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## Biosorption of Cr(VI) from Aqueous Solutions Using *Trametes Versicolor Polyporus* Fungi

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**Abstract:** Removal of chromium(VI) from aqueous solution was studied using abundantly available *trametes versicolor polyporus* fungi as biosorbing medium under equilibrium and column flow conditions. Various sorption parameters such as contact time, effect of pH, concentration of Cr(VI) and amount of biomass on the adsorption capacity of the biosorbent were studied. The equilibrium adsorption data were fitted to Freundlich and Langmuir adsorption isotherm models and the model parameters are evaluated. In addition, the data were used to predict the kinetics of adsorption. The results indicated that the adsorption of Cr(VI) on fungi followed second order kinetics. The column flow adsorption data were used to predict break through curves. The fungi loaded with Cr(VI) was regenerated with 0.1 M NaOH solution and the regenerated biomass was used in the subsequent adsorption-desorption cycles. The experimental results demonstrated that the *trametes versicolor polyporus* fungi could be used as sorbent for immobilizing Cr(VI).

**Keywords:** Biosorption, Chromium, *Polyporus* fungi, Isotherm models.

### Introduction

The presence of toxic heavy metals in aqueous streams, arising from the discharge of untreated metal containing effluent into water bodies, is one of the most important environmental issues<sup>1</sup>. Their presence in aquatic ecosystem poses human health risks and causes harmful effect to living organisms<sup>2</sup>. Chromium is one of the toxic contaminants, which exists in hexavalent and trivalent forms. Hexavalent form is more toxic<sup>3</sup> than trivalent and requires more concern. Exposure to Cr(VI) causes cancer in digestive tract and lungs<sup>4</sup> and may cause epigastric pain, nausea, vomiting, severe diarrhea and hemorrhage<sup>5</sup>. It is therefore, essential to remove Cr(VI) from wastewater before disposal. The main sources of

chromium(VI) are tannery, paint, ink, dye, and aluminium manufacturing industries *etc.* Several International Environmental Agencies have introduced strict regulations with regard to metal discharge, especially from industrial activities. According to USEPA, the discharge of Cr(VI) and its other forms is regulated<sup>6</sup> to below 2 mg/L.

The conventional methods for removing heavy metal from wastewaters include chemical precipitation, electrochemical treatment, evaporation, and exchange, membrane processing, evaporation, and solvent extraction. However, the application of these treatment processes has been found to be sometimes restricted, because of expensive investment, operational costs and the potential generation of secondary pollution. Furthermore such processes may be ineffective or extremely expensive when the initial heavy metal concentrations are in the range of 10-100 mg/L.

In recent years, the biosorption has been studied extensively using microbial biomass as a biosorbent for heavy metal removal. Both living and dead biomass may be utilized in biosorptive processes as it often exhibits marked tolerance towards metals and other adverse conditions such as low pH<sup>7, 8</sup>. Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy metals<sup>9-12</sup>. The interest in the potential utilization of fungal biomass as a biosorbent is increasing due to the need for economical and efficient adsorbents to remove chromium ions from wastewater. However, information available on interaction of fungi with chromium ions is still limited. Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake<sup>14,15</sup>. The high surface to volume ratio of microorganisms and their ability to detoxify metals are among the reasons that they are considered as potential alternative to synthetic resins for remediation of dilute solutions of metals and solid wastes<sup>16, 17</sup>. Fourest and Roux<sup>13</sup> have used fungal mycelial biomass to remove heavy metals from water. Biosorption of hexavalent chromium from aqueous solution by a tropical basidiomycete BDT-14 was reported by Trivedi *et al.*<sup>18</sup>. The effect of copper(II), lead(II) and chromium(VI) ions on the growth and bioaccumulation properties of *aspergillus niger* was investigated as a function of initial pH and initial metal ion concentration<sup>19</sup>. Brenda *et al.*<sup>20</sup> compared the biosorptive capacity of dried biomass fungus *rhizopus oryzae* for metal sorption with commercially available sources of chitin, chitosan and chitosan cross-linked with benzoquinone. A modified fungal biomass of *penicillium chrysogenum* with positive surface charges, prepared by grafting polyethylenimine (PEI) onto the biomass surface, was used for removal of Cr(VI)<sup>21</sup>. Bai and Abraham<sup>22</sup> reported the biosorption of Cr(VI) by chemically modified biomass of *rhizopus nigricans* and the possible mechanism of Cr complexation to the adsorbent. The simultaneous biosorption of Cr(VI) and Fe(III) ions in single component and binary systems has been studied using *rhizopus arrhizus*, a filamentous fungus, in a semi-batch reactor<sup>23</sup>. The thermodynamics of chromium ions sorption from aqueous solution on biomass fungi have been investigated<sup>24</sup>.

