BIOSORPTION OF HEXAVALENT CHROMIUM USING SPENT BIOMASS OF OLEAGINOUS *Pythium* sp.: KINETICS STUDIES IN BATCH MODE

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ABSTRACT

The effectiveness of low cost, spent biomass of oleaginous *Pythium* sp (solid biomass left over after extraction of oil from *Pythium* biomass) was evaluated for removal of Cr(VI) by biosorption as a function of time; initial Cr(VI) concentration and temperature. Batch studies indicated that Cr(VI) biosorption capacity ($q_e$) of spent biomass achieved equilibrium within 60 minutes under the mixing condition employed. The biosorption of Cr(VI) ions increased as the initial concentration of Cr(VI) ions increased in the aqueous solution. The maximum biosorption capacity of spent biomass at initial Cr(VI) concentration of 100, 200, 300, 400 and 500mg/L was 10.86, 20.87, 26.04, 37.17 and 45.87mg per gram of dry biomass respectively. The activation energy ($E_a$) of Cr(VI) adsorption by spent *Pythium* sp. biomass was determined using Arrhenius equation and was found to be -23.18 KJ/mole in the temperature range of 30 to 50°C. Using the equilibrium constant value obtained at different temperatures, the thermodynamics parameters of the biosorption ($\Delta H$ and $\Delta S$) were calculated, indicating the endothermic nature of biosorption. Two kinetic models viz. the Lagergren first-order and pseudo-second-order were used to analyse the biosorption data and the results suggested that the pseudo-second-order model represented the best correlation ($R^2>0.9$). The fitness of biosorption equilibrium data for Freundlich and Langmuir adsorption isotherm model indicated that both the models were suitable for biosorption of Cr(VI) onto low cost tested biomass.

Key words: Oleaginous fungus, biosorption, kinetic models, thermodynamics, activation energy, adsorption isotherms.

INTRODUCTION

An extensive use of hexavalent chromium in various industrial processes such as electroplating, leather tanning, paints, pigments, textiles, steel fabrication and tanning industries has led to the discharge of chromium to the environment well above the trace limits [1]. The maximum permissible limit of Cr(VI) in wastewater has been recommended as 0.005mg/L by World Health Organization (WHO). The toxicological effect of Cr(VI) originates due to the action of Cr(VI) as an oxidizing agent [2]. Inhalation and retention of Cr(VI) containing material can cause perforation of the nasal septum, asthma, bronchitis, pneumonia, inflammation of larynx and liver and increased incidence of bronchogenic carcinoma [3 & 4]. The contact of Cr(VI) compounds with skin may lead to skin allergies, dermatitis, dermal necrosis and dermal corrosion.

Thus, the removal of Cr(VI) ions from wastewater has become an important and widely studied area where a number of chemical and biological technologies have been developed over the years. The most conventional methods for removing Cr(VI) ions from wastewaters include, physico-chemical treatment technologies such as ion-exchange, electrodialysis, membrane filtration, reverse osmosis and chemical precipitation [5]. These methods have found limited application because they often involve high capital and operational costs.

In contrast, adsorption is an effective and widely used method for removing Cr(VI) from wastewater. In this context, natural materials like microbial biomass or waste products from industrial or agricultural operations may serve the purpose of low-cost, inexpensive biosorbents [6]. Literature survey reveals that several biological materials such as non-living biomass like coconut shell, lignin and scales of fish as well as living biomass like fungi, bacteria, algae have been investigated for the removal of Cr(VI) ions [7-10]. However, the research on Cr(VI) biosorption is still insufficient to cover the investigations of Cr(VI) biosorption using locally available and cheap biosorbents to eliminate Cr(VI) from contaminated waters.

Several species of fungi are reported for their oleaginous property [11 & 12]. Large amount of underutilized waste biomass is obtained after oil extraction and such dead fungal biomass obtained after oil extraction (referred as spent biomass) may be useful as a low cost, efficient biosorbent for Cr(VI).

Biosorption of metals from liquid waste would result in concentration of heavy metals in solid form and thus it is not the ultimate solution to the environmental concern regarding heavy metal pollution and associated toxicity. In this regards, biological detoxification would be obviously preferred over biosorption. For detoxification of Cr(VI) containing wastewaters, the only known biological mechanism is its reduction to Cr(III) which is insoluble and thereby less toxic [1]. However, for certain wastewaters such as effluents from chrome-plating industries, biosorption based removal of Cr(VI) would be preferable owing to its highly acidic pH, at which biological reduction would get severely inhibited. Therefore, we have been working on development of more effective biosorbents for removal of Cr(VI) from liquid wastewaters.

