



RESEARCH ARTICLE

ALGAE BIOTECHNOLOGY

EQUILIBRIUM AND KINETIC STUDIES ON BIOSORPTION OF Cr (VI) BY NON-LIVING MYCELIAL SUSPENSIONS OF *ASPERGILLUS NIGER*.

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ABSTRACT

Equilibrium and kinetic studies on biosorption of chromium (VI) from aqueous solution was studied using the dead biomass of *Aspergillus niger* (*A. niger*) as a feasible biosorbent under batch method at 30°C. Various sorption parameters such as contact time, initial concentration of chromium (VI) ion, effect of pH and effect of biosorbent dosages were studied. The adsorbent was effective for the quantitative removal of chromium (VI) ions in acidic conditions and equilibrium has been achieved in 240 min with a maximum removal of 90% is achieved at pH 2.0. The equilibrium adsorption data were fitted to Langmuir, Freundlich and Dubinin-Radushkevich (DR) adsorption isotherm models and the parameters were evaluated. The kinetic study showed that the first order, second order rate equation and power function equations were studied. Comparatively first order and second order kinetic equations are better described for this biosorption process. This method is found to be quite promising, feasible, economic and time saving.



KEY WORDS

Biosorption, Hexavalent chromium, *Aspergillus niger*, Dubinin-Radushkevich

1. INTRODUCTION

Water enriched with various pollutants like toxic heavy metals from municipal and industrial waste streams play an important role in waste water treatment. Metal species released into the environment by technological activities tend to persist indefinitely circulating and eventually accumulating throughout the food chain, being a serious threat to the environment¹. In recent years growing interest focused the attention towards the research of new, economical and easily available biosorbents such as algae, bacteria, fungi and yeast found to have potentialities in solving environmental problems². The commonly applied methods for the treatment of industrial effluents are by precipitation, ion exchange, electrochemical process, membrane processes, and adsorption³. Biosorption of heavy metals is one of the most promising technologies involved in the removal of toxic metals from industrial waste streams and natural water. It is a potential alternative to conventional process for the removal of metals. If the biomass employed is a waste material, then adsorption represents a cheap alternative to conventional process, due to the use of low cost sorbing materials. Many adsorbents have been used for the sorption of heavy metals. The sorption of heavy metals using agricultural products and by products such as peanut skin, cotton seed hull⁴, mango nut powder, wool, rice straw, pea nut hulls, sugarcane bagasse, tea⁵, coffee powder, sawdust, tamarind fruit shell⁶ etc., were proved to be effective biosorbents. Biological approaches on other hand, offer the potential for the highly selective removal of toxic metals coupled with considerable operational flexibility. Many such processes utilizes microorganism that have key role in the sorption of toxic metals. This mechanism is based on the metal binding capacities of various biological materials. Among various microbes, fungus has proved to be potential metal sorbent and its advantages lie in both

the good performance in metal removal and cost effectiveness. Metal sorption performance depends on some external factors such as pH, ionic strength, biomass concentration, temperature and presence of different metal ions in solutions etc. This technique is widely used in treatment of effluents coming from tanneries, textiles, metal polishing industries, fertilizer factories etc. The metal chromium is mainly found in the effluent discharged from industries like leather tanning, paint, ink, aluminum, textile, electroplating, metal finishing, petroleum refining, chrome tanning and wood preserving. The two predominant forms of chromium, trivalent and hexavalent are found in industrial waste waters among which hexavalent chromium is more hazardous when compared to trivalent chromium. The chromium concentration present generally in waste water is 50-290mg/l. The chromium permissible limit on surface water⁷ is 0.1mg/l. Chromium has been found in many specimens of blood and teeth of human beings and animals. Chromium causes dermatitis on the skin, bleeding, scabbing, dryness, superficial ulceration on the nasal mucosa, inflammation, lung carcinoma and liver injury in humans. Toxic effect of chromium in animals includes stunting of growth, thickening of alveolar walls and proliferation of cells along the blood vessels etc.,