The primary objective of this research was to study the biosorption of chromium(VI) from aqueous environments by *trametes versicolor polyporus* fungi under equilibrium and column flow conditions. The secondary objective was to investigate the effect of pH, contact time, concentration of Cr(VI) and amount of biomass on the extent of adsorption. The tertiary objective included the fitting of the experimental data to Freundlich and Langmuir adsorption isotherms and Lagergren first order, pseudo second order and Weber-Morris intraparticle diffusion kinetic models.

## Experimental

### *Materials*

All the chemicals used in the experimental work were of analytical grade. An initial stock solution of 1000 ppm of Cr(VI) was prepared by dissolving requisite amount of potassium dichromate (2.829 g) in 1000 mL of deionized distilled water. Standard solutions of Cr(VI) were obtained by diluting appropriate volumes of the stock solution. Sulfuric acid (1:1), potassium permanganate (0.1 N) and sodium azide (0.5%) were prepared using double distilled water. Chromium complexing reagent was prepared by dissolving 200 mg of diphenyl carbazide in 100 mL of methanol. *Trametes versicolor polyporus* fungi species was collected from Tirumala Hills, Tirupati, India. The fungi were washed with tap water and deionized water to remove dust and other impurities. The fungi was sundried and then dried in an oven at 70°C. After that the biosorbent was crushed and sieved through 300 µm sieve for uniform particle size and used as an adsorbent.

### *Method*

The sorption and reduction capacities of *trametes versicolor polyporus* fungi was studied by adding 0.1 g of adsorbent to 100 mL of Cr(VI) solutions of different concentrations in 125 mL Erlenmeyer flasks. The flasks, sealed with glass stoppers and para film, were placed in an oscillating shaker and shaken for 4 hours at 150 rpm. Initial adsorption experiments showed that this period of time was adequate to ensure equilibrium between chromium adsorbed and chromium unadsorbed. After the equilibrium period, samples were filtered through Whatmann No. 5 filter paper. The first portion of the filtrate was discarded to eliminate the effect of any adsorption of chromium on filter paper.

The filtrate was then divided into two parts and first part was used to determine equilibrium concentration of Cr(VI) and the second part was used to determine Cr(VI) reduced to Cr(III). The concentration of Cr(VI) was determined spectrophotometrically by using diphenyl carbazide as a complexing agent. 10 mL of solution from first part was pipette into 100 mL standard flasks and made upto the mark with 1:1 H<sub>2</sub>SO<sub>4</sub>, diphenyl carbazide and double distilled water. The concentration of Cr(VI) was determined spectrophotometrically at 540 nm.

From the second part, 10 mL of the solution was pipette into a conical flask followed by addition of 90 mL double distilled water. The contents were heated to boil and about 6 drops of 0.1 N potassium permanganate solution was added to oxidize Cr(III) to Cr(VI). To this about 4-5 drops of 0.5% sodium azide was added to remove excess potassium permanganate. The concentration of Cr(VI) was determined spectrophotometrically, which gave the total unadsorbed chromium. Chromium adsorbed was equal to the difference between the initial concentration of Cr(VI) and total unadsorbed chromium and chromium reduced was equal to the difference between the total chromium unadsorbed and Cr(VI) present in solution.

