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BIOSORPTION OF Cr (VI) BY RESTING CELLS OF *FUSARIUM SOLANI*

***[1] M. Sen ,[2] M. Ghosh Dastidar**

[1]Department of Applied Chemistry, Amity School of Engineering & Technology (ASET) , Bijwasan, New Delhi, India
[2]Centre for Energy Studies, Indian Institute of Technology, Hauz Khas, New Delhi, India

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ABSTRACT

Chromium is one of the toxic heavy metals which exists in nature as stable hexavalent and trivalent forms. The hexavalent form of chromium is more toxic than trivalent chromium as it persists indefinitely in the environment complicating its remediation. The conventional physical and chemical treatment techniques used for the removal of Cr(VI) are expensive and highly energy intensive, moreover they produce harmful by-products, ultimate disposal of which again causes secondary pollution. Removal of Cr(VI) from aqueous solution using biological sources as biosorbent has assumed advantageous over the existing conventional physico-chemical techniques for the treatment of metal contaminated wastes. The present batch biosorption study was undertaken with an aim to examine the Cr (VI) removal potential of the resting cells of *Fusarium solani* (isolated from soil) from aqueous solution. The specific Cr (VI) removal decreased with increase in pH and increased with increase in initial Cr(VI) concentration , up to 500 mg/L . The specific Cr(VI) removal remained almost constant by increasing biomass concentration from 2.4 to 5.2 g/L. The studies also carried out by using the resting cells obtained from various stages of growth and the maximum specific Cr(VI) removal (60 mg/g) was achieved at 500 mg/L initial Cr(VI) concentration and by using cells (36 h old). The Langmuir adsorption isotherm constants, Q^0 and b were observed to be 57.1 mg/L and 0.06 l 1/mg, respectively.

Key words: Batch biosorption; Cr (VI); *Fusarium solani*; Langmuir adsorption isotherm; Resting cells

INTRODUCTION

Cr(VI) is one of the major pollutants in the environment and is frequently present in wastewaters generated in industries such as dye, electroplating, metal cleaning, leather, wood preservation, alloy preparation, pigment manufacturing, and tanneries (Germain and Patterson, 1974; Patterson, 1977; Stratten *et al*., 1987; Komari *et al*., 1989; Faisal and Hassain, 2004). The conventional treatment techniques used for removal of Cr(VI) from wastewaters are expensive, 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 Cr(VI)/L (Aksu *et al.*, 2001). The potential application of microorganisms as biosorbent for heavy metals has been recognized as an alternative to the existing conventional methods for detoxification of industrial wastewaters (Volesky, 1990). Removal of Cr(VI) from aqueous solution is reported by using growing, resting and non-living cells of microorganisms (Llovera *et al.,* 1993; Nourbaksh *et al*.,1994; Campos *et al.,* 1995; Pattanapipitpaisal *et al.,* 2001; Aksu *et al*.,2001; Sudha Bai and Abraham, 2001; Muter *et al.*, 2001; Srinath *et al.*, 2002; Selvaraj *et al*., 2003; Sarah *et al*., 2003; Dursan *et al*., 2003; Penacastro *et al.,* 2004; Barkhordar and Ghaisseddin, 2004; Sen *et al*., (2002, 2005

*Corresponding author: E-mail: mgdastidar@hotmail.com
Tel:+91 11 26591267, Fax : + 91 11 26581121

and 2007) , Sen and Dastidar, 2007; Khanafari *et al*., 2008; Sen *et al*., 2010). However, most of the work to remove Cr (VI) have been carried out using non-living fungal cells (Nourbaksh *et al.*, 1994; Sag and Kustal, 1995; Prakasham *et al*., 1998; Gupta *et al.*, 2001; Barkhordar and Ghaisseddin, 2004; Sen *et al*., 2005; Sen and Dastidar, 2007; Sen *et al*., 2010) which have advantages over growing and resting cells due to the absence of both toxicity limitations and requirements for growth media and nutrients. The metal ion uptake by the growing cells (Muter *et al.*, 2001; Dursan *et al.*, 2003; Sarah *et al.*, 2003; Panacastro *et al*., 2004; Sen *et al*., 2005) as well as the resting cells (Llovera *et al.,* 1993; Campos *et al.,* 1995; Pillicsanmer *et al.,* 1995; Pattanapipitpaisal *et al.,* 2001; Pattanapipitpaisal *et al.,* 2001;Srinath *et al.*, 2002) 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 metabolically active. Moreover, they can sequester metal through a combination of surface reactions, intracellular and extracellular precipitation and extracellular complexation reactions.

