amount of Pb (II) taken up. The increase in initial Pb (II) concentration could increase the mass transfer driving force of the ions between the aqueous and solid phases (Aksu, 2002; Fawzi and Sameer, 2000; Malkoc and Nuhoglu, 2005). However, at a higher concentration the percentage of Pb (II) removed decreases. For an initial Pb (II) ion concentration of 58 mg/L, 96% of the ions were removed within 2 hours, whereas only 39% of the ions were removed from a 300 mg/L solution in the same time period. Thus, at higher metal concentrations, the available sites at which the biosorption process occurs are limited and the biosorption yield declines (Malkoc and Nuhoglu, 2005)

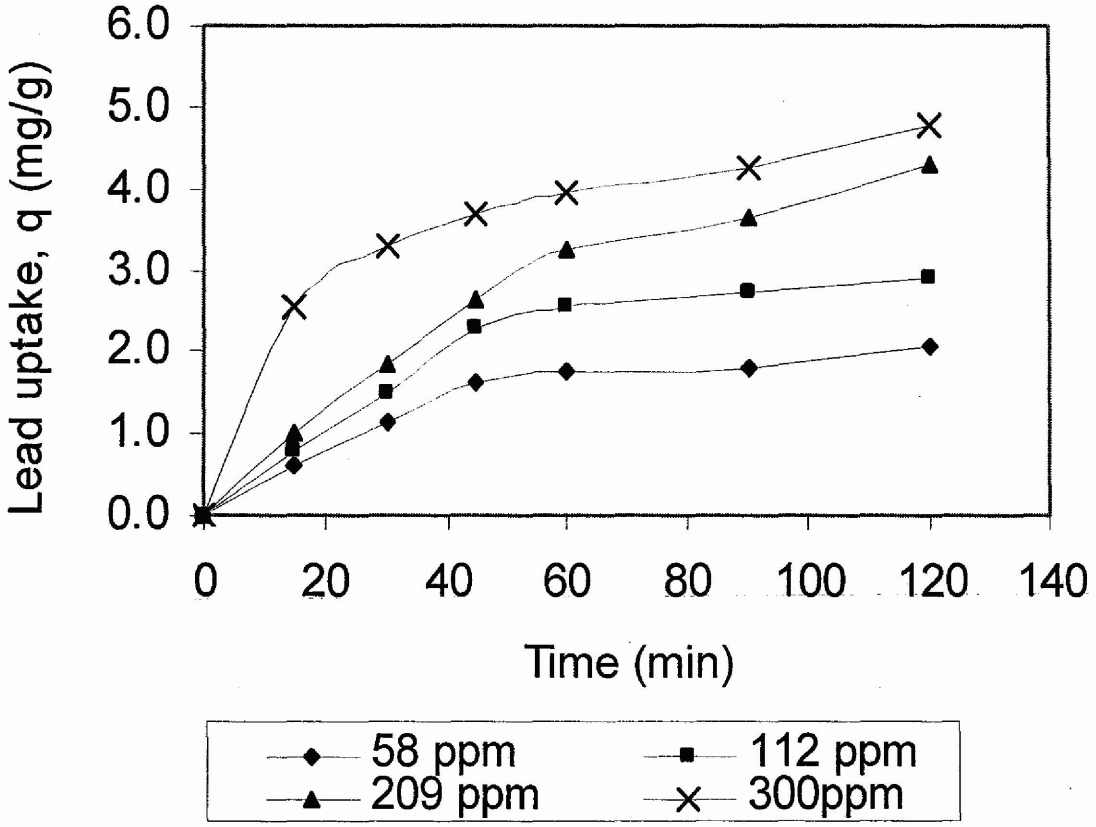


Figure 2. Effect of initial metal concentration on the biosorption of Pb (II) ions by immobilized cells of *P. sanguineus* (Condition: pH 4.0; biomass loading 3 g; agitation 150 rpm).

3.1.3 Effects of biosorbent loading.

The biosorption capacity for Pb (II) ions varies with the biosorbent loading (Figure 3). Pb (II) ion removal increased from 23% to 99% when the amount of immobilized cells of *P. sanguineus* increased from 1.0 to 6.0 g. This increase could be attributed to the presence of more binding sites for binding the Pb (II) ions (Malkoc and Nuhoglu, 2005; 10]. However, the Pb (II) uptake decreased from 2.89 to 1.95 mg/g as the biosorbent dosage increased. The maximum metal uptake capacity occurred at 3.0 g of immobilized cells. The high biosorbent loading may produce a 'screen' effect on the cell wall, protecting the binding sites and thus lowering the Pb (II) uptake (Malkoc and Nuhoglu, 2005; Mashitah et al., 1999; Pons and Futse, 1993)

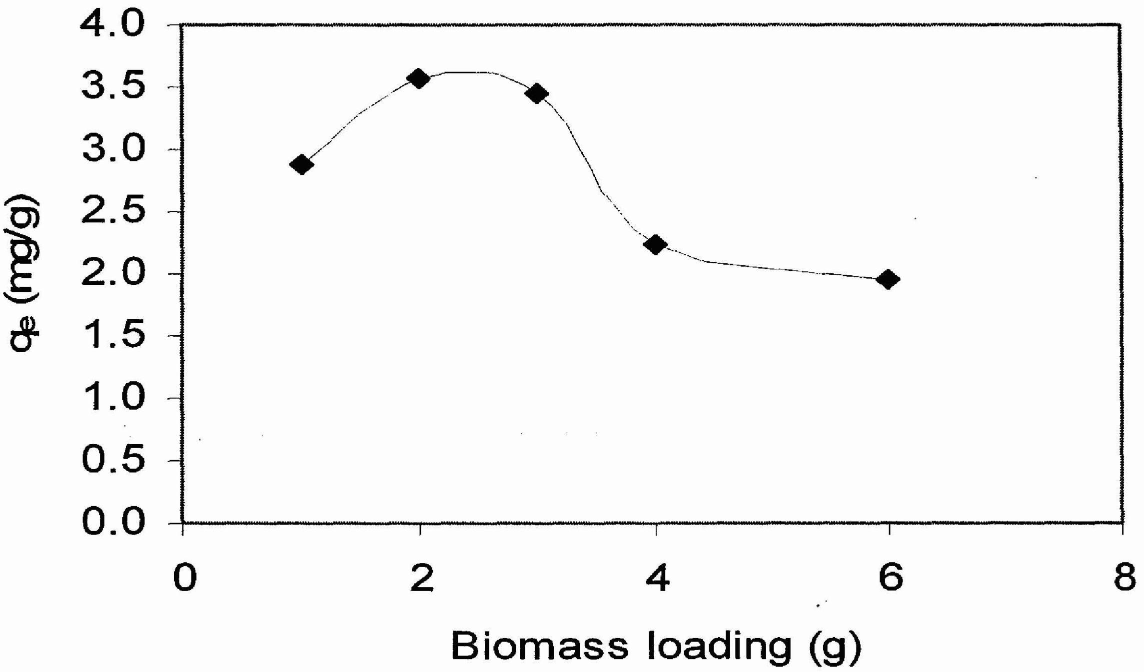


Figure 3. Effect of biomass loading on the uptake of Pb (II) ions by immobilized cells of *P. sanguineus* (Condition: pH 4.0; 100 mg/L Pb (II) ions; agitation 150 rpm).

3.1.4 Equilibrium isotherms

The linearised Langmuir, Freundlich and Redlich-Peterson equilibrium isotherm constants for Pb (II) ion biosorption were obtained at 30°C (303 K), 35°C (308 K) and 40°C (313 K) (Table 2). Biosorption of the metals to the immobilized cells of *P. sanguineus* could be represented well by any of the three isotherm models, as all had correlation coefficient (R^2) of approximately 1. The Langmuir constants K_b and q_{max} were determined using Eq. (3) (not shown here). The equilibrium sorption capacity, q_{max} increased from 3.62 to 4.43 mg/g when the temperature increased from 30 to 40°C. The higher the K_b value, the higher the affinity for binding metal ions. The highest K_b occurred at 313 K. The increase in K_b with temperature shows that a chemical interaction occurs between the metal ions and the biosorbent. (Malkoc and Nuhoglu, 2005).

The K_f values for the Freundlich isotherms also increased with increased in temperature (Table 2). Since the n values obtained were > 1.0, the Pb (II) ions were favorably adsorbed by the biosorbent at all temperatures studied (Dursun, 2005). For the Redlich-Peterson constant K_{rp}, the values also increased with reaction temperature and most values of β were between 0.95-1.0.

Table 2: Biosorption equilibrium constant obtained from Langmuir, Freundlich and Redlich-Peterson isotherms for the biosorption of Pb (II) ions onto immobilized cells of *P. sanguineus*

T	Langmuir			Freundlich			Redlich-Peterson			
(^{o}C)	q_{max} (mg/g)	K_b (dm^3/mg)	R^2	K_f	n	R^2	K_{rp} (dm^3/mg)	a_{rp} $(dm3/mg)^{\beta}$	β	R^2
30	3.62	1.341	1.000	1.553	4.713	0.933	3.928	1.075	1.000	1.000
35	4.00	1.416	0.999	1.840	5.025	0.962	9.955	2.630	0.990	0.999
40	4.43	1.569	0.999	2.179	5.313	0.949	25.234	7.059	0.950	0.999

3.1.5 Biosorption kinetics modeling

To evaluate the biosorption kinetics of Pb (II) ions, the pseudo-first order, pseudo-second order and intraparticle diffusion equation were applied to the experimental data. The corresponding constants for all three models obtained were presented in Table 3. The pseudo-second order model fitted the experimental data better than intraparticle diffusion and pseudo-first order models with correlation coefficient (R^2) approximately to 1.

Table 3: Kinetic constants of pseudo first, pseudo second-order and intraparticle diffusion models for the biosorption of Pb (II) ions onto immobilized cells of *P.sanguineus* at different temperatures

T (°C)	First-order kinetic model		Second-order kinetic model		Intraparticle diffusion	
	k_1 (min^{-1})	R^2	k_2 (g/mg min)	R^2	K_s	R^2
30	0.039	0.988	0.147	0.947	1.515	0.916
35	0.035	0.979	0.198	0.972	1.556	0.907
40	0.008	0.768	0.333	0.990	1.717	0.873

3.1.6 Thermodynamic parameters

The free Gibbs energy change (ΔG^o) obtained for the biosorption of Pb (II) ions to the immobilized cells of *P. sanguineus* were -739.03, -891.49 and -1172.57 J/mol when the temperature increased from 30 to 40°C. The negative values of free Gibbs energy change (ΔG^o) indicated that biosorption process of Pb (II) ions onto immobilized cells of *P. sanguineus* was spontaneous and favorable as the temperature increased (Nollet et al., 2003). The standard enthalpy (ΔH^o) and entropy change (ΔS^o) of biosorption calculated were found to be 12.42 kJ/mol and 43.35 J/mol K, respectively. The positive values of ΔH^o and ΔS^o indicated that the biosorption process was endothermic in nature and increased randomness at the solids/solution interface during the biosorption of Pb (II) ions onto biosorbent (Sarin et al., 2006).

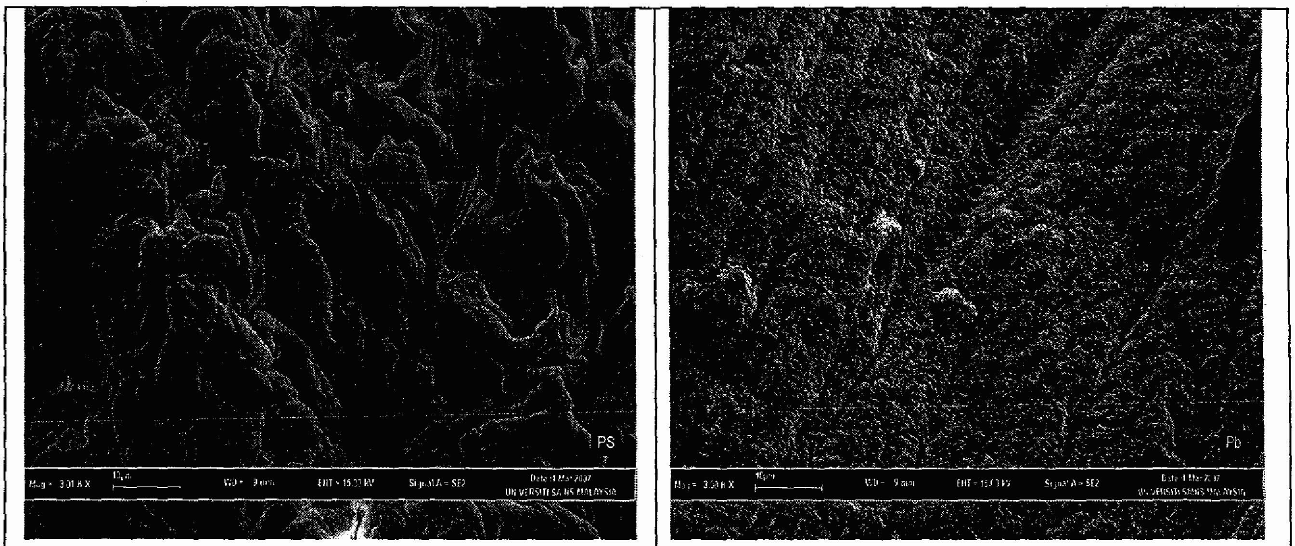
The pseudo second-order constant of Pb (II) biosorption is expressed as a function of temperature by Arrhenius equation:

$$\ln k_2 = \ln A_o - \frac{E_a}{RT} \qquad (6)$$

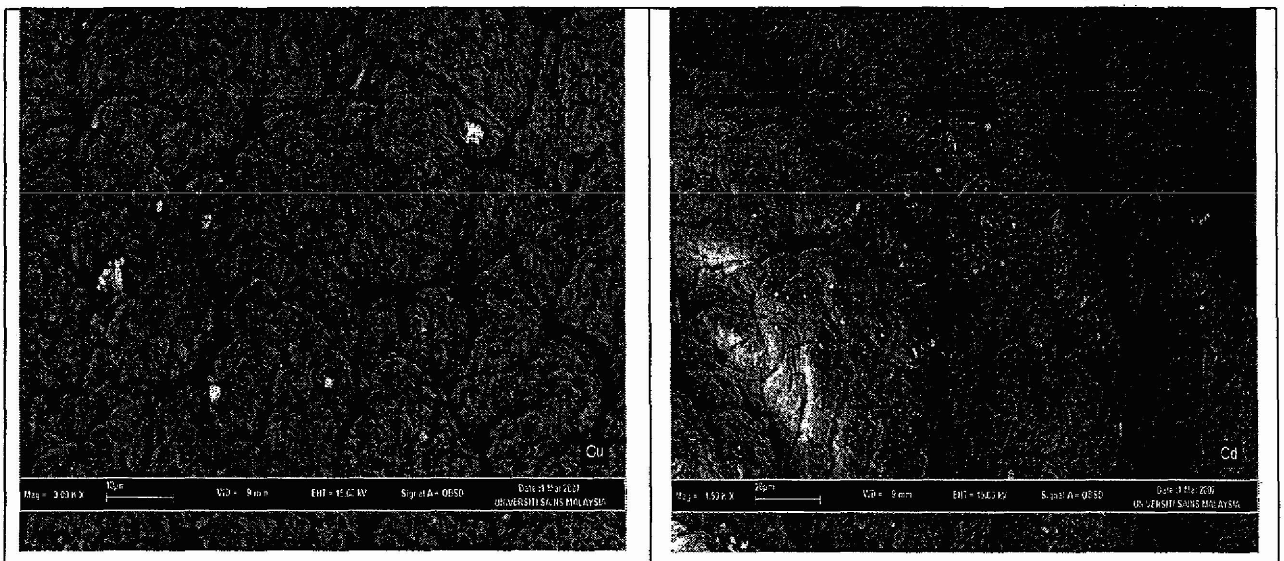
where A_o is the temperature independent factor called 'frequency factor', E_a is the Arrhenius activation energy of sorption (kJ/mol), R is the gas law constant (8.314 J/mol K) and T is the absolute temperature (K). The activation energy in the range of (5-40 kJ/mol) suggests a physisorption process, while at higher activation energy (40-800 kJ/mol) suggests chemisorption (Nollet et al., 2003). In the present study, the activation energy, E_a was 64.19 kJ/mol, indicating that the process followed chemisorption mechanism.