### *Batch adsorption studies*

In order to explore the effect of influencing factors, such as solution pH, contact time, quantity of adsorbent and the initial concentration of Cr(VI), a series of batch experiments were conducted with solutions of initial concentrations of 50, 100, 200, 300 ppm. The pH of the suspension at the start of the experiment was adjusted using 0.1 N HCl and 0.1 N NaOH. After agitating the samples for 4 hours, the samples were filtered through Whatmann No.5 filter papers to eliminate any fine particles. Then the concentrations of Cr(VI) and Cr(III) in the filtrate were determined as discussed earlier. The adsorption on the glassware was

found to be negligible and was determined by running blank experiments. The amount adsorbed per unit mass of adsorbent at equilibrium was obtained using the equation,

$$Q_e = \left( \frac{C_o - C_e}{m} \right) V \quad (1)$$

Where  $Q_e$  (mg/g) is the adsorption capacity at equilibrium,  $C_i$  and  $C_e$  denoted respectively the initial concentration of Cr(VI) and equilibrium concentration of unadsorbed Cr(VI) and Cr(VI) reduced to Cr(III).  $V$  is the volume of the solution in liters and  $m$  is the mass of the adsorbent in grams.

### *Column adsorption studies*

Dynamic column flow studies were carried out in a column made of Pyrex glass. The column was filled with *trametes versicolor polyporous* fungi by tapping so that the column is filled without gaps. The influent solution containing known concentration of Cr(VI) was allowed to pass through the bed at a constant flow rate of 1 mL min<sup>-1</sup>, in down flow manner. The complete cycles of operation of each column experiment included three steps: pH pre conditioning, Cr(VI) injection, and Cr(VI) adsorption until column exhaustion occurred. The effluent solution was collected at different time intervals and concentration of Cr(VI) and Cr(III) was determined. All experiments are carried out at room temperature.

### *Desorption studies*

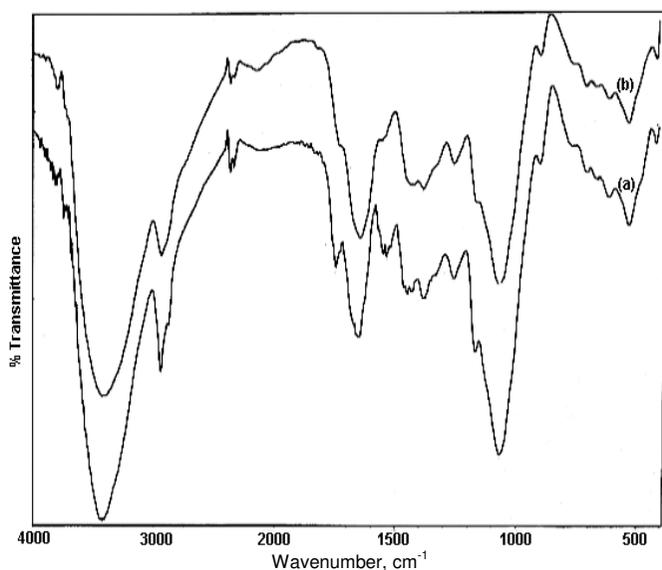
Desorption (recovery) studies are very important since the success of adsorption process depends on the regeneration of adsorbent. After the column was completely exhausted, the remaining aqueous solution in the column was drained off by pumping air. Desorption of solute from loaded adsorbent was carried out by solvent elution method, using 0.1 M NaOH solution as an eluent through the column maintained at constant temperature at a fixed flow rate (1 mL min<sup>-1</sup>). From the start of the experiment effluent samples at different time intervals (5, 10, 20, 30 min and so on) were collected at the bottom of the column for analysis. After the regeneration, the column was washed with distilled water to remove methanol from the column before the influent Cr(VI) solution was reintroduced for the subsequent adsorption-desorption cycles. Three adsorption-desorption cycles were performed using the same bed to check the sustainability of the bed for repeated use.