In this present study, the ability of the fungi *Aspergillus niger*⁸ to remove Cr (VI) ion from synthetic solution were carried out. Parameters such as contact time, initial metal ion concentration, pH of the metal solution and different biomass dosages have been investigated and optimized. The effect of initial metal ion concentration during the growth and its efficiency in removing Cr (VI) ions are also estimated. The maximum concentration of biomass produced and the maximum specific uptake of hexavalent



chromium were also evaluated. Equilibrium modeling was carried out using Langmuir⁹, Freundlich¹⁰ and Dubinin-Radushkevich¹¹ adsorption isotherms and the data were fitted. The kinetics of hexavalent chromium biosorption process using *A. niger* as a function of initial metal ion concentration have been evaluated with respect to first order, second order and power function kinetic equations and arranged based on its adsorption capacity.

2. MATERIALS AND METHODS

2.1. Biosorbent preparation

Aspergillus niger (NCIM 565), a filamentous fungi was used for this study based on its ability to remove chromium (VI) heavy metal¹². The culture was purchased from National Collection of Industrial Microorganisms (NCIM), Pune. The culture *Aspergillus niger* was maintained in the agar media. For growing the media on a large scale, fresh culture was transferred to 100ml of liquid media containing nutrient broth medium (Himedia-Bombay). The media were left for growth of two days. The 100 ml media was transferred into 500ml media and culture was used for further studies. In all cases, the media was autoclaved at 121°C for 15 minutes and strict aseptic conditions were maintained. Throughout the experiment precautions were taken while inoculating and transferring the culture.

2.2. Preparation of chromium solution

The stock solution of aqueous chromium of concentration 1000 mg/l was prepared by dissolving 2.829 g of potassium dichromate salt of AR grade in 1000 ml deionized water. The test solution containing chromium was prepared by diluting 1 ml of stock solution of metal to the desired concentrations. The chromium concentration was varied in the range of 25 to 150 mg/l.

2.3. Batch Experiments

Batch experiments were carried out using non-living mycelial suspensions. Batch experiments were carried out in Erlenmeyer flasks by adding 1g of suspended *Aspergillus niger* in 100ml of aqueous chromium solution

at desired concentrations. The flasks were agitated on a reciprocating shaker at constant temperature and at constant pH. The samples were taken at regular intervals of 15 min until the equilibrium was reached. When equilibrium was reached, the biosorbent was separated and the supernatant liquid was analyzed using spectrophotometer at 540 nm for the remaining chromium concentrations and the amount of metal ion adsorbed onto per unit weight of biomass, q_e was calculated¹³.

2.3.1. Effect of contact time

The effect of contact time were carried out in a batch reactor for different contact times (15, 30, 45, 60, 120, 180, 240, 300 min) at an initial concentration of 25 mg/l of Cr (VI) ion at pH 6.0. The adsorbent dosage of 1g in 100 ml solution in 250 ml conical flask at room temperature (30°C) were taken and then agitated in a rotary shaker at 150 rpm and at regular intervals the samples were filtered and analyzed spectrophotometrically at 540 nm¹⁴. Each determination was repeated three times in order to get accurate results.

2.3.2. Effect of initial metal ion concentration

The effect of initial chromium concentrations were carried out for the initial chromium (VI) concentration (25, 50, 100 and 150 mg/l) in a batch reactor at pH 6.0 at room temperature (30°C). 1g biomass was added in 100 ml solution in 250 ml conical flask and kept in a rotary shaker at 150 rpm. The samples were drawn at regular intervals until equilibrium was reached and analyzed for remaining chromium concentration as mentioned earlier.

2.3.3. Effect of pH

The chromium solution of 100ml of 150 mg/l was adjusted to various pH (1, 2, 3, 4, 5, 6 and 7) for the equilibrium batch experiments to study the effect of pH. The experiment was carried out by adding 1g of biomass at room temperature and kept in a rotary shaker at 150 rpm. The samples were withdrawn at regular intervals until equilibrium



was reached and analyzed for remaining chromium concentration as mentioned earlier.

