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Biosorption of Cr (VI) By *Trichoderma harizianum* A Novel Biosorbent

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A B S T R A C T

Industrial effluents containing heavy metals may consider a major source of contamination causes serious environmental problems. Decontamination of heavy metals from wastewater has been a challenged for a long time. A number of methods have been developed for removal of toxic metal ions from wastewaters such as precipitation, evaporation, electroplating, ion exchange, membrane processes, etc. However, these conventional technologies are providing expensive due to non- regenerable materials used, high cost and generation of toxic sludge. Biosorption is a process which represents a biotechnological innovation as well as a cost-effective excellent tool for removing heavy metals from aqueous solutions. It represents a typical technique for using economical alternate biological materials for the purpose. Today, biosorption is one of the main components of environmental and bioresource technology. Application of microorganisms (specifically bacteria, algae, yeasts and fungi) as biosorbents for heavy metal removal have received growing interest due to high surface to volume ratio; large availability, rapid kinetics of adsorption and desorption and low cost. In this work, Dried biomass of *Trichoderma harizianum* powder was taken as a low cost biosorbent for the sorption of Chromium (VI). The various parameters like Initial metal ion concentration, Initial pH, Temperature and Biosorbent dosage were studied in a batch reactor. Equilibrium was reached after 24 h of contact time. The optimum values of initial Chromium concentration, initial pH, temperature and biomass loading are found to be 50mg/l, 2,30°C and 5g/l. Under this optimized condition a maximum percentage removal of 95% and specific uptake of 11mg/g was obtained for Cr (VI) sorption.

***Key words*:** Biosorption, *Trichoderma harizianum*, Initial concentration, Biomass load, Equilibrium model

Chromium as one of the major pollutants of the environment is available in nature as an odourless, steel grey hard metallic element. It is the seventh most abundant element on the earth and twenty first most abundant element in the rocks [1]. Elemental chromium is not usually found pure in nature and principally occurs as the mineral chromite $FeOCr_2$ O_3 or chrome iron stone in which form it is extremely stable. Chromium exists in nature as stable hexavalent and trivalent forms. The hexavalent form of chromium is more toxic than trivalent chromium and is often present in wastewater as chromate (CrO_4 2-) and dichromate (Cr_2 O_7 2-). This is of serious environmental concern as Cr(VI) persists indefinitely in the environment complicating its removal. The persistant nature makes it accumulate in the food chain which with time reach harmful levels in living beings resulting in serious health hazards such as irritation in lungs and stomach, cancer in digestive tract, low growth rates in plants and death of animals. Therefore, removal of Cr(VI) from wastewater prior to its discharge into natural water systems, adjoining landmasses and sewer systems. requires serious and immediate attention.

The conventional physico-chemical techniques used for the removal of Cr(VI) include chemical reduction followed by precipitation with caustic soda. This process requires a large excess of chemicals and produces voluminous sludges, disposal of which again create secondary pollution. Other available treatments include ion-exchange, electrolysis and reverse osmosis. which are not only expensive and high energy processes, but are also ineffective in removal of metal ions present at lower concentration in large volume of wastewaters [2].

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Environmentally friendly processes, therefore, need to be developed to clean-up the environment without creating harmful waste by-products. Biosorption involves application of microorganisms in removal of heavy metals and has been recognized as a potential alternative to the conventional methods for treatment of contaminated wastewaters [3-4]. The growing, resting and non-living cells of microorganisms are reported to remove Cr(VI) from aqueous solutions [5-7]. However, most of the works to remove Cr(VI) have been carried out using non-living fungal cells.

Many types of biomaterials, including marine algae, bacteria, fungi and yeast, can be used for the removal of heavy metals [8-9]. Fungi are chosen for biosorption because of their special physiology and adsorbing capacity. Chitin and chitosan present in fungi are well known metal ion adsorbers due to the presence of both carboxyl and amine groups [10]. The applicability of fungi as biosorbent has some advantages due to their small size, ubiquity, ability to grow under controlled conditions and resilience to a wide range of environmental situations. Both viable and non-viable biomasses are able to bind metal ions. By-products or waste materials from large-scale industrial processes can therefore serve as an economical and reliable source of biomass for biosorption processes.