The present work reports the potential of unexploited spent biomass of *Pythium* sp for removal of Cr(VI) from aqueous solution. The potential of spent biomass was evaluated as a function of time; initial Cr(VI) concentration and temperature. Further, the kinetic model (viz. the Lagergren first-order and pseudo-second-order) and adsorption isotherms (viz. Langmuir and Freundlich isotherms) were used to analyse the Cr(VI) biosorption data.

MATERIALS AND METHODS

Chemicals

All the chemicals/reagents used in the present study were of analytical reagent grades belonging to the brands of Merck, Germany; Hi-Media, India and Sisco Research Laboratory (SRL), India.

Preparation of Biosorbents:

For biosorption studies, spent biomass obtained after extraction of oil from *Pythium* sp. cultivated under solid-state
condition using boiled rice (1 g per 250 mL flask) as a substrate was used. Since it was very difficult to separate the biomass from the substrate, the biomass along with substrate was dried and powdered using mortar and pestle. This powdered spent biomass was preserved in airtight polyethylene containers for further use. The uninoculated rice substrate processed similarly was used as a control.

**Effect of initial Cr(VI) concentration on Cr(VI) biosorption**

10mg/mL of dried and ground spent biomass *Pythium* sp (along with unutilized solid substrate i.e. rice) was mixed with 100mL of test Cr(VI) solution. The biomass was used as such and no prior treatment was given to the spent biomass. Test solutions containing Cr(VI) ions were prepared from analytical grade potassium dichromate. The concentration of Cr(VI) prepared from stock solution ranged from 100-500mg/L. Before mixing the biomass, the pH of each test solution was adjusted to pH:1.0 by using 1N HCl. After mixing, the experimental set was kept on shaker (150 rpm) at 30°C. Samples of 1mL were collected from conical flasks at regular time intervals and were filtered through Whatman No. 1 filter paper. The filtrates were analyzed for residual Cr(VI) concentration in the solution.

**Effect of pH on Cr(VI) biosorption**

The spent biomass (10 mg/mL) was added to 100 mL Cr(VI) solution (100 mg/L) with varying pH (pH 1.0 to 8.0). The pH of the solution was adjusted using 0.1N HCl /0.1N NaOH. At all pH values, controls without biomass addition were kept in order to compensate the effect of pH on Cr(VI). The pH of the solution was adjusted to pH:1.0 by using 1N HCl. After mixing, the experimental set was kept on shaker (150 rpm) at 30°C. Samples of 1mL were collected from conical flasks at regular time intervals and were filtered through Whatman No. 1 filter paper. The filtrates were analyzed for residual Cr(VI) concentration in the solution.

**Thermodynamic Studies**

Effect of temperature on Cr(VI) removal was studied by agitating 100mL of 100mg/L solution of Cr(VI) at different temperatures (25º-50ºC) for different agitation times till equilibrium was attained and then the results were analyzed to determine the rate of biosorption at different temperatures. The activation energy of the biosorption process was calculated by employing Arrhenius equation as follows

\[ \ln k = -Ea/R T + \ln Ao \]  

(1)

Where, \( E_a \) is activation energy and \( Ao \) is constant called the Frequency factor. Value of \( E_a \) can be determined from the Slope (-Ea/R) of \( \ln k \) versus 1/T plot [13].

The thermodynamic parameters of the biosorption i.e. the enthalpy change (\( \Delta H \)) and entropy change (\( \Delta S \)) were calculated using the Van’t Hoff’s plot (\( \ln K_c \) Vs 1/T) given as;

\[ \ln K_c = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \]  

(2)

Where, \( T \) is the temperature in Kelvin, \( R \) is the universal gas constant (0.0083 KJ/mol) and \( K_c \) is equilibrium constant calculated as,

\[ K_c = \frac{Q_{eq}}{C_{eq}} \]  

(3)

Here, \( Q_{eq} \) is the amount of Cr(VI) adsorbed per unit biomass (mg/g biomass) and \( C_{eq} \) is the Cr(VI) concentration in solution at equilibrium [14 & 15].

**Kinetics of Cr(VI) biosorption**

Experimental data can be tested using kinetic models in order to investigate the mechanism of biosorption and potential rate controlling steps such as mass transport and chemical reaction processes. The commonly studied kinetic model for modelling the kinetics of Cr(VI) ion biosorption includes, Lagergren-first order and pseudo-second order rate equation [16].

