Lead removal from aqueous solutions by *P. chrysosporium*

Upasana Singh¹, B. P. Singh¹ and Kishor Kumar Singh²*

¹Department of Botany, Post Graduate College, Ghazipur, Uttar Pradesh, India
²Department of Chemistry, Govt. Post Graduate College, Obra, Sonebhadra, India

ABSTRACT

The dead biomass of *P. chrysosporium* has been used for the removal of lead (II) from an aqueous solution. The effects of various parameters such as contact time, adsorbate concentration, pH of the medium and temperature were examined. Optimum removal at 20°C was found to be 98.4% at pH 6.5, with an initial Pb (II) concentration of 100 mg L⁻¹. Dynamics of the sorption process and mass transfer of Pb (II) to *P. chrysosporium* were investigated and the values of rate constant of adsorption, rate constant of intraparticle diffusion and the mass transfer coefficients were calculated. Different thermodynamic parameters viz., changes in standard free energy, enthalpy and entropy were evaluated and it was found that the reaction was spontaneous and exothermic in nature. The adsorption data fitted the Langmuir isotherm. The data were subjected to multiple regression analysis and a model was developed to predict the removal of Pb (II) from an aqueous solution.

Keywords: Adsorption, monolayer, lead (II), *P. chrysosporium*, Exothermic, multiple regressions, isotherm.

INTRODUCTION

The presence of heavy metals like Pb, Cd, Hg, Cr, Ni, Cu, Zn and Co are, at elevated concentrations, in the environment specifically in various water resources is of major concern because of their toxicity, non-biodegradable nature and threat to human, animal and plant life. WHO recommend in their guidelines for drinking water quality a threshold limit for lead is 10 μg L⁻¹. In order to fulfill the threshold criteria and to reduce pollution contaminated waters need to be cleaned. To minimize this problem, biosorption can be part of the solution. Biosorption of heavy metals by bacterial fungal or algal biomass (live or dead cells) and agricultural waste biomass [1-12] has been recognized as a potential alternative to existing technologies such as precipitation, ion exchange, solvent extraction and liquid membrane for the removal of heavy metals from industrial wastewater because all these processes have the limitations of technical and/or economical viability.

The literature reveals two distinct approaches to use of living organisms and the use of a non-viable biomass [13-14]. There are significant practical limitations to systems, which employ living microorganisms. The most significant limitation is that microbial growth is inhibited when the concentrations of metal ions are too high or when significant amount of metal ions are adsorbed by microorganisms [13]. Dead cells or agricultural wastes accumulate heavy metal ions to the same or to a greater extent than living cells [13-14]. The reason for this is that the changes, which occur in the cell structure after the cells are dry- killed, affect adsorption in a positive manner [15]. For metal removal applications, the use of dead biomass or agricultural waste may be preferable as large quantities are readily and cheaply available as a byproduct of various industries [16]. Therefore, *P. chrysosporium* was used for the removal of Pb (II) from aqueous solution.

EXPERIMENTAL SECTION

2.1 Media and Culture condition

The following medium and culture conditions were used for maintenance of cultures.
2.1.1. Maintenance of Culture
Nutrient agar medium were used as the maintenance media for the microorganisms. P. chrysosporium strain was maintained by transfer every three weeks on slants containing Malt Extract Agar- Blakeslee’s formula. The medium had the composition: Malt Extract- 20 gL\(^{-1}\), Glucose- 20 gL\(^{-1}\), Peptone- 1 gL\(^{-1}\), Agar- 20 gL\(^{-1}\), Distilled water- 1000 mL, pH- 5.5.

2.1.2 Growth Medium
P. chrysosporium was grown on the broth medium having the composition: D-glucose- 10 gL\(^{-1}\), KH\(_2\)PO\(_4\)- 2 gL\(^{-1}\), MgSO\(_4\).7H\(_2\)O- 0.5 gL\(^{-1}\), NH\(_4\)Cl- 0.1 gL\(^{-1}\), CaCl\(_2\).2H\(_2\)O- 0.1 gL\(^{-1}\), Thiamine- 0.001 gL\(^{-1}\), Distilled water- 1000 mL, pH- 4.5.

