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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_{eq}) 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 (ΔH and ΔS) 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, pneumonitis, 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, electro dialysis, 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 regard, 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

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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 amount of chromium adsorbed was monitored by determining residual Cr(VI) in the solution at different time intervals and subtracting it from the initial chromium.

Thermodynamic Studies

Effect of temperature on Cr(VI) removal was studied by agitating 100mL of 100mg/L solution of Cr(VI) at different temperatures (25°-50°C) 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

$$\ln k = -Ea/R T + \ln A_0 \quad (1)$$

Where Ea is activation energy and A_0 is constant called the Frequency factor. Value of Ea 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 (ΔH) and entropy change (ΔS) were calculated using the Van't Hoff's plot ($\ln Kc$ Vs $1/T$) given as;

$$\ln Kc = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad (2)$$

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

$$Kc = \frac{Q_{eq}}{C_{eq}} \quad (3)$$

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;

$$\log(q_{eq} - q_t) = \log q_{eq} - \frac{k_1 t}{2.303} \quad (4)$$

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 rate equation is expressed as

$$\frac{t}{q_t} = \frac{1}{k_2(q_{eq})^2} + \frac{t}{q_{eq}} \quad (5)$$

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 $1/k_2 q_{eq}^2$ 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].

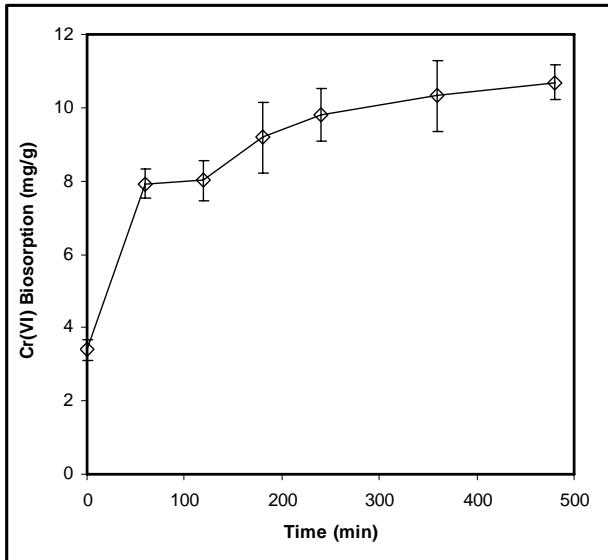


Fig. 1 Time course for Cr(VI) biosorption using spent biomass of *Pythium* sp.

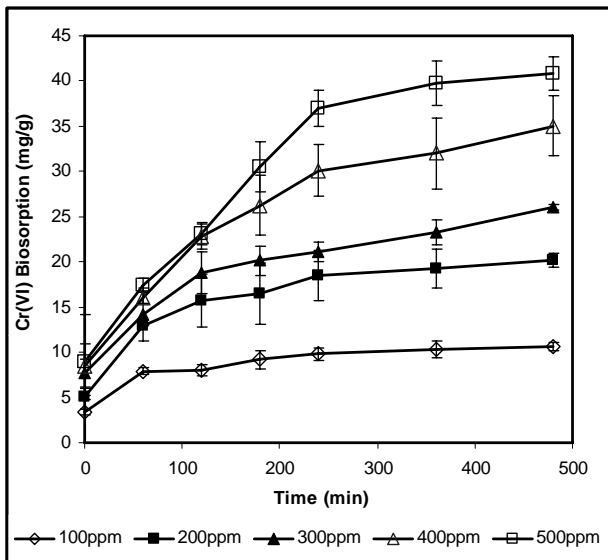


Fig. 2 Effect of initial Cr(VI) ion concentration on biosorption efficiency(mg/g) of spent biomass *Pythium* sp. cultivated using boiled rice as substrate under solid state conditions.

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].

