BIOSORPTION OF CR (VI) BY RESTING CELLS OF *ASPERGILLUS SP.*

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ABSTRACT
Biosorption of Cr(VI) from aqueous solution was studied in a batch bioreactor using the resting cells of filamentous fungal biomass (*Aspergillus* sp.) isolated from industrial wastewaters. The specific Cr(VI) removal (mg/g of dried biomass) decreased with increase in pH and increased with increase in initial Cr(VI) concentration, upto 500 mg/L. By increasing biomass concentration from 2.4 to 5.2 g/L, the specific metal removal remained almost constant. The studies carried out by using the resting cells from various stages of growth indicated maximum Cr(VI) removal of 34.8 mg/g using the biomass from the beginning of the stationary phase. The adsorption equilibrium constants Q^{o} (42.9 mg/g) and b (0.0091/mg) were obtained from the Langmuir adsorption isotherm model.

Key words: *Aspergillus sp*., batch biosorption, chromium (VI), Langmuir adsorption isotherm, resting cells

INTRODUCTION
Hexavalent chromium (Cr (VI)) is one of the major pollutants in the environment and is frequently present in wastewaters from industries such as dyes, electroplating, metal cleaning, leather industries, plating, photography, tanneries etc. The conventional treatment techniques used for removal of Cr (VI) ions from wastewaters are expensive and result in the production of harmful by-products and are not efficient when initial Cr (VI) concentration is in the range of 10-100 mg/L (Aksu *et al.,* 2001). The potential application of microorganisms as biosorbent for the removal of heavy metals has been recognized as an alternative to the existing conventional methods for detoxification of industrial wastewaters. Removal of Cr (VI) from aqueous solution is reported by using the growing, resting and non-living cells of microorganisms (Muter *et al.,* 2001, Srinath *et al.,* 2002). However, most of the work to remove Cr (VI) have been carried out using non-living fungal cells (Nourbaksh *et al.,* 1994, Gupta *et al.,* 2001, Srinath *et al.,* 2002) having advantages over growing and resting cells due to the absence of toxicity limitations, requirements of growth media and nutrients. The metal uptake by the growing as well as the resting cells, though is a function of cell age, composition of growth media and pH of the solution, the cells can be maintained biologically active to remove Cr (VI) from aqueous solution by maintaining the suitable cell energetic biological reaction conditions, whereas biological reactions are no longer continued in case of non-living biomass as the cells are dried. Resting cells have the advantage that they require very low maintenance energy to remain biologically active. The present study has been conducted to evaluate the potential of the resting cells of the *Aspergillus* sp. as a sorbent for Cr (VI) removal from aqueous solution. Effects of pH, initial Cr (VI) concentration, biomass concentration and age of the culture on Cr (VI) removal from aqueous solutions were studied using synthetic Cr (VI) solution in batch bioreactors. The adsorption constants were determined from the equilibrium Langmuir adsorption isotherm.

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MATERIALS AND METHODS
Microorganism and growth conditions
The fungus *Aspergillus* sp. isolated from industrial wastewaters was grown in 500mL erlenmeyer flasks in a shaking incubator at 30^{0} C and 180 rpm using 100 mL of liquid media of the following

composition (g/L): Glucose, 10; K_2HP0_4, 0.5; NaCl, 1.0; $MgS0_4$, 0.1; NH_4N0_3, 0.5; and yeast extract, 5.0. The pH of the growth media was 6.0.

After 36 h of growth, the fungal cells were centrifuged at 5000 rpm for 5 mins at 30 ºC and then washed thrice with sterile double distilled water. Dry cell weight was estimated gravimetrically by taking separately the amount of cells used in the experiment and drying it at 80 ºC for 24 h. A weighed amount of washed resting cells (4.5 g/L, on dry wt. basis) was used as a biosorbent in all the experiments.

Preparation of Cr (VI) solutions

Cr (VI) solutions of different concentrations (50-500 mg/L) were prepared by diluting a stock potassium dichromate solution (1000 mg/L) made by dissolving the required quantities of potassium dichromate in distilled water.

Batch biosorption

The biosorption experiment was conducted in an Erlenmeyer flask (500mL) containing 100 mL of the Cr (VI) solution at 30 ºC. The pH of the solution was adjusted to the required value with 1N H_2SO_4 solution and a small quantity (0.05 g/L) of glucose was added to the flask for maintenance of the cells. A weighed amount of the resting cells (4.5 g/L, on dry wt. basis) was added to the flask containing the Cr (VI) solution of a known concentration. The flask was incubated in a shaker at 150 rpm for 24 h. Periodically samples were withdrawn and centrifuged at 5000 rpm for 5 mins. The separated supernatant liquid was analysed for the residual Cr (VI) concentration. All the biosorption experiments were conducted in a similar manner to study the effect of pH (1.0-6.0), initial metal concentration (0-500 mg/L), biomass concentration (2.4-5.2 g/L) and culture age (12-48 h). All experiments were performed in triplicates.