* Same results were obtained for Cd (II) and Cu (II) biosorption by free and immobilized cells of *Pycnoporus sanguineus.*

3.1.7 Characterization of biosorbent before and after metals biosorption using SEM analysis



(a) (b)



(c)

(d)

Figure 4: SEM micrograph of (a) immobilized cells of *Pycnoporus sanguineus* (b) Pb (II) loading (c) Cu (II) loading and (d) Cd (II) loading

3.2 Column studies

3.2.1 Effect of Bed Height

Figure 5 presents a breakthrough curve for Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different bed height 5, 9 and 13 cm, respectively. Concentration of Pb (II) solution was fixed at 100 mg L^{-1} and pH 4 for all bed height studies. It was fed into the column and maintained at 4 ml min^{-1}. Results show that Pb (II) uptake increased from 11.51 to 19.65 mg g^{-1} as the bed height increased from 5 to 13 cm. As the bed height increased, more binding sites were available for the biosorption to occur (Vijayaraghavan *et al.*, 2005). The saturation of immobilized cells of *P. sanguineus* was achieved nearly 38 hours at 5 cm bed height. However, at higher bed height 13 cm, the saturation was obtained after 144 hours. Malkoc and Nuhoglu, (2006) stated that higher bed height would resulted in broaden mass transfer zone, thus increase the saturation period for the metals onto immobilized cells.

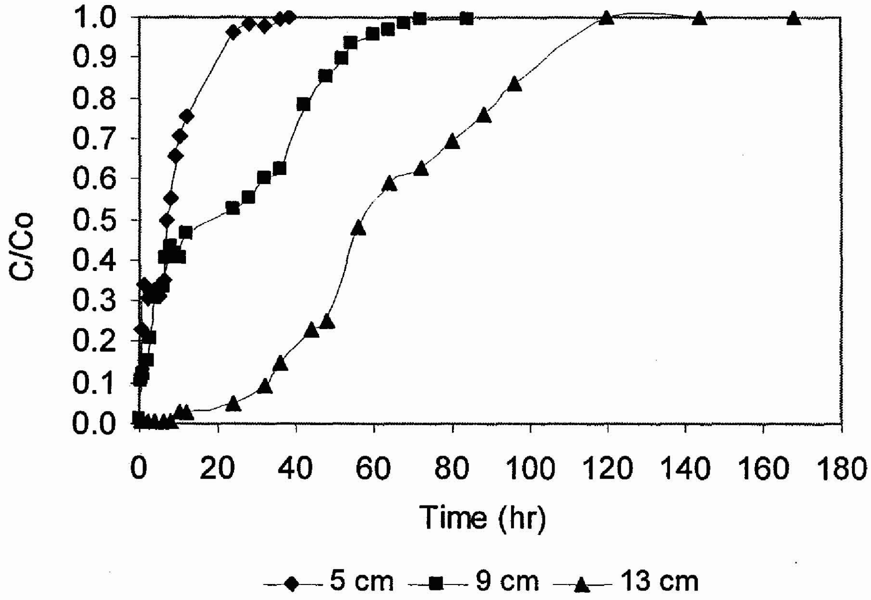


Figure 5: Breakthrough curves of Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different bed height (Condition: 100 mg L^{-1} Pb (II), flow rate: 4 ml min^{-1}, pH 4.0)

3.2.2 *Effect of Flow Rate*

Figure 6 presents the breakthrough curve of Pb (II) biosorption in a column at different flow rate from 4 to 12 ml min^{-1}. The experiments were carried out at constant initial Pb (II) concentration (100 mg L^{-1}) and 9 cm bed height. Result showed Pb (II) uptake decreased with the increase in flow rate. This is due to insufficient contact time for the Pb (II) ions to be adsorbed by the immobilized cells of *P. sanguineus* (Ko *et al.*, 2000; Vijayaraghavan *et al.*, 2005). As seen in Fig. 3, a steeper breakthrough curve was observed at flow rate 12 ml min^{-1} as the breakthrough time decreased. Similar phenomenon was reported on cobalt (II) and nickel (II) biosorption by seaweeds and heavy metals removal in column by *P. sanguineus*, respectively (Zulfadhly *et al.*, 2001; Vijayaraghavan *et al.*, 2005).

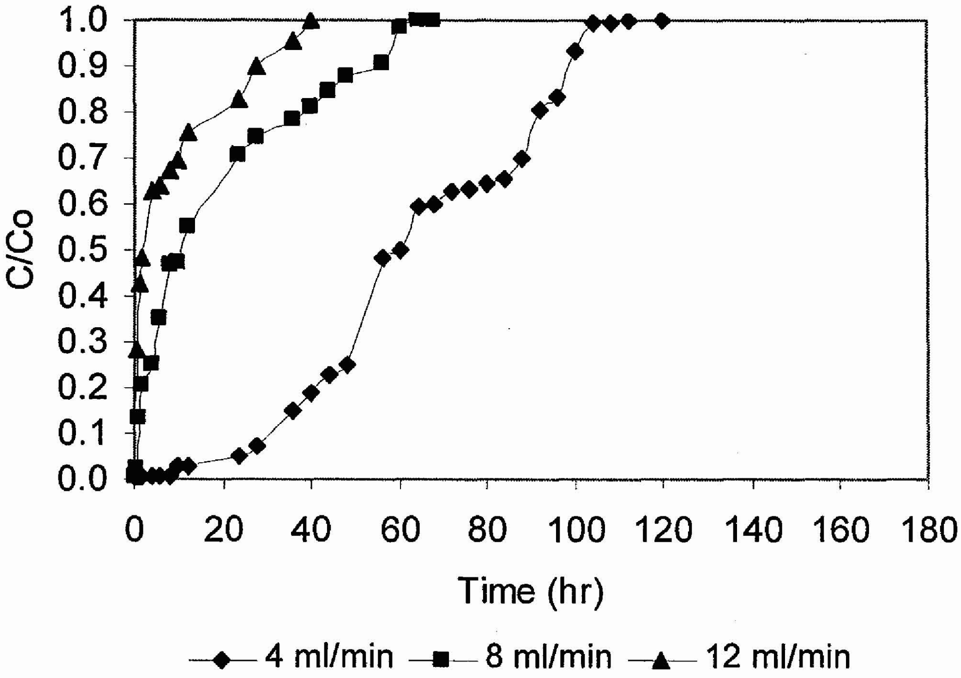


Figure 6: Breakthrough curves of Pb (II) biosorption onto immobilized cells of *P.sanguineus* at different flow rates (Condition: 100 mg L^{-1} Pb (II), bed height= 9 cm, pH 4.0)

3.2.3 Effect of Initial Metals Concentration

Figure 7 shows the breakthrough profiles of Pb (II) biosorption at different initial Pb (II) concentration. As the initial Pb (II) concentration increased from 50 to 300 mg L^{-1}, the Pb (II) uptake increased from 21.74 to 25.73 mg g^{-1}. At higher initial Pb (II) concentration, the biosorbent gets saturated early thus resulted a faster breakthrough and exhaustion time (Zulfadhly *et al.*, 2001; Malkoc and Nuhoglu, 2006). For 50 mg L^{-1} of Pb (II) solution, less driving force occurred between metal ions and the immobilized cells of

P. sanguineus which resulted in a broadened mass transfer zone (Malkoc and Nuhoglu, 2006).

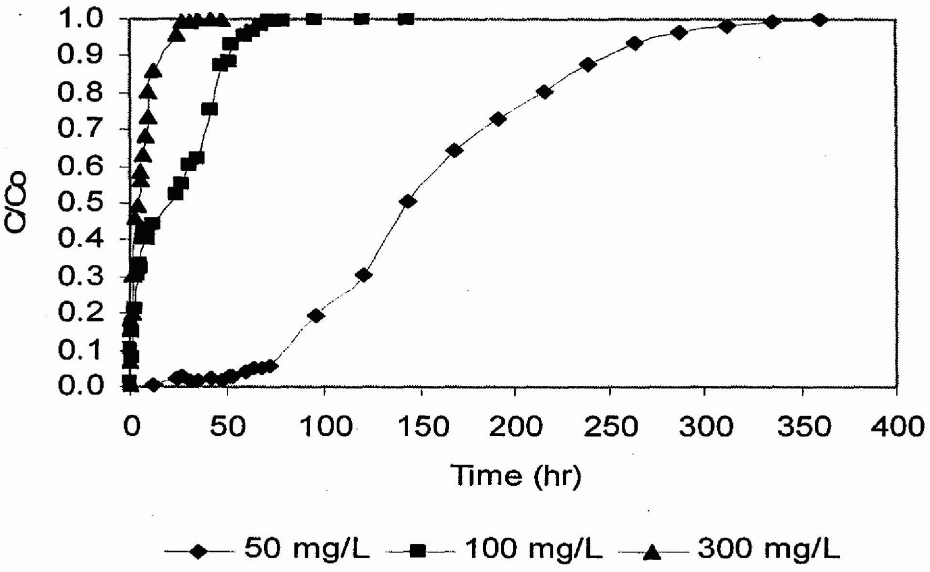


Figure 7: Breakthrough curves of Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different initial Pb (II) concentration (Condition: 100 mg L^{-1} Pb (II), bed height= 9 cm, pH 4.0)

3.4 Regeneration

Regeneration of biosorbent after biosorption process is very important in order to reduce the process cost in a continuous operation (Vijayaraghavan *et al*, 2005). Figure 8 shows a desorption curve of Pb (II) ions through a packed bed of *P. sanguineus* by passing 0.1 M HCl as an elution agent. The biosorbent were reused up to two biosorption-desorption cycles. It was observed that the elution efficiency was up to 85% for a complete recovery of Pb (II) ions and more than 20 L of 0.1 M HCl is being used. After two biosorption-desorption cycles, a significant biosorbent weight loss was observed and seemed that it was not suitable to be used in the next cycle. As reported by a few researchers, cells that were exposed to an acidic elutant might faced physical-chemical damage of the biosorbent structure which resulted in weight loss and reduction of the biosorption capacity in a subsequent cycles (Tuzun *et al.*, 2005; Vijayaraghavan *et al.*, 2005). These can be seen as tabulated in Table 4.

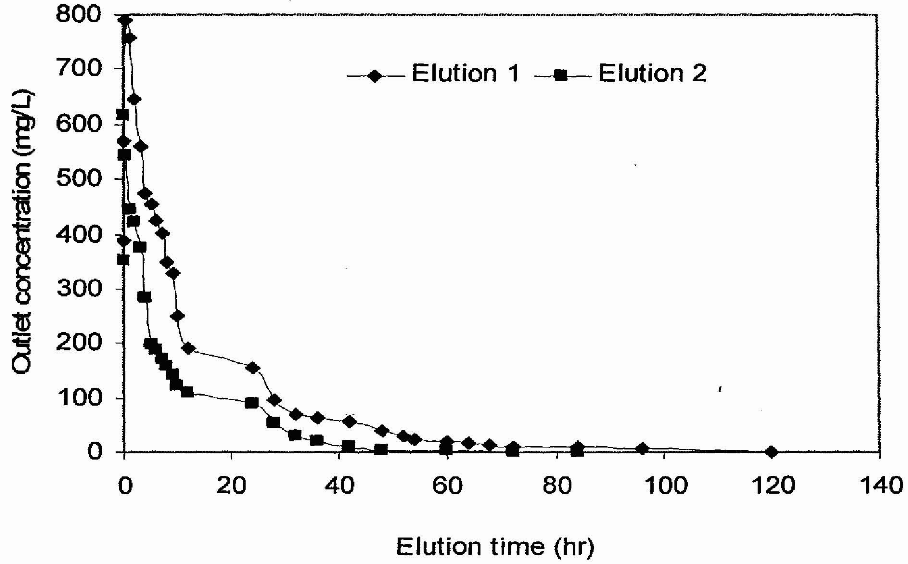


Figure 8: Breakthrough curves of Pb (II) desorption

Table 4: Elution parameters for two biosorption-desorption cycles.

Metal	Cycle No	Uptake capacity (mg g-1)	Metal removal (%)	Time for Elution (hr)	Elution efficiency (%)
Pb	1	34.50	88.84	120	84.83
	2	28.14	75.62	84	50.17

* Same results were obtained for Cd (II) and Cu (II) biosorption by immobilized cells of *Pycnoporus sanguineus* in column studies.