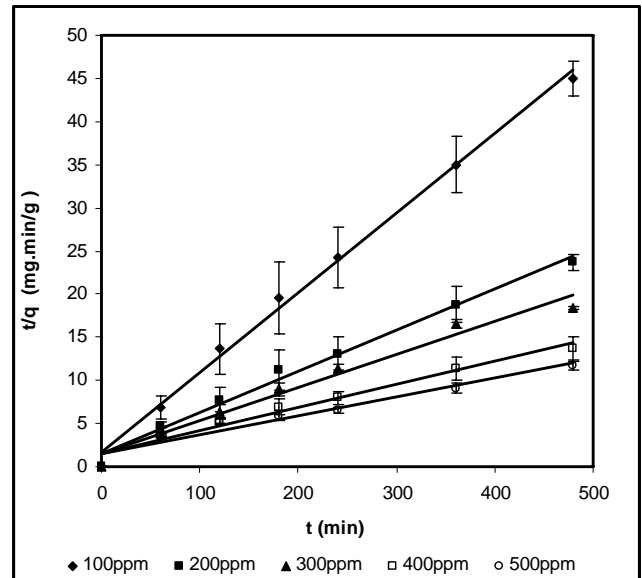


Fig. 3 Linearized pseudo second-order kinetic plots at varying initial concentrations of Cr(VI) ions (100-500 mg/L).

Table - 1 Pseudo-second order kinetic parameters for Cr(VI) biosorption at varying initial concentrations of Cr(VI) ions.

Cr(VI); mg/L	Kinetic Parameters			R ²
	q _{eq} : (mg/g)		K ₂ : (g/mg.min)	
	Experimental	Calculated.		
100	10.6 ± 0.476	10.8	0.00529	0.9955
200	20.2 ± 0.765	20.8	0.001582	0.9893
300	26.03 ± 0.290	26.04	0.00095	0.9726
400	35.04 ± 3.3	37.17	0.000493	0.9711
500	40.9 ± 1.83	45.87	0.000318	0.9508

Effect of pH and Temperature

The Cr(VI) biosorption was found to be maximum at pH 1.0 and remained fairly constant upto pH 6.0, while biosorption efficiency decreased significantly with further increase in pH upto 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 biosorbent surface would become negative and consequently due to respective charge repulsion of negatively charged Cr ions like HCrO_4^- , $\text{Cr}_2\text{O}_7^{2-}$, 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, CrO_4^{2-} and $\text{Cr}_2\text{O}_7^{2-}$. 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 upto 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 (E_a) 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 in biosorption process through different dependence on temperature.

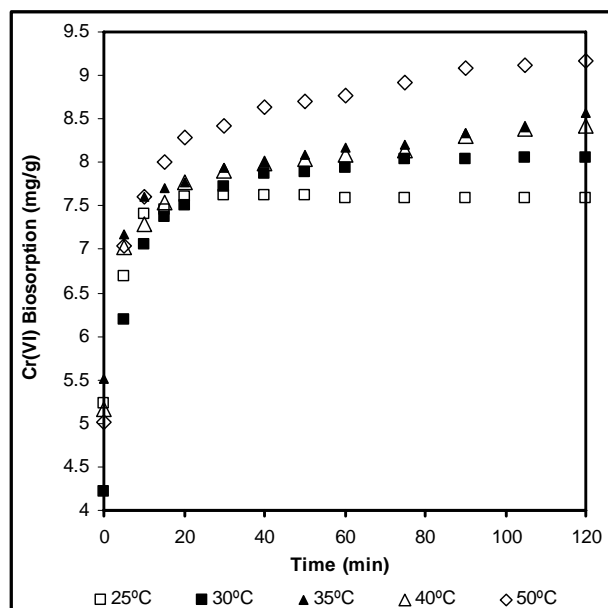


Fig. 4 Effect of temperature (25-50°C) on Cr(VI) biosorption efficiency (mg/g) of spent biomass *Pythium* sp. cultivated using boiled rice as substrate under solid state conditions.

