Journal Home page : www.jeb.co.in ★ E-mail : editor@jeb.co.in

JEB
Journal of Environmental Biology

ISSN: 0254-8704
CODEN: JEBIDP

Biosorption of lead by *Kluyveromyces marxianus* immobilized in alginate beads

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Publication Info

Paper received:
03 April 2012

Revised received:
12 December 2012

Accepted:
24 December 2012

Abstract

The uptake and recovery of Pb (II) ions were investigated by using sodium alginate beads. Biosorption experiments are carried out in batch mode. The experimental results showed that the beads were effective in removing Pb (II) ions from solution. Biosorption equilibrium was approached within 16hrs. Pseudo first order was applicable to all the sorption data over the entire time range. The sorption data conformed well for the Langmuir isotherm model. The maximum adsorption capacity (qmax) onto alginate beads was 62.5 mg g^{-1} for Pb (II) ions. The maximum uptake of metal ions was obtained at pH 7. At temperature 35°C, the biosorption of metal ions was found to be highest, with increase or decrease in temperature resulted in a decrease in the metal ions uptake capacity. The maximum removal efficiency of 94.02% was obtained at 100 mg l^{-1} of metal concentration with 200 numbers of immobilized beads. The results suggested that alginate beads can be used as a biosorbent for an efficient removal of Pb (II) ions from aqueous solution.

Key words

Alginate beads, Biosorption, Immobilized beads, Isotherms, *Kluyeromyces marxianus,* Lead

Introduction

Various human activities like ore mining and industrial processes have disrupted the natural biogeochemical cycles, causing increased depositions of heavy metals in the environment (Kim *et al*.,2005). Many industries especially metal industries, electroplating, battery manufacturing, pigment and dye industries, Pb smelting and internal combustion engine fueled with Pb petroleum etc. discharge Pb as a waste in the environment (Majumdar *et al*., 2010). Pb has a detrimental effect on the environment where it accumulates throughout the food chain (Suh *et al*., 1998).

Conventional methods such as chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, evaporation etc. for removing metal ions from aqueous solution have been suggested (Li *et al*., 2010, Topuz and Macit.,2010). However, these methods are ineffective, especially when metal ion concentration in aqueous solution is in the range of 1 to100 mg l^{-1}, also produce large quantity of sludge and are extremely expensive (Domnez and Aksu., 1999). In this endeavor, microbial biomass has emerged as an option for developing economic and eco-friendly waste water treatment process (Chau *et al*., 1995). A wide variety of fungi, algae and bacteria are now under study or are already in use as biosorbents for heavy metal remediation (Rani *et al*., 2010, Goksungur *et al*., 2005, Majumdar *et al*., 2010).

Uptake of heavy metals by microbial cells is a result of the mechanisms of bioaccumulation and biosorption. The term biosorption, sometimes referred to as physical adsorption, describes the ability of inactive, dead or living biomass to bind to heavy metals or contaminants present in dilute solutions. The cell-wall structure is mainly responsible for this property. The term bioaccumulation refers to the metabolically driven uptake by active living cells (Skountzou *et al*., 2003; Sarri *et al*., 2009).

Yeasts are an inexpensive, readily available, easily cultivated using simple fermentation techniques and inexpensive growth media. Furthermore, yeast cells retain their ability to accumulate a broad range of heavy metals to varying degrees under a wide range of external conditions (Aksu and Donmez 2000). The yeast has been studied by many investigators as a biosorbent for removal of heavy metals (Kim *et al*., 2005, Goksungur *et al*., 2005, Skountzou *et al*., 2003, Suh *et al*., 1998).

K. marxianus is an inexpensive yeast and readily available source of biomass for heavy metal removal from wastewater. Investigations conducted by several researchers demonstrated that *K. marxianus* is capable of accumulating heavy metals such as Cu (II) and U(VI) (Donmez and Aksu,1999; Anagnostopoulos *et al*., 2010; Bustard *et al*.,1997; Sarri *et al*., 2009).

