

A Comparative Study on Biosorption of Cr(VI) by *Fusarium solani* under Different Growth Conditions

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ABSTRACT

Biosorption of Cr(VI) from aqueous solution was studied in a batch bioreactor using the resting cells of *Fusarium solani* isolated from soil. The specific Cr(VI) removal decreased with increase in pH from 2.0 to 6.0 and increased with increase in initial Cr(VI) concentration, upto 500 mg·l⁻¹. By increasing biomass concentration from 2.0 - 5.0 g·l⁻¹, the specific metal removal remained almost constant. The maximum specific Cr(VI) removal was 60 mg·g⁻¹ achieved at 500 mg·l⁻¹ initial Cr(VI) concentration and by using resting cells (36 h old). The Langmuir adsorption isotherm constants, Q^0 and b were observed to be 57.1 mg·g⁻¹ and 0.06 l·mg⁻¹ respectively. These results were compared with the Cr(VI) removal obtained in earlier studies conducted by the present authors using non living and growing cells of *F. solani*.

Keywords: Biosorption; Cr(VI); *Fusarium solani*; Resting Cells

1. Introduction

Chromium(VI) is one of the toxic heavy metals that are present as chromate (CrO₄²⁻) and dichromate (Cr₂O₇²⁻) in aqueous waste water [1] of many industries such as dye, electroplating, metal cleaning, leather, tanneries metal plating, metal cleaning and processing, manufacture of anticorrosive agents, wood preservation, wood processing, alloy preparation, pigment manufacture, leather tanning, manufacturing of dyes, printing, etc. [2-4]. The persistent nature of Cr(VI) makes it accumulate in the food chain which with time reach harmful levels in living beings resulting in serious health hazards. Therefore, removal of Cr(VI) from waste water prior to its discharge into natural water systems, adjoining land-masses, sewer systems, etc. requires serious and immediate attention. 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⁻¹ [5]. Bioremediation involves potential 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 [6]. Several researchers reported removal of Cr(VI) from aqueous solution using growing, resting and non-living cells of different microorganisms [7-19].

However, most of the work to remove Cr(VI) have

been carried out using non-living fungal cells 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 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 biochemical reaction conditions, whereas biochemical reactions are no longer continued in case of non-living biomass as the cells are dried. Further, resting cells have the advantage over growing cells in that the former require very low maintenance energy to remain biologically active. The earlier studies were conducted by the present authors on biosorption of Cr(VI) by using *F. solani* isolated from soil under different growth conditions *i.e.*, resting cells [20] growing cells [21,22] as well as non living biomass [23]. Significant Cr(VI) removal was observed using growing cells in batch and continuous modes of operations and using non living biomass in batch bioreactor.

The present study has been conducted to evaluate the potential of the resting cells of the *F. solani* for Cr(VI) removal from aqueous solution with an aim to develop suitable operational strategy for treatment of Cr(VI) contaminated wastewaters. The effects of pH, initial Cr(VI) concentration, biomass concentration and age of the culture on Cr(VI) removal from aqueous a solutions were studied using synthetic Cr(VI) solution in batch bioreac-

tors. An attempt was made to fit the equilibrium data to Langmuir and Freundlich adsorption isotherms. The adsorption constants were determined from the adsorption isotherms. An attempt was also made to compare these results with those obtained using both growing cells and non living cells of *F. solani* obtained earlier.

2. Materials and Methods

2.1. Microorganisms and Inoculums

The fungus *F. 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⁻¹): Glucose, 10.0; K₂HPO₄, 0.5; NaCl, 1.0; MgSO₄, 0.1; NH₄NO₃, 0.5 and Yeast extract, 5.0. The pH of the media was 6.0. An inoculum of 10% (v/v) of a 36 h old culture was used for the growth of the organism.

2.2. Preparation of Biomass

After 36 h of growth, the fungal cells were centrifuged at 5000 rpm for 5 minutes at 30°C and then washed thrice with distilled water. A weighed amount of washed resting cells (4.5 g·l⁻¹, 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 80°C for 24 h.