2.1.3 Culture Condition
The culture condition of the P. chrysosporium was given as: temperature- 28 ºC, pH- 4.5, Aerobic, agitation rate-100 rpm.

Initially, biomass of microorganisms was obtained through the cultivation in broth media followed by centrifugation, then autoclaved to obtain the dead biomass.

2.2 Experimental Procedure
In the present investigation batch mode of operation was selected in order to measure the progress of adsorption. Thus in a representative experiment 1.0 gm dead biomass of P. chrysosporium was shaken with 50 ml of lead nitrate of desired concentration (100, 125 and 150 mg L\(^{-1}\)) at different temperatures (20, 30 and 40 \(^{0}\)C) and pH (3.2, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) in different glass bottles in a shaking thermostat set at 20, 30 and 40\(^{0}\)C at a constant speed of 125 rpm. The pH of the adsorbate solution was adjusted by adding 0.1M HCl or 0.1M NaOH. The progress of the adsorption process was observed at different time intervals till the attainment of saturation. At the completion of predetermined time intervals, the adsorbate and adsorbent were separated by centrifugation at 15000 rpm and the supernatant liquid was analysed by AAS to determine the residual concentration of lead ion.

RESULTS AND DISCUSSION

3.1 Effect of contact time and concentration
A series of experiments were performed at different adsorbate concentration viz., 100, 125 and 150 mg L\(^{-1}\) and time interval. The removal of Pb (II) was found to be 96.85, 92.15 and 85.75 % respectively, at 30 \(^{0}\)C and pH 6.5. The extent of adsorption increased rapidly in the initial stages and became slower at later stages until the attainment of equilibrium. Equilibrium time for the adsorption of Pb (II) on P. chrysosporium at various adsorbate concentrations was found to be 100 minutes, which showed that equilibrium time was independent of the initial adsorbate concentration. The following correlation had been developed between percentage removal and initial adsorbate concentration (C\(_{o}\)) to predict the percentage removal of Pb (II) by P. chrysosporium at any initial concentration.

\[
\text{Percentage removal of Pb (II)} = 33.5 C_{o}^{-0.63} 
\]

3.2 Dynamics Study
The rate constant \(k_{ad}\) (min\(^{-1}\)) for lead (II) adsorption on P. chrysosporium was determined by using the first order rate kinetic equation [17].

\[
\log (q_{e} - q) = \log q_{e} - \frac{k_{ad}}{2.303} t 
\]

(1)

Where \(q\) (mgg\(^{-1}\)) and \(q_{e}\) (mgg\(^{-1}\)) are the amounts of adsorbate at time \(t\) (min.) and at equilibrium respectively. The linear plots of \(\log (q_{e}-q)\) versus \(t\) (Fig. 1) suggest the first order kinetics of the uptake of Pb (II). The values of \(k_{ad}\) (5.675 \(\times\) 10\(^{-2}\), 5.148 \(\times\) 10\(^{-2}\) and 4.665 \(\times\) 10\(^{-2}\) mgg\(^{-1}\) min\(^{-1}\)) at different temperatures were calculated from the slopes of these plots.

The data was also tested for pore diffusion using the following equation [18]:

\[
q = k_{ad} t^{1/2} 
\]

(2)

