



Dead Fungal Biomass of *Rhizopus Arrhizus* for Decontamination of Hexavalent Chromium: Biosorption Kinetics, Equilibrium Modelling and Recovery

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ABSTRACT

The kinetics and equilibrium of biosorption of hexavalent chromium from aqueous solution was carried out using the dead physico-chemically treated biomass of *Rhizopus arrhizus* in a batch system. The biosorption characteristics of Cr(VI) ions were studied with respect to well-established parameters including pH, temperature, rotational speed, biosorbent dosage, initial metal ion concentration and contact time. The uptake of Cr(VI) decreased with an increase in pH and biomass concentration whereas it increased with an increase in the Cr(VI) concentration, temperature and rotational speed. Biosorption equilibrium was established in about 180 min. The adsorption data were analysed using the first and the second-order kinetic models as well as intra-particle rate expressions. The first-order equation was the most appropriate equation to predict the biosorption capacities of the fungal biosorbent. The sorption data obtained at pH 2.0 conformed well to both the Langmuir and Freundlich isotherm models. The reusability of the biosorbent was tested in five consecutive adsorption-desorption cycles and the regeneration efficiency was above 95%. From the practical viewpoint, the abundant and inexpensive dead fungal biomass of *Rhizopus arrhizus* could be used as an effective, low cost and environmental friendly biosorbent for the detoxification of Cr(VI).

INTRODUCTION

Presence of toxic levels of heavy metals in wastewaters from various industries has become a major cause of environmental concern due to their toxicity, serious health impacts associated with them and biomagnification in the food chain (Anjana et al. 2007). Chromium is introduced into natural waters from a variety of industrial wastewaters including those from the dyes, leather tanning, mining, electroplating, aircraft, textile, film and photography, petroleum refining, galvanometry, etc. (Sahin & Ozturk 2005). Chromium exists in nine valence states ranging from -2 to +6. However, only Cr(VI) and Cr(III) are ecologically important because of their stable oxidation forms. Both valences of chromium are potentially harmful but Cr(VI) is 100 times more toxic and 1000 times more mutagenic than Cr(III). The United States Environmental Protection Agency lists Cr(VI) as a priority pollutant. The EU Directive, WHO and US EPA have set the maximum contaminant concentration level for Cr(VI) in domestic water supplies as 0.05 mg/L (Directive 98/83/EC, Drinking Water Quality Intended for Human Consumption). Removal of Cr(VI) from waters and wastewaters is thus obligatory in order to avoid water pollution. Conventional methods for removing Cr(VI) ions from wastewaters include; chemical reduction and precipitation, evaporation, coagulation, electrochemical treatment, ion exchange, membrane processing and adsorption. Nevertheless, these

methods have several disadvantages, such as high installation and operating costs, requirement of preliminary treatment steps, difficulty of treating the subsequently generated solid waste, low efficiency at low metal concentration (less than 100 mg/L) and unpredictable metal ion removal (Zahoor & Rehman 2009).

With the increase in environmental awareness and governmental policies and the penalties imposed for the discharge of untreated wastewater causing large financial pressures on industrialists, there has been an emphasis on the development of new environmental friendly ways to decontaminate waters using low-cost methods and materials (Oliveira et al. 2005). In this endeavour, biosorption has emerged as a complementary, economic and eco-friendly device for controlling the mobility and bioavailability of metal ions in wastewater treatment processes because of its economy, analogous operation to conventional ion exchange technology, efficiency, reusability of the biomaterial, improved selectivity for specific metals, short operation time and no production of toxic secondary compounds. The use of living and non-living microorganisms such as fungi, yeast, bacteria and algae in the removal and possible recovery of toxic or precious metals from industrial wastes, has gained important credibility during recent years (Anjana et al. 2007, Aksu & Donmez 2006). The use of non-living microbial cells in industrial applications may offer some advantages over

living cells, such as lower sensitivity to toxic metal ions and adverse operating conditions, absence of requirements for growth media, easy desorption and recovery, and ease of mathematical modelling (Bayramoglu et al. 2005). Geometrically and chemically heterogeneous surface of dead microbial biomass in a rapid, non-metabolically mediated process may passively sequester metal(s) by the process of biosorption from dilute solutions (Loukidou et al. 2003). Recent biosorption experiments have focused attention on by-products or the wastes from large-scale industrial operations. The food and industrial fermentation processes use filamentous fungi to produce metabolites such as enzymes, flavourings or antibiotics. This provides a cheap and constant supply of thousands of tons of residual biomasses each year containing poorly biodegradable biopolymers (cellulose, chitin, glucans, etc.) and therefore, makes bad fertilizers for agricultural use. To date, incineration is the main way of destroying this by-product. Fungal biomass thus is an excellent candidate for detoxification of wastewater containing metals in dilute concentrations (Fourest & Roux 1992). This potential biosorbent can usually be obtained relatively free of charge in rather substantial quantities, from the respective producers since they already present disposal problems to them. The only costs incurred should be those of drying, if required and transport (Sag, 2001, Wang & Chen 2009).