2.3.4. Effect of biomass loading

For the effect of biomass loading studies 100 ml of 150 mg/l concentration of chromium solution was loaded with different biosorbent dosages (1, 2, 3, 4 and 5g) by keeping pH and temperature as 6.0 and 30⁰C respectively.

2.3.5. Analysis of chromium

The concentration of unadsorbed chromium (VI) ions in medium was determined spectrophotometrically at 540nm using diphenyl carbazide as the colour complexing agent, where diphenyl carbazide reagent combines with hexavalent chromium to form a complex which gives a beautiful magenta colour in acidic medium.

3. RESULTS AND DISCUSSION

3.1. Effect of contact time

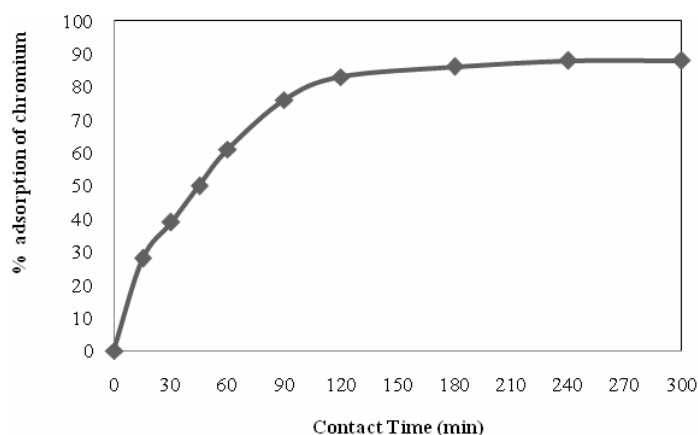


Fig. 1

Effect of contact time on bisorption of Cr(VI) using *A. niger*, 100 ml aqueous metal solution, pH=6.0, initial metal ion concentration (C_0) = 25 mg/l, weight of biosorbent = 1 g, and temperature = 30⁰C

3.2. Effect of initial chromium ion concentration

The percentage removal of chromium decreases with increase in initial chromium concentration as shown in Fig.2. The percentage removal of chromium decreased from 88% (at 25 mg/l) to 78.4% (at 150 mg/l) respectively. The decrease in the extent of

The effect of contact time on Cr (VI) biosorption using dead *Aspergillus niger* was studied and the results were shown in Fig. 1. It was found that the adsorption quantity of Cr (VI) ion increases as the contact time increased. The biosorption of chromium into dead biomass was found to be 76% at 90 min and equilibrium was reached at 240 min (88%). Hence, in the present study, 240 min was chosen as the equilibrium time. Initially the removal rate of sorbate was rapid, but it gradually decreases with time until it reaches equilibrium. The rate in percent of metal removal was higher in the beginning due to the larger surface area of the adsorbent being available for the adsorption of the metals¹³. The adsorption of the Cr (VI) was greater at earlier stages due to the availability of more number of active sorption sites. As time passes the metal uptake by the sorbent surface slows down as the competition for the decreasing availability of active sites intensifies by the metal ions remaining in the solution¹⁴.

removal of chromium percentage with increase in the initial concentration may be due to the reduction in immediate adsorption due to the lack of available active sites. For higher concentration of chromium the percentage removal decreases mainly due to the saturation of binding sites.