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Biosorption of cadmium (II) ions by immobilized cells of *Pycnoporus sanguineus* from aqueous solution

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Abstract

Biosorption of cadmium (II) ions from aqueous solution onto immobilized cells of *Pycnoporus sanguineus* (*P. sanguineus*) was investigated in a batch system. Equilibrium and kinetic studies were conducted by considering the effect of pH, initial cadmium (II) concentration, biomass loading and temperature. Results showed that the uptake of cadmium (II) ions increased with the increase of initial cadmium (II) concentration, pH and temperature. Langmuir, Freundlich and Redlich–Peterson isotherm models were used to analyze the equilibrium data at different temperatures. Langmuir isotherm model described the experimental data well followed by Redlich–Peterson and Freundlich isotherm models. Biosorption kinetics data were fitted using pseudo-first, pseudo-second-order and intraparticle diffusion. It was found that the kinetics data fitted well the pseudo-second-order followed by intraparticle diffusion. Thermodynamic parameters such as standard Gibbs free energy (ΔG^0), standard enthalpy (ΔH^0) and standard entropy (ΔS^0) were evaluated. The result showed that biosorption of cadmium (II) ions onto immobilized cells of *P. sanguineus* was spontaneous and endothermic nature.

Keywords: Biosorption; Cadmium; *Pycnoporus sanguineus*; Isotherm; Kinetics

1. Introduction

The presence of industrial effluent containing heavy metals into freshwater poses serious problems to the ecological system including humans as they are toxic even at low concentration (Malkoc and Nuhoglu, 2005; Tuzun et al., 2005). One of the most common toxic metals found in industrial effluents is cadmium. Cadmium identifies as a soft, blue–white malleable, lustrous metal or a grayish-white powder that is insoluble in water and reacts readily with dilute nitric acid. It may come from various industrial sources such as electroplating, fertilizers, mineral processing and battery manufacturing (Arica et al., 2001; Malkoc and Nuhoglu, 2005). Cadmium contamination in human was first reported in Japan in the 1950s where the municipal sewage sludge was used as a fertilizer through the rice crop (Kaneta et al., 1986). Exposed to cadmium may result in adverse effects such as cancer, lung insufficiency, disturbances in cardiovascular system, liver and kidney damage (Yin and Blanch, 1989; Sharma, 1995; Cruz et al., 2004). The removal of this substance from water and industrial wastewater has become a challenge to the researchers. Conventional methods used to remove this substance from wastewater are activated carbon adsorption, precipitation, membrane separation and reverse osmosis (Kapoor et al., 1999; Arica et al., 2001; Malkoc and Nuhoglu, 2005). However, these techniques are ineffective and expensive, especially when metal ions present in wastewater is at low concentrations (1–100 mg/L) (Sharma, 1995; Arica et al., 2001). Also the toxic sludge produced from the treatment will cause a regeneration problem (Akar et al., 2005; Tuzun et al., 2005).

For the past few decades, the metal biosorption by various biological materials including fungi, algae, bacteria and yeast have received great attention as it offer a low cost biosorbent, high efficiency and can be regenerated (Arica

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Nomenclature

a_{rp}	Redlich–Peterson isotherm constant $(L/mg)^{\beta}$	K_{rp}	Redlich–Peterson isotherm constant (L/mg)
b	intraparticle diffusion constant	K_s	intraparticle diffusion constant $(mg/(g/min^{0.5}))$
β	Redlich–Peterson isotherm constant	n	Freundlich constant
C_e	equilibrium concentration (mg/L)	q	metal ions biosorbed per g of biomass (mg/g)
C_i	initial concentration (mg/L)	q_{max}	maximum specific uptake corresponding (mg/g) to the sites saturation
C_f	final or equilibrium concentration (mg/L)		
E_a	activation energy (kJ/mol)	q_e	amount of metal ions uptake at equilibrium (mg/g)
ΔG^0	free energy change (J/mol)		
ΔH^0	standard enthalpy (kJ/mol)	q_t	amounts of adsorbed Cd (II) ions on the (mg/g) biosorbent at time t
k_1	rate constant of first-order biosorption (min^{-1})		
k_2	rate constant of second-order biosorption (g/mg/min)	R	percent metal adsorbed (%)
		ΔS^0	standard entropy (J/mol/K)
K_b	equilibrium constant (L/mg)	V	volume of metal solution in the flask (L)
K_c	equilibrium constant (L/mg)	W	weight of biosorbent (g)
K_f	Freundlich constant		

et al., 2001; Akar et al., 2005). These biological materials could be used in biosorption process to remove metal ions in wastewater (Kapoor et al., 1999). The biosorption process is a passive uptake that utilizes cell wall of biomass to sequester the metal ions from aqueous solutions (Vijayaraghavan et al., 2005; Dursun, 2006). The presence of functional groups on biomass cell wall such as carboxyl, hydroxyl, ketones and amino groups will involve a physico-chemical interaction between the metal ions during the biosorption processes (Mashitah et al., 1999). In previous studies, it has pointed out the capabilities of fungal biomass to remove heavy metal ions from aqueous solutions (Aloysius et al., 1999). Fungi is better suitable for the removal of metals from wastewater than other microbes because of their great tolerance towards heavy metals and other adverse conditions such as low pH, high cell wall binding capacity and high intracellular metal uptake capacity (Gadd, 1987). Several fungal biosorbents (*Aspergillus niger*, *Rhizopus* spp., *Saccharomyces* spp., and *Mucor* spp.) were studied as a potential biosorbent in heavy metals removal from aqueous solution (Kapoor and Viraraghavan, 1997; Kyung Kim et al., 2003; Yan and Viraraghavan, 2003). *Ganoderma* species have been also reported as an efficient biosorbent in copper removal compared to other macrofungi species (Mashitah et al., 1997). These fungi could remove metal ions from aqueous solution by adsorbing metal ions on their mycelium (Bayramoglu et al., 2003).

Cell immobilization is one of the methods used to overcome the incorporating free suspended cell in industrial operations. It offers several advantages include minimal clogging in continuous systems (Ting and Sun, 2000; Arica et al., 2001; Bayramoglu et al., 2003), easy to separate from the reaction system (Annadurai et al., 2007) and can be regenerated and reused the immobilized cells for a few cycles (Arica et al., 2001). Natural polymers mostly used as the matrix for the immobilization of microbial cells are alginate, chitosan, chitin and cellulose derivatives (Arica et al., 2001; Bayramoglu et al., 2003).

This study was carried out to determine the potential of immobilized living cells of *Pycnoporus sanguineus* to adsorb cadmium (II) ions in a batch adsorber. Adsorption isotherm and kinetics models are proposed. The experimental data were compared with the simulated data using proposed models.

2. Methods

2.1. Microorganism and production medium

P. sanguineus capable to adsorb heavy metals was obtained from Forest Research Institute of Malaysia (FRIM), Kepong, Selangor (Mashitah et al., 1999). It was maintained by weekly transfer on malt extract agar slant incubated at 30 °C for 6 days, after which were stored at 4 °C until required. The composition of the production medium comprised of (g/L): glucose 20, yeast extract 10 and malt extract 10, respectively. The pH of the medium was adjusted to 9.0 prior autoclaving at 121 °C (1.5 bar) for 15 min.

2.2. Immobilized cells preparation

Cell suspension was prepared by inoculating a stock culture of *P. sanguineus* onto malt extract agar plates and incubated at 30 °C for 6 days. The mycelial mat formed was scraped by a sterile blade and mixed with 10 ml sterile Tween 20 solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was then vortexed for 3 min so that the mycelium was evenly distributed in the liquid.

Fifteen milliliter of cell suspension was inoculated into an Erlenmeyer flask containing 135 ml of the production medium. The flask will then be incubated in a rotary shaker at 30 °C, 150 rpm for 66 h. The harvested sample was centrifuged at 3500 rpm for 4 min. Sodium alginate beads were prepared by dropping a mixture of sodium alginate solution and *P. sanguineus* cells into 2% (w/v) calcium chloride solution under magnetic stirring (slow) at room temperature. The beads were stirred slowly for 30 min, then collected by filtration, washed three times with sterile deionized water and stored in Tris–HCl buffer pH 7 at 4 °C until used. Plain beads without *P. sanguineus* cells were also prepared for comparison.

2.3. 3 Preparation of metal ions

The stock cadmium (II) solution was prepared by dissolving 1 g of cadmium chloride in 1 l of deionized water. The stock cadmium (II) solutions were adjusted so as to produce solution between 50 and 300 mg/L for equilibrium studies. For each of the solution, the initial concentrations of the metals and samples after biosorption treatment were determined using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

2.4. Batch biosorption procedures

The biosorption of cadmium (II) ions on the plane alginate beads and of immobilized cells of *P. sanguineus* from aqueous solution was investigated in batch biosorption equilibrium experiments. The effect of initial cadmium (II) ions concentration, solution pH, biomass loading and temperature on the biosorption capacity of cadmium (II) onto immobilized cells were studied. The plane alginate beads were used as a control system or blank. The effect of initial cadmium (II) concentration in the biosorption studies was carried out at pH 6 (which was adjusted with HCl or NaOH at the beginning of the experiment and not controlled throughout the experiments). The concentration of cadmium (II) ions was varied between 50 and 300 mg/L. Biosorption data were obtained in a set of Erlenmeyer flasks (250 mL) at constant temperature where metal ions with different concentration (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 solution pH on the biosorption rate of the alginate beads and immobilized *P. sanguineus* preparation with cadmium (II) ions (100 ml) was investigated in the range of 2–6 and similar procedures were repeated. The effect of biomass loading was varied between 1 and 6 g. For equilibrium study, the initial cadmium (II) concentration was 100 mg/L and pH was at 6.0. The contact time was varied between 15 and 240 minutes at different temperatures 30 °C (303 K), 35 °C (308 K) and 40 °C (313 K), respectively.

The amount of cadmium (II) ions bound by the biosorbent was calculated using the following equation:

$$q = \frac{V(C_i - C_f)}{W} \tag{1}$$

where q is mg of metal ion biosorbed per g of biomass (mg/g), C_i (mg/L) is the initial concentration, C_f (mg/L) is the final or equilibrium concentration, V (L) the volume of metal solution in the flask and W (g) is the weight of biosorbent. Each of the experiment was repeated three times and the average values were obtained. Samples taken after the desired incubation period were analysed for concentration of cadmium (II) ions using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

Kinetics experiments were carried out with a known biosorbent loading with varied temperature and agitating at 150 rpm. At different interval time, samples were withdrawn, filtered and cadmium (II) concentration was measured using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

2.5. Equilibrium isotherm and kinetics studies

2.5.1. Equilibrium isotherm models

Langmuir, Freundlich and Redlich–Peterson models were used to determine the sorption equilibrium between the solid biosorbent and metals ions. The isotherm equations for all models proposed are listed in Table 1. The Langmuir model assumes that a monomolecular layer is formed when biosorption takes place without any interaction between the adsorbed molecules (Aksu, 2002; Malkoc and Nuhoglu, 2005).

Freundlich isotherm is an empirical equation based on a heterogeneous surface (Arica et al., 2001; Koumanova et al., 2002). The Redlich–Peterson isotherm (Dursun, 2006) incorporates the features of the Langmuir and the Freundlich isotherms and has three parameters K_{rp}, a_r and β. The isotherm constants for the three models were obtained by non-linear regression method (Dursun, 2006).

Table 1
Equilibrium isotherm models proposed in the biosorption of cadmium (II) ions onto immobilized *P. sanguineus*

Models	Equation	Linear equation	References
Langmuir	$q_e = \frac{q_{max} K_c C_e}{1+K_c C_e}$	$\frac{C_e}{q_e} = \frac{1}{q_{max} K_c} + \frac{C_e}{q_{max}}$	Malkoc and Nuhoglu (2005), Aksu, (2002)
Freundlich	$q_e = K_f C_e^n$	$\ln q_e = \frac{1}{n} \ln C_e + \ln K_f$	Arica et al. (2001), Koumanova et al. (2002)
Redlich–Peterson	$q_e = \frac{K_{rp} C_e}{1+a_r C_e^\beta}$	$\ln\left(K_{rp} \frac{C_e}{q} - 1\right) = \beta \ln(C_e) + \ln a_r$ $\beta(0 < \beta < 1)$ For $\beta = 1$, the equation are converts to the Langmuir form.	Dursun (2006)

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2.5.2. Kinetic modeling

Different types of kinetic models including pseudo-first-order, pseudo-second-order and intraparticle diffusion were used to investigate the mechanism of biosorption and potential rate controlling steps such as mass transport and chemical reaction processes. The first-order rate equation of the Lagergren is given as (Lagergren and Svenska, 1898; Ho and McKay, 1999; Arica et al., 2001; Cheung et al., 2001)

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t \qquad (2)$$

where q_e and q_t are the amounts of adsorbed cadmium (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^{-1}).

The pseudo-second-order equation is also based on the sorption capacity of the solid phase is given as (Arica et al., 2001)

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

where k_2 is the second-order biosorption rate constant (g/mg min); q_e is the biosorption capacity calculated by the pseudo-second-order kinetic model (mg/g).

When the biosorbent is treated as a porous material in aqueous solution, the diffusion process can affected the biosorption process (Weber, 1985). Therefore the intraparticle diffusion equation was introduced to indicate the behaviour of intraparticle diffusion as the rate limiting step in the biosorption (Sharma and Foster, 1994). The equation is given as

$$R = K_s t^b \qquad (4)$$

where R is the percent metal adsorbed, t is the contact time (min), b is the gradient of linear plots and K_s is the intraparticle diffusion constant.

2.5.3. Thermodynamic parameters

In order to determine the thermodynamic parameters, experiments were carried out at different temperature 303–313 K for cadmium (II) biosorption. The free energy change (ΔG^0) of the sorption reaction is given as

$$\Delta G^0 = -RT \ln K_c \qquad (5)$$

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. The values of enthalpy (ΔH^0) and entropy change (ΔS^0) can be obtained from the slope of the plot of ΔG^0 versus T.

3. Results and discussions

3.1. Effect of initial cadmium (II) ions concentrations

Fig. 1 shows the effect of initial cadmium (II) concentration on biosorption of cadmium (II) ions onto immobilized cells of *P. sanguineus* and plain alginate beads. The uptake of cadmium (II) ions onto plain alginate beads had little effect compared to immobilized cells of *P. sanguineus*. The biosorption capacity of cadmium (II) ions was studied at different initial cadmium (II) concentration in a range of 50–300 mg/L at pH 4. Results showed that higher the initial cadmium (II) concentration, greater was the biosorption capacity of the metal ions. According to a few researchers, an initial metals concentration provides a driving force to overcome mass transfer resistance of cadmium (II) ions between the aqueous and solid phases (Aksu, 2002; Malkoc and Nuhoglu, 2005). An increase in the initial cadmium (II) concentration increased the mass transfer driving force of the metal ions between the aqueous solution and biosorbent phases which lead an increase in metal ions uptake (Salinas et al., 2000; Nollet et al., 2003; Malkoc and Nuhoglu, 2005). However, the percentage of cadmium (II) ions removal decreased as the initial cadmium (II) ions concentration increased. In fact, for an initial cadmium (II) ions concentration 50 mg/L, the ions removal achieved 79% compared to 37% for 300 mg/L within 4 h. Thus, indicating that at higher metal concentrations, the available sites for a biosorption process to occur were limited, consequently, decreased the biosorption yields (Malkoc and Nuhoglu, 2005).