The use of freely suspended biomass may be plagued with operational difficulties, but immobilized microbial cell systems could provide additional advantages: ease of regeneration and reuse of the biomass, easier solid liquid separation and minimal clogging in continuous flow systems (Wang *et al*., 2010). Materials that have been successfully used for cell entrapment include agar, agarose, alginate, k-carrageenan, polyacrylamide, polyurethane, cellulose, collagen, chitin, chitosan, polysulfone and epoxy resins (Akhtar *et al*.,2009). Ca-alginate has been one of the most extensively investigated biopolymers for binding heavy metals from dilute aqueous solutions (Sag *et al*., 1995; Yakup *et al*., 2003; Wang *et al*., 2010; Aksu *et al*.,1998).

Information is available on the use of immobilized *Kluyveromyces marxianus* biomass for other applications (Guo *et al*., 2010, Panesar *et al*., 2007), but no study has been conducted on the use of the immobilized biomass for removal of heavy metal ions. Therefore there is a need to study the performance of the immobilized yeast system with the other heavy metal ions. Therefore the present study focused on removal of heavy metal lead (II) ions using the immobilized *Kluyveromyces marxianus* under batch system.

Materials and Methods

The *Kluyveromyces marxianus* (MTCC Code 95) used in the present study was obtained from MTCC (Microbial Type Culture and Gene Bank), Institute of Microbial Technology, Chandigarh, India. The composition of the culture medium used was 1% Dextrose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract. All chemicals and reagents used for experiments and analyses were of analytical grade. Stock solutions of 1000 mg l^{-1} Pb (II) ions were prepared from Pb nitrate in distilled water. The solutions were diluted as required to obtain working solutions.

In the stationary phase of growth, yeast cells were centrifuged and resuspended in 3% sodium alginate. The ratio of Na-alginate to biomass was 100:3. This mixture was dropped into 0.5M calcium chloride solution forming as spheres which was stored in calcium chloride solution at 4ºC for 24hrs to complete gel formation. (Chatterjee and Ray, 2008; Banerjee and Nayak, 2007).

Viability cell count were determined by submerging a single bead in 1ml of saturated phosphate buffer solution at room temperature until the alginate cell suspension was mixed in the vortex mixer. 100ml sample were taken for each concentration of sodium alginate, it was serial diluted and spread plating was done in malt agar plates. The plates were incubated for 48 hrs.

Batch experiments were carried out by shaking the flasks at 150rpm using a rotary shaker. The optimization parameters such as pH (5-9), temperature (15-45ºC) were studied. Effect of immobilized bead number on Pb removal was studied by inoculating 100ml of Pb solution (100 mg l^{-1}) with immobilized beads ranging from 50-300 beads and incubated at optimized condition (35°C, pH 7). The pH was adjusted to 7 and incubated at 35°C in rotary shaker at 150rpm. The effect of initial metal concentration on the Pb removal was studied by inoculating optimum number of beads in 100ml of Pb solutions at various concentrations ranging from 100-500 mg l^{-1}. It was then incubated under the optimized conditions (35°C, pH 7.0).

Adsorption isotherms and kinetic study : The adsorption isotherm study was performed using the Langmuir and Freundlich isotherms. The kinetics study was evaluated by pseudo first order and pseudo second order equations. The adsorption isotherm study was performed using the Langmuir and Freundlich isotherms. The kinetics study was evaluated by pseudo first order and pseudo second order equations. The initial and final concentration of lead ions in adsorption experiments were determined by Perkin-Elmer atomic absorption spectrophotometer model 2380. The amount of lead adsorbed onto the biomass at equilibrium were calculated by the folloeing formuls

$$q_e = \frac{(C_o - C_e)V}{m} \quad \text{..................... (Eq. 1)}$$

Where, C_o = initial metal concentration (mg l^{-1}), C_e = metal concentration at equilibrium (mg l^{-1}), V = working solution volume (ml), m = weight of biomass (g), q_e = the equilibrium concentration of the adsorbed lead ion per gram of adsorbent at equilibrium (mg g^{-1}), respectively.

Results and Discussion

The density of a single immobilized bead was determined as 4.8×10^{-3}g ml^{-1}. The size of the immobilized bead was found to be 0.30cm. The viable cells immobilized in a single bead were found to be 2×10^9 CFU ml^{-1}.