The purpose of this study was to investigate the sorption of Cr(VI) and to study the kinetics of biosorption by physico-chemically treated dead fungal biomass of *Rhizopus arrhizus*. *Rhizopus arrhizus* is used in production of lipase, cellulolytic and pectolytic enzymes, lactic acid, fumaric acid, malic acid, etc. by Indian biotechnology industry. Thus, cheap and abundant availability of the biosorbent from such industries would make the process of biosorption economically viable. Experiments were done in a batch system and the sorption of Cr(VI) was investigated with respect to initial pH, temperature, rotational speed, amount of biomass, initial Cr(VI) ion concentration and process kinetics. The adsorption equilibrium was modelled using the Langmuir and Freundlich isotherm models. The selection of Cr(VI) in order to examine its removal by biosorption is due to the fact that it is a toxic metal requiring immediate priority for the application of novel treatment methods. The results of the adsorption equilibrium using the Langmuir and Freundlich isotherm models will contribute to better understanding of the designing of the sorption system and for selecting optimum operating conditions for full-scale batch process.

MATERIALS AND METHODS

All chemicals (AR grade) were procured from Hi Media Laboratories, Mumbai.

Metal solution preparation: A stock solution (1000 mg/L) of Cr(VI) used in this study was prepared by dissolving 2.828 g of $K_2Cr_2O_7$ in 100 mL deionized distilled water. Stock solution was then appropriately diluted to get the test solutions of 50 mg/L. The desired pH was maintained by the addition of 1 M HCl or NaOH at the beginning of the experiment without further control and was measured at the end of each experiment. The change in the working volume due to the addition of HCl or NaOH was negligible.

Preparation of the fungal biosorbent: A pure strain of *Rhizopus arrhizus* (NCIM 997) obtained from National Chemical Laboratory, Pune, was grown and maintained on Potato Dextrose Broth (g/L: Potato infusion from 200 g potatoes, Dextrose 20g, pH 5.0) containing 0.25% Tween 80 (to prevent sporulation) and Potato Dextrose Agar respectively. The cultures were grown aseptically at $30 \pm 1^\circ C$ under static conditions with intermittent shaking. The biomass harvested after 7 days was washed thoroughly with generous amounts of distilled water and dried at $80^\circ C$ in an oven for 24h, hereafter referred to as the native biomass. The protonated biomass was obtained by contacting 5g of native biomass with 0.5 M HNO_3 solution (500 mL), agitated on a rotary shaker at 180 rpm for 24h. This chemically treated biomass was further subjected to physical treatment of autoclaving at 10 lbs for 30 min. The biomass after each treatment was washed several times with deionized water; vacuum filtered using Whatman No.1 filter paper, followed by drying at $60^\circ C$ for 24h in a hot air oven. Care was taken to keep the particle size of the native and pretreated biomass uniform, by grinding into powder and sieving through a 150-mesh sieve for use in biosorption studies.

Biosorption experiments: The biosorption experiments were carried out in 250 mL Erlenmeyer flasks on an orbital shaker at 120 rpm at $30^\circ C$ after 1h of contact time. The optimum pH for the biosorption of Cr(VI) was investigated by equilibrating the native biomass (0.05 g) and 50 mg/L Cr(VI) solutions (100 mL) in the pH range 1.0-8.0. To elucidate other optimum conditions i.e., temperature, rotational speed, concentration of biomass, initial Cr(VI) concentration and contact time, the rest of the batch experiments were carried out using the physico-chemically treated biosorbent at the optimum pH obtained. The effect of temperature was studied by contacting the biosorbent with Cr(VI) solution (50 mg/L) in an environmental incubator shaker at $10-60^\circ C$ with an interval of $10^\circ C$. The effect of turbulence was evaluated by varying the rotational speed from 60-210 rpm with an interval of 30 rpm. The effect of biosorbent concentration on biosorption of Cr(VI) was investigated by employing biomass concentrations of 0.5, 1.00, 1.50, 2.00, 2.50 and 3.00 g/L under optimum conditions. The effect of the initial Cr(VI) concentration on the biosorption was studied under

optimum conditions as determined above, except that the concentration of Cr(VI) was varied between 50 and 500 mg/L.

The adsorption yield is defined as the ratio of sorbed Cr(VI) ions concentration at equilibrium to the initial concentration of Cr(VI) ions and is calculated from the equation:

$$\% \text{ Adsorption} = \frac{(C_i - C_f) \times 100}{C_i} \quad \dots(1)$$

The sorption capacity of Cr(VI) ions i.e., the concentration of the Cr(VI) on the fungal biomass at the corresponding equilibrium conditions was determined using a mass balance equation expressed as in eq. (2):

$$q = \frac{V(C_i - C_f)}{m} \quad \dots(2)$$

Where, q is Cr(VI) uptake (mg/g cell dry weight), V is the volume of metal-bearing solution contacted (batch) with the biosorbent (L), C_i is the initial concentration of Cr(VI) in the solution (mg/L), C_f is the final concentration of the Cr(VI) in the solution (mg/L) and m is the dry weight of the biosorbent added (g). Blanks without biosorbent were run simultaneously as control. Cr(VI) adsorption losses to the flask wall and the filter paper were negligible.

Kinetics of Cr(VI) sorption: The kinetic modelling of Cr(VI) biosorption process was studied using time dependent removal of Cr(VI) under optimized conditions over a period of 300 min using first and second-order kinetic equation models and intra-particle diffusion. Samples were taken at definite intervals of 15 min for determination of the residual Cr(VI) ion concentrations in the solution after filtering the samples using Whatman No. 1 filter paper. Blanks without biosorbent were run simultaneously as control. The total volume of withdrawn samples never exceeded 2% of the working volume. Cr(VI) adsorption losses to the flask wall and the filter paper were negligible. The first-order rate equation of Lagergren, one of the most widely used equations for the sorption of solute from a liquid solution is expressed as follows:

$$\log \frac{q_{eq}}{q_{eq} - q_t} = \frac{k_1 t}{2.303} \quad \dots(3)$$

where, k_1 is the rate constant of first-order biosorption (1/min) and q_{eq} and q_t denote the amounts of Cr(VI) sorbed per unit weight of sorbent at equilibrium and at time t , respectively (mg/g dry biomass). Ritchie's (1977) second order rate equation is expressed as:

$$1/q_t = 1/k_2 q_{eq} t + 1/q_{eq} \quad \dots(4)$$

Where, k_2 (g/mmol/min) is the rate constant of the second order adsorption.