## **Results and Discussion**

### *Fourier transform infrared analysis*

Numerous chemical functional groups such as carboxyl, hydroxyl, amide, ether, *etc.* have been identified as potential adsorption sites to be responsible for binding metallic ions to fungi. Their potential for metal uptake depends on factors such as the abundance of sites, their accessibility, chemical state and affinity between adsorption site and metal<sup>25</sup>. FTIR spectrum of fungi (Figure 1 a) shows peaks at 3550-3400 cm<sup>-1</sup> indicating the presence of OH or -NH groups. The absorption peaks at 1654-1540 cm<sup>-1</sup> corresponded to the 1° amide and 2° amide bands, respectively<sup>26</sup>. The bands at 1740 corresponds to C=O of carboxyl group present in fungi. Similarly band between 1540 and 1500 cm<sup>-1</sup> are due to 2° amide stretching frequency. The above three bands indicate the presence of amide group with hydrogen bond linkage in the fungi. The region between 3200 and 3500 cm<sup>-1</sup> represented the overlapping peaks of stretching vibration of O-H and N-H<sup>27</sup>. The region between 3000 and 2800 cm<sup>-1</sup> exhibits the C-H stretching vibrations of -CH<sub>3</sub> and >CH<sub>2</sub> functional groups, and 1300 - 1470 cm<sup>-1</sup> is the deformation stretching of C-H, -CH<sub>3</sub> and >CH<sub>2</sub> functional groups<sup>26</sup>.

The finger print region band of  $1063\text{ cm}^{-1}$  is due to C-O bands of polysaccharide, found in natural form of fungi. The FTIR spectrum of fungi loaded with Cr(VI) is shown in Figure 1b. The shift in position and change in the intensity of the peaks could be observed in the spectrum after exposing to Cr(VI). The enhancement of the intensity in the region  $3200\text{--}3500\text{ cm}^{-1}$ , indicating an increase of the free hydroxyl group on the fungus. This may be due to hydrolyzing of some polysaccharides on the cell wall to shorter saccharides such as oligosaccharides, dises and monoses<sup>28</sup>. The disappearance of bands at  $1740$  and  $1529\text{ cm}^{-1}$  after exposing to Cr(VI) indicates deprotonation of amide group and also carboxyl group present in the adsorbent.



**Figure 1.** FTIR spectra of fungus (a) Before adsorption and (b) After adsorption of chromium

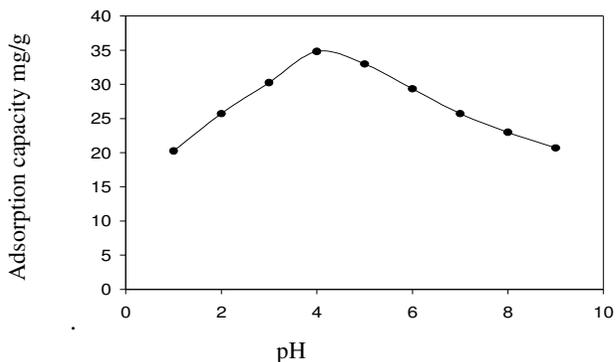
#### Effect of pH

The pH of the solution is an important parameter affecting biosorption of metal ions. The effect of initial pH on the adsorption and reduction of Cr(VI) using dried *trametes versicolor* polyporous fungi was studied. The results are presented in Figure 2. As evidenced from Figure, the adsorption of Cr(VI) increases with increase in pH from 1.0 to 4.0 and decreases from there on. The maximum removal was occurred at pH 4.0. This behavior can be explained by the nature and speciation of the adsorbate at different pH values, the ionic state of functional groups of sorbent. The reduction of Cr(VI) to Cr(III) increases with increase up to pH 5.0 and then decreases. At pH 5.0 about 10% of Cr(VI) is reduced to Cr(III). Depending on the pH of the solution Cr(VI) forms stable complexes such as  $\text{Cr}_2\text{O}_7^{2-}$ ,  $\text{Cr}_2\text{O}_4^{2-}$  and  $\text{HCr}_2\text{O}_4^-$  in solution<sup>29</sup>. The actual species of chromium present in solution depends on pH of the solution and the concentration of chromium. In a solution of acidic pH the predominant species present are  $\text{Cr}_2\text{O}_7^{2-}$  and  $\text{HCr}_2\text{O}_4^-$ . The electrostatic forces of attraction between negatively charged dichromate and adsorbent surface is the driving force for enhanced adsorption<sup>29</sup>.

