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Parameters of Biosorption and Bioaccumulation of Cr (VI) Ion from Aqueous Solutions by *Aspergillus niger*

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Abstract. Biosorption and bioaccumulation of Cr (VI) ion by *Aspergillus niger* were investigated in a batch system. Growth conditions for this strain were optimized using potato dextrose broth (PDB). The effects of some important parameters such as initial metal concentrations, temperature and inoculum concentration on biosorption capacity were also studied. In batch biosorption studies, *A. niger* removed 93.02% Cr (VI) ion at 30 °C at pH 6.0 and biosorbent dose of 1.0×10^3 spores/cm^3 solution containing 25 mg/L Cr (VI) ion at 100 rpm agitation. Biosorption equilibrium was established in 150 min and the kinetics of the process fitted well with pseudo second order kinetic model. The biosorption process was best explained by Langmuir isotherm.

Keywords: bioaccumulation, *Aspergillus niger,* equilibrium, kinetic model, Langmuir isotherm

Introduction

Environmental contamination by toxic metals is a serious problem worldwide due to their incremental accumulation in the food chain and continued persistence in the ecosystem. Conventional technologies such as; ion exchange or lime precipitation are often ineffective and/or expensive, particularly for the removal of heavy metal ions at low concentrations (below 50 mg/L). Furthermore, most of these techniques are based on physical displacement or chemical replacement, generating yet another problem in the form of toxic sludge, the disposal of which adds further burden on the techno-economic feasibility of the treatment process.

The Cr (VI) ion is a major toxic pollutant which enters the water streams through various industrial operations. Its potential sources include: effluents from metallurgy, electroplating, leather tanning, textile dyeing, paint, ink and aluminum manufacturing industries. Cr (VI) ion is carcinogenic to both human and animals (Mungasavalli *et al.,* 2007). Strong exposure to Cr (VI) ion causes cancer in the digestive tract and lungs and may also cause gastric pain, nausea and vomiting, severe diarrhoea and haemorrhage (Mohanty *et al.,* 2005). According to the United States Environmental Protection Agency (USEPA) the general permissible limits of total Cr ions in water is 1.0 mg/L(EPA, 1991).

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Conventional processes used in the removal of heavy metals from industrial wastewater include; chemical precipitation, oxidation reduction, filtration, electrochemical techniques and other sophisticated separation processes, using membranes. However, these processes are usually expensive and environmentally invasive. In this era of environmental protection, the use of microorganisms for the recovery of metals from waste streams, as well as employment of plants for landfill applications, has generated growing attention (Kotrba and Ruml, 2000). There are wide varieties of microorganisms, which include; bacteria, fungi, yeast and algae that can interact with metals and radionuclides through several mechanisms to transform them (Volesky and Holan, 1995). Among them fungi can be grown easily in substantial amounts using unsophisticated fermentation techniques and inexpensive growth media (Kapoor *et al.,* 1999).

Biosorption is regarded as an innovative technology to remove metals from aqueous solution because it has some obvious advantages such as; high efficiency and selectivity for absorbing metals at low concentrations, energy-saving, broad operational range of pH and temperature, easy reclamation of metals and easy recycling of the biosorbent (Kratochvil and Volesky, 1998). In the recent years, many agricultural based biosorbent materials have been utilized for heavy metal

biosorption. These include; coconut husk and shell (Amuda *et al.,*2007; Tan *et al.,*1993), sea weeds (Senthilkumar *et al.,*2007) and bagasse ash (Gupta *et al.,* 1998).

In this study, *A. niger* isolates have been used as a biosorbent for the removal of Cr (VI) ion from aqueous solution. The effects of solution pH, biosorbent dose, initial Cr (VI) ion concentration and contact time on Cr (VI) ion removal were investigated in a batch system. The experimental results provided useful information on application of this biosorbent in the treatment of Cr (VI) ion contaminated wastewater.

Materials and Methods

Equipment. A Jenway 3015 precision pH meter, a Mettler MT5 electronic balance, a Clifton oscillator, with temperature control were used in the experiments. The concentration of the residual Cr (VI) ion in the solution was determined with atomic absorption spectrometer AAS (Buck Scientific model 210 VGP).

Preparation of chromium (VI) ion. Aqueous solutions of Cr (VI) ion of different concentrations were prepared from an Analar grade of potassium dichromate salt - $K_2Cr_2O_7$ obtained from BDH. These were used as adsorbate and were not purified prior to use. Double distilled water was employed for the preparation of all the solutions and reagents. The concentration of Cr (VI) ion was spectrometrically estimated at λ540 nm (Eaton *et al.,* 1995).