The most-widely applied intra-particle diffusion equation for sorption system is:

$$q_t = k_i t^{0.5} \quad \dots(5)$$

Where, q_t (mg/g) is the amount of metal adsorbed at time t , k_i the intra-particle rate constant (mg/g min^{1/2}).

The biosorption experiments were producible within at most 5% error. Mean values from three independent experiments are presented and standard deviation and error bars are indicated wherever necessary.

Adsorption isotherms: All the data were analysed using Langmuir and Freundlich equilibrium isotherms to determine the feasibility of adsorption treatment. The Langmuir adsorption model is based on the sorption on a homogeneous surface by monolayer sorption without interaction between sorbed species. It assumes (i) reversible adsorption; (ii) no change in the properties of the adsorbed molecules; (iii) adsorption of molecules at affixed number of well-defined sites, each of which can hold one molecule and (iv) energetically equivalent sites distant to each other so that there are no lateral interactions between molecules adsorbed to the adjacent sites (Langmuir 1916, Sahin & Ozturk, 2005). The Freundlich isotherm is a special case applied to non-ideal sorption on heterogeneous surfaces and also to multilayer sorption, suggesting that binding sites are not equivalent and/or independent (Freundlich 1907, Aksu & Donmez 2006).

Desorption of Cr(VI) from the biosorbent: The Cr(VI) loaded biomass of *Rhizopus arrhizus* after 60 min of contact with 0.05 g/L of adsorbate under optimized conditions was filtered using Whatman No.1 filter paper and washed several times with deionized water, followed by drying to a constant weight at 60°C for 24 h in a hot air oven. Desorption of Cr(VI) was primarily optimized over a range of solid/liquid ratio (S/L) of 2, 5, 8, 10, 15, 20, 25, 30, 40 and 50 by using 0.05 g/L of this biomass and 0.1M NaOH as eluant at 150 rpm. At the end of the 60 min contact time of the eluant with Cr(VI) loaded biomass, the biomass was separated by filtration and the concentration of Cr(VI) released into the filtrate was determined. The reusability of *Rhizopus arrhizus* biosorbent was then determined using five consecutive biosorption-desorption cycles at optimum S/L using the same biomass. The eluted biosorbent was washed repeatedly with deionized water to remove any residual desorbing solution and placed into metal solution for the succeeding biosorption cycle after drying to a constant weight. Desorption efficiency was calculated by using following equation:

$$\text{Desorption efficiency} = \frac{\text{Amount of Cr(VI) desorbed}}{\text{Amount of Cr(VI) adsorbed}} \times 100 \dots(6)$$

Estimation of Cr(VI) concentration: The residual Cr(VI) concentration in the aqueous samples was determined using 1, 5-diphenylcarbazide. The pink coloured complex formed by the reaction of with Cr(VI) in acidic solution was spectrophotometrically analysed at 540 nm (Shimadzu UV1800 UV/VIS) (Greenberg 1985).

RESULTS AND DISCUSSION

Effect of initial pH on biosorption: Earlier studies on heavy metal biosorption have shown that solution pH is the single most important parameter affecting the biosorption process (Vinodhini & Das 2010). In order to establish the effect of pH on the biosorption of chromium (VI) ions, batch sorption studies were carried out at pH range 1.0-8.0. The variation of equilibrium Cr(VI) sorption by the native biomass of *Rhizopus arrhizus* at 50 mg/L initial Cr(VI) ion concentration was strongly affected by pH and the highest values (20.56 mg Cr(VI) /g of biomass) were found at pH 2.0 (Fig. 1). The biosorption of Cr(VI) decreased significantly with further increase in pH showing barely 0.66 mg/g sorption at pH 8.0. It is well known that both the cell surface metal binding sites and the availability of metal in solution are affected by pH. The *Rhizopus* biomass contains abundant chitin-chitosan units, which serve as a matrix of -COOH and -NH₂ groups, that take part in binding of metal ions (Tsezos & Volesky 1982). Decrease in the pH of the solution causes the formation of more polymerized chromium oxide species such as HCrO_4^- , $\text{Cr}_2\text{O}_7^{2-}$, $\text{Cr}_4\text{O}_{13}^{2-}$ and $\text{Cr}_3\text{O}_{10}^{2-}$, which interact more strongly with the positively charged groups like the amines of the chitin in the cell wall resulting in high Cr(VI) uptake. Reduction in the biosorption of Cr(VI) at pH value lower than 2.0 is probably due to the change in the surface characteristics including availability of surface area of *Rhizopus arrhizus* due to hydrolytic activity of the acid (Tewari et al. 2005, Vinodhini & Das 2010, Shroff & Vaidya 2012). Control of pH at an optimal value is critical to attaining maximum performance. Unchanged final pH of the solution at the end of each experiment indicated that the adjustment of pH during adsorption is unnecessary, thereby reducing the overall cost of the treatment. Thus, in the present study, Cr(VI) removal from aqueous solution by *Rhizopus arrhizus* biomass seems to follow anionic adsorption mechanism similar to results obtained by Kavita et al. (2011).