Linear form of Lagergren -first order rate equation is expressed as follows;

\[ \log(q_{eq} - q_t) = \log q_{eq} - \frac{k_1 t}{2.303} \]  

(4)

Where, \( q_t \) and \( q_{eq} \) is sorption capacity at time \( t \) and at equilibrium respectively and \( k_1 \) is pseudo-first order rate constant.

In case the biosorption follows Lagergren -first order rate equation, a plot of \( \log q_{eq} - q_t \) vs \( t \) should generate straight line with intercept of \( \log q_{eq} \) and slope of \(-k_1/2.303\). Similarly, linear form of pseudo-second order rate equation is expressed as

\[ \frac{t}{q_t} = \frac{1}{k_2(q_{eq})^2} + \frac{t}{q_{eq}} \]  

(5)

Where, \( k_2 \) is pseudo second order rate constant.

In case the biosorption follows pseudo-second order rate equation, a plot of \( t/q_t \) vs \( t \) should generate a straight line with intercept of \( 1/k_{2q_{eq}} \) and slope of \( 1/q_{eq} \).

The shape (linearity) of graph and comparison of experimental and calculated \( q_{eq} \) values can help in deciding which kinetic model is followed by biosorption process. Another important factor which influences the kinetic model is the value of coefficient of determination; \( R^2 \). A value of \( R^2 > 0.9 \) shows the suitability of model for describing the kinetics.

**Equilibrium model for Cr(VI) biosorption**

Adsorption isotherm, based on equilibrium data are basic requirements for the design of adsorption systems. Classical adsorption models (Langmuir and Freundlich isotherms) were used to describe the equilibrium between adsorbed metal ions on the spent biomass of *Pythium* sp (\( q_{eq} \)) and metal ions in solution (\( C_{eq} \)) as a function of different Cr(VI) concentrations.

The Freundlich isotherm equation is an empirical equation based on the biosorption on a heterogeneous surface suggesting that the binding sites are not equivalent or dependent [17], whereas Langmuir isotherm equation is based on monolayer sorption onto a surface with finite number of identical sites, which are homogeneously distributed over the sorbent surface [18].

**Analysis of Cr(VI) ions**

The concentration of the Cr(VI) ions was determined spectrophotometrically after complexation of the Cr(VI) ion with 1, 5-diphenylcarbazide [19]. The absorbance was recorded...
at 540nm and concentration was determined from the calibration curve.

RESULTS AND DISCUSSION

Effect of contact time on Cr(VI) ion biosorption by spent biomass of *Pythium* sp.

The present study on time dependent profile of Cr(VI) biosorption by spent biomass indicated that a rapid major part of Cr(VI) biosorption occurred in the first 240 minutes of incubation (Fig. 1). There was no considerable increase in the Cr(VI) biosorption after 240 minutes. The biosorption became slow in later stage because in the initial stage of Cr(VI) biosorption, a large number of unoccupied surface sites were available for biosorption, whereas in the later stages the remaining vacant surface sites probably became inaccessible. These results are at par with the reports on two phase Cr(VI) biosorption by *Mucor hiemalis* and *Rhizopus nigricans* [20, 21].

![Fig. 1 Time course for Cr(VI) biosorption using spent biomass of *Pythium* sp.](image1.png)

**Fig. 1** Time course for Cr(VI) biosorption using spent biomass of *Pythium* sp.

Effect of initial concentration of Cr(VI) ions

Fig. 2 shows that Cr(VI) biosorption increased from 10.6 to 40.9 mg/g biosorbent with the increase in initial Cr(VI) concentration from 100mg/L to 500mg/L. The maximum biosorption of Cr(VI) was found to be 40.9 mg Cr(VI)/g of spent biomass at an initial concentration of 500mg Cr(VI)/L. The increased Cr(VI) biosorption from concentrated Cr(VI) solution can be attributed to an increase in the number of Cr(VI) ions competing for the available functional groups present on the surface of biomass [22]. This in turn would increase the higher probability of collision between Cr(VI) ions and biosorbents thereby providing the driving force to overcome all mass transfer resistance of metal ions between the aqueous and solid phase.