Equilibrium modelling

Biosorption isotherms are used to describe equilibrium data and are important for developing equations that can be used to compare different biosorbents under different operational conditions. Sorption equilibrium provides fundamental physicochemical data for evaluating the applicability of sorption processes as a unit operation. Sorption equilibrium is usually described by an isotherm equation whose parameters express the surface properties and affinity of the sorbent, at a fixed temperature, pH and initial metal concentration. The simplest forms of these isotherms are Freundlich and Langmuir isotherms which in most cases are used to obtain maximum biosorption capacity of the bio-sorbent.

$$\%Removal = \frac{(C_o - C)X100}{C_o} \qquad \text{…….. (1)}$$

$$q = \frac{(C_o - C)V}{W} \qquad \text{…….. (2)}$$

Where C_o is the initial concentration of metal ions (mg/l), V is the volume of metal solution (l), W is the weight of biosorbent (g) and C is the final concentration of metal ions (mg/l).

The Langmuir adsorption isotherm

The equilibrium of the process is often described by fitting the experimental points with models usually used for the representation of adsorption isotherms. The Langmuir model suggests, as a hypothesis, that uptake occurs on a homogeneous surface by monolayer sorption without interaction between adsorbed molecules. The basic assumptions on which the model is based are: 1) metal ions are chemically adsorbed at a fixed number of well- defined sites, 2) each site can hold one sorbate ion, 3) all sites are energetically equivalent and 4) there is no interaction between ions adsorbed on neighboring sites. This model is described by the equation [11-12].

$$q_{eq} = \frac{Q^o bC_{eq}}{1 + bC_{eq}} \qquad \text{…….. (3)}$$

Where q_{eq} and C_{eq} are the amount of adsorbed metal per unit weight of biosorbent and unadsorbed metal concentration in solution at equilibrium, respectively. Q^o is the maximum amount of metal per unit weight of biomass to form a complete monolayer on the surface bound and b is a constant related to the affinity of the binding sites. Q^o and b can be determined from a plot of $1/q_{eq}$ and $1/C_{eq}$.

The Freundlich adsorption isotherm

The Freundlich model proposes a monolayer sorption with a heterogeneous energetic distribution of active sites, and with interactions between sorbed molecules, as described by the equation:

$$q_{eq} = K_F C_{eq}^{1/n} \qquad \text{…….. (4)}$$

Where K_F and n are the Freundlich constants characteristics of the system. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively. Eq. (4) can be linearized in logarithmic form and Freundlich constants can be determined. Both models are developed for a single-layer metal sorption [11-12].

MATERIALS AND METHODS

Microbial Biosorbent

Isolation of Trichoderma from different soil samples

The soil samples were collected in clean collection bottles from different spots of Annamalai Nagar, Tamil Nadu, India. The samples were transferred immediately to the laboratory for further analysis.

Trichoderma fungi were isolated from different soil samples by using PDA medium by a dilution plate method. The soil sample were serially diluted up to 10^{-3} to 10^{-4} and plated in Petri plates containing PDA medium. The suspensions were distributed uniformly over the medium by horizontal shaking and incubated for 25ºC for 7 days. The culture plates were examined daily and each colony that appeared was considered to be one colony forming unit (cfu). An individual colonies were isolated from the same plates and each uncommon colony was reisolated onto a fresh Potato Dextrose Agar (PDA) plate. Distinct morphological characteristics were observed for identification and the plates were stored at 4 °C. Two techniques, visual observation on Petri dishes and micro-morphological studies in slide culture, were adopted for identification of *Trichoderma* species. For visual observation, the isolates were grown on PDA agar for 3-5 days. The mode of mycelia growth, colour, odour and changes of medium colour for each isolate were examined every day. For micromorphological studies, a slide culture technique was used [13]. Examination of the shape, size, arrangement and development of conidiophores or phialides provided a tentative identification of *Trichoderma* sp. Samples were compared to a taxonomic key for the genus *Trichoderma* [14]. The culture is purified by either single spore isolation or single hyphal tip method and transferred into PDA slants and kept at 4ºC for further use. The Isolates are named as Isolate-1, Isolate-2, Isolate-3, Isolate-4 and Isolate-5. *In vitro* tolerance of *Trichoderma* sp. to different