Effect of temperature on biosorption: The effect of temperature on Cr(VI) sorption by the biomass of *Rhizopus arrhizus* was studied over a range of 10-60°C with an interval of 10°C. The biosorption of Cr(VI) appeared to be

temperature dependent though its effect was less significant than pH of the solution. In this work the maximum initial removal of Cr(VI) was found to be 34.79 mg Cr(VI)/g of biomass at 50°C (Fig. 2). Adsorption is mostly an exothermic process, although few examples of endothermic adsorption have also been reported (Bai & Abraham 2001). In cases where the interaction between the metal ions and microbial cell wall is exothermic, binding is promoted at lower temperature while, for endothermic reaction, higher temperature enhances the binding. Compared to the ambient temperature (30°C), effective removal of Cr(VI) ions took place at higher temperatures of 40°C (32.24 mg/g) and 50°C (34.79 mg/g), indicating that the adsorption of Cr(VI) ions is of endothermic nature. Similar observations were also reported by the other researchers (Bai & Abraham 2002, Ajay Kumar et al. 2009). The favourable effect of higher temperature on Cr(VI) biosorption can also be attributed to higher affinity of sites for Cr(VI), an increase in binding sites on biosorbent surfaces as a result of re-orientation of cell wall components of the fungal biomass, rise in kinetic energy of the sorbent particles, increased collision frequency and ionization of chemical moieties on the cell wall (Bayramoglu et al. 2005). However, adsorption was found to decrease at 60°C (23.27 mg/g) possibly due to the damage of active binding sites in the biomass.

Effect of rotational speed on biosorption: The effect of the rotational speed (60-210 rpm) of the sorbent/sorbate system on Cr(VI) adsorption is shown in Fig. 3. Rotational speed increased the removal efficiency until it reached the maximum at 150 rpm (39.38 mg/g) followed by a decrease in the sorption capacity at higher speeds of agitation. This is because agitation facilitates proper contact between the metal ions in solution and the biomass binding sites thereby promoting effective transfer of sorbate ions to the sorbent sites (Bai & Abraham 2001, Ahalya et al. 2005). These results indicated that the contact between solid and liquid is more effective at moderate agitation (150 rpm). This observation agrees with the previously reported biosorptive removal of Cr(VI) (Sepehr et al. 2005). At higher rotational speed the decrease in efficiency may be due to improper contact between the metal ions and the binding sites (Ajay Kumar et al. 2009).

Effect of the concentration of the biomass on biosorption: The influence of sorbent/solute ratio expressed by biomass dosage ranging from 0.5 to 3.0 g/L at a fixed Cr(VI) concentration of 50 mg/L on percentage sorption and uptake is depicted in Fig. 4. The removal of Cr(VI) ions was dependent on the concentration of biosorbent preparation; more the biomass used, higher the removal efficiency obtained. At a lower dose (0.50 g/L) the removal of Cr(VI) was 35.91% while at a higher dose (3 g/L), the removal of Cr(VI) by

Rhizopus arrhizus increased up to 79.22%. This appears due to an increase in the availability of binding sites and increased adsorbent surface area for complexation of Cr(VI) ions resulting in higher removal of Cr(VI) ions at higher concentration levels. At high sorbent doses (2.5 and 3.0 g/L) a significant improvement in adsorption was not seen probably due to the binding of almost all Cr(VI) ions to the sorbent and the establishment of equilibrium between the ions bound to the sorbent and those remaining unadsorbed in the solution (Bai & Abraham 2001). Biomass concentration in solution seems to influence the specific uptake capacity, which is a measure of the amount of Cr(VI) bound by unit weight of the sorbent (Fourest & Roux 1992). The specific uptake capacity decreased from 37.09 mg/g to 13.63 mg/g with increase in the biomass concentration. Various reasons have been suggested to explain the reduced uptake capacity at increasing biomass concentrations. These include the competition of the solute ions for limited available sites, electrostatic interactions, overlapping or aggregation of adsorption sites resulting in a decrease in the total adsorbent surface area, interference between binding sites and reduced mixing at higher biomass densities (Tewari et al. 2005). Similar trend was also reported by other authors for the sorption of Cr(VI) and other metal ions on *Chlorella vulgaris*, *Senedesmus obliquus*, *Synectiocystis* sp., *Rhizopus nigricans* and *Mucor hiemalis* (Bai & Abraham 2001, Tewari et al. 2005, Shroff & Vaidya 2011).

Effect of the concentration of Cr(VI) ions on biosorption:

The concentration of both the sorbent and the metal ions play a significant role in determining the feasibility and efficiency of a biosorption process. It determines the sorbent/sorbate equilibrium of the system (Aksu & Akpinar 2000). As seen in Fig. 5, the percentage removal of Cr(VI) ions by the biomass decreased whereas the specific uptake capacity displayed an opposite trend with an increment in the initial metal ion concentration from 50 to 500 mg/L. At lower concentrations (50 mg/L), the metal ions present in solution interacted with the binding sites and thus facilitated 35.69% adsorption which further reduced to 8.77% at 500 mg/L of Cr(VI). At initial Cr(VI) concentrations, adsorption sites on the biosorbent remain unsaturated during the adsorption reaction, while at higher concentrations of Cr(VI), the number of ions competing for the available binding sites on the biomass increase and hence, there is lack of binding sites for complexation of Cr(VI) ions. Aggregation/agglomeration of adsorbent particles at higher concentrations leads to a decrease in the total surface area of the adsorbent particles available for adsorption and an increase in the diffusional path length (Ajay Kumar et al. 2009). With the increase in initial concentration of Cr(VI), the uptake capacity increased from 36.8 to 87.0 mg/g. The increase in uptake capacity of the

sorbent with the increase of Cr(VI) ion concentration is due to higher availability of Cr(VI) ions for sorption. Higher initial adsorbate concentration provides a higher driving force to overcome all mass transfer resistances of the metal ions from the aqueous to the solid phase resulting in higher probability of collision between Cr(VI) ions and the active sites resulting in higher uptake of Cr(VI) for the given amount of treated biomass (Tewari et al. 2005). This trend is in agreement with the earlier work on sorption of Cr(VI) and other metals (Bai & Abraham 2001, Shroff & Vaidya 2011).