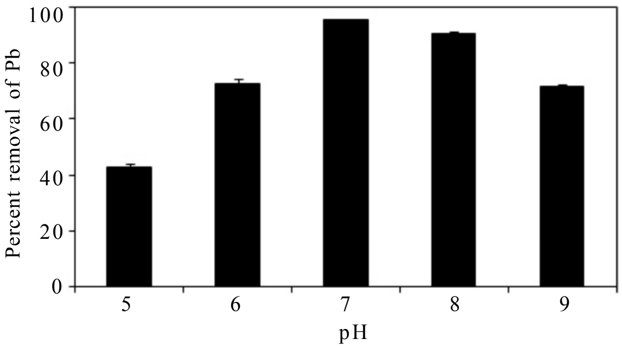

Fig. 1 : Effect of pH on removal of Pb (Initial concentration of lead= 100ppm, Temperature=35°C, Bead number 100)

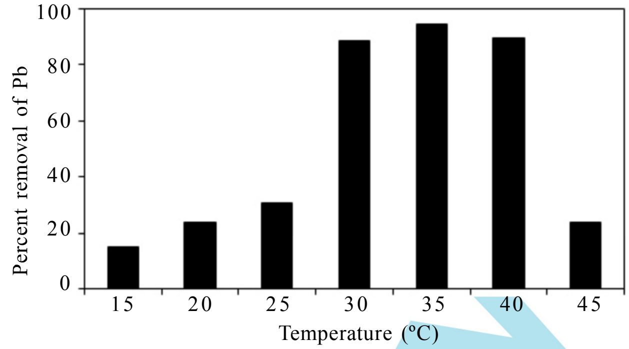

Fig. 2 : Effect of Temperature on removal of Pb (Initial concentration of lead= 100ppm, Temperature=35°C, pH=7)

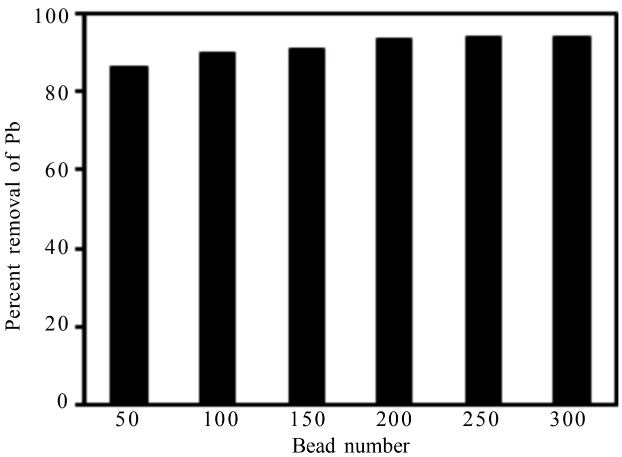

Fig. 3 : Effect of bead number on removal of Pb (Initial concentration of lead= 100ppm, Temperature=35°C, pH=5)

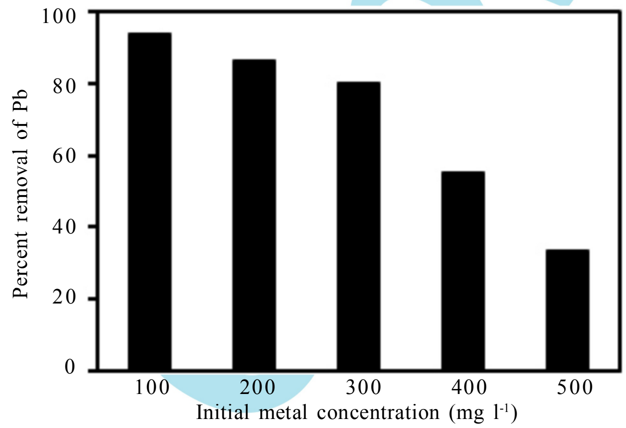

Fig. 4 : Effect of initial metal concentration on removal of Pb (Temperature=35°C, pH=5, Immobilized bead number=200)

The influence of pH on Pb removal by calcium alginate immobilized *Kluveromyces marmaxianus* was investigated by varying the pH from 5-9 and the results obtained are shown in Fig. 1. The maximum removal of Pb was observed at pH 7.0. The variation in pH affected the enzymatic activity of the yeast and pH 7-8 showed more than 90% reduction efficiency than other pH conditions. Any variation in the pH of the medium causes conformational and structural modifications which are also influenced by the ionic status of the active sites of the enzyme. The maximum Pb ion removal by immobilized organisms was observed at pH 7. (Poopal and Laxman, 2009)

The influence of temperature on the removal of Pb ions is very important to arrive an optimum temperature for the process. The metabolic activity, enzymatic activity and growth of the microbial population are significantly influenced by the temperature. The influence of temperature on removal of the metal by immobilized cells was investigated by varying the temperature from 15-45°C and the results obtained are shown in Fig 2. The metal removal was higher at 35°C after 180 hrs of incubation above that temperature the adsorbent loss its binding sites which attribute to reduction in removal efficiency (Majumdar *et al*., 2010).