concentrations of chromium was determined by the poisoned food technique [15]. Among the five isolates, Isolate-3 recorded highest mycelial growth so it was used for further study.

Preparation of the biosorbent

Trichoderma harizianum culture from agar slants was transferred to 50 ml of liquid medium containing potato extract, dextrose and yeast extract. The culture was grown initially in filamentous form with white fluffy mycelia and finally it is matured to dark colonies with black spores. At the deceleration phase of the growth, the contents of the vessels are harvested by filtering through nylon mesh. The recovered biomass was washed extensively with tap water. After washing, the biomass was dried at 60°C for 12-15h and powdered using mortar and pestle. The powdered biomass was sieved through a sieve with openings of 75 µm and the undersize particles were used for the study. The oversize particles are used as suspended dried cells for subsequent use.

Preparation of stock chromium solution

1000 mg/l of stock chromium solution was prepared by dissolving 2.83 g of Potassium dichromate in 1 litre of double distilled water.

Batch biosorption studies

Batch experiments were carried out in Erlenmeyer flasks by adding known amount of by dried biomass of *Trichoderma harzianum* in 100 ml aqueous Potassium dichromate solution. The flasks were agitated on a shaker with a constant shaking rate at 150 rpm for 135 min until equilibrium was attained. Samples were taken from the solution at regular time intervals for the residual metal ion concentration in the solution. The residual concentration of chromium ions in the solutions was determined spectrophotometrically at 540 nm using Diphenylcarbazide as the colour complexing agent.

The effect of initial Chromium ion concentration on percentage removal of Chromium was studied by conducting experiments with different initial Chromium ion concentrations namely 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l and 250 mg/l under identical conditions of temperature, pH and biomass loading and the experiment was carried out as described above.

The effect of initial pH on percentage removal of chromium was studied by conducting experiments with different initial pH namely 2,3,4,5 and 6 under identical conditions of initial Cr(VI) ion concentration, temperature and biomass loading and the experiment was carried out as described above.

The effect of temperature on percentage removal of copper was studied by conducting experiments with different temperature namely 25°C, 30°C, 35°C and 40°C under identical conditions of initial Cr (VI) ion concentration, initial pH and biomass loading and the experiment was carried out as described above.

The effect of biomass loading on percentage removal of chromium was studied by conducting experiments with different biomass load namely 1g/l, 2g/l, 3g/l, 4g/l and 5g/l under identical conditions of initial Cr (VI) ion concentration, initial pH and temperature and the experiment was carried out as described above.

RESULTS AND DISCUSSION

The *Trichoderma* sp were isolated from different soil samples by dilution plate method and the results are given the (Table 1). Five isolates of *Trichoderma* sp were isolated. The Isolates were named as Isolate-1, Isolate-2, Isolate-3, Isolate-4 and Isolate-5. The Isolate-1 and Isolate-4 were showed that initially the colony colour was observed to be whitish to light green, watery in centre. Later the colony gradually became deep grass green in colour and looked soft and leathery to the naked eye. The conidiophores were erect, smooth, penicillate branched, asymmetrical branches singly or vertically arranged at different levels, phialides were flask-shaped, coverage toward the main branch, emphasizing the penicillate branching. Phialospores were sub-globose to elliptical, smooth-walled identified as *Trichoderma virens*.