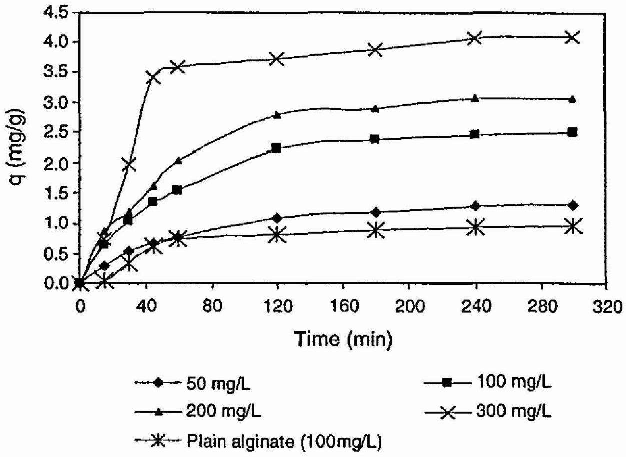

Fig. 1. Effect of initial Cd (II) concentration onto immobilized cells *P. sanguineus* (Condition: pH 6.0; biomass loading 3 g; agitation 150 rpm).

3.2. Effect of pH

Heavy metals biosorption significantly dependent on pH. In order to study the effect of pH on biosorption of cadmium (II) onto immobilized cells of *P. sanguineus*, experiments were carried out in the range of pH 2–6. Results showed that pH appreciably affected the biosorption of cadmium (II) ions onto immobilized cells of *P. sanguineus*. It was found that the biosorption capacity of cadmium (II) was lower at low pH and started to increase as the pH solution increased. The maximum biosorption of cadmium (II) ions were observed at pH 6. No experiments

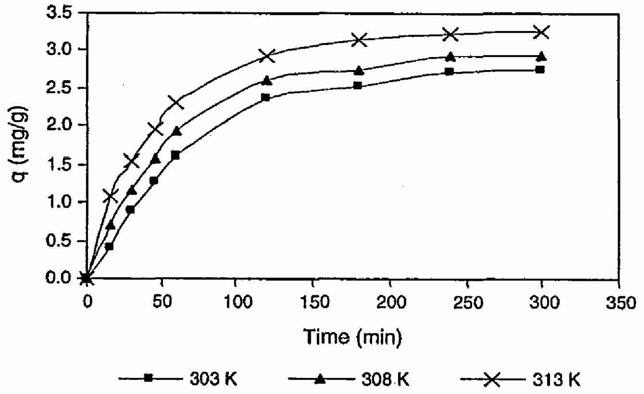

Fig. 2. Effect of temperature on the uptake of Cd (II) ions by immobilized cells of *P. sanguineus* (*condition*: pH 6.0; 100 mg/L Cd (II) ions; agitation 150 rpm).

were conducted at pH beyond 6.0 as precipitation will occur. According to Malkoc and Nuhoglu (2005), H^+ ions would compete with metal cation for the exchange site at low pH values (pH 2), which results in lower uptake of metal ions. Previous researchers reported that the functional groups such as carboxyl, amine group and phosphate were responsible for biosorption of heavy metals (Kapoor and Viraraghavan, 1997; Dursun, 2006). At pH which is above the isoelectric point, the surface charge became negative and the ionic state of ligands such as amino groups, carboxyl and phosphate will promote the reaction with cadmium (II) ions, resulting in rapid binding efficiency (Arica et al., 2001).

3.3. Effect of biosorbent loading

The experiments were carried out by varying the biosorbent loading from 1.0 to 6.0 g. It was found that the cadmium (II) ion uptake decreased from 3.42 to 1.36 mg/g as the biosorbent loading increased from 1.0 to 6.0 g. The higher biosorbent loading could produce a ‘screen’ effect on the cell wall, protecting the binding sites, thus resulting in lower cadmium (II) uptake (Mashitah et al., 1999; Malkoc and Nuhoglu, 2005). A similar phenomenon was also observed for the biosorption of copper (II) onto *P. sanguineus* (Mashitah et al., 1999).

3.4. Effect of temperature

The effects of temperature on biosorption of cadmium (II) onto immobilized cells of *P. sanguineus* were carried out by varying a series of temperature from 30 (313 K) to 40 °C (303 K). Results presented in Fig. 2 shows that the biosorption capacity of 100 mg/L cadmium (II) ions increased from 2.75 to 3.27 mg/g as the temperature increased from 30 to 40 °C. Similar results were reported on biosorption of copper (II) and lead (II) onto dried *A. niger* (Dursun, 2006). The increase in the biosorption capacity with the increase in temperature indicates that the biosorption of cadmium (II) onto immobilized cells of *P. sanguineus* is endothermic. A similar trend was observed for the biosorption of cadmium (II) onto *Rhodotorula rubra* (Salinas et al., 2000).

3.5. Equilibrium isotherms

The Langmuir constants (q_{max}, b and R^2) for the cadmium (II) biosorption onto immobilized cells of *P. sanguineus* are presented in Table 2. The maximum capacity, q_{max} determined from the Langmuir isotherm defines the total capacity of the immobilized cells for cadmium (II) ions. The biosorption capacity of cadmium (II) onto immobilized cells of *P. sanguineus* increased with increase in temperature. High values of K_c indicate the affinity for binding of cadmium (II) ions (Dursun, 2006). The magnitude of K_f and n were calculated and the values are presented in Table 2. It was found that the values of K_f also increased as the temperature increase even the correlation coefficient was less than 0.9 for all the temperatures studied. The n value was greater than unity at all studied temperatures indicating a favorable biosorption processes (Wang et al., 2007). Another equation used in the analysis of isotherms was proposed by Redlich–Peterson. The Redlich–Peterson (R–P) constants were determined by non-linear regression method. The R–P isotherm constants K_{rp}, a_{rp} and β are also presented in Table 2. Based on the values of correlation coefficients, R^2 listed in Table 2, Langmuir isotherm model best described the experimental data for immobilized cells of *P. sanguineus* at different temperatures followed by Redlich–Peterson and Freundlich isotherm models.

3.6. Biosorption kinetics modeling

In order to analyze the biosorption kinetics of cadmium (II) ions onto the immobilized cells of *P. sanguineus*, the pseudo-first-order, pseudo-second-order and intraparticle diffusion models were tested using experimental data. The corresponding parameters were determined by linear regression and are listed in Table 3. Result obtained

Table 2
Biosorption equilibrium constants obtained from Langmuir, Freundlich and Redlich–Peterson isotherms in the biosorption of cadmium (II) ions onto immobilized *P. sanguineus*

T (K)	Langmuir		Freundlich				Redlich–Peterson			
	q_{max} (mg/g)	K_c (L/mg)	R^2	K_f	n	R^2	K_{rp} (L/mg)	a_{rp} (L/mg)$^\beta$	β	R^2
303	3.18	1.17	1.000	1.70	6.74	0.834	2.68	0.83	1.0	0.996
308	3.27	1.19	1.000	1.76	6.79	0.844	3.22	0.98	1.0	0.998
313	3.32	1.34	1.000	1.83	7.01	0.860	4.26	1.28	1.0	1.000

Table 3
Kinetic constants using pseudo-first-order, pseudo-second-order and intraparticle diffusion models at various temperatures in the biosorption of cadmium (II) ions onto immobilized *P. sanguineus*

T (K)	Pseudo-first-order		Pseudo-second-order		Intraparticle diffusion	
	k_l (min^{-1})	R^2	k_2 (g/mg min)	R^2	K_s (mg/(g min$^{0.5}$)	R^2
303	0.0076	0.9583	0.0278	0.9662	2.0773	0.8570
308	0.0088	0.9758	0.0258	0.9642	2.0253	0.8685
313	0.0099	0.9703	0.0286	0.9706	1.9570	0.8809

indicates that the pseudo-first-order best described the data for biosorption of cadmium (II) ions onto immobilized cells of *P. sanguineus* at 308 and 313 K followed by pseudo-second and intraparticle diffusion.

3.7. Thermodynamic parameters

The Gibbs free energy changes (ΔG^0) for the biosorption of cadmium (II) onto immobilized cells of *P. sanguineus* were calculated using Eq. (4). Results obtained for the Gibbs free energy change values were −404.94, −435.78 and −757.58 J/mol when the temperature increased from 303 to 313 K. This indicated the spontaneous nature of the biosorption (Dursun, 2006) and highly favorable at high temperature. The standard enthalpy (ΔH^o) and entropy change (ΔS^0) of biosorption calculated were found to be 10.329 kJ/mol and 35.265 J/mol/K, respectively. The positive value of ΔH^0 indicates the biosorption process was endothermic nature and the positive value of ΔS^o suggest increased randomness at the solids/solution interface during the biosorption of metal ions onto biosorbent (Mashitah et al., 1999; Bayramoglu et al., 2003).

The pseudo-first-order constant of cadmium (II) biosorption is expressed as a function of temperature by Arrhenius equation:

$$\ln k_1 = \ln A_0 - \frac{E_a}{RT} \tag{6}$$

where A_0 is the temperature independent factor called 'frequency factor', E_a is the Arrhenius activation energy of sorption (kJ/mol), R is the gas law constant (8.314 J/(mol K)) and T is the absolute temperature (K). The activation energy in the range of 5–40 kJ/mol suggests a physisorption process, while at higher activation energy 40–800 kJ/mol suggests chemisorption (Nollet et al., 2003). In the present study, the activation energy, E_a was 20.88 kJ/mol, indicating that the process followed physisorption mechanism.

4. Conclusions

The ability of immobilized cells of *P. sanguineus* to adsorb cadmium (II) ions from aqueous solution was investigated in a batch studies. Results showed that the initial cadmium (II) concentration, pH and biomass loading highly affected the cadmium (II) biosorption. The biosorption capacity increased as the initial cadmium (II) ion concentration and temperature increased. Langmuir. Freundlich and Redlich–Peterson isotherm models fitted well the experimental data with $R^2 \sim 1$. The kinetics of cadmium (II) ions sorption on different loading of immobilized cell of *P. sanguineus* was best described using pseudo-first-order followed by pseudo-second and intraparticle diffusion models. Thermodynamic constant such as ΔG^0, ΔH^0 and ΔS^0 calculated indicates that the biosorption of cadmium (II) ions onto immobilized cells is spontaneous process and endothermic in nature.

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BIOSORPTION OF CHROMIUM BY IMMOBILIZED *PYCNOPORUS SANGUINEUS* IN FIXED-BED COLUMN

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ABSTRACT

Biosorption of chromium (Cr) from an aqueous solution on an immobilized brown rot-macrofungi, *Pycnoporus sanguineus* was studied in a fixed bed column, with respect to initial Cr concentration, ratio of biosorbent to sodium alginate concentration, column bed height and flow rate of metal solution into the column. The optimum biosorption conditions were found to be initial Cr concentration 100 ppm, biosorbent to sodium alginate ratio 1:21, column bed height 15 cm and Cr solution flow rate 1.5 ml min^{-1}, respectively. The biosorption rate of the system was also investigated as a function of flow rate. Increasing the flow rate decreased the external mass transfer resistance, but at higher flow rates lower adsorption rate were observed due to insufficient contact times for the establishment of adsorption equilibria between immobilized cells and Cr ions. .

Keywords: Biosorption; immobilized cell; *Pycnoporus sanguineus;* chromium; fixed bed column

INTRODUCTION

The utilization of biosorption technology for the treatment of heavy metal contaminated wastewaters and for the recovery of precious metals from industrial wastes or in metallurgical effluents is importance [1]. It involves an interaction between toxic metal ions and the functional groups such as carboxylate, hydroxyl and amino groups present on the cell wall surface composed of polysaccharides, proteins and lipids [2,3]. This technology which utilizes live, dead and chemical pretreated biological cells has become an alternative method to conventional separation technique applied to the treatment of industrial effluents [1,2,4] or aqueous media [5]. It could remove heavy metals from sludges at costs much lower than conventionally used in ion exchange systems [6].

Since Malaysia is a tropical country, great diversity of macro-fungi was established as they could exploit a wide range of substrates, having different behaviours and genetically easy to adopt changes of environmental conditions. Brierley and Gadd [7,8] reported that micro-organism such as fungi removed heavy metals in many ways. In the case of live cells, under the effect of metabolic cycle, some heavy metal ions will go through cell membranes and enter the cells. This metal uptake is referred to as an active uptake or intracellular uptake. The passive and active uptake consists of what is termed as "bioaccumulation". However, once heavy metal ions are entrapped in the cellular structure of a micro-organism, they will bind to the binding sites. This passive uptake, independent of the biological metabolisms is termed as "biosorption" which is a non-directed physico-chemical interaction that may occur between metal species and the cellular compounds of biological species [9]. Although it is well understood what some macro-fungi does, its biosorption pattern and physiological functions either freely or immobilized in fixed-bed column is lessly reported. In fact, biosorption models in column studies let alone to be exploited.

For this reason, a study was conducted to investigate the ability of immobilized macro-fungi *Pycnoporus sanguineous* to remove Cr ions from an aqueous solution in fixed bed column. The influences of different parameters such as initial Cr concentration, biosorbent to sodium alginate ratio, column bed height and flow rate of Cr solution into the column on the sorption of Cr were examined. The biosorption rate, k_p of Cr ions at various flow rates will also be looked at.

MATERIALS AND METHODS

Microorganism

Pycnoporus sanguineus capable to adsorb heavy metals [5] was obtained from Forest Research Institute Malaysia (FRIM), Kepong, Selangor. Culture was maintained by weekly transfer on malt extract agar slant incubated at 30°C for 6 days, after which they were stored at 4°C until required.

Preparation of production media

The composition of the medium that will be used comprised (gl^{-1}): glucose 20, yeast extract 10 and malt extract 10, respectively. The pH of the medium was adjusted to pH 9 prior autoclaving at 121°C (1.5 bar) for 15 minutes.