2.3. Preparation of Cr(VI) Solutions

Cr(VI) solutions of different concentrations [100 - 1000 mg·l⁻¹] were prepared by diluting a stock solution [2.82 g·l⁻¹] prepared by dissolving the required quantities of potassium dichromate in distilled water.

2.4. Batch Studies

A weighed amount of the resting cells (4.5 g·l⁻¹ on dry wt. basis) was added to the flask containing 100 ml of Cr(VI) solution of a known concentration. Before mixing the biomass the pH of the solution containing Cr(VI) was adjusted to the required value with 1N H₂SO₄ solution and a small quantity of glucose (0.05 g·l⁻¹) was added to the flask which was required only for maintenance of the cells. The flask was inoculated and incubated in a shaker at 150 rpm for 24 h at 30°C. 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 in triplicates. The effect of pH (2.0 - 6.0) was studied at initial Cr(VI) concentration of 500 mg·l⁻¹. A parallel control run was carried out at the same concentration and at same pH values

to examine the chemical reduction of Cr(VI) to Cr(III) in the absence of *F. solani*. The effect of initial Cr(VI) concentration [100 - 1000 mg·l⁻¹], biomass concentration (2.0 - 5.0 g·l⁻¹) and culture age (12 - 48 h) were studied at pH 4.0. The effluent contained multi metal ions: Cr(VI) 500; zinc 9 and nickel 10 mg·l⁻¹ was procured from an electroplating industry. The initial pH of the effluent was 2.0. The batch biosorption studies using resting cells were carried out similarly as described above.

2.5. Assay Techniques

The residual Cr(VI) concentration was determined spectrophotometrically (Systronics, UV-VIS Spectrophotometer 117) at 540 nm using di-phenyl carbazide as the complexing agent. The concentration of zinc and nickel present in the sample was estimated using atomic absorption spectrophotometer (Perkin-Elmer Model-Analyst 200 AAS), [24].

3. Results and Discussion

Figure 1(a) shows the effect of pH (2.0 - 6.0) on removal of Cr(VI) both in the presence and in the absence (control) of the resting cells (36 h) of the *F. solani* at initial 500 mg·l⁻¹ concentration using the biomass concentration of 4.5 g·l⁻¹ (dry wt. basis). In the absence of *F. solani* the concentration of Cr(VI) removed chemically decreased from 12.5 to 5 mg·l⁻¹ with an increase in pH from 2 to 3 and no removal was observed beyond pH 3.0. Increased availability of H⁺ ions at lower pH (upto 3.0) favours a chemical redox reaction between the dichromate anions and the H⁺ ions resulting in conversion of Cr(VI) to Cr(III). In the presence of *F. solani* a significant decrease in the biological removal of Cr(VI) from 287.5 mg·l⁻¹ to 225.5 mg·l⁻¹ was observed with an increase in pH from 2.0 to 6.0. 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. The values of specific Cr(VI) removal (mg·g⁻¹) as shown in **Figure 1(b)** decrease with increase in pH.

The specific Cr(VI) removal obtained in the present study using resting cells at different pH values are compared in the **Table 1** with the values obtained in earlier studies conducted by the present authors using the same fungi (*F. solani*) under growing condition [21,22] and as adsorbent using non-living cells [23]

The **Table 1** shows no Cr(VI) removal using growing cells at pH 2.0. This is due to the fact that growth of the organism was inhibited at pH below 3.5, whereas significant specific Cr(VI) removal could be achieved using the resting and non living cells at the same pH. Again, higher Cr(VI) removal observed in case of resting cells