The Isolate-2, Isolate-3 and Isolate-5 were showed that at the early stage whitish to greenish mycelia appeared. Next a deep green colour developed in central part and gradually extended to the periphery. Finally, it appeared a whitish green colour. Mostly globose conidia developed on phialides produced in the opposite direction in each point identified as *Trichoderma harzianum*. The results of screening of chromium tolerant *Trichoderma sp* was given in the (Table 2). The highest mycelial growth was observed at 50ppm, while increasing the concentration of chromium the mycelial growth was decreased. The highest radial growth rate (66.0mm) was observed at 50ppm by Isolate-3, 38 followed by Isolate -1 recorded (65.3mm). The lowest mycelial growth rate (38.8mm) was observed at 200ppm by the Isolate-5 when compared to control. Among the five isolates, the *Trichoderma harzianum*-isolate 3 were showed highest mycelial growth so it was used further work. The effects of different concentrations of Ni on mycelia growth of the fourteen *Trichoderma* isolates were tested and the maximum radial growth was shown by UBT-18(70.8mm) at a Ni concentration of 40ppm, followed by isolate MT-4(66.0mm) these results are in lined with our study [16].

The biosorption of Cr(VI) using dried biomass of *Trichoderma harzianum* in a batch process depends on both contact time between the adsorbate and adsorbent particles and initial metal ion concentration. The effect of initial metal ion concentration on contact time, percentage removal and specific uptake of chromium was given in (Fig 1-2) respectively. (Fig 1) shows that equilibrium is attained in 24 h, also the sorption of chromium on biosorbent increases with increasing contact time. The chromium removal efficiency was affected by the initial metal ion concentration, with decreasing removal percentages as concentration increases from 50 mg/l to 250 mg/l. As the initial chromium concentration increases from 50 mg/l to 250 mg/l, the percentage removal of chromium decreases from 95% to 89% and the specific uptake of chromium increases from 11mg/g to 55mg/g respectively. The specific uptake increases with increase in initial metal ion concentration, at 250 mg/ litre the percentage removal of chromium is reduced to 89% suggesting the competition effects between the metal ions for the binding sites in biomass is very strong whereas at low concentration it is found to be less.

The effect of pH on percentage removal of chromium metal sorption varies with pH of the medium which is given in (Fig 3). The percentage removal of chromium decreases from 92% to 64% as the pH increased from 2.0 to 6.0**.** Biosorption of chromium was low at alkaline condition. The

maximum percentage removal is found to be 92% at pH 2.0 and selected as the optimum pH. At higher pH the surface of the biosorbent will have hydroxyl group which will not attract chromate ions that compete with the hydroxyl ions. At very low pH, the functional groups remain in protonated form and create less conductive binding charges condition for the biosorption due to the reduction in negatively charged surface. However, with the increase in pH, the negative charge density on the cell surface increases due to deprotonation of the metal-bindings sites, thus increasing the attraction of metallic ions with positive charge and allowing the biosorption on the cell surface [17].

Temperature is an important factor in all biosorption studies of heavy metals. Temperature affects the interaction between the biomass and the metal ions, usually by influencing the stability of the metal-sorbent complex, and the ionization of the cell moieties [18]. The effect of temperature on percentage removal of chromium was studied in Erlenmeyer flasks with 100 ml of aqueous chromium solution at different controlled temperatures namely 25ºC, 30ºC, 35ºC and 40ºC. The effect of temperature on percentage removal of chromium by dried biomass of *Trichoderma harzianum* was given in (Fig 4). A maximum chromium removal of 70% is obtained at 30°C because the number of binding sites is more at this temperature. The percentage removal of chromium by dried biomass of *Trichoderma harzianum* is higher at room temperature and it decreases with further increase in temperature due to the destruction of the cell walls expected, and a reduction in chromium removal is observed.

The effect of biomass loading on percentage removal of chromium was studied by conducting the experiments in Erlenmeyer flasks with 100 ml of aqueous chromium solution with different biomass loading namely 1g/l, 2g/l, 3g/l, 4g/l and 5g/l. The results of effect of biomass loading on contact time and percentage removal of chromium during the biosorption process are given in (Fig 5). It was observed that the percentage removal of chromium increased from 78 to 91% as the biomass loading increased from 1 g/l to 5 g/l. this is mainly due to an increasing the number of available active adsorption sites and surface area with increase in biomass loading and resulting in the increase of adsorbed metal ion concentration. A maximum chromium removal of 91% was observed at a biomass loading of 5 g/l.