In order to understand the mechanism of Cr(VI) biosorption, the adsorption data were fitted to Lagergren first-order and pseudo-second order kinetic model. It was found that Cr(VI) biosorption by spent biomass follows a pseudo-second order rate kinetics (Fig. 3). The pseudo-second order kinetic model demonstrates that the metal biosorption process is dependent on the number of metal ions present in the solution as well as the free biosorption sites on the biosorbent surface. Additionally, the second order rate constant \( K_2 \) and \( q_{eq} \) calculated from the intercept and slope of the plots are summarized in Table 1. It clearly appears from the data that with increase in Cr(VI) ion concentration, value of \( q_{eq} \) increases linearly. The theoretical \( q_{eq} \) value shows a lot of resemblance to the experimental \( q_{eq} \) values. The correlation coefficient \( (R^2) \) for the second order kinetics were greater than 0.9 \( (R^2>0.9) \) for all Cr(VI) concentration tested. Some of the very recent investigations concerning the kinetics of Cr(VI) adsorption onto various biosorbents have also reported higher correlations for pseudo-second order model [23 & 24].

![Fig. 3 Linearized pseudo second-order kinetic plots at varying initial concentrations of Cr(VI) ions (100-500 mg/L).](image3.png)

**Fig. 3** Linearized pseudo second-order kinetic plots at varying initial concentrations of Cr(VI) ions (100-500 mg/L).
Effect of pH and Temperature

The Cr(VI) biosorption was found to be maximum at pH 1.0 and remained fairly constant up to pH 6.0, while biosorption efficiency decreased significantly with further increase in pH up to 8.0 (data not shown). The increase in adsorption with decrease in pH may be due to protonation of functional groups involved in biosorption of negatively charged chromate ions. At alkaline pH the overall charge on the biosorbed surface would become negative and consequently due to respective charge repulsion of negatively charged Cr ions like HCrO$_4^-$, Cr$_2$O$_7^{2-}$, CrO$_4^{2-}$, resulting into lower adsorption efficiency [20]. Hence, electrostatic attraction probably plays an important role in biosorption of negatively charged chromium ions at low pH. Additionally, the dominant form of Cr(VI) at pH 1.0 is the acid chromate ion species (HCrO$_4^-$) and increasing pH shifts the concentration of HCrO$_4^-$ to other forms, Cr$_2$O$_7^{2-}$ and CrO$_4^{2-}$. Since there is an increase in sorption of Cr(VI) as pH decreases to 1.0, it may be suggested that HCrO$_4^-$ is the active form of Cr(VI) which is being absorbed by the acid treated fungal biomass.

Temperature plays a critical role in biosorption of metal ions. Temperature, under certain range can have an influence (positive/negative) on the biosorption of metal ions [10]. Fig. 4 shows the effect of temperature (25-50°C) on the biosorption of Cr(VI) by the spent biomass of *Pythium* sp. as a function of time. The Cr(VI) biosorption efficiency (mg/g) of the biosorbent increased with increase in temperature from 25–50°C. However the initial rate of biosorption was found to decrease with increase in temperature. The initial rate of Cr(VI) biosorption decreased drastically with increase in temperature from 25°C to 30°C, while it decreased slowly and linearly with further increase in temperature up to 50°C. This may be due to presence of two types of biosorbents in spent biomass, viz. fungal biomass and residual substrate.

Based on the values of initial biosorption rates obtained at these five different temperatures, the activation energy (Ea) from the Arrhenius equation was found to be -23.18 KJ/mole (Fig. 5). This suggested that rate of Cr(VI) biosorption was not significantly influenced by process temperature in the temperature range of 30-50°C. The reactions exhibiting these negative activation energies may be considered as typically barrierless reaction. Such reaction either relies on the capture of the molecules in a active site or the biosorption of Cr(VI) on spent fungal biomass may follow a two step reaction and might involve a transition where rate constants show different dependence on temperature resulting in net negative activation energy [25]. This observation can be further substantiated by the fact that the spent biomass used in the present study consisted of both substrate and biomass and both of these can contribute to biosorption process through different dependence on temperature.