Where, \(q\) is the amount sorbed at time \(t\) and \(t^{1/2}\) is the square root of the time. The values of \(k_{ad}\) (3.582 \(\times\) 10\(^{-2}\), 3.245 \(\times\) 10\(^{-2}\) and 2.825 \(\times\) 10\(^{-2}\) mgg\(^{-1}\) min\(^{-1/2}\)) at temperatures 20, 30 and 40 \(^{0}\)C respectively, were calculated from the slopes of respective plot \(q\) versus \(t^{1/2}\) (Fig. 2) at later stages. The dual nature of the curves was obtained due to the varying extent of sorption in the initial and final stages of the experiment. This can be attributed to the fact that in the initial
stages, sorption was due to boundary layer diffusion effect whereas, in the later stages (linear portion of the curve) was due to the intraparticle diffusion effects. However, these plots indicated that the intraparticle diffusion was not the only rate controlling step because it didn’t pass through the origin. This was further supported by calculating the intraparticle diffusion co-efficient ($D$, cm$^2$ sec$^{-1}$) using the following equation [19].

$$D = 0.03r^2/t_{1/2} \quad (3)$$

where $r$ (cm) is the average radius of the sorbent particle and $t_{1/2}$ (min.) is the time for half of the sorption. According to the Michelsen et al. [20] a $D$ (cm$^2$ sec$^{-1}$) value of the order of $10^{-11}$ is indicative of intraparticle diffusion as rate determining step. In this investigation, the values of $D$ (8.142 x 10$^{-9}$, 7.255 x 10$^{-9}$ and 5.778 x 10$^{-9}$ cm$^2$ sec$^{-1}$ at 20, 30 and 40ºC respectively) obtained was in order of $10^{-9}$ cm$^2$ sec$^{-1}$ which was more than two order of magnitude higher, indicated that the intraparticle diffusion was not the only rate controlling step. It was concluded that both boundary layer and intraparticle diffusion might be involved in this removal process.

3.3 Mass Transfer Study

Mckay et al. model [21] has been used for the present investigation:

$$\ln\left(\frac{C_A}{C_{A0}} - \frac{1}{1+mK}\right) = \ln\left(\frac{mK}{1+mK}\right)\beta_1S_s t \quad (4)$$

where, $m$ is the mass of the biosorbent per unit volume, $K$ is the constant obtained by multiplying $Q^0$ and $b$ (Langmuir's constants), $\beta_1$ is the mass transfer coefficient, $S_s$ is the outer specific surface of the biosorbent particles per unit volume of particle-free slurry. The values of $m$ and $S_s$ were calculated using the following relations:

$$m = \frac{W}{V}, \quad (5)$$

$$S_s = \frac{6m}{dp}\delta\rho(1-\epsilon_p), \quad (6)$$

where, $W$ is the weight of the adsorbent, $V$ the volume of particle-free slurry solution, and $dp$, $\delta\rho$ and $\epsilon_p$ are the diameter, density and porosity of the adsorbent particles, respectively. The values of $\beta_1$ (5.585X 10$^{-5}$, 4.965X 10$^{-5}$ and 4.126X 10$^{-5}$ cm sec$^{-1}$) calculated from the slopes and intercepts of the plots (Fig. 3) of $\ln (Ct/C0 – 1/1+ mK)$ versus $t$ (min.) at different temperatures (20, 30 and 40 ºC). The values of $\beta_1$ obtained show that the rate of transfer of mass from bulk solution to the biosorbent surface was rapid enough so it cannot be rate controlling step [22]. It can also be mentioned that the deviation of some of the points from the linearity of the plots indicated the varying extent of mass transfer at the initial and final stages of the sorption.

3.4 Thermodynamic Study

This was again confirmed by thermodynamic parameters such as free energy ($\Delta G^0$, k cal mol$^{-1}$), enthalpy ($\Delta H^0$, k cal mol$^{-1}$) and entropy ($\Delta S^0$, cal mol$^{-1}$ k$^{-1}$) changes during the process. These parameters (Table 1) were calculated by the method described by Singh et al. [22] at 20, 30 and 40ºC temperatures. The negative and small values of free energy change ($\Delta G^0$) were an indication of the spontaneous nature of the adsorption process. The negative values of standard enthalpy change ($\Delta H^0$) for the intervals of temperatures was indicative of the exothermic nature of the adsorption process and the negative values of $\Delta S^0$ for the corresponding temperature intervals suggested the probability of favourable adsorption.