The present study has been conducted to evaluate the potential of the resting cells of the *Fusarium solani* for Cr(VI) removal from aqueous solution. The 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 equilibrium constants were determined from the equilibrium Langmuir adsorption isotherm.

MATERIALS AND METHODS

Microorganisms and inoculum

The fungus *Fusarium solani* (isolated from soil) used in the present study was grown in 250 mL Erlenmeyer flasks in a shaking incubator at 30° C and 180 rpm using 100 mL liquid media of the following composition (g/L) : Glucose, 10.0 ; 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 media was 6. An inoculum of 10 % (v/v) of a 36 h old culture was used for the growth of the organism.

Preparation of biomass

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

Preparation of Cr(VI) solutions

Cr (VI) solutions of different concentrations [100-1000 mg Cr(VI)/L] were prepared by diluting a stock solution [2.82 g Cr(VI)/L] prepared by dissolving the required quantities of potassium dichromate in distilled water.

Batch Biosorption

A weighed amount of the resting cells (4.5 g/L on dry wt. basis) was added to the flask containing 100 mL of Cr(VI) solution of a known concentration. Before mixing the biomass, pH of the solution containing Cr(VI) was adjusted to the required value with 1N H_2SO_4 solution, and glucose (0.05 g/L) needed only for maintenance of the cells was added to the flask, which was inoculated and incubated in a shaker at 150 rpm for 24 h at 30ºC. Periodically samples were withdrawn and centrifuged at 5000 rpm for 5 minutes. The separated supernatant liquid was analyzed for the residual Cr(VI) concentration. All the batch experiments were carried out in a similar manner to study the effect of pH (2–6), initial Cr (VI) concentration [100-1000 mg Cr(VI)/L], biomass concentration (2.4–5.2 g/L) and culture age (12–48 h). All experiments were performed in triplicates.

Assay techniques

The residual Cr(VI) concentration was determined spectrophotometrically (Systronics, UV-VIS Spectrophotometer 117) at 540 nm using Di-

Phenyl Carbazide (DPC) as the complexing agent (Standards methods for determination of water and wastewater, American Public Health Association (APHA, 1989), 17th edition,1989).

RESULTS

Fig. 1 shows the effect of pH (2-6) on specific removal of Cr(VI) by the resting cells of the *Fusarium solani* at initial 500 mg Cr(VI) /L concentration using the biomass concentration of 4.5 g biomass/L (dry wt. basis).

Fig. 2 shows the specific Cr(VI) removal (mg Cr(VI)/g of dried biomass) at pH=4.0 and at different initial Cr(VI) concentrations ranging from 100-1000 mg Cr(VI)/L.

The removal of Cr (VI) with respect to biomass concentrations(2.4-5.2 g biomass/L) of the resting cells at initial 500 mg Cr(VI)/L concentration and at pH=4.0 is shown in Fig. 3.

The cells harvested from various stages of growth (12, 24, 36 and 48h) of *Fusarium solani* in the absence of Cr(VI) were maintained under resting condition (by the addition of low maintenance energy in the form of glucose) and were used to study the effect of culture age (physiological state of growth) on Cr(VI) removal at initial 500 mg Cr(VI)/L concentration and at pH=4.0 by the same resting cells of the organisms, results of which are shown in Fig. 4. The relation between the amount of metal ion

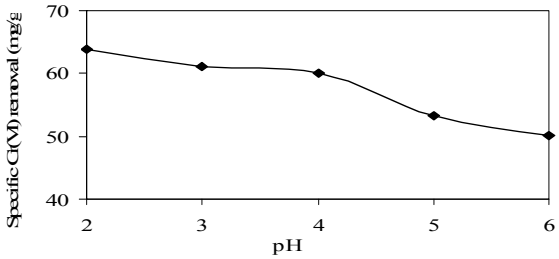


Fig. 1: Effect of pH on specific Cr(VI) removal by the resting cells of the *Fusarium solani*

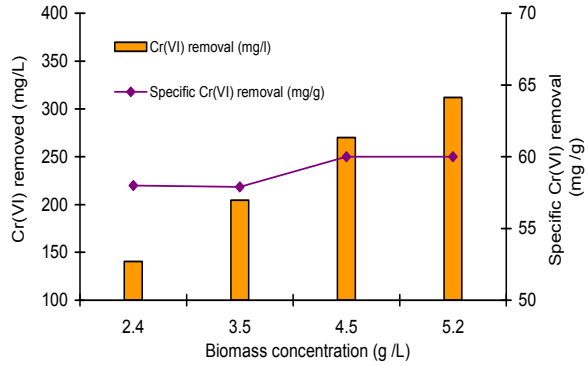


Fig. 3: Removal of Cr (VI) with respect to biomass concentrations at 500 mg Cr(VI)/L concentration and pH=4.0