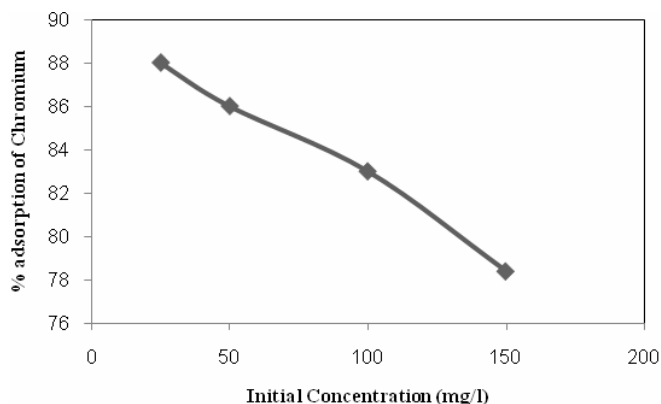


Fig.2

Effect of initial metal ion concentration on biosorption of Cr(VI) using *A. niger*; 100 ml aqueous metal solution of initial concentration = 25 mg/l, 50 mg/l, 100 mg/l and 150 mg/l; pH=6.0; weight of biosorbent = 1 g, and temperature = 30°C

3.3. Effect of pH

The effect of pH on the percentage removal of chromium at 150 mg/l chromium concentration was studied as a function of pH at constant fungal doses and the result is shown in Fig. 3. It is clear from the figure the percentage removal of chromium increases with increase in pH from 1.0 to 2.0 and thereafter decreases with further increase in pH. This behavior can be explained by considering the nature of the biosorbent at different pH in chromium biosorption. The cell wall of *Aspergillus* species contains a large number of surface functional groups. The pH dependence of chromium biosorption can

largely be related to the type and ionic state of these functional groups and also on the metal chemistry in solution. Maximum sorption of chromium occurs at pH 2.0 which suggests that the negatively charged chromium species bind through electrostatic attraction to positively charged functional groups on the surface of fungal cell wall because at this pH more functional groups carrying positive charges would be exposed¹⁵. But at pH above 3.0, it seems that fungal cell wall possess more functional groups carrying a net negative charge which tends to repulse the anions.

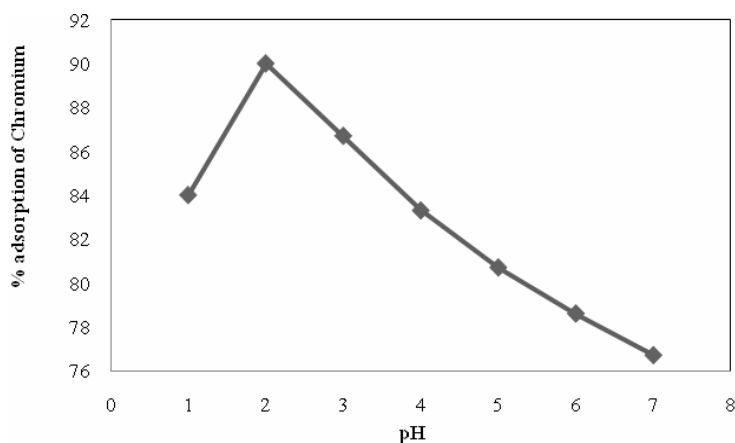


Fig.3

Effect of pH on adsorption of chromium using *A. niger*; pH = 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0; volume of solution = 100 ml; initial Cr(VI) concentration = 150 mg/l; weight of biosorbent = 1 g; temperature = 30°C.



3.4. Effect of biomass loading

The effect of biomass loading on percentage removal of chromium was studied is depicted in Fig. 4. It indicates that increase in the cell concentration increases the initial rate of sorption as well as the chromium ion

removal. Similar metal uptake with variation in biosorbent dosages has been reported. The increase in Cr (VI) adsorption may possibly due to the increased number of binding sites for chromium ions at higher cell loading.

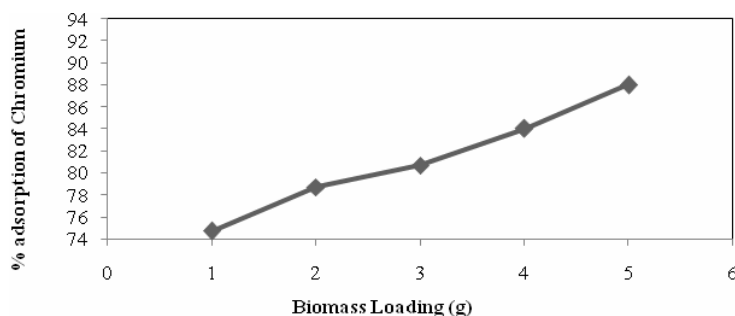


Fig.4

Effect of biomass loading on adsorption of chromium using *A. niger*; weight of biosorbent = 1.0, 2.0, 3.0, 4.0, and 5.0 g; volume of solution = 100 ml; initial Cr(VI) concentration = 150 mg/l; weight of biosorbent = 1 g; pH = 6.0; temperature = 30°C.