3.5 Isotherm Study

The experimental data was found to fit the Langmuir isotherm [23]. The basic assumption of Langmuir adsorption isotherm is based on monolayer coverage of the adsorbate on the surface of adsorbent. The saturated monolayer is represented by the following equation.

$$\frac{C_e}{q_e} = \frac{1}{Q^0 b} + \frac{C_e}{Q^0} \quad (7)$$

Where, $C_e$ (mg L$^{-1}$) is the equilibrium concentration of the adsorbate, $q_e$ (mg g$^{-1}$) is the amount of adsorbate adsorbed.
at equilibrium: $Q^0$ (mg g$^{-1}$) and $b$ (L mg$^{-1}$) is the Langmuir constants related to the capacity and energy of the adsorption respectively. The linearity of the plots $C_e/q$ versus $C_e$ (Fig. 4) showed the applicability of the Langmuir isotherm for the present system. $Q^0$ and $b$ were determined from the slopes and intercepts of the respective plots. The decrease in their values (Table 1) with temperature increase also supported that removal of Pb (II) on $P$. chrysosporium was exothermic in nature [22, 24]. High $Q^0$ values (Table 1) also showed that the adsorbent had a good capacity to remove Pb (II).

The equilibrium parameter $R_L$ which is defined as $R_L = 1/(1+bC_0)$ in the range $0 < R_L < 1$ reflects a favourable adsorption process [25] where $b$ (L mg$^{-1}$) is the Langmuir’s constant and $C_0$ (mg L$^{-1}$) is initial adsorbate concentration. In the present investigation the equilibrium parameter (Table 1) was found to be in the range $0 < R_L < 1$ indicating that the adsorption process was favourable and the Langmuir isotherm was applicable [25].

3.6 Effect of pH
Experiments were performed at different pH values (3.2, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0). The percentage removal increased from 23.60 to 96.85 % with an increase of pH from 3.2 to 6.5 and thereafter removal decreased from 96.85 to 31.20 % with an increase of pH from 6.5 to 8.0, at 30°C and Pb (II) concentration of 100 mg L$^{-1}$. The optimum pH for the removal of Pb (II) on $P$. chrysosporium was found 6.5 (Fig. 5). Furthermore, the adsorption process can be explained on the basis of the nature of adsorbent used which contained several metal oxides. These oxides when mixed up with adsorbate solution undergo surface hydroxylation and form hydroxyl compounds on the surface which gives positively or negatively charged surface as a result of subsequent acid base dissociation [26, 27]. It can be seen that adsorption increased in the pH range 3.0-6.5 and beyond pH 6.5, it started to decrease. The increase in adsorption correlated with the presence of Pb (II) ions up to pH 6.5. Beyond pH 6.5, the formation of hydroxide of Pb (II) occurred which resulted in a decrease in adsorption. This fact was supported by the distribution of lead species at various pH values [28].

Multiple Regression Analysis
The effect of initial adsorbate concentration, contact time, temperature and pH of the system on Pb (II) removal by $P$. chrysosporium had been examined. The cumulative effect of all these independent variables (lead removal) are given by the following relation:

$$Y = 6.5593 + 0.6385a_1 + 0.5168a_2 - 0.4679a_3 + 0.2835a_4 - 0.0959a_5$$

Where, $Y$ is the predicted value of Pb (II) removal, $a_1$, concentration of adsorbate, $a_2$, contact time; $a_3$, temperature; $a_4$, pH; $a_5$, agitation rate of the system. The model values calculated with the help of equation (8) and the experimental values are given in Table 2. It may be seen that predicted values were pretty close to the experimental values. From these results it is concluded that all independent variables have cumulative effect on lead removal by $P$. chrysosporium.