Preparation of media for microbial growth. Potato dextrose agar and potato dextrose broth were used in this study. Potato dextrose agar was prepared by weighing accurately 10 g of potato dextrose agar powder dissolved in 250 mL of sterile distilled water. The mixture was then melted in a water bath and sterilised in the autoclave at 121 °C for 15 min.

The broth was prepared from 200 g of potato tuber, 20 g of dextrose and a drop of antibiotic. The potato was peeled, weighed and boiled till soft (1 h). It was mashed and extracted with water; the extract was kept in conical flask and distilled water, dextrose agar and lactic acid were added. The resulting solution was sealed with cotton wool and foil in a conical flask and sterilized in the autoclave at 121 ° C for 15 min.

Isolation of microorganism. *Sub-culturing*. *Aspergillus niger* was obtained from the Department of Microbiology, University of Agriculture, Abeokuta. The organism was picked with a sterile wire loop from the pure culture and sub cultured by streaking onto a freshly solidified media. The microbial counting was done by slide culture technique.

Batch equilibrium studies. Adsorption isotherms were performed in a set of 43 Erlenmeyer flasks (100 mL), solutions of Cr (VI) ion with different initial concentrations (25-100 mg/L) were prepared in these flasks. 1.0×10^3 spore/cm^3 of *A. niger,* 10 mL of potato dextrose broth and distilled water used to make up the solution to mark. The solution was kept on an isothermal shaker (Clifton bath/shaker) at 25 ± 1 °C up to 72 h to reach equilibrium. Similar procedure was followed for another set of Erlenmeyer flask containing the same Cr (VI) ion concentration without adsorbate to be used as a blank. The pH was adjusted to 7.0 by adding either few drops of diluted hydrochloric acid or sodium hydroxide (0.1 mol/L). The flasks were then removed from the shaker and the final concentrations of Cr (VI) ion in the solutions were determined. The concentration of Cr (VI) ion in the supernatant solution before and after adsorption was determined using an atomic absorption spectrophotometer (Buck Scientific model 200 VGP). The samples were filtered prior to analysis in order to minimize interference of the biosorbent with the analysis. The amount of adsorption at equilibrium, q_e (mg/g), was calculated by

$$q_e = \frac{(C_o - C_e)V}{W} \quad \text{.....(1)}$$

where:

C_o and C_e (mg/L) are the liquid-phase concentrations of Cr (VI) ion at initial and equilibrium, respectively. V is the volume of the solution (L) and W is the mass of dry adsorbent used (g).

Bioaccumulation studies. The procedure was similar to that of equilibrium studies, except that samples were prepared and withdrawn at an interval of 72 h for the determinations of residual Cr (VI) ion in the solution. The amount of Cr (VI) ion absorbed at interval was expressed as percentage.

Batch kinetic studies. The procedures of kinetic experiments were basically identical to those of equilibrium tests. The aqueous samples were taken at preset time intervals, and the concentration of Cr (VI) ion were similarly measured. The amount biosorbed at time t, q_t (mg/g), was calculated by below equation:

$$q_t = \frac{(C_o - C_t)V}{W} \quad \text{.....(2)}$$

where, C_o and C_t (mg/L) are the liquid phase concentrations of Cr (VI) ion at initial and any time t, respectively. V is the volume of the solution (L) and W is the mass of dry adsorbent used (g).

Results and Discussion

Effect of agitation time and concentration on biosorption of Cr (VI) ion. A series of contact time experiments for Cr (VI) ion have been carried out at different initial concentrations (25-100 mg/L) and at temperature of 25 °C. Fig. 1 shows that the necessary contact time for Cr (VI) ion with initial concentrations of 25-100 mg/L to reach equilibrium is about 30 min. The amount of Cr (VI) ion biosorbed into the organism increases with time and at some point in time, reaches a constant value beyond which no more is removed from solution. At this point, the amount of the Cr (VI) ion desorbing from the adsorbent is in a state of dynamic equilibrium with the amount of the Cr (VI) ion being biosorbed by the organism. The time required to attain this state of equilibrium is termed as the equilibrium time, and the amount of Cr (VI) ion adsorbed at the equilibrium time reflects the maximum adsorption capacity of the adsorbent under those operating conditions.