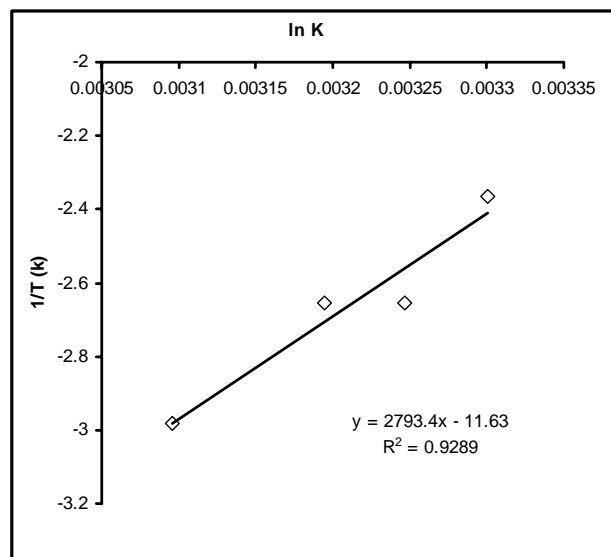


Fig. 5 Arrhenius plot for Cr(VI) biosorption using spent biomass of *Pythium* sp.

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 (13.56KJ/mol) suggests the biosorption process as a net endothermic process. Likewise, the positive value of ΔS (0.034kJ/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.

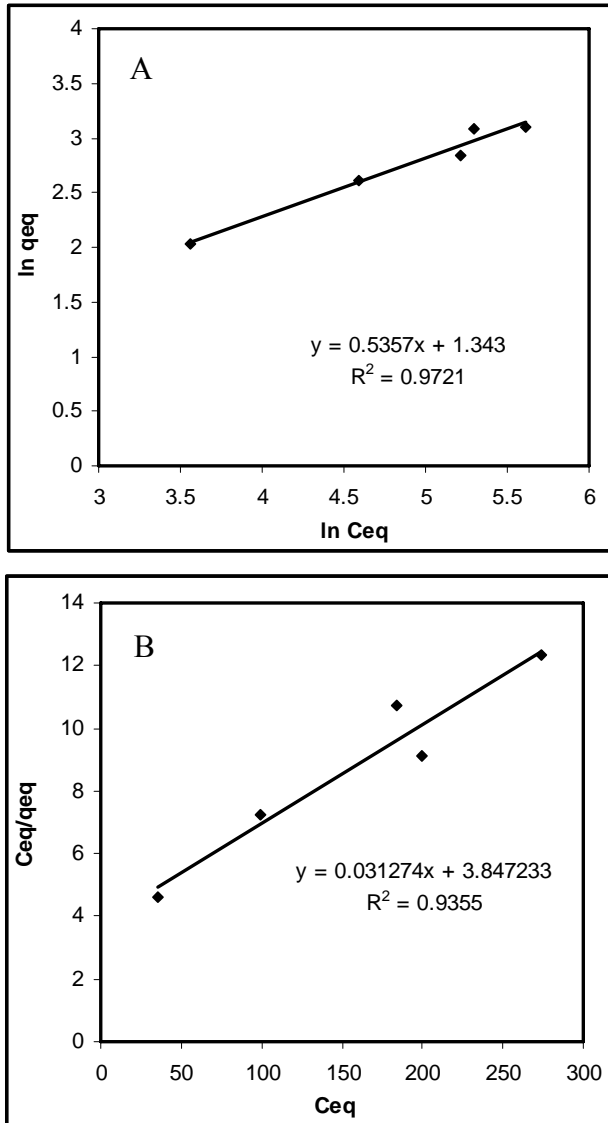


Fig. 6 Assessment of Cr (VI) biosorption by (A) Langmuir isotherm, (B) Freundlich isotherm at varying initial Cr(VI) ion concentration (100 – 500mg/L).

The correlation coefficients (R^2) of both the isotherm 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 biosorbent 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 - 2 Cr(VI) biosorption isotherm parameters for Cr(VI) biosorption by spent biomass of *Pythium* sp.

Freundlich Isotherm Constants			Langmuir Isotherm Constant		
n	K_f ; mg/g	R^2	b	Q; mg/g	R^2
1.86	3.83	0.9721	0.0081	32.05	0.9355

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 an 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.