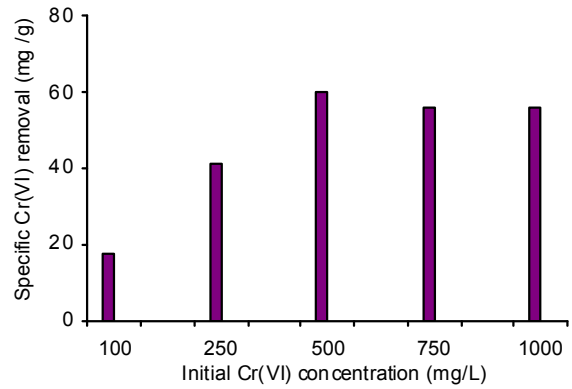


Fig. 2: Specific Cr (VI) removal at different initial Cr(VI) concentrations at pH=4

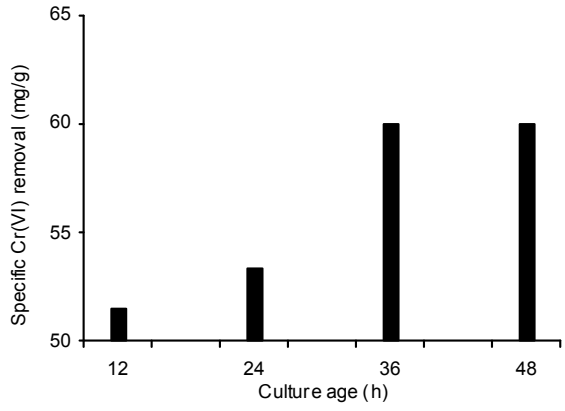


Fig. 4: Effect of culture age on specific Cr(VI) removal at 500 mg Cr(VI)/L concentration and pH=4.0

Table 1: Comparison of Cr(VI) removal using resting cells of different organisms

Organism	Reactor/conditions	Tolerance limit (mg/L)	Maximum Cr(VI) removed (mg/g)	Reference
Fusarium Solani	Batch, 30 ºC, 24 h, 150 rpm, pH 2.0 pH 4.0	1000	63.9 60.0	Present study
Bacillus circulans *Bacillus megaterium*	Batch, 28 ºC, pH=4.0, 24 h, 150 rpm	50	34.5 32.0	Srinath *et al.,* 2002
Bacillus circulans *Bacillus megaterium*	Batch, 28 ºC, pH=2.5, 24 h, 150 rpm	50	23.8 15.7	Srinath *et al.,* 2002
Zgyomycete (Mucor hiemalis MP/92/3/4)	Batch, 30 ºC, pH=1.0,	50	4.3	Pillicsanmer *et al.,* 1995
Agro bacterium radiobacter EPS-916	Batch, 25-30 ºC, pH=7-7.5, 6 h	26	Complete removal	Llovera *et al.,* 1993

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

$$q_e = \frac{Q^o b C_e}{1 + b C_e}$$

or

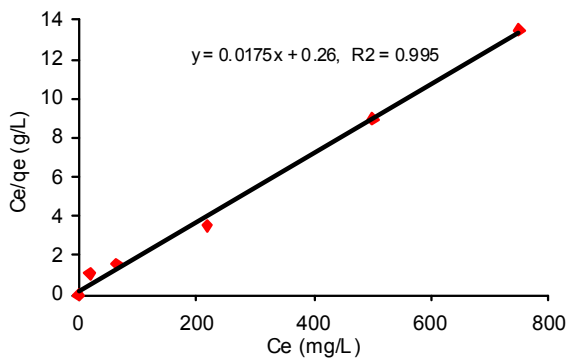
$$\frac{1}{q_e} = \frac{1 + b C_e}{Q^o b C_e}$$

or

$$\frac{C_e}{q_e} = \frac{1 + b C_e}{Q^o b} = \frac{1}{Q^o b} + \frac{Ce}{Q^o}$$

Where: q_e is the amount of metal ion adsorbed per gram of dried biomass at equilibrium [mg Cr(VI)/g of dried biomass] and C_e is the residual (equilibrium) metal ion concentration remaining in the solution after sorption [mg Cr(VI)/L]. The Langmuir constants, Q^o and b, indicate the maximum amount of metal ion bound per gram of sorbent to form a monolayer and the affinity of the binding sites, respectively (Aksu *et al.*, 2001 and Gupta *et al.*, 2001).