Kinetic modelling of the biosorption process: The rate of biosorption is important for designing batch biosorption experiments. Therefore, the effect of contact time on the biosorption of Cr(VI) was investigated. The time course profile of Cr(VI) sorption indicated that the contact time had a significant impact on the sorption equilibrium. The uptake increased rapidly in first 60 min, after which there was a gradual increase. Nearly 35.42 % of sorption occurred at the end of 60 min reaching a plateau value after about 180 min. As seen in Fig. 6, it can be observed that the maximum adsorbed amount of the metal ions was achieved within 210-225 min (49.51 mg/g for Cr(VI)). This behaviour verified the fact that sorption occurred in two stages: the first was rapid surface binding and the second was slow intracellular diffusion (Kahraman et al. 2005). The first-order and second-order equations were employed to model the sorption data over a period of 300 min as shown in Fig. 7 (a) and 7 (b) respectively. A plot of $\log(q_{eq} - q_t)$ against t should give a straight line to confirm the applicability of the first order kinetic model (Bayramoglu et al. 2005), while a plot of $1/q_t$ versus $1/t$ should give a linear relationship for the applicability of the second-order kinetics. The rate constant k_2 and adsorption at equilibrium q_{eq} can be obtained from the intercept and slope, respectively (Bayramoglu et al. 2005). Fig. 7 (b) shows the plot of $1/q_t$ versus $1/t$ and the relationship was linear over the entire time range. The comparison of experimental biosorption capacities and the theoretical values estimated from the first and second-order rate equations indicated that the q_{eq} value of the first order equation matched closely with the experimental value than the q_{eq} value of second order kinetics (Table 1). Therefore, the first-order kinetic model best described the experimental data.

If the movement of the metal ion from the bulk liquid film surrounding the particle is ignored, the adsorption process can be divided into boundary layer diffusion, sorption of ions onto sites and intra-particle diffusion. A functional relationship common to most treatments of intra-particle diffusion is that uptake varies almost proportionately with the half-power of time $t^{0.5}$, rather than t . According to Weber & Morris (1963), if the rate limiting step is intra-particle diffusion, a plot of solute sorbed against the square root of the

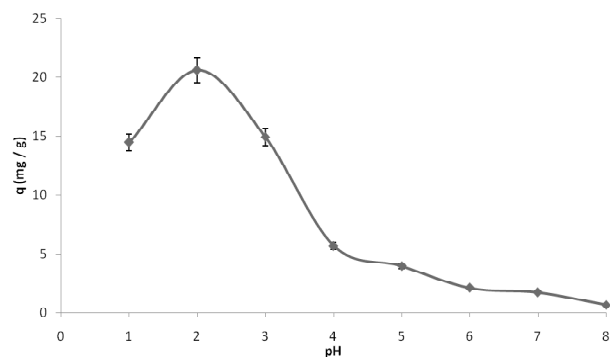


Fig. 1: Effect of pH on sorption of Cr(VI) onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; temperature = 30°C; rotational speed = 120 rpm; contact time = 60 min).

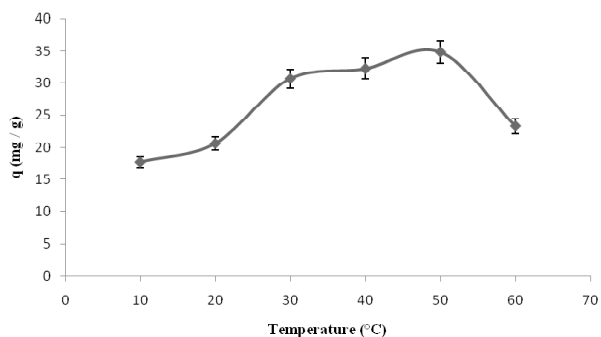


Fig. 2: Effect of temperature on sorption of Cr(VI) onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; pH = 2.0; rotational speed = 120 rpm; contact time = 60 min).

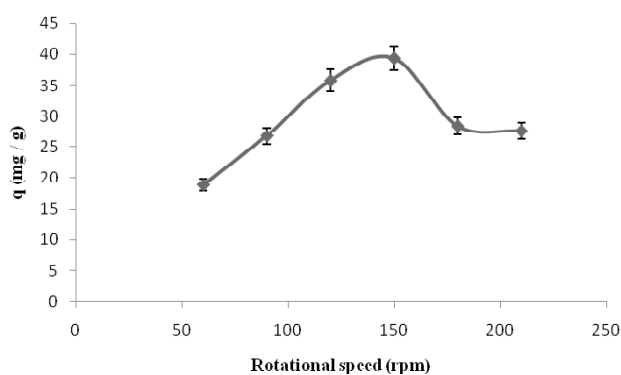


Fig. 3: Effect of rotational speed on sorption of Cr(VI) onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; pH = 2.0; temperature = 50°C; contact time = 60 min).