3.5. Equilibrium models for the biosorption of chromium

Adsorption isotherm shows the relation between the amounts of metal adsorbed per unit weight of adsorbate remaining in a test medium at equilibrium¹³. The capacity of *A.niger* to remove hexavalent chromium was evaluated using Langmuir, Freundlich and Dubinin-Radushkevich isotherm equations.

The well known expression of the Langmuir model⁹ is valid for a monolayer adsorption is given below.

$$q_e = \frac{q_{\max} b C_{eq}}{1 + bC_{eq}}$$

Where, q_e and C_{eq} are the amount of adsorbed metal per unit weight of biosorbent at equilibrium (mg/g) and unadsorbed metal concentration in solution at equilibrium (mg/g), respectively. q_{\max} is the maximum amount of metal per unit weight of biomass to form a complete monolayer on the surface bound (mg/g) and b is a constant related to the affinity of the binding sites (l/mg). Fig.7 depicts the Langmuir plot for adsorption of chromium into *A.niger* and the parameters evaluated were given in Table.1.

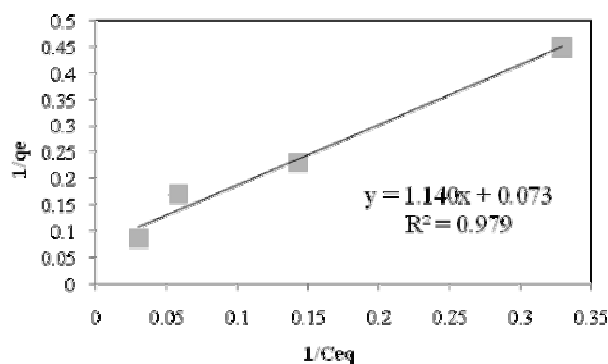


Fig.7

Langmuir isotherm plot for Cr(VI) adsorption into *A.niger*

Table 1
Freundlich, Langmuir and Dubinin-Radushkevich isotherm constants for the hexavalent chromium ions adsorption into dead *Aspergillus niger*.

Adsorption Isotherm	q_{max} (mg/g)	b (l/mg)	R^2
Langmuir	9.23	0.1072	0.9872
Freundlich	K_F 1.4768	n 2.1413	R^2 0.9345
Dubinin-Radushkevich	q_D 10.32	B_D 1.1×10^{-3}	R^2 0.8804

The essential characteristics of Langmuir isotherm can be explained in terms of dimensionless constant separation factor (R_L) defined by ¹⁶

$$R_L = \frac{1}{1 + bC_0}$$

Where, C_0 is the initial concentration of metal ion. The calculated R_L values are given in Table.2. Fig. 8 represents the plot of calculated R_L values versus the initial concentration of Cr (VI) at 30°C. From the Fig. 8. it is observed that, as the initial

concentration increased from 25 to 150 mg/l, the R_L values started to decrease from 0.2717 to 0.0585. All the R_L values are between 0 and 1 indicates that the adsorption of Cr (VI) on the dead *Aspergillus niger* was favorable at the conditions being studied.

Table. 2
Parameters used to relate separation factor with type of adsorption.