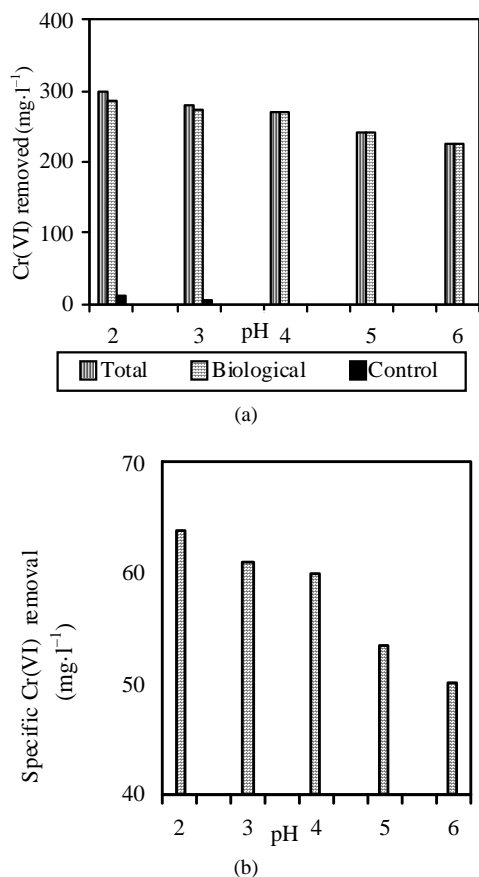


Figure 1. (a) Effect of pH on removal of Cr(VI) at 500 mg·l⁻¹ concentration; (b) Effect of pH on specific Cr(VI) removal (mg·g⁻¹) at 500 mg·l⁻¹ concentration.

Table 1. Comparison of maximum specific Cr(VI) removal (a) during growth; (b) resting condition and (c) as adsorbent at initial 500 mg Cr(VI) l⁻¹ concentration.

pH	Specific Cr(VI) removal (mg·g ⁻¹)		
	Resting cells	Cells during growth [21,22]	Non living cells [23]
2.0	63.9	No growth	47.5
3.0	61.1	No growth	18.9
4.0	60.0	57	8.0
5.0	53.4	71	3.1
6.0	50.1	69	No removal

as compared to non-living cells of *F. solani* could be due to the metabolism independent extracellular as well as intracellular accumulation of Cr(VI). An intracellular accumulation of Cr(VI) by the resting cells is mediated via cell activity induced by the membrane redox enzymes produced while growing *F. solani* (separately for resting cell production) in the absence of Cr(VI) in a growth media and maintaining the cells under resting condition

using only a small quantity of glucose as maintenance energy source. The lower Cr(VI) removal by the non living cells is only due to the extracellular accumulation.

At higher pH value (5.0), the specific Cr(VI) removal by *F. solani* during its growth was found to be maximum (71 mg·g⁻¹) when glucose was completely utilised. At the same pH a significantly lower (53.4 mg·g⁻¹) Cr(VI) removal was observed using the resting cells and a very poor removal of 3.1 mg·g⁻¹ was obtained using the non living cells. In case of resting cells and non living cells the pronated cell functional groups are assumed to be responsible for higher Cr(VI) removal at lower pH, availability of which decreases with increase in pH resulting in decreased Cr(VI) removal. On the other hand, growth associated enzymatic activity at higher pH value might have played a key role in higher Cr(VI) removal by growing cells of *F. solani*. Although at higher pH availability of pronated cell functional groups is decreased, enzymatic activity is responsible for significant removal of Cr(VI) by the resting cells. The absence of enzymatic activity and also decreased pronated cell functional groups at pH 5.0 resulted in poor Cr(VI) removal when non living cells of *F. solani* were used as adsorbent. As the cells were dried for Cr(VI) adsorption, enzymatic activity was no longer perused. However, the highest Cr(VI) removal was observed with *F. solani* at pH 5.0 when the cells were growing and removing Cr(VI) from the broth. A complex mechanism of intracellular/extracellular accumulation or intracellular/extracellular reduction can be expected by the *F. solani* during its growth. The above results strongly indicate that different mechanisms are involved in Cr(VI) removal by *F. solani* under different growth conditions (non living, resting and growing cells). The mechanism of Cr(VI) removal appears to be highly dependent on pH conditions. While a simple mechanism of physical adsorption is involved in case of non living cells and a combination of extracellular and intra cellular accumulation is suggested using the cells under resting conditions, a complex mechanism of growth associated intracellular/extracellular reduction along with intracellular/extracellular accumulation is expected by the *F. solani* during its growth. However, more in-depth studies are needed to elucidate the exact mechanism of Cr(VI) removal.