Cell suspension preparation

Cell suspension was prepared by inoculating a stock culture of *Pycnoporous 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 sterile Tween 20 solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was then vortexed for 3 minutes so that the mycelium will be evenly distributed in the liquid.

Cultivation condition

15 ml of cell suspension was inoculated into an Erlenmeyer flask containing 135 ml of the production medium. The flask will then be incubated in a rotary shaker at 30°C, 150 rpm for 24 hr. The sample will then be harvested and centrifuged at 3000 rpm for 10 minutes.

Preparation of immobilized gel beads

Sodium alginate beads were prepared by dropping a mixture of sodium alginate solution and *Pycnoporus sanguineus* cells into 2% (w/v) $CaCl_2$ solution under magnetic stirring (slow) at room temperature. The beads were stirred in this solution for 30 minutes. Successively, they was than collected by filtration, washed three times with sterile deionized water and stored in Tris-HCl buffer pH 7 at 4°C until used.

Preparation of metal solution

Metal solutions were prepared by diluting 1000 ppm chromium nitrate solutions with deionized water to a desired concentration range between 10 to 50 ppm. For each of the solution, the initial concentrations of the metals were determined using an Atomic Absorption Spectrometer (AAS).

Biosorption studies

Biosorption studies were performed in a jacketed glass column (length 60 cm, i.d. 4 cm) at room temperature (Figure 1). Immobilized beads were packed into it by a wet packing technique. The bed was supported and closed by glass wool plugs to ensure good liquid distribution. The effective length of the column was 15 cm which was rinsed with sterile deionized water and left overnight to ensure a closely packed arrangement of particles without voids, channels or cracks. The metal ion solution was fed from the top at a fixed flow rate of 1.5 ml min^{-1} keeping a constant head of 16 cm. Samples of the eluate were collected periodically and analyzed for the Cr concentration (C) using AAS. The flow through the column was continued till the outlet and inlet concentrations were equal. The breakthrough curve was obtained by plotting C/C_0 against time.

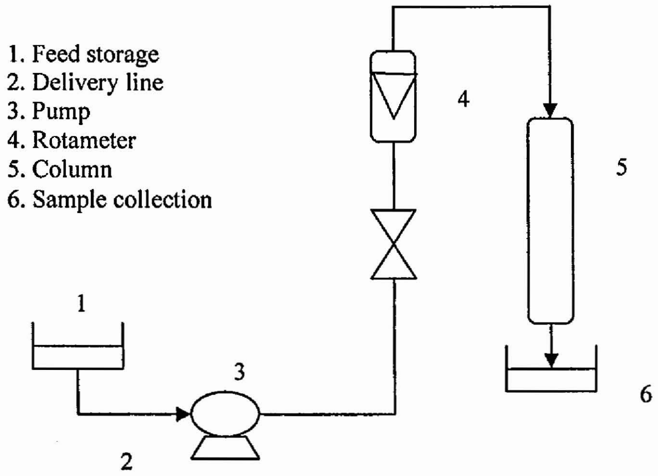

Figure 1: Experimental set up for biosorption studies in fixed bed column

RESULTS AND DISCUSSION

Effect of flow rate

The effect of flow rate on the chromium (Cr) sorption characteristics of immobilized *Pycnoporus sanguineus* in a fixed bed column was examined at room temperature by varying the flow rate from 1.5 to 8 ml min^{-1}, while the bed height and inlet Cr concentration was held constant at 15 cm and 50 ppm, respectively. The plot of C/C_0 versus time at different flow rates are shown in Figure 2. The results show that the biosorption of Cr ions onto the biosorbent beads was dependent on flow rate. From the figure, at the lower flow rate of 1.5 ml min^{-1}, the breakthrough curve followed the typical S-shape curve for column operation. Relatively higher uptake values were observed for Cr biosorption at the beginning of column operation for 4 h due to the magnitude of concentration driving force and number of active sites. But, as solution continued to flow, the concentration of Cr in the effluent suddenly rose until it become constant (~ 10 h). As the flow rate increased, the breakthrough curves became steeper. This behavior can be explained that Cr biosorption by the immobilized macro-fungi was affected by insufficient residence time of the Cr in the column. According to Aksu, Egretli and Kutsal [10] this might be due to diffusion phenomena in which at lower flow rate or longer contact time, Cr biosorption by a column of immobilized *Pycnoporus sanguineus* cells were highly effective.

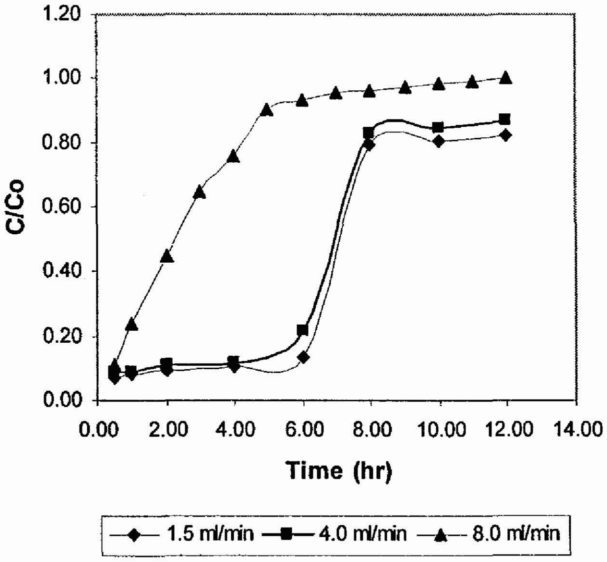

Figure 2: Breakthrough curve of chromium biosorption at different flow rate. Condition: 50 ppm of Cr solution, ratio of Na-alginate to biomass 21:1 and bed height 15 cm.

Effect of inlet metal concentration

The column biosorption performance of immobilized *Pycnoporus sanguineus* for Cr was also tested at various inlet metal concentrations. The breakthrough curves obtained by changing inlet Cr concentration from 10 ppm to 50 ppm at 1.5 ml min^{-1} flow rate are given in Figure 3. As expected, a decreased inlet Cr concentration gave a later breakthrough curve since the lower concentration gradient caused a slower transport. At 10 ppm of inlet Cr concentration, the sorption was very efficient in the initial steps of the process. Similar observation was also obtained for inlet Cr concentration (25 ppm). Much sharper breakthrough curves, an indicator of shortened mass transfer zone were obtained especially at higher inlet Cr concentration. The breakthrough point for a higher Cr concentration was earlier than that for a lower influent Cr concentration as the binding sites became more quickly saturated in the systems. Meaning that, the driving force for biosorption is the concentration difference between the solute on the sorbent and the solute in the solution [11]. A high concentration difference provides a high driving force for the biosorption process and this may explain why higher biosorption capacities was achieved in the column fed with higher Cr concentration.

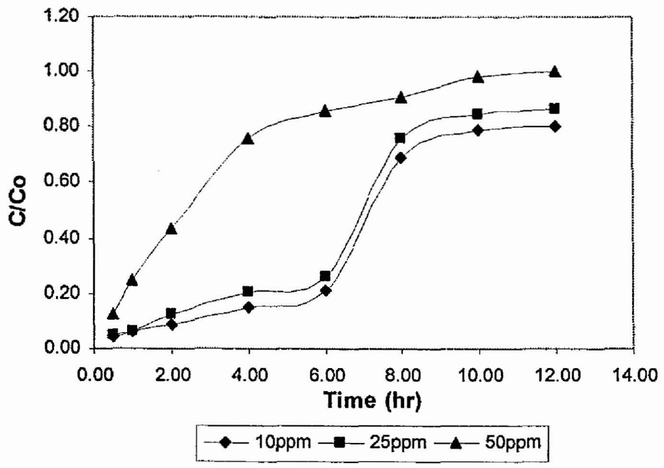

Figure 3: Breakthrough curves of *Pycnoporus sanguineus* at different inlet chromium solution. Condition: Flow rate 1.5 ml min^{-1}, ratio of Na-alginate to biomass 21:1 and bed height 15 cm.

Effect of biosorbent to sodium alginate concentration

To investigate the effect of biosorbent to sodium alginate concentration, the biosorption of Cr onto immobilized *Pycnoporus sanguineus* was measured at three different ratios (biosorbent: Na-alginate at 1:21, 1:28 and 1:47) for initial Cr concentration of 10 ppm, and the breakthrough curves were presented in Figure 4. It was shown that the adsorbed Cr concentration increased with decreased in biosorbent to sodium alginate ratio. It is readily understood that the number of available biosorption sites increases with a decrease in Na-alginate concentration and this results in an increase of adsorbed Cr concentration. The decrease in adsorption capacity with increasing biosorbent to Na-alginate ratio (1:47) is mainly due to unsaturation of adsorption sites throughout the reaction. Another reason may be due to lesser cell to metal interaction, resulted from high Na-alginate concentration. Such condition would lead to decrease in total surface area of the sorbent (cell) and an increase in diffusional path length.

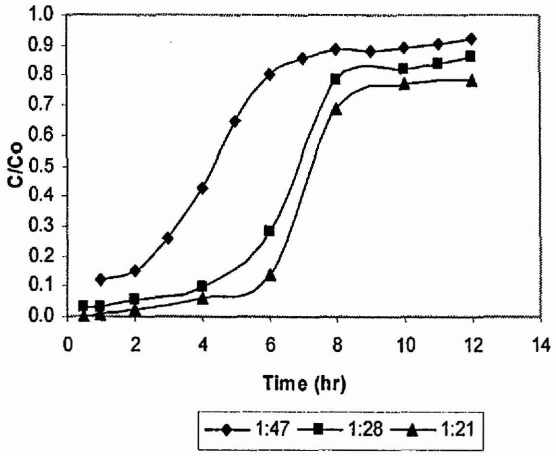

Figure 4: Breakthrough curves of *Pycnoporus sanguineus* at different ratio of biomass to Na-alginate. Condition: Flow rate 1.5 ml min^{-1}, 10ppm of Cr solution and bed height 15 cm.

Effect of bed height

Figure 5 shows the breakthrough profiles of Cr adsorption for different bed height at flow rate 1.5 ml min^{-1}, initial inlet Cr concentration 10 ppm and ratio of Na-alginate to biosorbent 21:1. The biosorption of Cr increased with the increased in the bed height from 5 to 15 cm. This was reflected from the breakthrough curves with a bed height of 5 cm; the biosorbent gets saturated early compared to other bed heights. The increase in the Cr biosorption with the increase of bed height in the column was due to the increase in the surface area of biosorbent (immobilized *Pycnoporus sanguineus*) which provided more binding site for the biosorption. The breakthrough time was also increased with an increase in bed height.

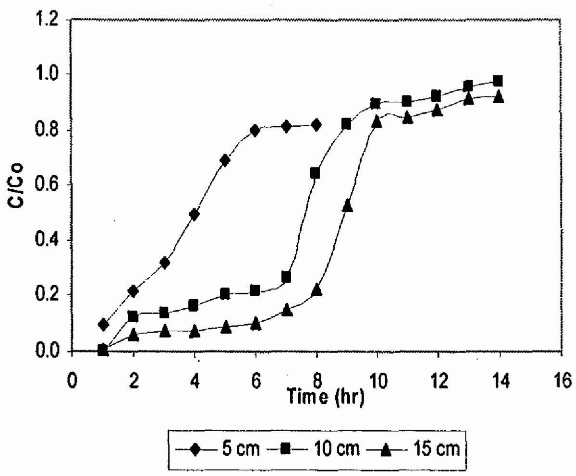

Figure 5: Breakthrough curves of *Pycnoporus sanguineus* at different bed height. Condition: Flow rate 1.5 ml min^{-1}, 10 ppm of Cr $(NO_3)_2$ and ratio of Na-alginate to biomass 21:1.

Biosorption Rate

The mass balance for chromium in packed bed column reactor is given by Equation 1 as described by Dursun and Aksu [12]. In the development of this equation, steady state, plug flow, no axial dispersion and spherical immobilized cell particles were assumed.

$$\left(\frac{HQ}{w}\right)\frac{dC}{dz}X6X10^{-2}=-q \qquad \text{Equation (1)}$$

where q is the biosorption rate (mg $g^{-1}hr^{-1}$), Q a volumetric flow rate (ml min^{-1}), H is the height of column (cm), w is the total amount of wet cells in the immobilized particles (g) and $\frac{dC}{dz}$ is the concentration gradient along the column length (mg cm l^{-1})

The relationship between the biosorption rate and the substrate (Cr) concentration in the column when reach equilibrium is given as

$$q=\frac{k_pC}{1+k_pC} \qquad \text{Equation (2)}$$

Assumed that the biosorption rate is first order (this assumption applied to lower substrate concentrations), the relationship between the biosorption rate and the substrate (chromium) concentration in the column become

$$q = k_p C \qquad \text{Equation (3)}$$

where k_p is a first order biosorption rate constant (l $g^{-1}hr^{-1}$)

Substituting Equation 3 into 1, Equation 4 will be obtained.

$$\left(\frac{HQ}{w}\right)\frac{dC}{dz} X6X10^{-2} = -k_p C \qquad \text{Equation (4)}$$

After integrating Equation 4 using boundary conditions at z = 0, C = C_o and at z = H: C = C

$$\ln\left(\frac{C_o}{C}\right) = \frac{W}{Q} k_p X \left(\frac{10^3}{60}\right) \qquad \text{Equation (5)}$$

where C_o is an inlet substrate concentration (mg l^{-1}) and C is an outlet substrate concentration (mg l^{-1}).

The observed first order biosorption (k_p) can be calculated from Equation 5 at different flow rates when constant wet cells quantity was immobilized in the gel beads.

Table 1 shows the calculated values of k_p at different flow rate. The higher the flow rate, the lower will be the value of k_p. According to Aksu and Kutsal [13], increasing the flow rate decreased the external mass transfer resistance but higher flow rates lowered the adsorption rates because of insufficient contact times for establishing adsorption equilibria between immobilized cells and metal ions.

Table 1: Values of k_p at various flow rates

Q (ml /min)	C/C_o	C_o/C	ln (C_o/C)	k_p
1.5	0.6667	1.9477	0.0486	0.00040
2	0.5000	1.6487	0.0416	0.00035
4	0.2500	1.2840	0.0060	0.00013
6	0.1667	1.1814	0.0149	0.00011
8	0.1250	1.1331	0.0038	0.00010

CONCLUSION

The living cell of *Pycnoporus sanguineus* immobilized in the Na-alginate was able to adsorb chromium ions at a flow rate of chromium solution 1.5 ml min^{-1}, ratio of Na-alginate to biomass 21:1 and the bed height was 15 cm, respectively. For the biosorption rate, the value of k_p tends to decrease when the flow rate of chromium solution through the bed increased. This could be due to lesser contact time at higher flow rate between the immobilized cells beads and chromium ions.