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Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Review Articles intended to provide concise in-depth reviews of both established and new areas and summarize recent insights in specific research areas within the scope of PRAJNA are solicited by the Editorial Board from leading researchers. The manuscript of this category should be limited to 5,000 words with an abstract of no more than 250 words, a maximum of 5 tables and figures (total), and up to 50 references. Word count includes only the main body of text (i.e., not tables, figures, abstracts or references).

Commentaries call attention to papers of particular note and are written at the invitation of the Editorial Board.

Perspectives present a viewpoint on an important area of research and are written only at the invitation of the Editorial Board. Perspectives focus on a specific field or subfield within a larger discipline and discuss current advances and future directions. Perspectives are of broad interest for non-specialists and may add personal insight to a field.

Letters are brief comments that contribute to the discussion of a research article published in the last issue of PRAJNA. Letters may not include requests to cite the letter writer's work, accusations of misconduct, or personal comments to an author. Letters are limited to 500 words and no more than five references. Letters must be submitted within 3 months of the publication date of the subject article.

Also announcement of forthcoming Seminars / Conferences / Symposia / Workshops etc. will be considered for publication in PRAJNA.

File format for soft copies:

Texts (should be of Times New Roman with 9 point for Abstract and 11 point for other matter) and Tables, if any, must be saved in *.doc (Word) or *.rtf (rich text) format, graphs in Excel and for illustrations (diagrams, maps, drawings, etc.), the TIF format (300 dpi minimal resolution) is the most appropriate (*.TIF or *.JPEG extension).

Instructions for preparation of manuscripts:

1. The paper should be written in English and neatly typed with double spacing.
2. The title of the paper and the name(s) of the author(s) be in capital letters. The name of the institution be given in small letters below the name (s) of the author(s).
3. The 'Abstract of the paper, in not more than 150 words, should be provided on a separate page along with 4-6 keywords.
4. The sub-titles, e.g. INTRODUCTION, should be written in capital letters.

5. Displayed formulae, mathematical equations and expressions should be numbered serially. Table should be with a title in addition to a serial number for it.
6. Photographs / Figures should be original with good contrast so as to be in a form suitable for direct reproduction / scanning.
7. Footnotes are not normally allowed, except to identify the author for correspondence.
8. All figures must be numbered serially as they appear in the text, and their legends / captions should necessarily be provided.
9. References should be numbered in brackets [] in the order of appearance in the text. All the references in the bibliographic list must correspond to in-text references and vice versa. Abbreviated periodical titles should follow standard subject Abstracts. Names which are not listed by any standard subject indexing organizations should be spelled out in full.
10. All references should be clear and follow the examples below:

Periodical articles

- [2] Sadqui, M., Fushman, D. and Munoz, V. (2006) Atom – by – atom analysis of global downhill protein folding. *Nature*, **442**: 317 – 321.

Books

- [16] Stebbins, G. L. (1974) *Flowering plants: Evolution above the species level*, Arnold Press, London, pp. 1 – 399.

Chapters from a book

- [19] Schafer, H. and Muyzer, G. (2001) Denaturing gradient gel electrophoresis in marine microbial ecology. In *Methods in Microbiology* (Ed. Paul, J. H.), Academic Press, London, Vol. 30, pp. 425 – 468.

Thesis or other diplomas

- [21] Nayaka, S. (2004) *The visionary studies on the lichen genus Lecanora sensu lato in India*. Ph. D. Thesis, Dr. R. M. L. Avadh University, Faizabad, India.

Conference proceedings

- [4] Mohapatra, G. C. (1981) Environment and culture of early man in the valley of rivers Chenab and Ravi, western sub-Himalayas. In *Proceedings X Congress of IUPPS*, Mexico, pp. 90 – 123.

Online documentation

- [9] Koning, R. E. (1994). Home Page for Ross Koning. Retrieved 26-6-2009 from *Plant Physiology Information Website*: <http://plantphys.info/index.html>.

Note:

Manuscripts prepared faithfully in accordance with the instructions will accelerate their processing towards publication; otherwise it would be delayed in view of their expected re-submission.

For and on behalf of Editorial Board, PRAJNA

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