Analysis of Cr (VI)

The residual Cr (VI) concentration in the cell free supernatant liquid was determined spectrophotometrically at 540 nm using diphenyl carbazide as the complexing agent (Standards methods for determination of wastewaters, American Public Health Association, 17th edition,1989).

RESULTS

Fig. 1 shows the effect of pH on specific uptake of Cr (VI) by the resting cells of *Aspergillus* sp. at 500 mg/L initial Cr (VI) concentration with biosorbent concentration of 4.5 g/L (dry wt. basis).

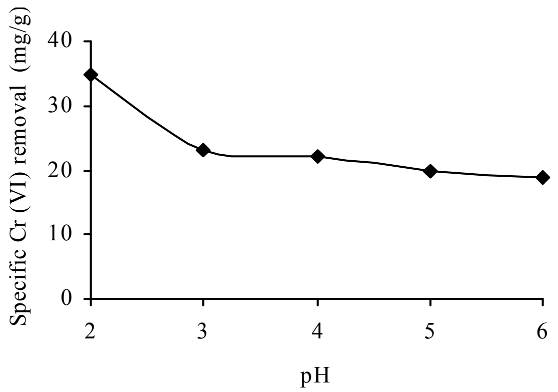

Fig. 1: Effect of pH on specific Cr(VI) removal by resting cells of *Aspergillus* sp.

Fig. 2 shows the effect of initial Cr (VI) concentration ranging from 50-500 mg/L on specific Cr (VI) removal (mg/g) at pH= 2. The increase in Cr (VI) removal upto 500 mg Cr (VI)/L is due to the availability of more and more Cr (VI) for bioaccumulation by the *Aspergillus* sp. The maximum specific removal was found to be 34.8 mg/g of biomass. The percent removal of Cr (VI) was decreased with increase in initial metal concentration and was 31 at initial metal concentration of 500 mg/L .

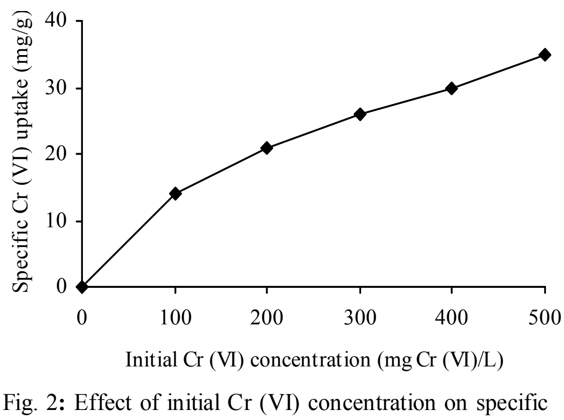

Fig. 2: Effect of initial Cr (VI) concentration on specific Cr (VI) removal (mg/g)

Fig. 3 shows the specific Cr (VI) removal at 500 mg/L initial metal concentration at pH= 2.0 using different concentrations (2.4-5.2 g/L, dry wt. basis) of the resting cells of the *Aspergillus sp.*

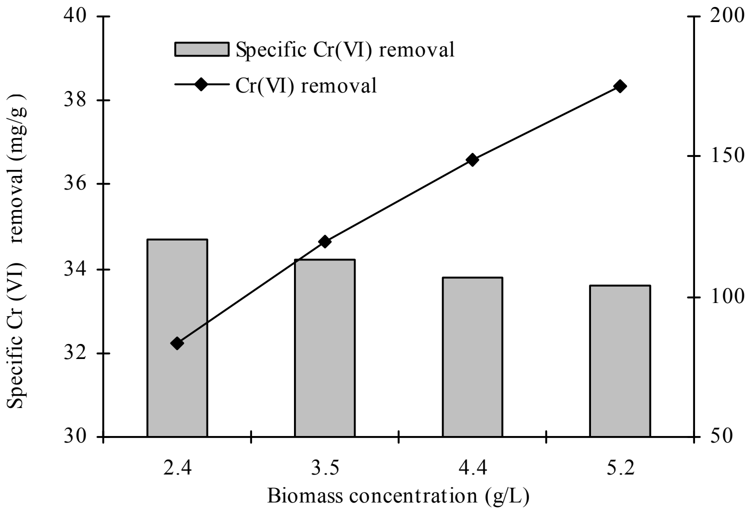

Fig. 3: Effect of biomass concentration on Cr (VI) removal

The effect of culture age on specific Cr(VI) removal by the resting cells of the organism taken from various stages of growth at the same metal concentration and pH 2.0 is shown in Fig. 4.