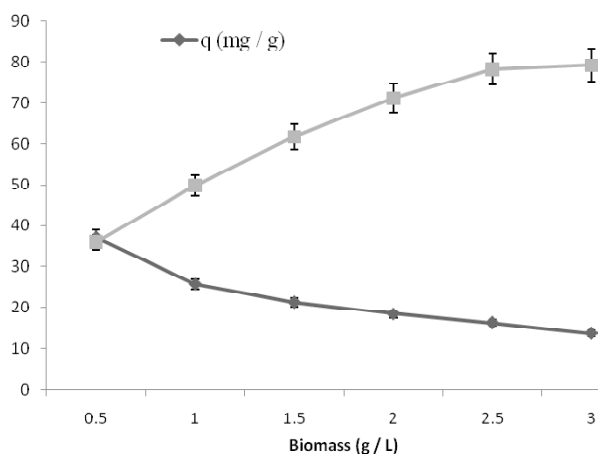


Fig. 4: Effect of biomass concentration on sorption of Cr(VI) onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; pH = 2.0; temperature = 50°C; rotational speed = 150 rpm; contact time = 60 min).

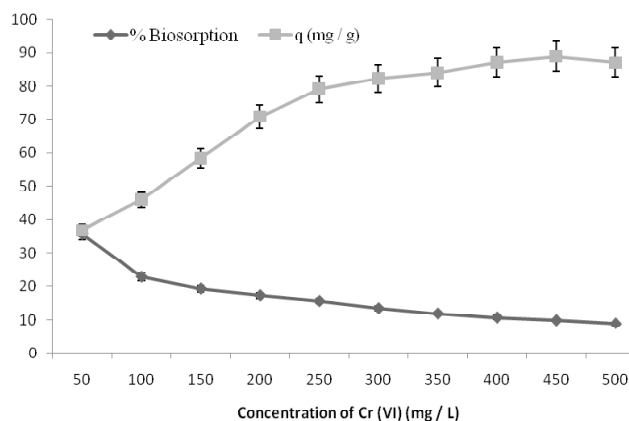


Fig. 5: Effect of initial concentration of Cr(VI) ions on its sorption onto *Rhizopus arrhizus* biomass (Biosorption conditions: biomass = 0.05 g; pH = 2.0; temperature = 50°C; rotational speed = 150 rpm; contact time = 60 min).

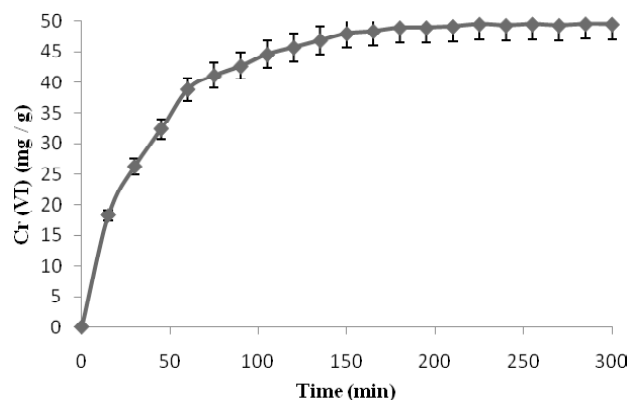


Fig. 6: Effect of contact time on sorption of Cr(VI) ions onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; pH = 2.0; temperature = 50°C; rotational speed = 150 rpm).

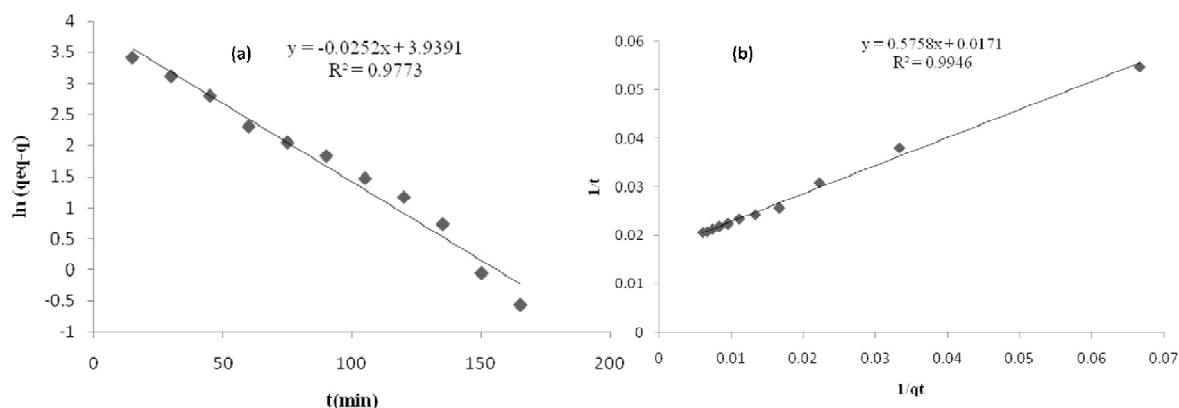


Fig. 7: (a) First-order (b) Second-order plot for sorption of Cr(VI) onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; pH = 2.0; temperature = 50°C; rotational speed = 150 rpm)