R_L value	Type of Fit	Estimated R_L
$R_L > 1$	Unfavourable	-
$R_L = 1$	Linear	-
$0 < R_L < 1$	Favourable	0.275
$R_L = 0$	Irreversible	-

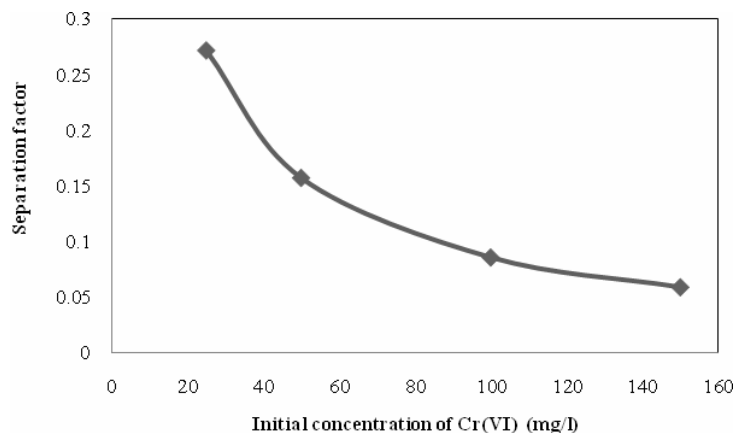


Fig.8
Effect of initial concentration of Cr(VI) on separation factor R_L



The next isotherm (Freundlich, 1906) used for fitting the data using Freundlich equation is given below:

$$q_e = K_F C_e^{\frac{1}{n}}$$

Where, q_e is the amount of metal ion adsorbed per gram of adsorbent (mg/g), C_e is the equilibrium concentration of metal ion in solution (mg/l). K_F and $\frac{1}{n}$ are Freundlich constants, indicating the adsorption capacity and adsorption intensity respectively. The model can be linearized in natural logarithmic form to evaluate the constants (Patil & Shrivastava, 2010). The Freundlich isotherm plot for Cr (VI) adsorption into *A.niger* is shown in Fig.9. The data were fitted to the isotherm and the constants were calculated from the Fig.9 are tabulated in Table. 1. The Freundlich constant n value between 1 and 10 indicates good adsorption. The value of n for adsorption of Cr (VI) using *A.niger* and correlation coefficient were found to be 2.1413 and 0.9345 respectively, which indicates that this isotherm is best fitted and is favourable. The tabulated values of K_F and n are given in Table. 1 shows easy separation of heavy metal ion from waste water and high adsorption capacity of the biosorbent to remove hexavalent chromium.

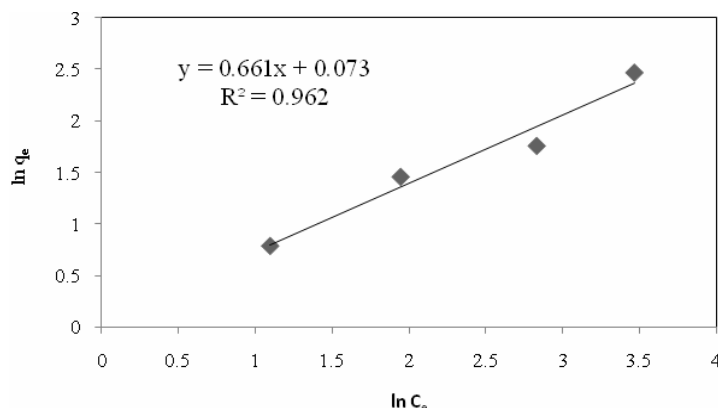


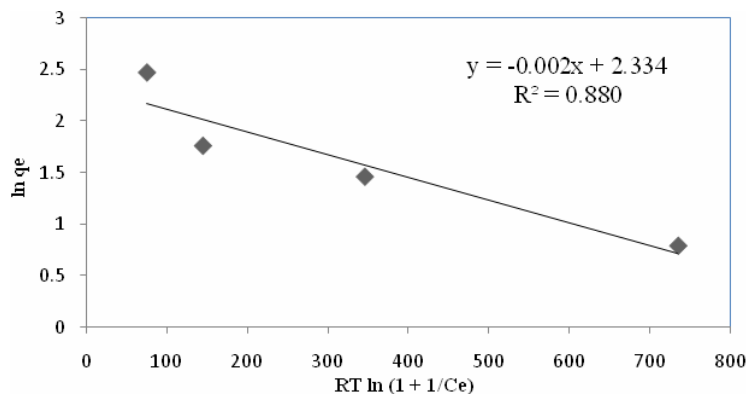
Fig.9
Freundlich isotherm plot for Cr(VI) adsorption into *A.niger*