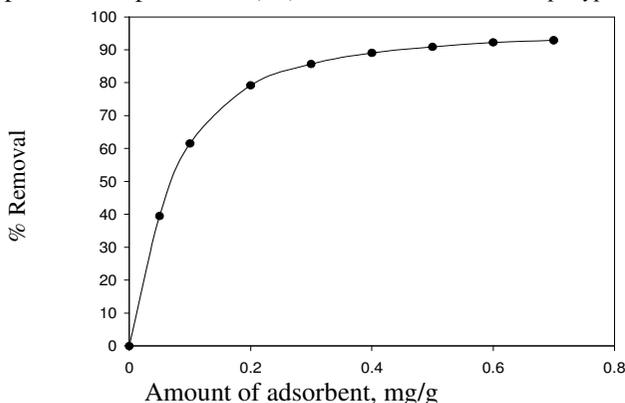
#### Effect of sorbent dose

Experiment were conducted with different weights of *trametes versicolor polyporous* fungi (0.05 – 0.7 g) keeping the concentration of Cr(VI), volume of adsorbate solution and

contact time. The results, presented in Figure 3, indicate that the percent removal Cr(VI) increases with increase in biomass content to some extent and there after remains constant. This may be attributed to the more number of adsorption sites available as a result of increase in amount of adsorbent.



**Figure 2.** Effect of pH on adsorption of Cr(VI) on *trametes versicolor polyporous* fungi



**Figure 3.** Effect of amount of *trametes versicolor polyporous* fungi on percent removal of Chromium

### Adsorption kinetics

#### First-order kinetics

The results of dependence of adsorption capacity of the fungi on time, determined at different initial concentrations, are graphically presented in Figure 4. Although a range of models are available to study kinetics of adsorption, Lagergren first order, pseudo second order and intraparticle diffusion models were used to study the kinetics of Cr(VI) adsorption on fungi. Lagergren first order model takes the form,

$$\log(Q_e - Q_t) = \frac{K_1}{2.303} t \tag{2}$$

Where  $Q_e$  and  $Q_t$  are amount of metal ion adsorbed per unit mass of a adsorbent at equilibrium and at time  $t$  (min).  $k_1$  is the first order rate constant of adsorption. The linear plots of time  $t$  versus  $\log(Q_e - Q_t)$  at different concentrations indicate the applicability of the equation. Values of  $k_1$  along with regression coefficients, obtained from a linear plot of  $\log(Q_e - Q_t)$  versus  $t$  (Figure 5), are presented in Table 1.

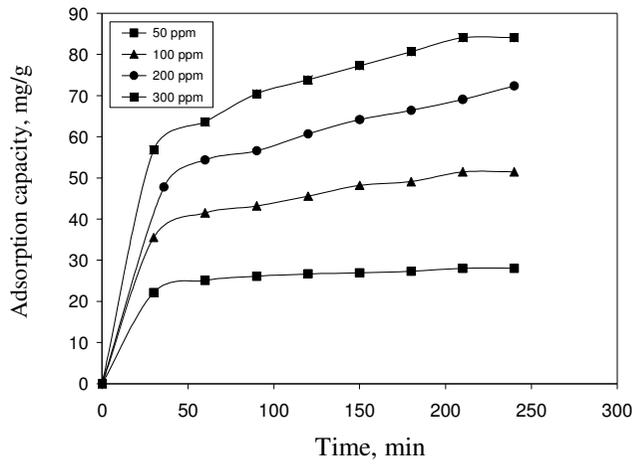


Figure 4. Effect of contact time on adsorption of Cr(VI) on *trametes versicolor polyporous* fungi.

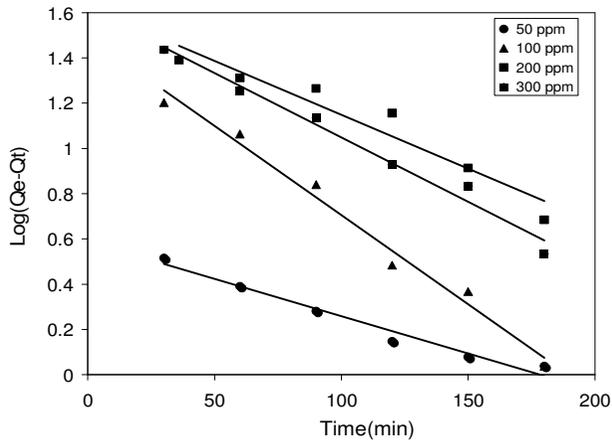


Figure 5. Pseudo first order plots for adsorption of Cr(VI) on *trametes versicolor polyporous* fungi