In the present study, as it was observed from the control experiment that no chemical reduction of Cr(VI) took place at pH beyond 3.0 and as the natural pH of dichromate solution was also found to be 4 at which a significant Cr(VI) removal (60 mg·g⁻¹) could be obtained using the cells under resting conditions, the further studies using resting cells were carried out at pH 4.0.

Figure 2 shows the specific Cr(VI) removal (mg·g⁻¹) at different initial Cr(VI) concentrations ranging from 100 - 1000 mg·l⁻¹ and at pH 4.0 [25]. As complete Cr(VI)

removal could be obtained at concentration lower than $100 \text{ mg}\cdot\text{l}^{-1}$, the results have been reported only in the range $100 - 1000 \text{ mg}\cdot\text{l}^{-1}$. The specific Cr(VI) removal increased with increase in Cr(VI) concentration upto $500 \text{ mg}\cdot\text{l}^{-1}$, then decreased and remained constant upto $1000 \text{ mg}\cdot\text{l}^{-1}$. The maximum specific Cr(VI) removal was found to be $60 \text{ mg}\cdot\text{g}^{-1}$. The increase in Cr(VI) removal upto $500 \text{ mg}\cdot\text{l}^{-1}$ is due to the availability of more and more Cr(VI) for bioaccumulation by the *F. solani*. A small decrease in Cr(VI) removal upto $750 \text{ mg}\cdot\text{l}^{-1}$ and no further decrease upto $1000 \text{ mg}\cdot\text{l}^{-1}$ could be due to the reduced accessibility of the binding sites of the *F. solani* by Cr(VI) at very high concentrations. The earlier batch studies conducted by the present authors under similar conditions showed Cr(VI) removal of $57 \text{ mg}\cdot\text{g}^{-1}$ using growing cells and $8.0 \text{ mg}\cdot\text{g}^{-1}$ of dried biomass using the non living cells of the *F. solani*.

A similar trend of Cr(VI) removal was observed using growing cells with a maximum specific Cr(VI) removal (71 mg/g) at pH 5.0 and at 500 mg/l initial Cr(VI) concentration [21,22].

At pH 2.0, using non living cells a similar trend was observed in Cr(VI) removal with maximum specific Cr(VI) removal of $47.5 \text{ mg}\cdot\text{g}^{-1}$ at $500 \text{ mg}\cdot\text{l}^{-1}$ initial Cr(VI) concentration [23]. The above results indicate that the process of Cr(VI) removal using growing cells can be operated at higher pH nearer to the natural pH (6.0) of the media, whereas using nonliving cells the process can be operated at lower pH. However, the resting cells are effective at both higher and lower pH values. Therefore, both resting and non living cells have potential in industrial applications where Cr(VI) contaminated waste waters are highly acidic in nature. However, for higher Cr(VI) removal growing cells can be used requiring an additional step of pH adjustment.

Figure 3 compares maximum Cr(VI) removal from an

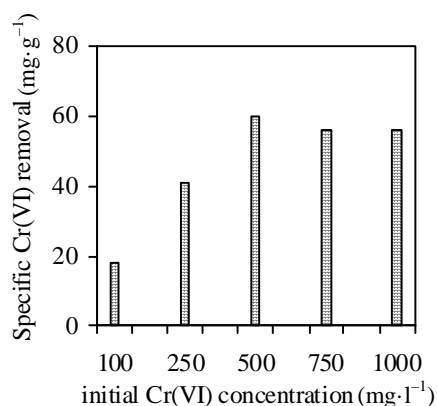


Figure 2. Specific Cr(VI) removal ($\text{mg}\cdot\text{g}^{-1}$) at different initial Cr(VI) concentrations at pH 4.0.

actual industrial effluent (pH 2.0) using growing cells after adjustment of pH to 5.0 [22] and using non living [24] and resting cells (present study) of *F. solani* at pH 2.0 at $500 \text{ mg}\cdot\text{l}^{-1}$ initial Cr(VI) concentration. The specific Cr(VI) removal from an actual effluent (**Figure 3**) was found to be lower than the values obtained using synthetic Cr(VI) solution (**Table 1**). This is due to the presence of other metals [zinc; $9 \text{ mg}\cdot\text{l}^{-1}$ and nickel; $10 \text{ mg}\cdot\text{l}^{-1}$] in the effluent, which might have interfered with the removal of Cr(VI). This was supported by the AAS analysis indicating complete removal of zinc and nickel from the effluent.