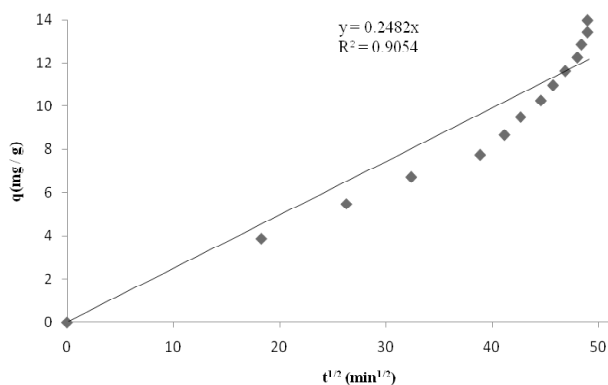


Fig. 8: Intraparticle diffusion for Cr(VI) sorption onto *Rhizopus arrhizus* biomass (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; pH = 2.0; temperature = 50°C; rotational speed = 150 rpm).

contact time should yield a straight line passing through the origin. The relationship between q and $t^{0.5}$ was not linear over the whole time range (Fig. 8). It may be concluded that the rate limiting step is intra-particle diffusion only in the initial period of the reaction. This indicated that there were several processes affecting the adsorption. Other researchers have also reported this non-linear relationship and considered that there were both boundary diffusion and intra-particle diffusion (Xiangliang et al. 2005, Krim et al. 2006).

Isotherm biosorption analysis: Both Langmuir and Freundlich isotherm models were evaluated to examine biosorption with increasing concentration of Cr(VI). The plots of $1/q_{eq}$ versus $1/C_{eq}$ (Fig. 9 (a)) and $\ln q_{eq}$ versus $\ln C_{eq}$ (Fig. 9 (b)) at 50°C were found to be linear, indicating the applicability of the classical Langmuir and Freundlich adsorption isotherms respectively to the sorbate-sorbent system. An adsorption isotherm is characterized by certain constants, the values of which express the surface properties and

Table 1: Comparison between the first-order and second-order kinetics constants for sorption of Cr(VI) onto *Rhizopus arrhizus* biomass.

Experimental q_{exp} (mg/g)	First order kinetic			Second order kinetic		
	k_1 (1/min)	R^2	q_{eq} (mg/g)	k_2 (g/mmol/min)	R^2	q_{eq} (mg/g)
48.93	0.057	51.37	0.97	0.029	58.47	0.9946

Table 2: Desorption of Cr(VI) from biomass of *Rhizopus arrhizus* (Biosorption conditions: $C_o = 50$ mg/L, biomass = 0.05g, adsorption pH = 2.0, temperature = 50°C; rotational speed = 150 rpm, S/L ratio = 50, adsorption and desorption period = 60 min).

Cycles	Biosorption (mg/g)	Desorption (mg/g)	Reduction in sorption capacity (%)	Desorption (%)
I	38.25	38.09	-	99.58
II	38.01	37.67	0.62	99.10
III	37.42	37.01	2.16	98.90
IV	36.67	36.06	4.13	98.33
V	35.83	35.05	6.32	97.82

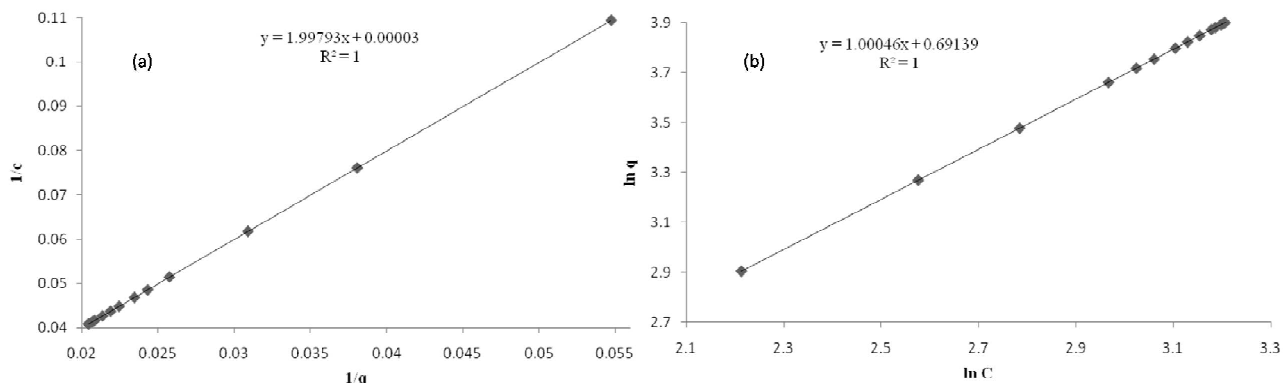


Fig. 9: Linearized (a) Langmuir and (b) Freundlich adsorption isotherm plots for Cr(VI) sorption onto *Rhizopus arrhizus* biomass.

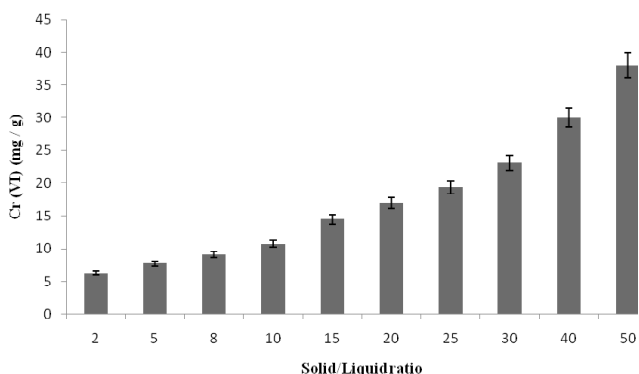


Fig. 10: Effect of Solid/Liquid ratio on recovery of Cr(VI) (Biosorption conditions: $C_o = 50$ mg/L; biomass = 0.05 g; temperature = 50°C; rotational speed = 150 rpm; desorption period = 60 min).

affinity of the sorbent and can be used to compare biosorptive capacities of biomass for Cr(VI) ions. Langmuir parameters appear to be high for the Cr(VI)-*Rhizopus arrhizus* biomass system. R_L values (Langmuir separation factor) between 0 and 1 indicate favourable adsorption (Ahalya et al. 2005). The R_L values in the present study were found to be 0.999. Thus, we can say that the Langmuir isotherm model fits the results reasonably well, suggesting that the surface of the sorbent could be homogenous.