Dubinini-Radushkevich isotherm model was chosen to estimate the characteristic porosity of the biomass and the apparent energy of adsorption of chromium. The model is represented by the equation as shown below:

$$q_e = q_D \exp\left(-B_D \left[RT \ln\left(1 + \frac{1}{C_e}\right)\right]^2\right)$$

Where, B_D is related to the free energy of sorption per mole of the sorbate as it migrates to the surface of the biomass from infinite distance in the solution and q_D is the DR isotherm constant related to the degree of sorbate sorption by the sorbent surface. The above equation can be linearized in natural logarithmic form to evaluate the constants. Fig. 10, represents the linearized DR isotherm plot for biosorption of hexavalent chromium

into *A.niger*. The data were fitted to the isotherm and the constants were calculated from the Fig.10 are tabulated in Table. 1. It was observed that, high values of q_D shows high sorption capacity and the apparent energy of adsorption shows that this is a physisorption process. Therefore, DR isotherm was also fitted to the data of sorption process.

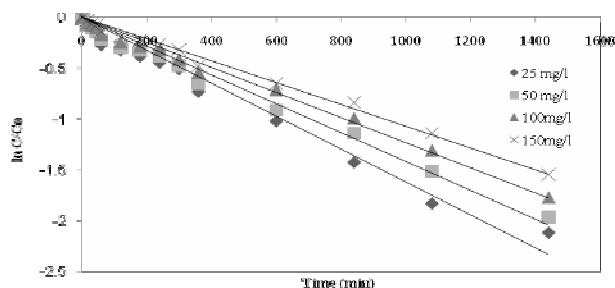
**Fig. 10**

The Dubinin-Radushkevich isotherm plot for Cr(VI) adsorption into *A.niger*.

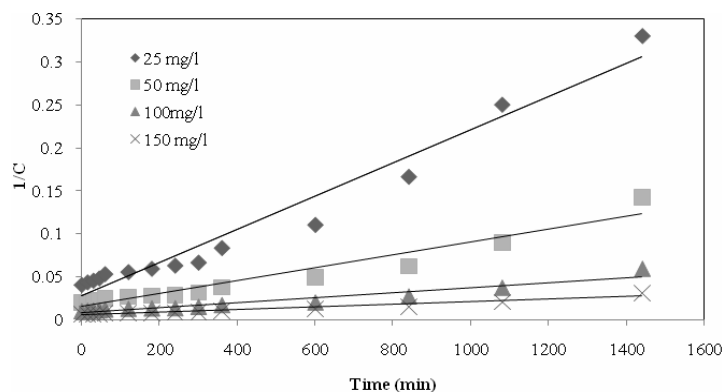
3.6. Adsorption kinetics

The time-dependent experimental adsorption data were used for kinetic modeling. The model equations used for fitting the data were first order equation, second order equation and power function equation are given in Table. 3. The present

Cr (VI) sorption data of *Aspergillus niger* at various initial concentrations are tested for kinetic modeling for first order equation, second order equation and power function equation by linear regression plots are shown in Fig.11, Fig. 12 and Fig. 13 respectively.

**Fig. 11**

First order kinetics Plot for biosorption of various initial concentrations of Cr(VI) into *A.niger* at 30°C and pH 6.0.

**Fig. 12**

Second order kinetics Plot for biosorption of various initial concentrations of Cr(VI) into *A.niger* at 30°C and pH 6.0.