### Table - 1

<table>
<thead>
<tr>
<th>Cr(VI) mg/L</th>
<th>q$_0$ (mg/g)</th>
<th>K$_2$ (g/mg.min)</th>
<th>R$^2$</th>
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<tr>
<td>100</td>
<td>10.6 ± 0.476</td>
<td>0.00529</td>
<td>0.9555</td>
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<tr>
<td>200</td>
<td>20.2 ± 0.765</td>
<td>0.001582</td>
<td>0.9893</td>
</tr>
<tr>
<td>300</td>
<td>26.03 ± 0.290</td>
<td>0.000955</td>
<td>0.9726</td>
</tr>
<tr>
<td>400</td>
<td>35.04 ± 3.3</td>
<td>0.000493</td>
<td>0.9711</td>
</tr>
<tr>
<td>500</td>
<td>40.9 ± 1.83</td>
<td>0.000318</td>
<td>0.9508</td>
</tr>
</tbody>
</table>

Temperature dependence of the biosorption process is associated with several thermodynamic parameters. The values of ΔS and ΔH for Cr(VI) biosorption using spent biomass were calculated from slope and intercept of the Vant Hoff’s plot (Figure not shown). The positive value of ΔH (13.56 KJ/mol) suggests the biosorption process as a net endothermic process. Likewise, the positive value of ΔS (0.034 KJ/mol) reflects the affinity of Cr(VI) for the biosorbent used [14 & 15]. In addition, positive value of ΔS also shows an increasing randomness at the solid/liquid interface during the biosorption of Cr(VI) on tested biomass.

### Analysis of adsorption isotherm:

The purpose of adsorption isotherm is to relate the metal concentration adsorbed on the sorbent with metal concentration...
in the bulk solution. The analysis of the isotherm data is important to develop an equation that accurately represents the biosorption results. The isotherm can be described by several sorption isotherm models, of which Langmuir and Freundlich are the most widely referred equation. Both Langmuir and Freundlich isotherm models were evaluated to examine Cr(VI) biosorption with increasing concentration of Cr(VI). Fig. 6 shows typical linearized plots of Langmuir and Freundlich isotherm models for increasing concentration of Cr(VI). The linear plots of $C_{eq}/q$ versus $C_{eq}$ and $\ln q_{eq}$ versus $\ln C_{eq}$ confirmed that adsorption follows both Langmuir and Freundlich adsorption model.

The correlation coefficients ($R^2$) of both the isotherms were greater than 0.9 ($R^2 > 0.9$) indicating a good mathematical fit. This in turn suggests the presence of two different binding sites with two different mechanisms. As mentioned earlier, the biosorbert employed in present study is a mixture of fungal biomass and residual solid substrate. Thus it is obvious, that two different components represent two different types of metal binding sites and each may differ in the way it interacts with the metal ions.

The Langmuir and Freundlich adsorption constants calculated from the corresponding isotherms are presented in Table 2. Freundlich isotherm constants $K_f$ and $n$ was calculated as 8.3 and 1.13 respectively. The high magnitude of $K_f$ and $n$ illustrate high adsorption capacity of biomass. Likewise, Langmuir constants related to bonding energy of the adsorption (b) and maximum adsorption capacity ($Q$) were calculated as 0.0081 and 32.05 mg/g respectively.

<table>
<thead>
<tr>
<th>Freundlich Isotherm Constants</th>
<th>Langmuir Isotherm Constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>k</td>
<td>$R^2$</td>
</tr>
<tr>
<td>1.86</td>
<td>3.83</td>
</tr>
</tbody>
</table>

Further, based on the linearity of the isotherms it can be hypothesized that spent biomass of *Pythium* sp. offers two different binding sites, which contribute in Cr(VI) binding differently. Adsorption of Cr (VI) at one site is a complex-process involving multilayer, interactive or multiple site type binding which follows Freundlich isotherm and at the same time, the other type of binding sites follow Langmuir isotherm, which is based on monolayer sorption onto surface with finite number of identical sites, which are homogeneously distributed over the sorbent surface [26]. The adsorption studies with only Pythium sp. biomass suggests that it follows Freundlich isotherm for Cr(VI) biosorption [27]. Thus it may be further extrapolated that residual rice substrate probably follows Langmuir isotherm for Cr(VI) biosorption.

**CONCLUSION**

Spent biomass obtained after extraction of oil from Cr(VI) tolerant *Pythium* sp. was used for biosorption of hexavalent chromium. The biosorption was found to follow pseudo-second order kinetics. The thermal kinetics suggests that biosorption in this case may be a endothermic two-step process with net negative activation energy for Cr(VI) sorption. Moreover, the biosorption followed both Langmuir and Freundlich isotherm models suggesting the presence of two different types of binding sites with two different mechanisms.

**ACKNOWLEDGEMENT**

Authors are thankful to Department of Science and Technology (DST) and University Grant Commission (UGC), New Delhi for financial assistance.

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