The removal of Cr(VI) with respect to biomass concentrations ($2.0 - 5.0 \text{ g}\cdot\text{l}^{-1}$) of the resting cells at $500 \text{ mg}\cdot\text{l}^{-1}$ initial concentration and at pH 4.0 is shown in **Figure 4**. The figure shows that Cr(VI) removal is dependent on biomass concentration of resting cells, although the specific Cr(VI) removal ($\text{mg}\cdot\text{g}^{-1}$) remained nearly the constant at all the biomass concentrations.

The cells harvested from various stages of growth (12, 24, 36 and 48 h) of *F. solani* in the absence of Cr(VI) were maintained under resting condition and were used to study the effect of culture age (physiological state of growth) on Cr(VI) removal at initial $500 \text{ mg}\cdot\text{l}^{-1}$ concentration and

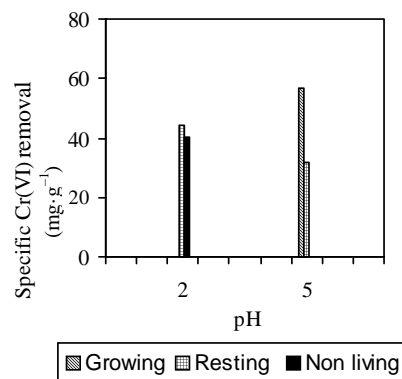


Figure 3. Comparison of maximum specific Cr(VI) removal from an actual industrial effluent using growing, non living and resting cells of *F. solani* at $500 \text{ mg}\cdot\text{l}^{-1}$ initial Cr(VI) concentration.

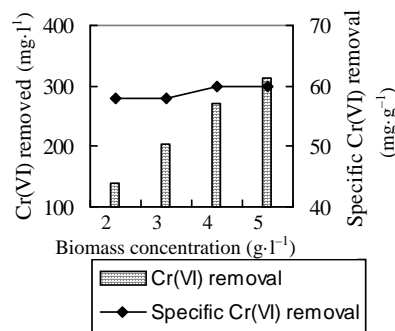


Figure 4. Removal of Cr(VI) with respect to biomass concentrations at $500 \text{ mg}\cdot\text{l}^{-1}$ concentration and pH 4.0.

at pH 4.0. The specific Cr(VI) removal is shown in **Figure 5**, 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. The resting cells harvested from the beginning of stationary phase appear to be most effective in maximum Cr(VI) removal per gram of dried biomass. Using 36 h old culture the maximum specific Cr(VI) removal was found to be $60 \text{ mg}\cdot\text{g}^{-1}$ of dried biomass.

The relation between the amount of Cr(VI) adsorbed by the adsorbent and unadsorbed Cr(VI) in solution at a constant temperature can be represented by Langmuir and Freundlich adsorption isotherm, which provide the equilibrium data required for the designing of the adsorption system. The Langmuir adsorption isotherm which is applicable to monolayer sorption onto a surface with a number of identical sites homogeneously distributed over the surface sorbent is given by

$$q_e = \frac{Q^0 b C_e}{1 + b C_e} \quad (1)$$

$$\frac{1}{q_e} = \frac{1 + b C_e}{Q^0 b C_e} \quad (1a)$$

$$\frac{C_e}{q_e} = \frac{1 + b C_e}{Q^0 b} = \frac{1}{Q^0 b} + \frac{C_e}{Q^0} \quad (1b)$$

where q_e is the amount of Cr(VI) adsorbed per gram of dried biomass at equilibrium [$\text{mg Cr(VI)} \text{ g}^{-1}$ of dried biomass] and C_e is the residual (equilibrium) Cr(VI) concentration remaining in the solution after sorption [mg l^{-1}]. The Langmuir constants, Q^0 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 [20,24-27].