The effect of bead number on the percent removal of Pb by the yeast is shown in Fig 3. The percent removal of Pb ions was found to increase with increase in the number of beads. The percent removal of Pb ions did not increase significantly there was an increase in bead numbes from 200 to 300. Hence, the optimum number of beads was fixed 200 and 94.02% of Pb was removed from the medium.

The effect of initial Pb concentration on the removal percent of Pb ions by the immobilized yeast is shown in the Fig. 4. As initial Pb concentration increased, the removal percentage decreased. The removal percentage of Pb ions varied from 33.4-94.02%. A removal efficiency of 90% was obtained by treating real waste water by immobilized cells of *Aspergillus niger* (Tsekova *et al*., 2010). The biosorption capacity of live and inactive immobilized *Phanerochaete chrysosporium* showed a saturation value of 500mg l^{-1} for lead and 300mg l^{-1} for zinc (Yakup *et al*.,2003).

Adsorption isotherm and kinetics : In order to determine if the yeast entrapped systems could be modeled using adsorption isotherms, the two most commonly used adsorption isotherms for biosorption of Pb were investigated. Fig. 5 shows the Langmuir plot for Pb (II)

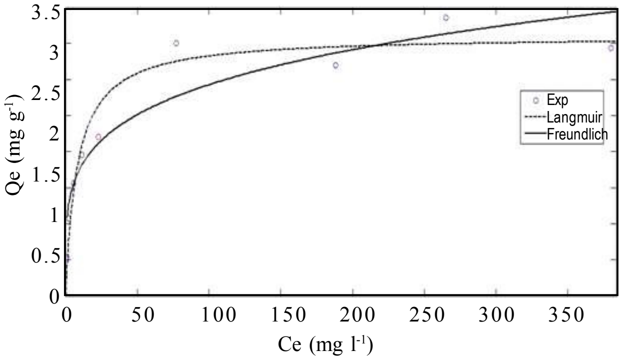

Fig. 5 : Langmuir and Freundlich isotherm

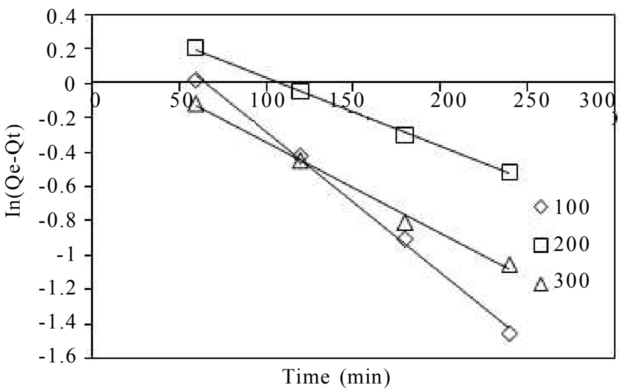

Fig. 6 : Pseudo first order kinetics

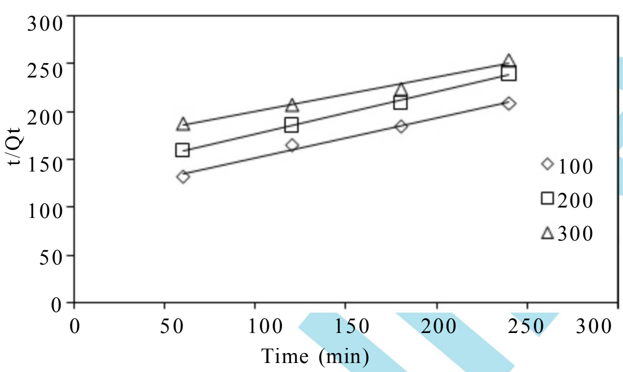

Fig. 7 : Pseudo second order kinetics

biosorption by entrapped yeast. The Langmuir constants (Q_{max} and b) along with correlation coefficients were calculated from the plot and the results are presented in Table 1. The maximum capacity determined from the Langmuir isotherm defines the total capacity of the beads for Pb (II) ions. The magnitudes of K_f and n (Freundlich constants) showed easy separation of metal ions from aqueous medium and indicate favorable adsorption. The intercept K_f value is an indication of the adsorption capacity of the adsorbent; the slope 1 n^{-1} indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. Fig. 5 shows the Freundlich plot for Pb (II) adsorption on the immobilized yeast. The magnitude of K_f and n showed easy uptake of Pb (II) from aqueous medium with a high adsorption capacity of the entrapped live yeast. As seen from Table 1, *n* value was found high enough for separation. The experimental equilibrium data fits both models well, thus illustrating the fact that the use of immobilized beads for biosorption of Pb could be modeled using both the Langmuir and Freundlich isotherms.

Table 1 : Isotherm fit values and correlation constants

Isotherm	Q_{max} (mg g^{-1})	b (l g^{-1})	n	K (l g^{-1})	R^2
Langmuir	3.601	0.0195	-	-	0.947
Freundlich	-	-	0.22	1.058	0.88

The ability of residual biomass from the yeast strain *Kluyveromyces marxianus* IMB3 to function as a biosorbent for uranium has been examined. The calculated value for the biosorption maximum, obtained by fitting the data to the Langmuir model was found to be 130mg g^{-1} d. wt. biomass (Bustard *et al*., 1997). The uranium sorption onto *Kluyveromyces marxianus* could be better described by a Freundlich isotherm (Sarri *et al*.,2009). The basidio spores of *Phanerochaete chryosporium* were immobilized in alginate gel beads. The experimental biosorption equilibrium data for Pb and Zinc ions were in good agreement with those calculated by Langmuir model (Yakup *et al*.,2003).