Table 1. Values of the parameters of kinetic models

Kinetic model	Parameter	Initial concentration of Cr(VI), mg/L			
		50	100	200	300
First order	$k_1$	0.0175	0.0182	0.0115	0.01312
	$R^2$	0.9716	0.9835	0.9259	0.9806
Second order	$k_2$	0.0041	0.0011	0.0006	0.0005
	$Q_e$	28.6	53.5	73.5	88.5
	$R^2$	1.000	0.9978	0.9967	0.9969
Weber-Morris	$k_{id}$	0.6554	1.6771	2.3156	3.0147
	$C$	19.08	27.26	35.45	40.64
	$R^2$	0.9254	0.9751	0.9916	0.9949

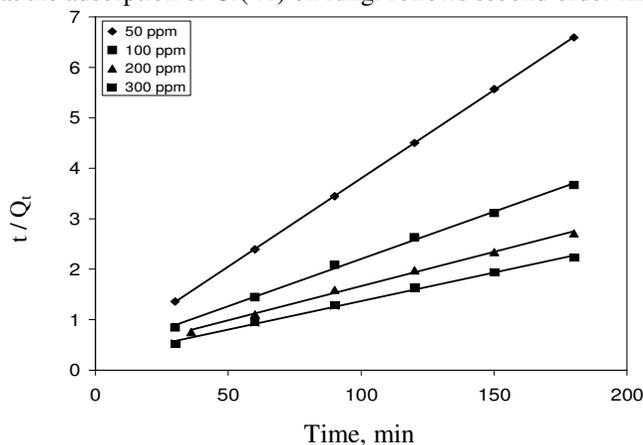
### Second order kinetics

Experimental data were also fitted to the pseudo-second-order kinetic model<sup>30</sup>,

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} + \frac{1}{Q_e} t \quad (3)$$

Where  $k_2$  (g/mg min) is the rate constant of the second-order equation,  $Q_t$  (mg/g) is the amount adsorbed at time  $t$  (min) and  $Q_e$  is the amount of adsorbed at equilibrium (mg/g).

This model is more likely to predict the kinetic behaviour of adsorption with chemical sorption being the rate-controlling step<sup>31</sup>. The values of rate constants ( $k_2$ ), calculated from the linear plots of  $t/Q_t$  versus  $t$  (Figure 6), along with correlation coefficient and  $Q_e$  values are also included in Table 1. Based on the correlation coefficient values, it may be concluded that the adsorption of Cr(VI) on fungi follows second order kinetic model.



**Figure 6.** Pseudo second order plots for adsorption of Cr(VI) on *trametes versicolor* polyporous fungi

### Weber and Morris intraparticle diffusion model

Weber and Morris<sup>32</sup> proposed intraparticle diffusion model, which can be written as,

$$Q_t = k_{id} t^{1/2} + C \quad (4)$$

Where  $Q_t$  (mg/L) is the amount adsorbed at time  $t$  (min),  $K_{id}$  ( $\text{mg g}^{-1} \text{min}^{-1/2}$ ) is the rate constant of intraparticle diffusion.  $C$  is the value of intercept which gives an idea about the boundary layer thickness, i.e. the larger intercept; the greater is the boundary layer effect. The plots of  $Q_t$  versus  $t^{1/2}$  obtained for the adsorption of chromium onto *trametes versicolor* polyporous fungi at different concentrations are shown in Figure 7. The intraparticle rate constant  $K_{id}$  ( $\text{mg g}^{-1} \text{min}^{-1/2}$ ) and intercept  $C$  ( $\text{mg g}^{-1}$ ) are given in Table 1. The linear plots of  $Q_e$  versus  $t^{1/2}$  with zero intercept indicates that intraparticle diffusion alone determines the overall rate of adsorption. In this case intraparticle diffusion is not the only determining mechanism, in addition other mechanisms such as electrostatic interaction, ion exchange may be operating to different extents.