Table 1 Isolation and characterization of *Trichoderma* sp

Isolate	Macro/Microscopic characteristics	Identified as
Isolate-1	Initially the colony colour was observed to be whitish to light green, watery in centre. Next the colony gradually became deep grass green in colour and looked soft and leathery to the naked eye. The conidiophores were erect, smooth, penicillately branched, asymmetrical branches singly or vertically arranged at different levels, philades were flask-shaped, coverage toward the main branch, emphasizing the peniclillate branching. Phialospores were sub-globose to elliptical, smooth-walled.	*Trichoderma virens*
Isolate-2	At the early stage whitish to greenish mycelia appeared. Next a deep green colour developed in central part and gradually extended to the periphery. Finally, it appeared a whitish green colour. Mostly globose conidia developed on phialides produced in the opposite direction in each point.	*Trichoderma harzianum*
Isolate-3	The colony was observed to be watery white at the early stage, bright green and dark green coloured mycelia mat exhibited at the central part of PDA plate at the later stage. Reproductive structures were very similar to Isolate-2.	*Trichoderma harzianum*
Isolate-4	Initially the colony colour was observed to be whitish to light green, watery in centre. Next it gradually became deep grass green in colour.	*Trichoderma virens*
Isolate-5	The colony colour was initially watery white and turned bright green to dark green and dull green with compact conidiophores throughout the petriplate. Reproductive structures were closely similar to Isolate-2.	*Trichoderma harzianum*

Table 2 Screening of chromium tolerant *Trichoderma sp*

Isolate	Growth rate (mm)				
	0ppm (control)	50ppm	100ppm	150ppm	200ppm
Isolate-1- Trichoderma virens	66.2	65.3	64.0	62.8	61.0
Isolate-2- *Trichoderma harzianum*	52.6	50.5	49.7	48.1	46.9
Isolate-3- *Trichoderma harzianum*	67.3	66.0	65.1	63.8	61.9
Isolate-4- *Trichoderma virens*	53.8	51.9	50.2	49.6	47.6
Isolate-5- *Trichoderma harzianum*	44.4	43.2	41.7	40.9	38.8

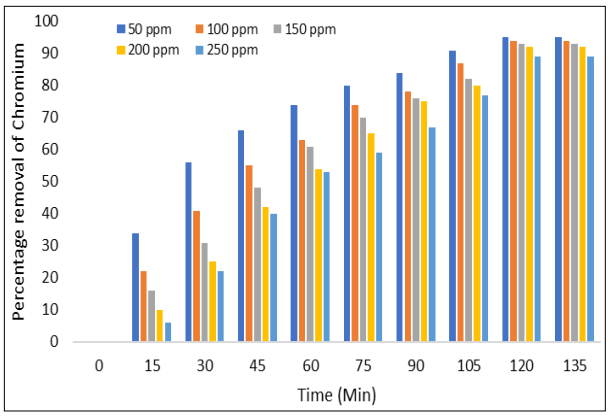

Fig 1 Effect of initial chromium concentration on percentage removal of chromium by dried biomass of *Trichoderma harzianum* powder

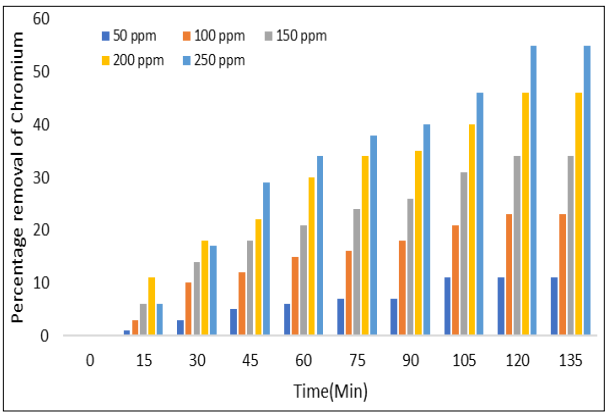

Fig 2 Effect of Initial metal ion concentration on specific uptake for the sorption of chromium by dried biomass of *Trichoderma harzianum* powder