ACKNOWLEGDEMENT

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EQUILIBRIUM AND KINETIC STUDIES OF PB(II) IONS BIOSORPTION BY IMMOBILIZED CELL OF *PYCNOPORUS SANGUINEUS.*

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ABSTRACT

The biosorption of Pb(II) ions from aqueous solution by immobilized cell of Pycnoporus sanguineus was investigated at 25°C, 150 rpm for 2 h. . The experimental results showed that the maximum pH for efficient sorption of Pb(II) ions was 4± 0.1 at which evaluated biosorbent dosage, initial Pb(II) concentration, temperature and contact time were 3g, 100 ppm and 120 min, respectively. Langmuir, Freundlich and Redlich-Peterson isotherm models fitted well to data of Pb(II) biosorption as represented by their high value of correlation coefficient ($R^2 \simeq 1$). The change in the biosorption capacity with time was found to fit the pseudo-second order model at different temperature.

Keywords: *Biosorption; Equilibrium; Kinetics; Lead, Pycnoporus sanguineus*

INTRODUCTION

The presence of lead in the environment such as soil and natural water will pose serious problems to the ecological system including humans. It may come from various industrial sources such as battery manufacturing, textile, mining and metal finishing. The removal of this substance from water and industrial wastewater has become a challenge to researchers. Conventional methods used to remove metal ions in industrial effluents such as chemical precipitation, membrane separation, activated carbon adsorption and ion exchange [1, 2] may be inapplicable for some disadvantages such as inefficient, high operational costs at a very low concentration of metal ions in treated water [3] and disposal of metal sludge [4]. For a past few decades, the metal biosorption by various biological materials including fungi, algae, bacteria and yeast have received great attention [5]. The biosorption process is a passive uptake that utilizes cell wall of biomass to sequester the metal ions from aqueous solutions [6, 7]. The presence of functional groups on biomass cell wall such as carboxyl, hydroxyl, ketones and amino groups will involves a physico-chemical interaction between the metal ions during the biosorption processes [8].

Fungi have been well known for many years used as a biosorbent in heavy metals biosorption. It may be better suitable for the removal of metals from wastewater than other microbes because of their great tolerance towards heavy metals and other adverse conditions such as low pH, high cell wall binding capacity and high intracellular metal uptake capacity [9]. Microbial metal uptake can often be divided into three main phases: extracellular accumulation/precipitation, cell surface sorption and intracellular accumulation [10]. Both living and dead fungal cells have a capability to uptake metal ions [11]. Several fungal biosorbents (*A. niger* spp., *Rhizopus* spp., Saccharomyces spp., and *Mucor* spp.) have already been studied as a potential biosorbent in heavy metals removal from aqueous solution [12, 13, 14]. *Ganoderma* species also have been reported as an efficient biosorbent in copper removal compared to other macrofungi species [15].

Cell immobilization is one of the methods used to overcome the incorporating free suspended cell in an engineering process [16, 17]. The several advantages of immobilized cell over free suspended cell include enhancing cell productivity and stability [16, 18, 19, 20, 21, 22], capacity for re-use of immobilized cell in continuous process operation [17] and can be easily separated from the reaction system even at high dilution rates [16]. Polyacrylamide, alginate and chitin are the most immobilization carrier in laboratory research studies [16, 17].

Though dead cell of *Pycnoporus sanguineus* was a potential biosorbent for Pb(II), Cu(II) and Cd(II) biosorption, no studies were reported on Pb(II) biosorption by the immobilized cells system [8]. Thus, this study was carried out to determine the potential of immobilized living cells of *Pycnoporus sanguineus* to adsorb Pb(II) ions in a shake flask culture. Adsorption isotherm and kinetics models were also looked at.

MATERIALS AND METHOD

2.1 Microorganism and production medium

Pycnoporus sanguineus capable to adsorb heavy metals [8] was obtained from Forest Research Institute of Malaysia (FRIM), Kepong, Selangor. It was maintained by weekly transfer on malt extract agar slant incubated at 30°C for 6 days, after which were stored at 4°C until required. The composition of the production medium comprised of (gl^{-1}): glucose 20, yeast extract 10 and malt extract 10, respectively. The pH of the medium was adjusted to 9.0 prior autoclaving at 121°C (1.5 bar) for 15 minutes.

2.2 Immobilized cells preparation

Cell suspension was prepared by inoculating a stock culture of *Pycnoporus sanguineus* onto malt extract agar plates and incubated at 30°C for 6 days. The mycelial mat formed was scraped by a sterile blade and mixed with 10 ml sterile Tween 20 solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was then vortex for 3 minutes so that the mycelium was evenly distributed in the liquid.

15 ml of cell suspension was inoculated into an Erlenmeyer flask containing 135 ml of the production medium. The flask will then be incubated in a rotary shaker at 30°C, 150 rpm for 66 hr. The harvested sample was centrifuged at 3500 rpm for 4 minutes. Sodium alginate beads were prepared by dropping a mixture of sodium alginate solution and *Pycnoporus sanguineus* cells into 2% (w/v) $CaCl_2$ solution under magnetic stirring (slow) at room temperature. The beads were stirred slowly for 30 minutes, then collected by filtration, washed three times with sterile deionized water and stored in Tris-HCl buffer pH 7 at 4°C until used.

2. 3 Preparation of metal ions

Metal solutions were prepared by diluting 1000 ppm of $Pb(NO_3)_2$ solutions with deionized water to a desired concentration range between 50 to 300 ppm. For each of the solution, the initial concentrations of the metals and samples after biosorption treatment were determined using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

2.4 Batch biosorption procedures

The biosorption of Pb(II) ions on the plane alginate beads and immobilized *Pycnoporus sanguineus* from aqueous solution was investigated in batch biosorption equilibrium experiments. Effect of initial Pb(II) ions concentration, solution pH, biomass loading and temperature on the biosorption rate and capacity were studied. The plane alginate beads were used as a control system or blank (result not shown).

The effect of solution pH on the biosorption rate of the alginate beads and immobilized *Pycnoporus sanguineus* preparation with Pb(II) ions (100 ml) was investigated in the range of 2 to 4 (which was adjusted with HCl or NaOH at the beginning of the experiment and not controlled throughout the experiments) at 150 rpm, 30°C for 2 hr. For initial Pb(II) concentration, the biosorption studies was carried out at pH 4 as described earlier except that the concentration of Pb(II) ions was varied between 58 to 300 mgL^{-1} . The effect of biomass loading was varied between 1 to 6 g and other procedure was as described before. For equilibrium study, the initial Pb(II) concentration was 100 mgL^{-1} and working pH was at pH 4. The contact time was varied between 15 and 120 min at different temperatures such as 30°C, 35°C and 40°C, respectively.

The amount of Pb(II) ions bound by the biosorbent was calculated using the following equation:

$$q = \frac{V(Ci - Cf)}{W} \tag{1}$$

where q is mg of metal ion biosorbed per g of biomass (mgg^{-1}), C_i (mgL^{-1}) is the initial concentration, C_f (mgL^{-1}) is the final or equilibrium concentration, V (L) the volume of metal solution in the flask and W (g) is the weight of biosorbent. Each of the experiment was repeated three times and the results given are the average values. Samples taken after the desired incubation period were measured using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

Kinetics experiments were carried out at a known concentration of Pb(II) with varied biosorbent loading and agitating at 150 rpm. At pre-determined interval time, samples were withdrawn, filtered and Pb(II) concentration was measured using an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

2.5 Equilibrium isotherm and kinetics studies

2.5.1 Equilibrium isotherm models

Langmuir, Freundlich and Redlich-Peterson models have been used to determine the sorption equilibrium between the solid biosorbent and metals ions. The Langmuir model assumes that a monomolecular layer is formed when biosorption takes place without any interaction between the adsorbed molecules [4, 23]. The Langmuir equation is given by Eq. (2)

$$q_e = \frac{q_{\max} K_b C_e}{1 + K_b C_e} \tag{2}$$

where q_{max} is the maximum specific uptake corresponding to the sites saturation, K_b is equilibrium constant (dm^3mg^{-1}) and both parameter can be determine from a linearised from Eq. (2)

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

where C_e is the equilibrium concentration (mgL^{-1}); q_e is the amount of metal ions uptake at equilibrium (mgg^{-1}); q_{max} is q_e for a complete monolayer (mgg^{-1}); K_b is equilibrium constant (dm^3mg^{-1}).

Freundlich isotherm model [24]:

$$q_e = K_f C_e^n \tag{4}$$

And the equation may be linearised and described by Eq. (5)

$$\ln q_e = \frac{1}{n} \ln C_e + \ln K_f \tag{5}$$

where q_e is the amount of metal ions uptake (mgg^{-1}); C_e is the equilibrium concentration (mgL^{-1}); K_f and n are the Freundlich constant and can be determined by plotting *ln* q_e versus *ln* C_e.

The Redlich-Peterson isotherm [25] has three parameters and incorporates the features of the Langmuir and the Freundlich isotherms. It can be described by the following equation:

$$q_e = \frac{K_{rp} C_e}{1 + b C_e^{\beta}} \tag{6}$$

where K_{rp}, b and β ($0 < \beta < 1$) are the isotherm constants. These isotherms can be evaluated from the linear plot described by Eq. (7) using a nonlinear regression method.

$$\ln(K_{rp} \frac{C_e}{q_e} - 1) = \beta \ln(C_e) + \ln b \tag{7}$$

2.5.2 Kinetic modeling

Kinetic models have been used to investigate the mechanism of biosorption and potential rate controlling step such as mass transport and chemical reaction processes. The kinetic models included the pseudo first-order and pseudo second-order equations by assuming the measured concentrations are equal to cell surface concentrations. The first-order rate expression of Lagergren [26] based on solid capacity was used:

$$\log(q_e - q_t) = \log(q_e) - \frac{k_1}{2.303}t \qquad (8)$$

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

The pseudo second-order equation is also based on the sorption capacity of the solid phase and could be obtained from the following equation:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e^2}t \qquad (10)$$

where k_2 is the rate constant of second-order biosorption ($gmg^{-1}min^{-1}$); q_e is the biosorption capacity calculated by the pseudo-second order kinetic model (mgg^{-1}).

RESULTS AND DISCUSSIONS

3.1 Effect of initial Pb(II) ions concentrations

The effect of initial Pb(II) ions concentration on its uptake was studied in a range 58 to 300 mgL^{-1} (Figure 1). The results showed that an increase in the initial Pb(II) concentration resulted in an increase in ions uptake. According to a few researchers, an increase in the initial Pb(II) concentration, would increase the mass transfer driving force of the ions between the aqueous and solid phases [4, 27, 28]. However, at higher concentration the percentage of Pb(II) removal become lower. In fact, for an initial Pb(II) ions concentration 58 mgL^{-1}, the ions removal achieved 96% compared to 39% for 300 mgL^{-1} within 2 h. Thus, indicating that at higher metal concentrations, the available sites for a biosorption process to occur were limited; consequently decreased the biosorption yields [4].

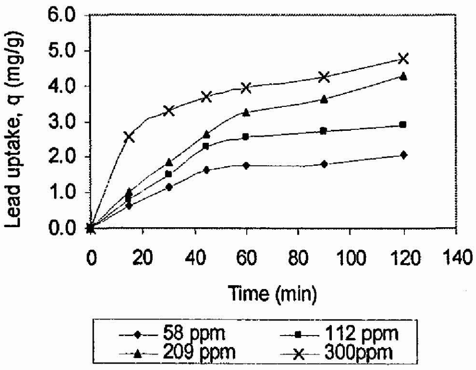

Figure1. Effect of initial metal concentration on the biosorption of Pb(II) ions by immobilized cells of *Pycnoporus sanguineus* (Condition: pH 4.0; biomass loading 3 g; agitation 150 rpm).

3.2 Effect of pH.

Heavy metals removal critically dependent on pH. In order to establish the effect of pH on Pb(II) removal by immobilized cells of *Pycnoporus sanguineus*, the biosorption studies were carried out in a range of pH from 2.0 to 4.0. It was found that solution with pH less than 4.0, the biosorption capacity decreased. As seen in Figure. 2, the maximum biosorption of Pb(II) ions were observed at pH 4.0. Malkoc and Nuhoglu [2005] stated that at lower pH value, the surface charge on the cells became positive and metal cations and proton compete for binding sites on cell wall, which results in lower Pb(II) uptake in medium. At pH which is above the isoelectric point, the surface charge became negative and the ionic state of ligands such as amino groups, carboxyl and phosphate will promote the reaction with Pb(II) ions, resulting in rapid binding efficiency [3]. In the present study, the percent removal of Pb(II) on immobilized cell of *Pycnoporus sanguineus* were more than 90% within 2 h compared to those at pH 2 and 3, respectively.

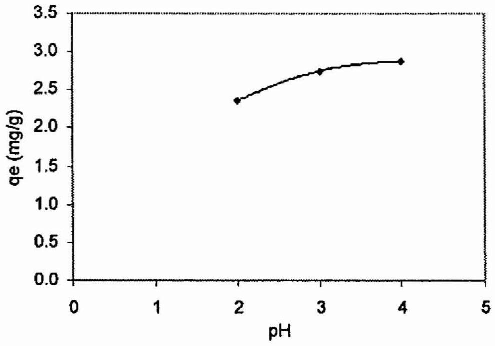

Figure 2. Effect of pH on the uptake of Pb(II) ions by immobilized cells of *Pycnoporus sanguineus* (Condition: biomass loading 3 g; 100 mgL^{-1} Pb(II) ions; agitation 150 rpm).

3.3 Effect of biosorbent loading.

Figure 3 showed the biosorption capacity of Pb(II) ions at varying biosorbent loading at 1.0 to 6.0 g. The Pb(II) ions removal increased from 23% to 99% when the immobilized cell of *Pycnoporus sanguineus* dosage increased from 1.0 to 6.0 g. This could be attributed to the presence of more binding sites for complexation of Pb(II) ions [4, 29]. However, the Pb(II) uptake were decreased from 2.89 to 1.95 mgg^{-1} as the number of biosorbent dosage increased. As seen in the figure, the maximum metal uptake capacity was observed at 3.0 g of immobilized cell of the tested fungi. As reported by previous workers, the high biosorbent loading could produce a 'screen' effect on the cell wall, protecting the binding sites, thus resulting in lower Pb(II) uptake [4, 8, 30].