In order to analyze the biosorption kinetics of Pb ions onto the immobilized cells of beads, the pseudo-first order and pseudo-second order models were tested using experimental data. The corresponding parameters were determined by linear regression and are listed in Table 2 Table 2 showed the adsorption capacities calculated by the model are closer to those calculated by the experiments. Result obtained indicates that the pseudo-first order model best described the data for biosorption of Pb ions onto immobilized beads followed by pseudo-second order model.

The linear form of the pseudo-first order model and the pseudo-second order model for the adsorption of Pb at various initial metal concentrations is given in Fig. 6 and 7, respectively. The correlation coefficients (R^2) for the linear plots using the pseudo-first order model ranged between 0.998 and 0.991 while the correlation coefficients for the linear plots for the pseudo-second order model ranged between 0.99 and 0.983. This result suggested that the pseudo-second order model is less suitable to describe this biosorption process and the pseudo-first order model can be adapted to describe this process as it fits accurately with the experimental data.

The adequacy of the pseudo-first order model suggested that metal uptake by the immobilized beads is controlled by diffusion, because the pseudo-first order model is mathematically equivalent to a mass action rate equation for sorption kinetics seen as a transfer process. By contrast, the pseudo-second order model assumes that the rate limiting step is chemisorption of metal ions onto sorbent binding sites. The interaction between metal ions

Table 2 : Kinetic models for uptake of lead (II) in batch mode by the immobilized beads

Initial concentration	Pseudo first order			Experimental	Pseudo second order		
of lead (ppm)	Q_e (mg g^{-1})	K_1 (min^{-1})	R^2	value q (mg g^{-1})	q_e(mg g^{-1})	K_2(min^{-1})	R^2
100	1.55	0.007	0.991	1.382	2.392	0.009	0.987
200	1.55	0.004	0.998	1.604	2.262	0.0075	0.99
300	1.20	0.005	0.996	1.595	2.793	0.006	0.983

and binding sites in beads must be a fast step in the sorption process, so that it is not rate-limiting. Biosorption of U (VI) ions by immobilized *Aspergillus fumigatus* beads was investigated in a batch system. The dynamic adsorption model conformed to pseudo-second order model (Wang *et al*., 2010). The sorption of Cr (Vl) on immobilized *Trichodrema viride* biomass follows pseudo-first order model (Bishnoi *et al*., 2007)

The result of the present study showed that *Kluyveromyces marxianus* was effective in removing Pb and possessed all intrinsic characteristics to be employed for the treatment of Pb bearing industrial effluents.

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