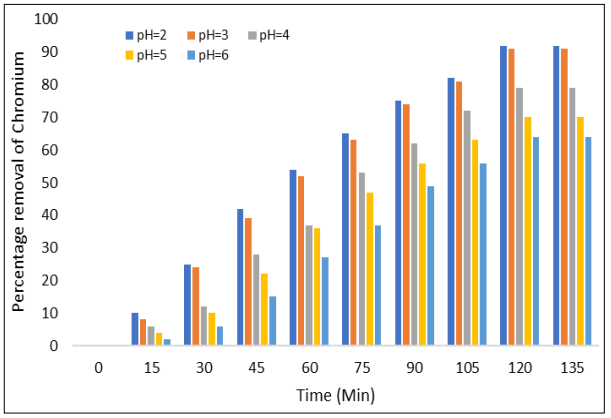

Fig 3 Effect of pH on percentage removal of chromium by dried biomass of *Trichoderma harzianum* (Isolate-3) powder

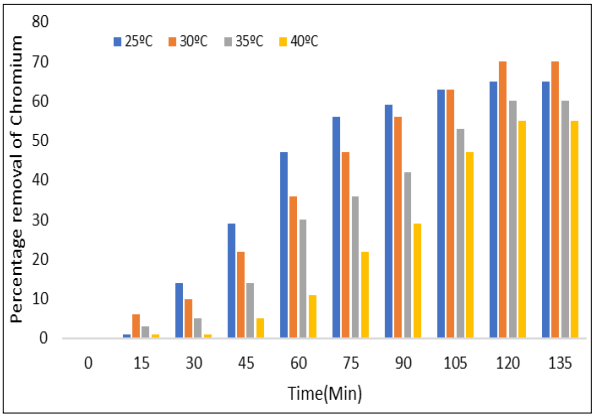

Fig 4 Effect of temperature on percentage removal of chromium by dried biomass of *Trichoderma harzianum* (Isolate-3) powder

The biosorption data is analyzed according to the linear form of the Langmuir adsorption isotherm. The linear adsorption isotherm constants (Q^o & b) with the correlation coefficients are presented in (Table 3). The plots of specific sorption ($1/q_{eq}$) against the equilibrium concentration ($1/C_{eq}$) for Chromium is shown in (Fig 6).

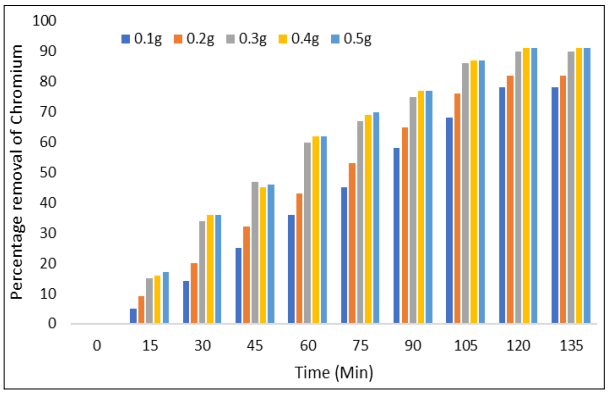

Fig 5 Effect of biomass loading on percentage removal of chromium by dried biomass of *Trichoderma harzianum* (Isolate-3) powder

(Fig 6) suggests that the linear equilibrium isotherm is a good model for the sorption of chromium. (Table 3) shows that the sorption constants, b, and sorption capacity, Q^o. The large value of b implies strong bonding of metals to the *Trichoderma harzianum* (Isolate-3) powder. (Table 2) also shows that a very high regression coefficient is found for chromium sorption. The higher correlation coefficients suggest that the Langmuir adsorption isotherm is found to be linear over the whole concentration ranges studied with homogeneous surface by monolayer sorption and provides a suitable model for the sorption of chromium.