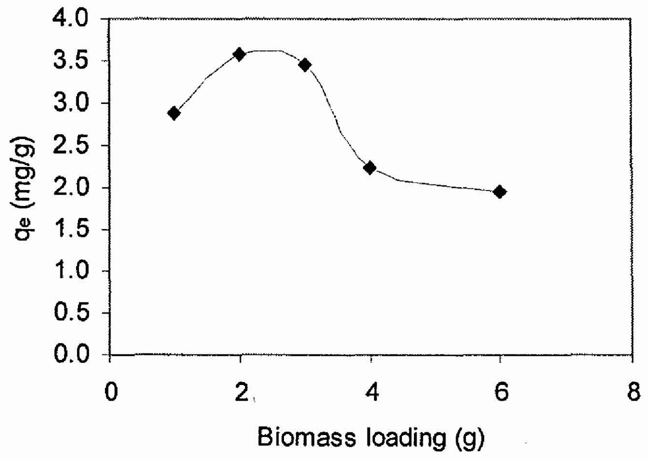

Figure 3. Effect of biomass loading on the uptake of Pb(II) ions by immobilized cells of *Pycnoporus sanguineus*. (Condition: pH 4.0; 100 mgL^{-1} Pb(II) ions; agitation 150 rpm).

3.4 Equilibrium isotherms

The linearised Langmuir, Freundlich and Redlich-Peterson equilibrium isotherms constant of Pb(II) ions biosorption were obtained at different temperatures such as 30, 35 and 40°C, respectively (Table 1). Results showed that biosorption data of the metals onto immobilized cells of *Pycnoporus sanguineus* fitted well on the three isotherm models with correlation efficiency $R^2 \simeq 1$. The Langmuir constants K_b and q_{max} have been determined using Eq. (3). The equilibrium sorption capacity, q_{max} was found to increase from 3.62 to 4.43 mgg^{-1} for an increase in the temperatures from 303 K to 313 K. A high K_b value indicates that the affinity for binding of metal ions and the highest K_b were observed at 40°C. According to Malkoc and Nuhoglu [2005], an increase in K_b with temperature showed that a chemical interaction between the metal ions and biosorbent took place.

As in Table 1, the K_f values of Freundlich isotherms increased with increased in temperature. Since the n values obtained were higher than 1.0, the Pb(II) ions were favorably adsorbed by the biosorbent at all temperature studied [7]. For the Redlich-Peterson constant K_{rp}, the values increased with increased in reaction temperature and most values of β were found between 0.9-1.0, respectively.

Table 1: Biosorption equilibrium constant obtained from Langmuir, Freundlich and Redlich-Peterson isotherms.

Temperature	Langmuir			Freundlich			Redlich-Peterson			
(K)	q_{max}(mg/g)	K_b(dm^3/mg)	R^2	K_f	n	R^2	K_{rp} (dm^3/mg)	b $(dm^3/mg)^\beta$	β	R^2
303	3.62	1.34	1.000	1.55	4.71	0.933	3.928	1.075	1	0.999
308	4.00	1.42	0.999	1.84	5.03	0.962	9.955	2.63	0.99	0.999
313	4.43	1.57	0.999	2.18	5.31	0.949	25.234	7.059	0.95	0.999

3.5 Biosorption kinetics modeling

In order to analyze the biosorption kinetics of Pb(II) ions, the pseudo-first order and the pseudo-second order equation were applied to the data. Plots for both models were shown in Figure 4 and Figure 5. The pseudo-second order model fitted well the experiment data compared to pseudo-first order model with $R^2 \simeq 1$. The pseudo-second order rate constants for biosorption of Pb(II) onto immobilized cells of Pycnoporus sanguineus increase with temperature. The values were found to increase from 1.472×10^{-1} to 3.327×10^{-1} g mg^{-1} min^{-1} with an increase in the temperature from 303 to 313 K.

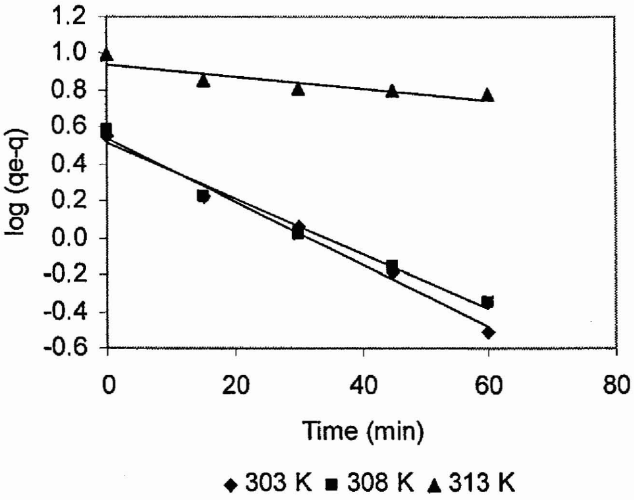

Figure 4. Linearized pseudo-first order kinetics model for Pb(II) ions uptake by immobilized cell of *Pycnoporus sanguineus* at different temperature

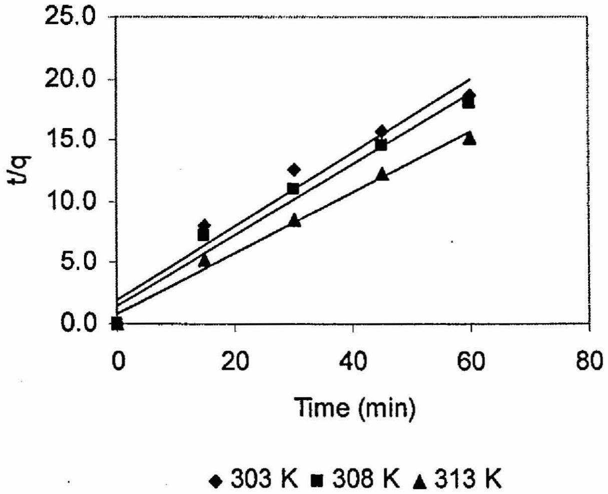

Figure 5. Linearized pseudo-second order kinetics model for Pb(II) ions uptake by immobilized cells of *Pycnoporus sanguineus* at different temperature

Table 2: Kinetic constants of pseudo first and pseudo second-order models at different temperature

T(K)	Pseudo first-order		Pseudo second-order	
	k_1 (min^{-1})	R^2	k_2 (g/mg min)	R^2
303	0.0389	0.9879	0.1472	0.9472
308	0.0345	0.9785	0.1980	0.9723
313	0.0076	0.7681	0.3327	0.9896

CONCLUSIONS

The ability of immobilized cells of *Pycnoporus sanguineus* to adsorb Pb(II) ions from aqueous solution was investigated in a batch studies. Results showed that the initial Pb(II) concentration, pH and biomass loading highly affected the Pb(II) biosorption. Langmuir, Freundlich and Redlich-Peterson isotherm fitted well the experimental data with $R^2 \simeq 1$. The kinetics of Pb(II) ions sorption onto immobilized cell of *Pycnoporus sanguineus* at different temperature was best described using pseudo second order model.

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NOMENCLATURE

b	Redlich-Peterson isotherm constant	
β	Redlich-Peterson isotherm constant	
C_e	equilibrium concentration	(mgL^{-1});
C_i	initial concentration	(mgL^{-1})
C_f	final or equilibrium concentration	(mgL^{-1})
k_1	rate constant of first-order biosorption	(min^{-1})
k_2	rate constant of second-order biosorption	(gmg^{-1}min^{-1})
K_b	equilibrium constant	(dm^{3}mg^{-1})
K_f	Freundlich constant	
K_{rp}	Redlich-Peterson isotherm constant	
n	Freundlich constant	
q	metal ions biosorbed per g of biomass	(mgg^{-1})
q_{max}	maximum specific uptake corresponding to the sites saturation	(mgg^{-1})
q_e	amount of metal ions uptake at equilibrium	(mgg^{-1})
q_t	amounts of adsorbed Pb(II) ions on the biosorbent at time t	(mgg^{-1})
V	volume of metal solution in the flask	(L)
W	weight of biosorbent	(g)

Biosorption of Pb (II) ions by immobilized cells of *Pycnoporous sanguineus* in a packed bed column

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ABSTRACT

Lead (Pb) (II) biosorption by immobilized cells of *Pycnoporus sanguineus* was investigated in a packed bed column. The experiments were carried out by considering the effect of bed height (5-13 cm), flow rate (4-12 ml min^{-1}) and initial lead (II) concentration (50-300 mg L^{-1}). The breakthrough profiles were obtained and results showed that the saturation of metal ions was achieved faster for 5 cm bed height and 12 ml min^{-1} influent flow rate. However, the breakthrough time decreased as the initial metal concentration increased from 50 to 300 mg L^{-1}. The column was regenerated using 0.1M HCl solution and biosorption-desorption studies were carried out for 2 cycles. Results showed that breakthrough time decreased as the number of cycles proceeds.

Keywords: *Biosorption; column; breakthrough curve; lead; Pycnoporus sanguineus;*

1. INTRODUCTION

Various treatment technologies have been introduced for an efficient removal of heavy metals from an industrial effluent. One of these technologies is biosorption process which utilized biological materials including fungi, algae, bacteria and yeast to accumulate metal ions from wastewater (Arica *et al.*, 2001). Conventional methods such as chemical precipitation, electrochemical treatment, membrane technology and ion exchange processes may be inefficient and expansive when operate at low metal concentration (1-100 mg L^{-1}) (Cruz *et al.*, 2004; Malkoc and Nuhoglu, 2006). Some of these treatments produced toxic sludge that caused further disposal problem. Since these biological materials are abundant and capable to adsorb metal ions, therefore biosorption process has emerged an alternative method in heavy metals removal over conventional methods (Cordero *et al.*, 2004; Cruz *et al.*, 2004). Application of fungi as a biosorbent in heavy metals removal has received a great attention (Mittar *et al.*, 1992; Arica *et al.*, 2003).

Many fungal species such as *Aspergillus niger*, *Rhizopus* spp., *Saccharomyces* spp., *Mucor* spp and *Phanerochaete crysosporium* have been extensively studied as a potential biosorbent in metal ions removal (Kapoor and Viraraghavan, 1997; Say *et al.*, 2001; Kim *et al.*, 2003; Yan and Viraraghavan 2003). For an efficient biosorbents usage in heavy metals removal in an industrial operation, these free fungal cell were immobilized in carbohydrate based polymers including alginate, chitin, chitosan and carboxymethyl cellulose (Jianlong *et al.*, 2000; Arica *et al.*, 2003). These immobilized cells offer several advantages including minimal clogging in continuous systems (Ting and Sun, 2000; Arica *et al.*, 2001; Bayramoglu *et al.*, 2003), easy to separate from the reaction system and can be regenerated and reused (Arica *et al.*, 2001; Annadurai *et al.*, 2007).

Mashitah *et al* (1999) reported that non-living biomass, *Pycnoporus sanguineus* (*P. sanguineus*), a white rot fungi is one of the potential biosorbent for Pb (II), Cu (II) and Cd (II) biosorption. However, the utilization of *P. sanguineus* cells in immobilized system has not been reported. Therefore, this study was carried out to determine the potential of live immobilized cells of *P. sanguineus* to adsorb Pb (II) ions in a packed bed column.

2. MATERIALS AND METHODS

2.1. Microorganism, Medium and Growth conditions

Pycnoporus sanguineus (*P.sanguineus*) capable to adsorb heavy metals was obtained from Forest Research Institute Malaysia (FRIM), Kepong, Selangor. Culture was maintained by weekly transfer 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 used comprised: glucose 20 g L^{-1}, yeast extract 10g L^{-1} and malt extract 10 g L^{-1} , respectively. The pH of the medium was adjusted to pH 9 prior autoclaving at 121°C (1.5 bar) for 15 minutes.

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 sterile Tween 20 solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was then vortexes for 3 minutes so that the mycelium will be evenly distributed in the liquid.

15 ml 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°C, 150 rpm for 66 hr. The sample will then be harvested and centrifuged at 3500 rpm for 4 minutes.

2.2. Preparation of immobilized cells

Sodium alginate beads were prepared by dropping a mixture of sodium alginate solution and *P. sanguineus* cells into 2% (w/v) $CaCl_2$ solution under magnetic stirring (slow) at room temperature. The beads were stirred in this solution for 30 minutes. Successively, they were collected by filtration, washed three times with sterile deionized water and stored in Tris-HCl buffer pH 7 at 4°C until used.

2.3. Preparation of Metal Ions

Metal solutions were prepared by diluting 1000 mg L^{-1} of Pb $(NO_3)_2$ solutions with deionized water to a desired concentration range between 50 to 300 mg L^{-1}. For each of the solution, the initial concentrations of the metals and samples after biosorption treatment were determined using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

2.4 Biosorption procedures

Biosorption studies were performed in a jacketed glass column (length 60 cm, i.d. 4 cm) at room temperature. Immobilized beads were packed into it by a wet packing technique. The bed was supported and closed by glass wool plugs to ensure good liquid distribution at the top and bottom of the column. Figure 1 shows the experimental set up used for the present study. The experiments were carried out to study the effect of the following variables such as:

1. Bed height (5-13 cm)
2. Flow rate (4-12 ml min^{-1})
3. Initial Pb (II) concentration (50-300 mg mL^{-1})

In a typical experiment, a known Pb (II) ion concentration feed (100 mg L^{-1}) was pumped at fixed flow rate into the column with a known bed height. Samples were collected periodically and analyzed for the Pb (II) concentration using atomic absorption spectrometer (AAS) until saturation was reached in the column. The breakthrough curve was obtained by plotting C/C_o against time or outlet concentration, C against time (t) . Operation of the column was stopped when the effluent Pb (II) concentration exceeded a value of 99.5% of initial feed concentration. Total quantity of metal mass biosorbed in the column (m_{ad}) was calculated from the area above the breakthrough curve (outlet concentration, C versus time, t) multiplied by the flow rate. Dividing the metal mass (m_{ad}) by the mass of biosorbent (M) leads to metal uptake capacity (Q) (Volesky *et a.l*, 2003; Padmesh *et al.*, 2005). The breakthrough time (t_b) is time at which metal concentration in the effluent reached 0.01% of initial feed concentration and exhaustion time ($t_{e,}$) time at which metal concentration exceeded 99.5 % of initial feed concentration, respectively.