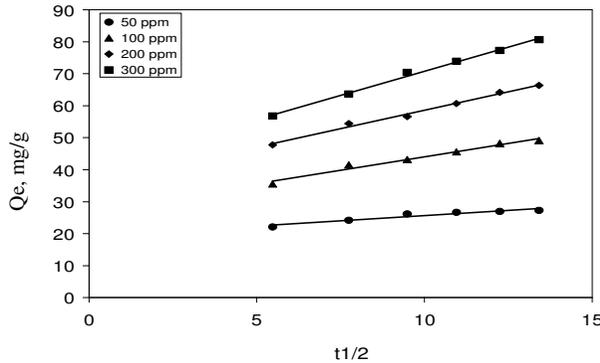
### Adsorption isotherms

The equilibrium adsorption isotherms are one of the most important tools to understand the mechanism of the sorption. Several isotherm equations are available and two isotherms, Langmuir and Freundlich, were used to evaluate the experimental data.

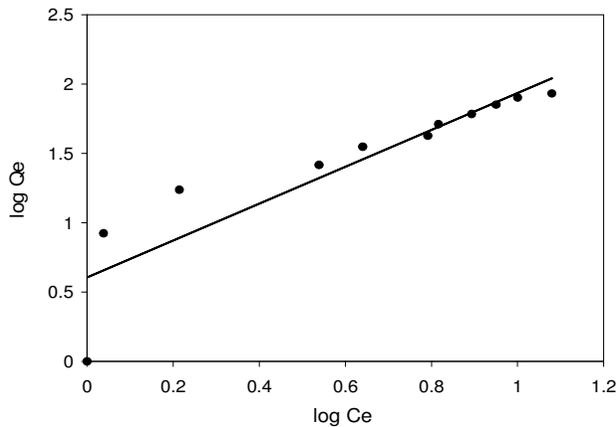
The Freundlich model takes the form,

$$Q_e = K_f C_e^{1/n} \tag{5}$$

where,  $K_f$  and  $n$  are Freundlich constants. Linear plots of  $\log C_e$  Vs  $\log Q_e$ , shown in Figure 8, were used to evaluate the values of  $K_f$  and  $n$ .



**Figure 7.** Weber-Morris model for adsorption of Cr(VI) on *trametes versicolor polyporous* fungi

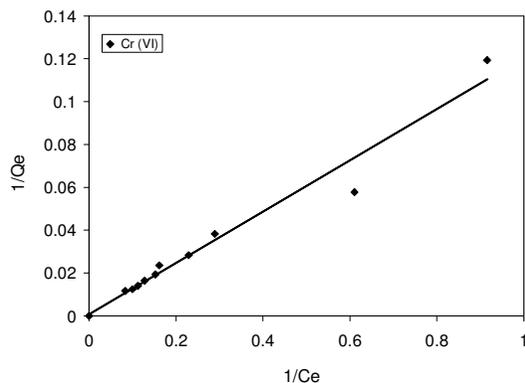


**Figure 8.** Freundlich isotherm for adsorption of Cr(VI) on *trametes versicolor polyporous* fungi

The Langmuir adsorption isotherm assumes that the adsorption can only occur at a fixed number of definite localized sites, each site can hold only one adsorbate molecule (monolayer), and the sites are homogeneous. The Langmuir equation is commonly written as,

$$Q_e = \frac{Q^o b C_e}{1 + b C_e} \tag{6}$$

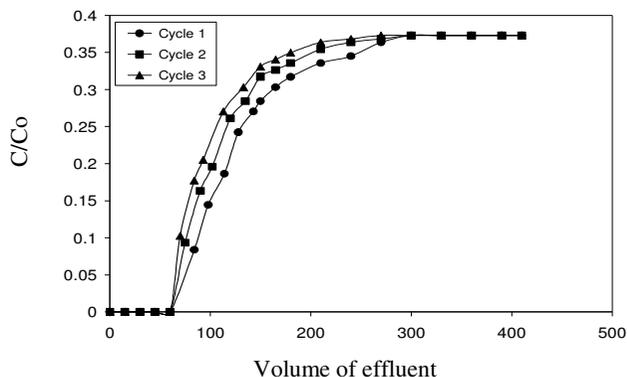
where  $Q^o$  is the maximum monolayer adsorption capacity per unit weight of fungi and  $b$  is related to affinity of the binding sites.  $Q^o$  and  $b$  are determined from the liner plots  $1/C_e$  Vs  $1/Q_e$  (Fig. 9). The Freundlich and Langmuir parameters are included in Table 2 along with regression coefficients. Among these models, Langmuir isotherm gives a better representation of adsorption of Cr(VI) on fungi.