The magnitude of the Freundlich constants, K_f (Freundlich isotherm adsorption capacity constant (L/g)) and n (Freundlich adsorption intensity constant), showed easy uptake of Cr(VI) with a high adsorptive capacity of *Rhizopus arrhizus* biomass. The results fitted well with the Freundlich isotherm model, which yielded a straight line with the regression coefficient (R^2) value of unity. The constants were found to be $K_f = 1.99571$ and $n = 0.9995$. The K_f is primarily related to the capacity of the adsorbent for the given ion; the higher the value of K_f , the larger is the capacity of sorption. *Rhizopus arrhizus* biomass compared favourably with some easily available and ecofriendly adsorbents like activated coconut shell carbon ($K_f = 2.20$), activated bagasse carbon

($K_f = 0.19$) and activated coconut jute carbon ($K_f = 1.55$). The value of n , which is related to the distribution of bonded ions on the sorbent surface, between 1 and 10 represent beneficial adsorption. For dead biomass of *Rhizopus arrhizus*, the value of n was found to be almost unity, thus indicating that adsorption of Cr(VI) could be favourable (Ahalya et al. 2005). The high correlation of the linearized Freundlich isotherm suggests that a degree of heterogeneity is possible for the existing ionic species in the solution and the surface. *Rhizopus arrhizus* biomass shrinks in acid solutions, causing compactness. Thus, diffusion steps become slower and should be rate determining in the sorption process (Oliveira et al. 2005). Conformity to Freundlich model suggests that the biomass was completely saturated and Cr(VI) ions were adsorbed onto the surface in a multilayered pattern (Bai & Abraham 2002). The applicability of both Langmuir and Freundlich isotherms to the biosorption of Cr(VI) ions expresses that both monolayer adsorption and heterogeneous energetic distribution of active sites on the surface of the adsorbent exist under the experimental conditions employed. Hence, it is not surprising that the biosorption data of the present study fitted both the models (Sag & Kutsal 2001).

Desorption and reusability studies: Biotechnological exploitation of biosorption technology depends on the efficiency of the regeneration of biosorbent with the possibility of recovery of metals after metal desorption. Therefore, non-destructive recovery by mild and cheap desorbing agents is desirable for regeneration of biomass for use in multiple cycles (Gupta et al. 2000). The stability and the potential reusability of the biosorbent were assessed by monitoring the changes in recoveries through five consecutive adsorption-desorption cycles of 60 min each. The same preparation of the biomass containing Cr(VI) loaded biosorbent and 0.1 M NaOH solution as an eluant (desorbing agent) was used for achieving sorption or desorption equilibrium in a batch system. The efficiency of the eluant is often expressed

by the S/L ratio, i.e., solid to liquid ratio. The solid represents the solid sorbent (mg dry wt) and the liquid represents the amount of eluant applied (mL). High values of S/L ratios are desirable for complete elution and to make the process more economical (Gupta et al. 2000). The S/L ratios for desorption of the bound Cr(VI) species were optimized in the range of 2 to 50 (Fig. 10). As the S/L ratio increased, the amount of Cr(VI) (mg Cr(VI)/g biomass) desorbed increased from 6.24 mg/g to 38.04 mg/g. Hence, S/L ratio of 50 was optimized for maximum desorption of Cr(VI) ions from the biomass of *Rhizopus arrhizus* in the following cycles. As given in Table 2, biosorption was completely reversible and more than 95 % of the adsorbed Cr(VI) ions were desorbed in all cases. The biomass undergoing successive adsorption-desorption processes retained good metal adsorption capacity even after five cycles and only a maximum of 6.32% decrease in sorption capacity was observed after five cycles. Thus, NaOH proved to be an effective eluant as has also been reported by other workers (Tewari et al. 2005, Bai & Abraham 2003). The results indicated that the physico-chemically treated biomass of *Rhizopus arrhizus* offers potential to be used repeatedly in Cr(VI) adsorption studies without significant loss in the total adsorption capacity.

CONCLUSION

In the present study, *Rhizopus arrhizus* proved to be a suitable low cost waste biomass from industrial fermentations to eradicate the pollution caused by Cr(VI). The results showed that pH, temperature, rotational speed, biomass dose, initial metal concentration and contact time highly affected the overall metal uptake capacity of the biosorbent. The suitability of the first-order chemical reaction for the sorption of Cr(VI) ions onto *Rhizopus arrhizus* biomass was apparent. The experimental data also showed that intra-particle diffusion is significant in determination of the sorption rate. The present results demonstrate that both the Langmuir and Freundlich models fitted the adsorption equilibrium data with good correlation coefficients. Desorption and reusability studies using loaded biomass by elution using 0.1 M NaOH, showed potential for the recovery and further containment of highly toxic species of Cr(VI). The biosorbent could be regenerated and reused at least five times in biosorption-desorption cycles successively.

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