The total amount of metal feed to the column (m_{total}) is calculated from Eq. (1):

$$m_{total} = \frac{C_o F t_e}{1000} \qquad (1)$$

Mass transfer zone can be calculated from Eq. (2)

$$\Delta t = t_e - t_b \qquad (2)$$

Total metal removal (%) with respect to flow volume is given as:

$$\text{Total metal removal } (\%) = \frac{m_{ad}}{m_{total}} x100 \qquad (3)$$

The metal mass desorbed (m_d) can be calculated from the elution curve (C versus t) and the elution efficiency is given as:

$$E(\%) = \frac{m_d}{m_{ad}} x100 \qquad (4)$$

Loaded biosorbents with metal ions were regenerated with 0.1M HCl by pumped it in down-flow operation of the column. After regeneration, the biosorption studies were carried out again. This biosorption-regeneration was repeated two times in order to investigate the biosorption capacity of the immobilized cells. The continuous experiments were conducted at room temperature (30°C).

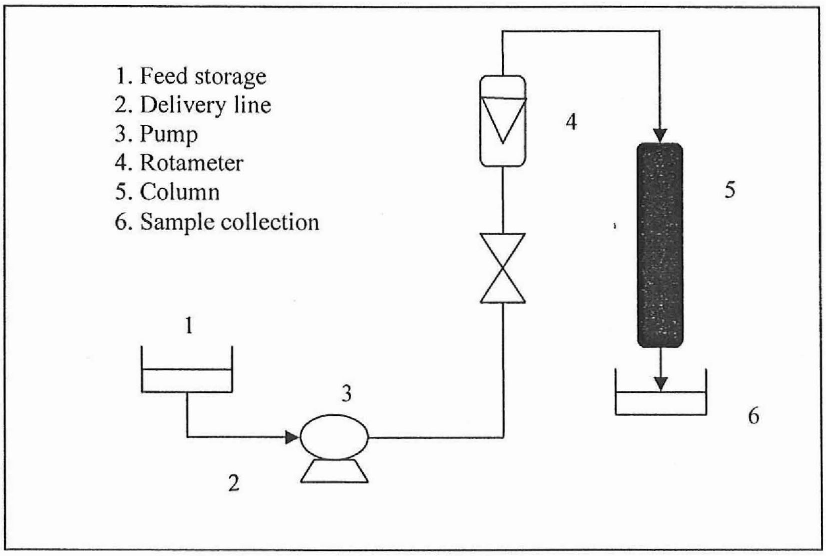

Figure 1: Experimental set up for biosorption studies in fixed bed column

3.0 RESULTS AND DISCUSSION

3.1 Effect of Bed Height

Figure 2 presents a breakthrough curve for Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different bed height 5, 9 and 13 cm, respectively. Concentration of Pb (II) solution was fixed at 100 mg L^{-1} and pH 4 for all bed height studies. It was fed into the column and maintained at 4 ml min^{-1}. Results show that Pb (II) uptake increased from 11.51 to 19.65 mg g^{-1} as the bed height increased from 5 to 13 cm. As the bed height increased, more binding sites were available for the biosorption to occur (Vijayaraghavan *et al.*, 2005). The saturation of immobilized cells of *P. sanguineus* was achieved nearly 38 hours at 5 cm bed height. However, at higher bed height of 13 cm, the saturation was obtained after 144 hours. Malkoc and Nuhoglu, (2006) stated that higher bed height would resulted in broaden mass transfer zone, thus increase the saturation period for the metals onto immobilized cells.

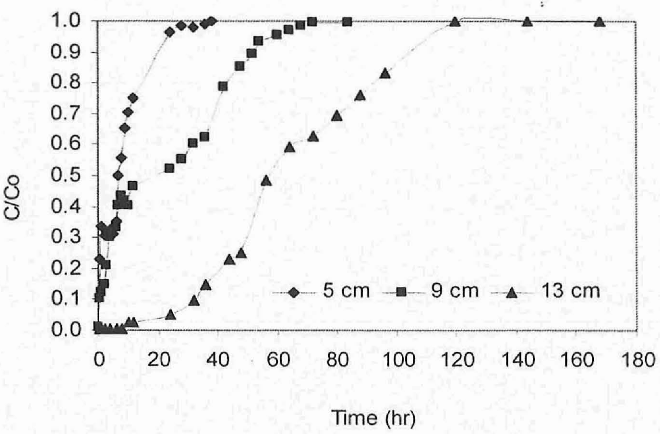

Figure 2: Breakthrough curves of Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different bed height (Condition: 100 mg L^{-1} Pb (II), flow rate: 4 ml min^{-1}, pH 4.0)

3.2 Effect of Flow Rate

Figure 3 presents the breakthrough curve of Pb (II) biosorption in column at different flow rate from 4 to 12 ml min^{-1}. The experiments were carried out at constant initial Pb (II) concentration (100 mg L^{-1}) and 9 cm bed height. Results show that Pb (II) uptake decreased with the increase in flow rate. This is due to insufficient contact time for the Pb (II) ions to be adsorbed by the immobilized cells of *P. sanguineus* (Ko *et al.*, 2000; Vijayaraghavan *et al.*, 2005). As seen in Fig. 3, a steeper breakthrough curve was observed at flow rate 12 ml min^{-1} as the breakthrough time decreased. Similar phenomenon was reported on cobalt (II) and nickel (II) biosorption by seaweeds and heavy metals removal in column by *P. sanguineus*, respectively (Zulfadhly *et al.*, 2001; Vijayaraghavan *et al.*, 2005).

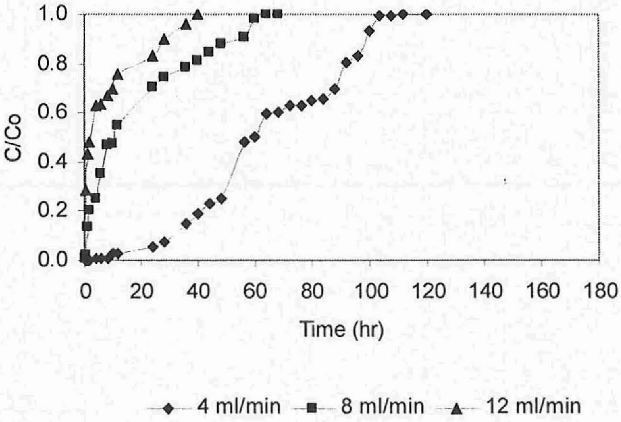

Figure 3: Breakthrough curves of Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different flow rates (Condition: 100 mg L^{-1} Pb (II), bed height= 9 cm, pH 4.0)

3.3 Effect of Initial Metals Concentration

Figure 4 shows the breakthrough profiles of Pb (II) biosorption at different initial Pb (II) concentration. As the initial Pb (II) concentration increased from 50 to 300 mg L^{-1}, the Pb (II) uptake increased from 21.74 to 25.73 mg g^{-1}. At higher initial Pb (II) concentration, the biosorbent gets saturated early thus resulted a faster breakthrough and exhaustion time (Zulfadhly *et al.*, 2001; Malkoc and Nuhoglu, 2006). For 50 mg L^{-1} of Pb (II) solution, less driving force occurred between metal ions and the immobilized cells of *P. sanguineus* which resulted in a broadened mass transfer zone (Malkoc and Nuhoglu, 2006).

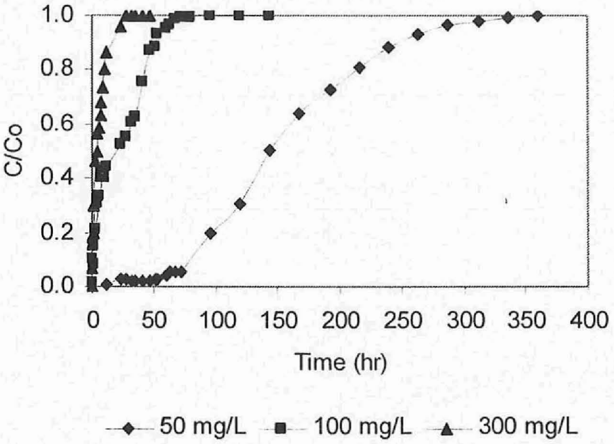

Figure 4: Breakthrough curves of Pb (II) biosorption onto immobilized cells of *P. sanguineus* at different initial Pb (II) concentration. (Condition: 100 mg L^{-1} Pb (II), bed height= 9 cm, pH 4.0)

3.4 Regeneration

Regeneration of biosorbent after biosorption process is very important in order to reduce the process cost in a continuous operation (Vijayaraghavan *et al*, 2005). Figure 5 shows a desorption curve of Pb (II) ions through a packed bed of *P. sanguineus* by passing 0.1 M HCl as an elution agent. The biosorbent were reused up to two biosorption-desorption cycles. It was observed that the elution efficiency was up to 85% for a complete recovery of Pb (II) ions and more than 20 L of 0.1 M HCl is being used. After two biosorption-desorption cycles, a significant biosorbent weight loss was observed and seemed that it was not suitable to be used in the next cycle. As reported by a few researchers, cells that were exposed to an acidic elutant might faced physical-chemical damage of the biosorbent structure which resulted in weight loss and reduction of the biosorption capacity in a subsequent cycles (Tuzun *et al.*, 2005; Vijayaraghavan *et al.*, 2005). These can be seen as tabulated in Table 1.

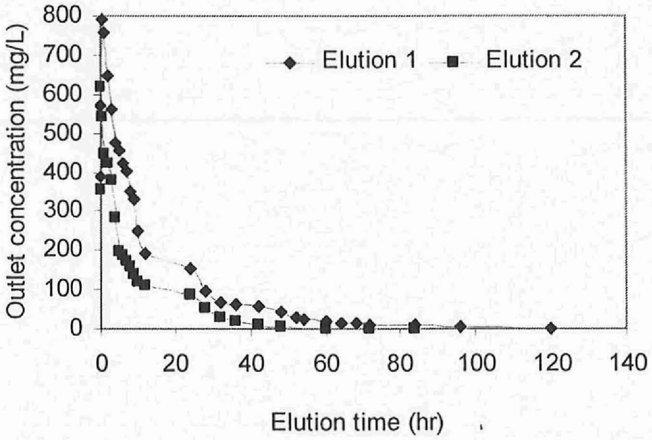

Figure 5: Elution breakthrough curve for Pb (II) during two biosorption-desorption cycles. (Condition: 5 ml min^{-1}, bed height= 9 cm).

Table 1: Elution parameters for two biosorption-desorption cycles.

Metal	Cycle No	Uptake capacity (mg g-1)	Metal removal (%)	Time for elution (hr)	Elution efficiency (%)
Pb	1	34.50	88.84	120	84.83
	2	28.14	75.62	84	50.17

CONCLUSIONS

The biosorption of Pb (II) ions has been examined using immobilized cells of *P. sanguineus* cell in a packed bed column and the following conclusions summarized:

- Immobilized cells of *P. sanguineus* cell were capable to remove Pb (II) ions from aqueous solutions.
- Increased in bed height and initial Pb (II) concentration increased the metals uptake in the column. Contact time in column at higher flow rate resulted in decreased of metal uptakes.
- Column regeneration using 0.1 M HCl was carried out for two biosorption-desorption cycles indicate a significant biosorbent weight loss.

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NOMENCLATURE

C_o	initial concentration	(mg L^{-1})
C	outlet concentration	(mg L^{-1});
M	mass of biosorbent	(g)
Q	metals uptake capacity	(mg g^{-1})
t_b	breakthrough time	(hr)
t_e	exhaustion time	(hr)

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CONTINUOUS BIOSORPTION OF CHROMIUM BY IMMOBILIZED *Pycnoporus sanguineus* IN FIXED BED COLUMN

By

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Thesis submitted in partial fulfillment of the requirements for the degree of Bachelor of Chemical Engineering

April 2006

ABSTRACT

Biosorption of chromium by immobilized *Pycnoporus sanguineus* on Na-alginate beads has been carried out in fixed-bed column studies. The effect of various parameters such as column bed height, cell biomass concentration, initial concentration and flow rate of chromium solution were investigated. Promising result was obtained when flow rate of chromium solution was 1.5 ml/min, chromium concentration 10 ppm, the ratio of Na-alginate to biomass 21:1 and the bed height was 15 cm, respectively. A first order biosorption rate has been assumed to determine the biosorption rate constant, k_p. It was found that higher the value of flow rate, Q, lower will be the k_p. This could be due to lesser contact time between immobilized cells and the chromium solution. As a result, less chromium was adsorbed into the cells. For SEM studies, the structure of the tested fungus that was entrapped in Na-alginate beads shrunk after biosorption process. A substantial amount of chloride, calcium and sodium were also detected in the beads before biosorption as shown on the EDX micrographs. After biosorption, all the metals were released into the solution. As a result, no peak was found on the micrographs.

Keywords: Biosorption; immobilized; *Pycnoporus sanguineus*; chromium; fixed-bed column

Biosorption of Copper Ions by Immobilized *Pycnoporus sanguineus* in Batch System

By

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ABSTRACT

Copper biosorption by non-living biomass of *Pycnoporus sanguineus* immobilized in sodium alginate has been studied. In batch equilibrium studies of this metal, copper adsorption has shown a very high affinity and fitted well with Freundlich isotherms model. An optimum condition for biosorption is at pH 5, initial copper concentration 200 ppm, 70°C, contact time 100min and accumulated biomass in gel bead is 1.5g *Pycnoporus sanguineus in* 450ml 1.5w/v% sodium alginate respectively. Higher the pH, temperature, contact time and copper ion concentration, the greater was the copper uptake capacity. When the accumulated biomass in gel beads and mass of gel beads increase, copper uptake capacity decreases.

In terms of thermodynamic studies, the value of ΔH^o and ΔS^o are 14,886.26Jmol^{-1} and 54.46Jmol^{-1}K^{-1}, respectively. Results also showed that as the temperature increased, the ΔG^o decreases.

Five different kinetic models namely Reversible reaction (Adsorption-desorption model), Irreversible reaction (Adsorption model), Intraparticle diffusion model, Pseudo 1st order kinetics and Pseudo 2nd order kinetics were used for rate data analysis. Besides, the Shrinking Core Model were used to indicate the kinetic limiting step is the particle diffusion controlling.

By using SEM and EDX methods, it shows the structure of gel bead change after biosorption and Cu^{2+} ions appear on the surface of gel beads. Besides, it indicated that Na^{+} may release and Cl^{-} may bind to Cu^{2+} after biosorption.