**Figure 9.** Langmuir isotherm for adsorption of Cr(VI) on *trametes versicolor polyporous* fungi

#### Column adsorption studies

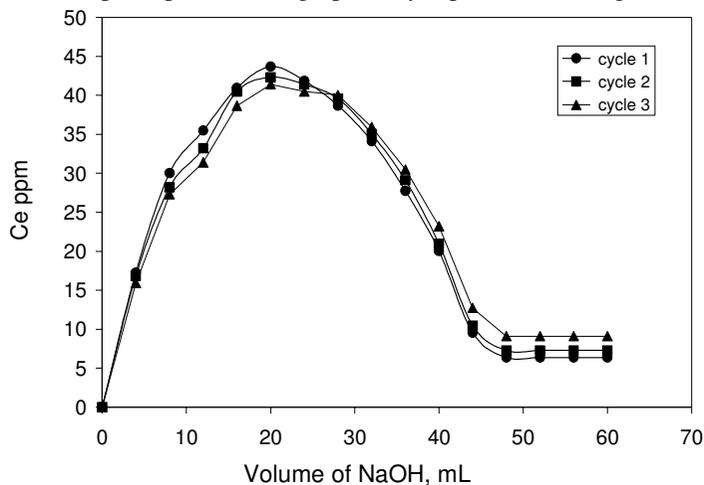
The results of dynamic flow experiments were used to obtain the breakthrough curves for chromium adsorption from aqueous solutions by plotting bed volume *versus* effluent concentration. The breakthrough capacity, which is the amount adsorbed until the effluent concentration of the adsorbate is equal to the influent solution concentration, were computed from the breakthrough curves shown in Figure 10. An examination of the curves indicates that no leakage of chromium was observed up to about 75 mL of the adsorbate solution.



**Figure 10.** Break through curves for adsorption of Cr(VI) on *trametes versicolor olyporous* fungi

To be useful in separation and removal processes, adsorbed species should be easily desorbed under mild conditions and adsorbents should be used many times in order to decrease material costs. When the bed gets exhausted or the effluent coming out of the column reaches the allowable maximum discharge level, the regeneration of adsorption bed to recover the adsorbed material and/or to regenerate the adsorbent becomes quite essential. The regeneration could be accomplished by a variety of techniques such as thermal desorption, steam washing, solvent extraction *etc.* Each method has inherent advantages and limitations. In this study several solvents were tried to regenerate the adsorption bed. 0.1 N NaOH solution was found to be effective in desorbing and recovering solvent solutions quantitatively from the adsorption bed. The fixed bed columns of *trametes versicolor polyporous* fungi saturated with chromium

was regenerated by passing 0.1 N NaOH solutions as an eluent at a fixed flow rate of  $1\text{ mL min}^{-1}$ . To evaluate the solvent recovery efficiency, the percentage of chromium recovered is calculated from the breakthrough and recovery curves. More specifically, maximum desorption occurred at 7 mL of 0.1 N NaOH solution and complete regeneration occurred at about 26 mL. These adsorption-desorption cycles were repeated thrice, which indicated that there is no apparent change in the adsorption capacity of the biosorbent. The desorption profiles are graphically represented in Figure 11.



**Figure 11.** Desorption curves of Cr(VI) from *trametes versicolor polyporus* fungi

## Conclusion

The results of the present study demonstrated that *trametes versicolor polyporus* fungi were excellent biosorbent for the removal of chromium ions. The maximum uptake of Cr(VI) ions occurred at pH 4. Increase in the amount of biosorbent increased the percent removal of the metal ions. Further, the biosorbent were characterized by FTIR spectral analyses. The data were fitted relatively well to Langmuir isotherm compared to Freundlich isotherm. The maximum monolayer adsorption capacity of *trametes versicolor polyporus* fungi was found to be 125.0 mg/g. The adsorption process followed second order kinetics. Further, results from the limited number of column adsorption-desorption cycles indicated that the adsorption capacity of the *trametes versicolor polyporus* fungi remains unchanged and the fungi could be used to remove chromium from wastewater in cycles.

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