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Process optimization studies of lead (Pb(II)) biosorption onto immobilized cells of *Pycnoporus sanguineus* using response surface methodology

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ABSTRACT

A central composite design (CCD) was employed to optimize the biosorption of Pb(II) ions onto immobilized cells of *Pycnoporus sanguineus*. The independent variables were initial Pb(II) concentration, pH and biomass loading. The combined effects of these variables were analyzed by response surface methodology (RSM) using quadratic model for predicting the optimum point. Under these conditions the model predicted a maximum of 97.7% of Pb(II) ions removal at pH 4, 200 mg/L of initial Pb(II) concentration with 10 g/L of biosorbent. The experimental values are in good agreement with predicted values within +0.10 to +0.81% error.

1. Introduction

Industrial effluents containing heavy metals when release to the environment without a proper treatment could harm the aquatic life including human beings (Tunali et al., 2006). According to agency of toxic substance and disease registry (ATSDR, 2005), lead is a naturally bluish-gray metal with its solubility is highest in soft, acidic water. Lead pollution may come from various sources e.g. battery manufacturing, textiles, mining wastes, automobiles and metal finishing (Lai et al., 2006; Tunali et al., 2006). The exposure to lead over the permissible limit could cause anemia, nephritic syndrome and attack on gastrointestinal track or nervous system (Lo et al., 1999; Ho and Ofomaja, 2005; Zulkali et al., 2006). Conventional methods used for heavy metals removal include activated carbon adsorption, evaporation, chemical precipitation and ion-exchange. These technologies have limitations such as high operating cost, incomplete metal ions removal and generation of toxic sludge (Zulkali et al., 2006; Cabuk et al., 2007; Malkoc and Nuhoglu, 2005).

Biosorption process has received great attention as an alternative method to remove toxic metals from wastewaters (Ahalya et al., 2003). Biosorption process offers several advantages such as low cost biosorbent, efficient and regeneration of biosorbent (Cruz et al., 2004; Naddafi et al., 2007). Microorganisms such as algae, yeast, bacteria and fungi have been widely evaluated as a biosorbent in order to remove metal ions from aqueous solution because of its low cost and abundant supply (Tunali et al., 2006). As reported in literature, biosorption of metal ions onto microorganisms is affected by several factors such as pH, metals concentration, biomass loading, temperature and surface of the microorganisms' cell wall (Gadd, 1993; Saglam et al., 2002; Ahalya et al., 2003; Arica et al., 2003).

Fungi have been investigated as a biosorbent and capable to sequester metal ions from aqueous solutions (Aloysius et al., 1999). Fungi could be chosen as an economical biosorbent for metals ion removal because it is available and could be easily grown in a low cost growth media (Tunali et al., 2006). Metal ions that adsorbed by fungal can be classified as: extracellular accumulation, cell surface sorption and intracellular accumulation (Ahalya et al., 2003; Cruz et al., 2004). Fungal immobilization onto natural polymers such as sodium alginate, chitin and chitosan can improve cell productivity and stability, regeneration in continuous operation and easy separation of the cells from the reaction system (Baklashova et al., 1984; Li et al., 1984; Federici et al., 1987, 1990; Federici, 1993; Annadurai et al., 2007).

The present study is focused to determine the optimum condition for Pb(II) removal by live immobilized cells of *Pycnoporus sanguineus* from aqueous solution using central composite design (CCD) combined with response surface methodology (RSM). In this study, the CCD was selected to determine effect of parameters and their interactions over removal of Pb(II) ions. The interactions between the factors that influence the percent of Pb(II) removal were established. The optimum value of the parameters was determined

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for removal of Pb(II) ions from the aqueous solution using response surface methodology.

2. Methods

2.1. Microorganism, production medium and immobilized cells preparation

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 (150 kN/m^2) for 15 min.

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 (Sigma) solution prior putting it into a sterile sampling bottle (100 ml). The sampling bottle was vortexed for 3 min 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 h. The harvested sample was centrifuged at 3500 rpm for 4 min at 25 °C. *P. sanguineus* beads were prepared by dropping a mixture of 1.5% (w/v) sodium alginate solution and *P. sanguineus* mycelial mat into a 2% (w/v) $CaCl_2$ solution stirring slowly at room temperature (25–30 °C). 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.