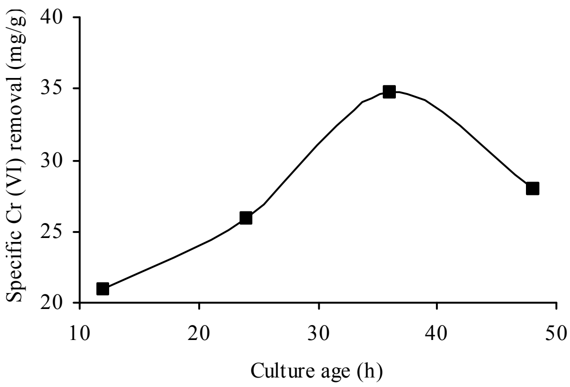

Fig. 4: Effect of culture age on specific Cr (VI) removal

The relation between the amount of metal adsorbed by the adsorbent and unadsorbed component in solution at a constant temperature can be represented by Langmuir adsorption isotherm which is expressed as:

$$q_e = \frac{Q^o b C_e}{1 + bC} \quad (1)$$

or:

$$\frac{1}{q_e} = \frac{1 + bC_e}{Q^o b C_e} \quad (2)$$

or:

$$\frac{C_e}{q_e} = \frac{1 + bC_e}{Q^o b} = \frac{1}{Q^o b} + \frac{1}{Q^o} C_e \quad (3)$$

where q_e is the amount of metal adsorbed per gram of dried biomass at equilibrium (mg/g) and Ce is the residual (equilibrium) metal concentration remaining in the solution after sorption (mg/L), Q^o and b are the Langmuir constants, indicating the maximum amount of metal bound per gram of sorbent to form a monolayer and the affinity of the binding sites, respectively (Aksu *et al.,* 2001, Gupta *et al.,* 2001). Fig. 5 shows the Langmuir adsorption isotherm of Cr (VI) obtained at 30 ºC by plotting Ce/qe against the residual concentration , Ce.

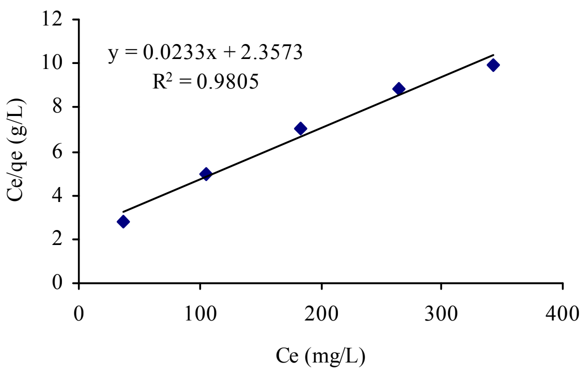

Fig. 5: The Langmuir adsorption isotherm of Cr (VI)

The constants Q^o and b were calculated from the slope and intercept of the straight line obtained The maximum amount of chromium(VI) adsorbed per gram of biosorbent to form a monolayer on the surface (Q^o) was 42.9 mg and the adsorption affinity (b) for binding the metal on the adsorbent sites was 0.009 (l m/g). The correlation coefficient (R^2) obtained from Langmuir adsorption isotherm was 0.98.

DISCUSSION

Fig. 1 indicates a sharp decrease in specific removal of Cr(VI) with an increase in pH value from 2 to 3 beyond which a slow decrease was observed in the removal with further increase in pH upto 6. The removal value at pH= 6 was found to be 19 mg/g. The decrease in removal with increase in pH is due to the decreased availability of hydrogen ions for the protonation of the cell wall functional groups, thus reducing the interaction between the metal ions and the available binding sites. The increased Cr(VI) removal at low pH is due to an interaction between the negatively charged dichromate ions and the positively charged cell protonated functional groups and also because of reduction of Cr(VI) to trivalent chromium (Rapport and Muter, 1994). As it shown in Fig. 2, the specific removal of Cr(VI) increased with increase in initial Cr(VI) concentration, upto 500 mg/L. The specific Cr(VI) removal remained nearly constant at all the biomass concentrations, although the Cr(VI) removal increased with increase in biomass concentrations (Fig. 3). The maximum Cr(VI) removal of 36 % and 17 % were obtained at 5.2 g/L and 2.4 g/L initial biomass concentrations, respectively. Fig. 4 indicates that the specific removal of Cr(VI) increased with increase in cell age, reached a maxima when 36 h old culture taken from the beginning of stationary phase was used for the biosorption and then decreased with further increase in cell age. The maximum removal 34.8 mg/g of biomass was observed using the 36 h old culture. Results of this study showed that the resting cells of *Aspergillus* sp. isolated from industrial wastewaters was able to significantly remove Cr(VI) from an aqueous solution. The biosorption process was dependent on pH and initial metal concentration. The maximum specific Cr(VI) removal (34.8 mg/g) was obtained at 500 mg/L initial Cr(VI) concentration at pH 2.0. The adsorption constants calculated from the Langmuir adsorption isotherm indicating high adsorption capacity of the *Aspergillus* sp. for removal of Cr(VI). These findings shall form the basis of development of suitable operational strategies for treatment of Cr(VI) contaminated wastewaters.

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