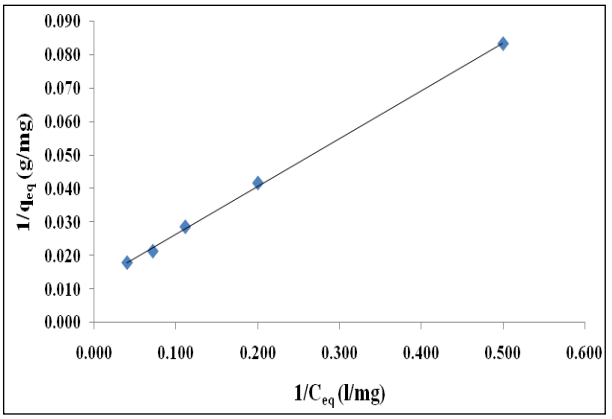

Fig 6 Langmuir adsorption isotherm for the biosorption of chromium by *Trichoderma harzianum* (Isolate-3) powder

The biosorption data is analyzed according to the linear form of the Freundlich adsorption isotherm. The linear Freundlich isotherm plots for the sorption of chromium on immobilized *Trichoderma harzianum* powder is presented in (Fig 7).

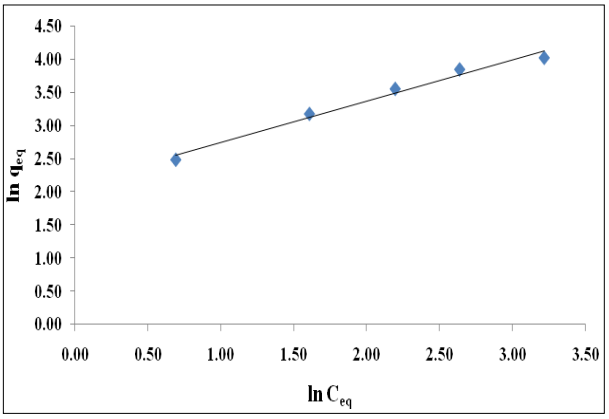

Fig 7 Freundlich adsorption isotherm for the biosorption of chromium by *Trichoderma harzianum* powder

Table 3 Langmuir constants

Q_o(mg/g)	b (l/mg)	R^2
83.15	0.072	0.9815
	Freundlich constants	
K_F	N	R^2
8.24	1.5	0.9786

The Freundlich adsorption isotherm constants (K_F & n) are given in (Table 2). From (Table 2), the Freundlich constants (K_F & n) shows monolayer uptakes of heterogeneous distribution of active sites of Cr(VI) with lower adsorptive capacity of *Trichoderma harzianum* (Isolate-3) powder. The adsorption intensity, n, is greater than unity for Cr(VI) and indicates that the forces between the surface layers are repulsive. The high R^2 values suggest that the Freundlich adsorption isotherm provides a good model of the sorption system with poor adsorption intensity for Chromium over the entire ranges of concentration.

CONCLUSION

Biosorption experiments were performed as a function of initial metal ion concentration, pH, temperature and biosorbent dosage. Biosorption was influenced by initial Chromium ion concentrations and it was found that as the initial Chromium concentration increases from 50 mg/l to 250 mg/l, the percentage removal of Chromium decreases from 95 % to 89% and the specific uptake of Chromium increases from 11 mg/g to 55 mg/g respectively. The effect of initial pH was also influence the sorption efficiency. The optimum conditions were found to be initial concentration 50mg/l, pH 2, temperature 30°C and biomass loading of 5g/l. The obtained results showed that dried biomass of *Trichoderma harzianum* powder was a good adsorbent for the removal of metal ions and had high adsorption yields for the treatment of aqueous solutions containing Chromium ions. The equilibrium data fitted very well to Langmuir and Freundlich adsorption isotherm model.

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