2.3. Preparation of metal stock solution

Metal solutions were prepared by diluting 1000 mg/L of Pb$(NO_3)_2$ (Mallinckrodt) solutions with deionized water to obtain concentration of 50–350 mg/L. For each solution, the initial lead concentration and the concentration in the samples after biosorption treatment were determined using an Atomic Absorption Spectrometer (Model Shimadzu AA 6650).

2.4. Experimental design for biosorption studies

In order to obtain the optimum condition for percentage of Pb(II) removal, three independent parameters were selected for the study and are presented in Table 1. The range of study for initial Pb(II) concentration (*A*), pH (*B*) and biomass loading (*C*) were chosen based on preliminary experiments. The relationship between the parameters and response were determined using central composite design (CCD) under Response Surface Methodology of Design Expert Software (version 6.0.6) Stat Ease Inc. USA. The CCD design was chosen in this study as it is efficient, flexible and robust. The parameters presented in Table 1 with 3 levels are coded as −1, 0 and +1, respectively. Twenty experiments were conducted with six star points ($\alpha = 1$) and six replicates a centre points according to CCD.

Table 1
Experimental independent variables

Factor	Units	Factor code	Levels and range (coded)		
			−1	0	1
Initial Pb(II) concentration	mg/L	A	50	200	350
pH		B	2	4	6
Biomass loading	g/L	C	2	6	10

The percent of Pb(II) removal was taken as a response (*Y*) of the experimental design and calculated as

$$\%\text{Pb(II)removal} = \left(\frac{C_i - C_f}{C_i}\right) \times 100\% \quad (1)$$

where C_i (mg/L) is the initial concentration and C_f (mg/L) is the final or equilibrium concentration. Each experiment was repeated three times and the results reported are the average values. Samples taken after the desired incubation period were analyzed with an Atomic Absorption Spectrophotometer (Model Shimadzu AA 6650).

The regression analyses, graphical analyses and analyses of variance (ANOVA) were done using the Design Expert Software (version 6.0.6), Stat Ease Inc, USA. The statistical significance of the coefficient was determined by Student's *t*-test and *p*-values (Zulkali et al., 2006). The proportion of variance obtained by the model was explained by the multiple coefficient of determination, R^2.

3. Results and discussion

3.1. Statistical analysis

In order to determine an optimum condition for Pb(II) ions removal, the parameters that have greatest influence over the response need to be identified. In the present study, the relationship between three independent variables and percent of Pb(II) ions removal fitted well with the quadratic model. The quadratic regression model for percent of Pb(II) removal (after biosorption process) obtained from CCD design in terms of coded factors is presented as

$$\text{Pb(II)removal}(Y) = +93.27 - 2.88A + 0.89B + 7.25C - 6.93B^2 - 2.84C^2 - 1.57AB + 2.72AC - 1.82BC \quad (2)$$

where *A*, *B* and *C* were the coded values of tested variables such as initial Pb(II) concentration, pH and biomass loading, respectively.

Table 2 presents the variations in the corresponding coded values of three parameters and response based on experimental runs

Table 2
Comparison of experimental and predicted values on Pb(II) ions removal (%)

Standard order	Coded values			Response (Y)		Residual
	A	B	C	Experimental value	Predicted value	
1	−1	−1	−1	76.7	77.6	−0.9
2	1	−1	−1	68.8	69.5	−0.7
3	−1	1	−1	87.5	86.1	1.4
4	1	1	−1	71.2	71.8	−0.6
5	−1	−1	1	90.5	90.3	0.3
6	1	−1	1	91.4	93.1	−1.7
7	−1	1	−1	92.0	91.6	0.4
8	1	1	1	88.7	88.1	0.6
9	−1	0	0	93.5	96.2	−2.7
10	1	0	0	91.2	90.4	0.8
11	0	−1	0	88.4	85.5	3.0
12	0	1	0	85.4	87.2	−1.8
13	0	0	−1	84.0	83.2	0.8
14	0	0	1	98.0	97.7	0.3
Repeated runs						
15	0	0	0	92.3	93.3	−0.9
16	0	0	0	94.7	93.3	1.4
17	0	0	0	92.5	93.3	−0.7
18	0	0	0	92.0	93.3	−1.3
19	0	0	0	94.7	93.3	1.5
20	0	0	0	94.0	93.3	0.8
Mean[a]				93.4[a]	93.3	

[a] Calculated statistic for repeated experiments.