Fig. 5 shows the Langmuir adsorption isotherm of Cr(VI) obtained at 30ºC by plotting C_e/q_e VS. the residual concentration , C_e. The maximum amount of Cr(VI) adsorbed per gram of biosorbent to form a monolayer on the surface (Q^o) was 57.1 mg and the adsorption affinity (b) for binding the metal ions on the adsorbent sites was 0.06



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

(l/mg). The correlation coefficient (R^2) obtained from Langmuir adsorption isotherm was 0.995.
dsorption isotherm of Cr(VI)

DISCUSSION

Fig. 1 shows a sharp decrease in specific removal of Cr(VI) from 63.88 mg Cr(VI)/g to 50.12 mg Cr(VI)/g with an increase in pH from 2 to 6. The higher Cr(VI) removal at lower pH is due to the increased availability of hydrogen ions for the protonation of the cell wall functional groups, thereby increasing the interaction between the negatively charged dichromate anions and the protonated cell functional groups and also because of reduction of Cr(VI) to trivalent chromium (Rapport and Muter, 1994).

As the natural pH of dichromate solution was found to be 4 and significant removal (60 mg Cr(VI)/g of dried biomass) was obtained at the same pH, the rest of the studies using resting cells were carried out at pH=4.0.

Fig. 2 shows the specific Cr(VI) removal (mg Cr(VI)/g of dried biomass) at pH 4.0 and at different initial Cr(VI) concentrations ranging from 100-1000 mg Cr(VI)/l .

As complete Cr(VI) removal could be obtained at concentration lower than 100 mg Cr(VI)/L ,the results have been reported only in the range 100-1000 mg Cr(VI)/L . The specific Cr(VI) removal increased with increase in Cr(VI) concentration upto 500 mg Cr(VI)/L, then decreased and remained constant upto 1000 mg Cr(VI)/L. The increase in Cr(VI) removal upto 500 mg Cr(VI)/l is due to the availability of more and more Cr(VI) ions for bioaccumulation by the *Fusarium solani*. A small decrease in Cr(VI) removal upto 750 mg Cr(VI)/L and no further decrease upto 1000 mg Cr(VI)/L could be due to the reduced accessibility of the binding sites of the *Fusarium solani* by Cr(VI) at very high concentrations.

The resting cells of the *Fusarium solani* used in the present study was found to be not only tolerant to very high concentrations of Cr(VI) but also effective in Cr(VI) removal. In Table 1, Cr(VI) removal potential of resting cells of the *Fusarium solani* is compared with the potential of resting cells of different organisms reported in the literature. Most of the studies reported with resting cells of different bacteria and fungi carried out at lower initial Cr(VI) ion concentrations, clearly indicates lower tolerance of the organisms for Cr(VI). In most of the cases the maximum tolerance limit was found to be 50 mg/l beyond which the growth as well as the Cr(VI) removal were inhibited. In the present study, *Fusarium solani* was found to be tolerant even upto 1000 mg/L Cr(VI) concentration. The maximum Cr(VI) removal capacity of *Fusarium solani* was found to be 63.9 mg/g which was sifnificantly higher than the capacities observed with other organisms as shown in Table 1.

The results of Fig. 3 suggest that Cr(VI) removal is dependent on biomass concentration of resting cells, although the specific Cr(VI) removal (mg Cr(VI)/g of dried biomass) remained nearly the constant at all the biomass concentrations .

The specific Cr(VI) removal is shown in Fig. 4, in which an increase in Cr(VI) removal was observed with an increase in cell age from 12 h (beginning of the exponential phase) to 36 h (beginning of the stationary phase).The specific Cr(VI) removal remained constant with further increase in cell age upto 48 h (stationary phase). These results suggest that Cr(VI) removal by the resting cells is also dependent on the age of the culture. Using 36 h old culture the maximum specific Cr(VI) removal was found to be 60 mg Cr(VI)/g of dried biomass .

The adsorption constants Q^o (57.1 mg/g) and b (0.06 l/mg), calculated from the Langmuir adsorption isotherm (Fig. 5) were found to be higher as compared to the values obtained using the non-living cells of the same fungal biomass [Q^o = 50.25 (mg/g) and b= 0.03 (l/mg) (Sen *et al*., 2005)]. From the above studies the resting cells appear to be more effective than the non-living biomass of the same fungal biomass. These above findings could be the basis of the development of suitable operational strategies for the removal of Cr(VI) from contaminated industrial wastewaters using the resting cells of *Fusarium solani*.

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