The Freundlich adsorption isotherm is applicable to

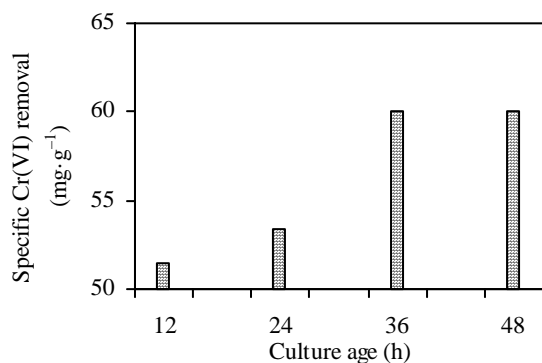


Figure 5. Effect of culture age on specific Cr(VI) removal ($\text{mg}\cdot\text{g}^{-1}$) at $500 \text{ mg}\cdot\text{l}^{-1}$ concentration and pH 4.0.

removal of Cr(VI) on a heterogeneous surface and is expressed as

$$q_e = K_F C_e^{1/n} \quad (2)$$

or

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (2a)$$

where K_F and n are the Freundlich constants and are related to the adsorption capacity and adsorption intensity of the adsorbent, respectively. Equation (2) can be linearized in logarithmic form and Freundlich constants n and K_F can be determined from the slope and intercept which are equal to $1/n$ and K_F , respectively [28-31]. **Figure 6** shows the Langmuir adsorption isotherm of Cr(VI) obtained at 30° by plotting C_e/q_e against the residual concentration, C_e . The maximum amount of Cr(VI) adsorbed per gram of biosorbent to form a monolayer on the surface (Q^0) was 57.1 mg and the adsorption affinity (b) for binding the metal ions on the adsorbent sites was $0.06 \text{ (l}\cdot\text{mg}^{-1})$. The correlation coefficient (R^2) obtained from Langmuir adsorption isotherm was 0.995.

The values of Q^0 and b using non-living cells of the same fungal biomass were found to be $50.3 \text{ [mg}\cdot\text{g}^{-1}$ of dried biomass] and $0.03 \text{ (l}\cdot\text{mg}^{-1})$ respectively, [6]. The higher values of adsorption constants in case of resting cells indicates higher amount of Cr(VI) bound per gram of sorbent to form a monolayer and the higher binding affinity of the cells sites.

However, the data did not fit the Freundlich isotherm and hence are not shown in the present paper.

4. Conclusion

Significant Cr(VI) removal can be achieved in batch operation using resting cells of *F. solani*. Cr(VI) removal is dependent on pH, initial Cr(VI) concentration, biomass concentration and culture age. pH plays an important role in Cr(VI) removal under different growth conditions (growing, resting and non living). Resting and non living cells are effective at lower pH thus having great potential in industrial applications where effluents generated are highly acidic in nature. pH adjustment is required using

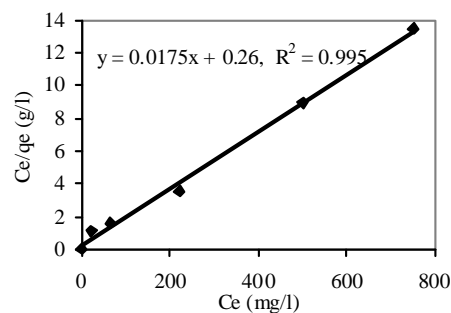


Figure 6. The Langmuir adsorption isotherm of Cr(VI).

cells under growing condition. The equilibrium adsorption data fitted well with Langmuir adsorption isotherm indicating the favorable monolayer adsorption on the cell surface. The findings from this comparative study provide useful basis for development of suitable operational strategies for treatment of Cr(VI) contaminated wastewaters.

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