### Table 1: Values of thermodynamic parameters, Langmuir constants and $R_L$ values of Pb (II) adsorption on $P$. chrysosporium at different temperatures

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>$-\Delta G^\circ$ (k cal mol$^{-1}$)</th>
<th>$-\Delta H^\circ$ (k cal mol$^{-1}$)</th>
<th>$-\Delta S^\circ$ (cal mol$^{-1}$K$^{-1}$)</th>
<th>$Q^0$ (mg g$^{-1}$)</th>
<th>$b$ (L mg$^{-1}$)</th>
<th>$R_L$</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1.470</td>
<td>142.86</td>
<td>0.959</td>
<td>0.0103</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.067</td>
<td>18.344</td>
<td>57.019</td>
<td>0.542</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.497</td>
<td>136.98</td>
<td>0.204</td>
<td>0.0467</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 2: Percentage removal at different conditions (experimental and predicted values at equilibrium time, pH 6.5 and agitation rate 125 rpm).

<table>
<thead>
<tr>
<th>Initial adsorbate concentration (mg L$^{-1}$)</th>
<th>Percentage removal</th>
<th>Temp. (°C)</th>
<th>Percentage removal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp. value</td>
<td>Predicted value</td>
<td>Exp. value</td>
</tr>
<tr>
<td>100</td>
<td>96.85</td>
<td>97.9</td>
<td>20</td>
</tr>
<tr>
<td>125</td>
<td>92.15</td>
<td>91.0</td>
<td>30</td>
</tr>
<tr>
<td>150</td>
<td>85.75</td>
<td>86.5</td>
<td>40</td>
</tr>
</tbody>
</table>
Fig. 1 RATE CONSTANT PLOT FOR ADSORPTION OF Pb (II) ON Bacillus subtilis
AT DIFFERENT TEMPERATURES
CONDITIONS: PARTICLE SIZE: < 178 µm; CONCENTRATION 100 mg L⁻¹;
pH: 6.5; TEMPERATURE: 20, 30 and 40°C.
Fig. 2 INTRAPARTICLE DIFFUSION PLOT FOR ADSORPTION OF Pb (II) ON Bacillus subtilis AT DIFFERENT TEMPERATURES
CONDITIONS: PARTICLE SIZE: <178 µm, CONCENTRATION: 100 mg L⁻¹, pH: 6.5.
Fig. 3 MASS TRANSFER PLOT FOR THE ADSORPTION OF Pb (II) ON Bacillus subtilis AT DIFFERENT TEMPERATURE CONDITIONS: CONCENTRATION: 100 mg L\(^{-1}\); PARTICLE SIZE: < 178 µm; pH: 6.5.
Fig. 4 LANGMUIR ISOTHERM PLOT FOR THE ADSORPTION OF Pb (II) ON Bacillus subtilis AT DIFFERENT TEMPERATURES.

CONDITIONS: PARTICLE SIZE: <178 μm; pH: 6.5; CONCENTRATION: 100, 110, 125, 140 & 150 mgL⁻¹.
**Fig. 5** EFFECT OF pH ON THE REMOVAL OF LEAD BY *Bacillus subtilis*.

**CONDITIONS:**
- CONCENTRATION: 100 mg L\(^{-1}\)
- PARTICLE SIZE: <178 µm
- TEMP.: 30°C
- pH: 3.2, 4.0, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0.

**CONCLUSION**

The dead biomass of *P. chrysosporium* was found to be an effective biosorbent for the removal of Pb (II) from an aqueous solution. The study showed that the temperature and pH of the solution strongly influenced the adsorption process. Adsorption in the initial stages was due to the boundary layer diffusion whereas in the later stages adsorption was due to intraparticle diffusion. Thermodynamic studies confirmed that the process was spontaneous and exothermic. The fit of the adsorption data into the Langmuir isotherm confirmed monolayer adsorption. Mass transfer studies confirmed that the rate of mass transfer from sorbate to adsorbent was rapid enough. The data thus obtained from this investigation would be useful in designing and fabricating an efficient treatment plant for Pb (II) rich effluents.

**REFERENCES**