The estimated statistical parameters of the model equations are presented in Table. 3. Based on linear regression values, the kinetics of Cr (VI) adsorption onto non-growing *Aspergillus niger* was best described in the order of first order equation which is

greater than second order equation which in turn is greater than power function equation. The results clearly indicate that the first order kinetics followed by second order model fits progressively well with increasing sorbate concentration.

Table.3
Parameters for First order, Second order and Power function kinetics equation

		25	50	100	150
1st order equation $-\ln \frac{C}{C_0} = K_1 t$	$K_1(\text{min}^{-1})$	1.6×10^{-3}	1.4×10^{-3}	1.2×10^{-3}	1.1×10^{-3}
	R_1^2	0.9724	0.979	0.9871	0.9907
2nd order equation $\frac{1}{C} - \frac{1}{C_0} = K_2 t$	$K_2(\text{g/mg min})$	0.2×10^{-3}	7E-05	3E-05	2E-05
	R_2^2	0.9609	0.9379	0.9286	0.9438
Power function equation $\log q = \log a + b \log t$	a	0.563	0.656	0.656	0.637
	b	0.2413	0.3049	0.3839	0.4399
	R^2	0.4257	0.6398	0.7754	0.7922

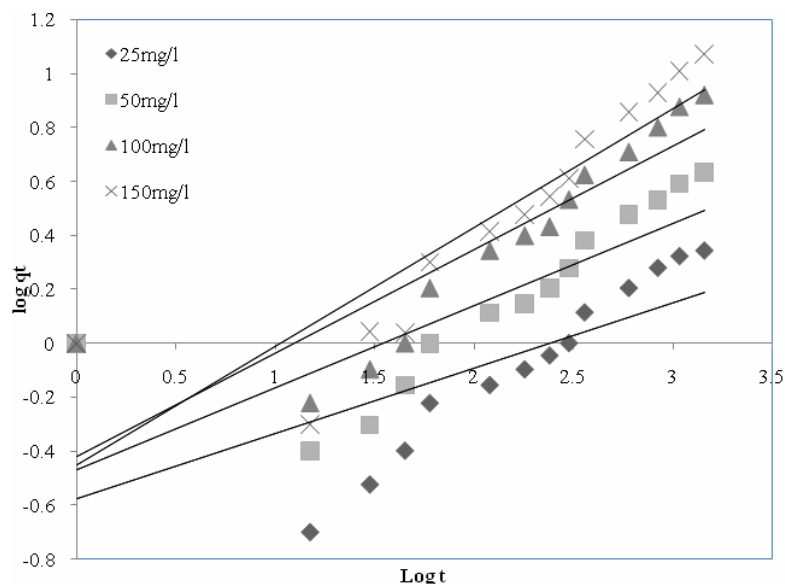


Fig. 13

Power function plot for biosorption of various initial concentrations of Cr(VI) into *A.niger* at 30°C and pH 6.0.

4. CONCLUSION

In this study, the biosorption of hexavalent chromium ions by *Aspergillus niger* was

investigated for various contact time, initial concentration of the metal ion, pH and sorbent dosages. The biosorbent was successful in removing hexavalent chromium



with 88% efficiency from aqueous solution and it was also observed that adsorption was pH dependent and maximum adsorption of 90% was observed at pH 2.0. The equilibrium data were analysed using the Langmuir, Freundlich and Dubinin-Radushkevich isotherms. The characteristics parameters for each isotherm were determined and proved Langmuir isotherm followed by Freundlich adsorption isotherm provided the best correlation for biosorption of chromium onto *Aspergillus niger* than DR isotherm. The results of the present study also proved that the biosorption of Cr

(VI) into *A. niger* fits first order kinetic model followed by second order kinetic model and then by power function equation. Based on the results it can be concluded that *Aspergillus niger* is having potential to remove hexavalent chromium from waste water and an alternative that can be easily produced and used as a low cost sorbent with considerably high biosorption capacity. It also leaves greater scope for further investigations to be carried out in order to improve the removal efficiency of *A. niger* using some pretreatment methods.

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