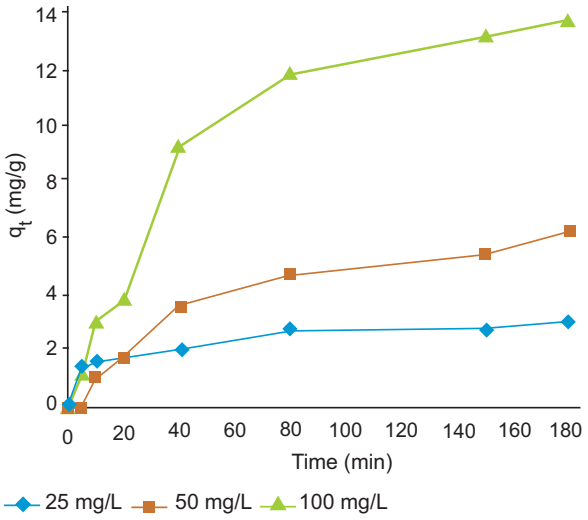

Fig. 1. The variation of adsorption capacity with biosorption time at various concentration of Cr (VI) ion at 25°C (pH 7, inoculum = 10^2 spore/cm^3).

The adsorption capacity at equilibrium increases from 1.8 to 10.5 mg/g with an increase in the initial Cr (VI) ion concentration from 20 to 100 mg/L. It is evident that *A. niger* is an efficient biosorbent for Cr (VI) ion in aqueous solution as the process attains equilibrium gradually.

Bioaccumulation of Cr (VI) ion by *A. niger*. As observed, earlier metals can be bioaccumulated by living organisms through complexation, coordination, ion exchange, chelation, and adsorption (Volesky, 1990). Bioaccumulation of Cr (VI) ion by *A. niger* increases with the numbers of days (Fig. 2), at the end of 15th day about 90% of the metal ions in the aqueous solution had been accumulated by the organism when the initial concentration was 25 mg/L. As the concentration of the metal ion increases from 25-100 mg/L the amount accumulated decreases from about 90% to 43.49% (Table 1). This indicates the viability of the organism to remove the metal ions at a very low concentration. Since the accumulation occurs gradually in the presence of living organism, adsorption on to the cellular structure is much more favoured than all other processes.

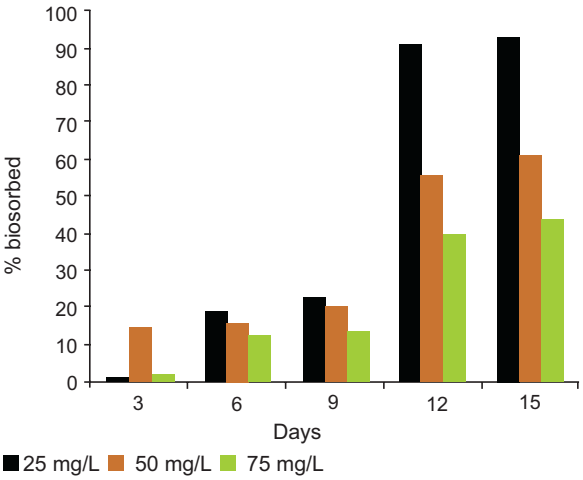

Fig. 2. The variation in biosorption capaciy of *A. niger* with time at various concentrations of Cr (VI) ion at 25 °C pH 7.

Biosorption kinetics. The rate constant of adsorption is determined from the pseudo first-order equation given by Langergren and Svenska (1998):

$$\ln (q_e - q_1) = \ln q_1 - k_1 t \quad \text{.....(3)}$$

where:

q_e and q_t are the amounts of Cr (VI) ion biosorbed (mg/g) at equilibrium and at time t (min), respectively and k_1 the rate constant adsorption (1/h). Values of k_1 were calculated from the plots of ln (q_e -q_t) versus t for different concentrations of Cr (VI) ion. The correlation coefficient values were around 0.9, however, the

Table 1. The influence of various parameters on bioaccumulation of Cr (VI) ion by *A. niger*

Parameters		Percentage bioaccumulation					
		Day 1	Day 3	Day 6	Day 9	Day 12	Day 15
Initial metal concentration							
(mg/L)	25	0.00	0.64	19.30	23.02	91.14	93.02
	50	0.00	14.75	16.21	20.38	55.73	60.49
	100	0.00	2.37	12.76	13.88	39.99	43.49
pH							
	2.0	0.00	15.35	64.90	68.79	69.43	79.61
	4.0	0.00	13.40	19.81	33.81	46.56	61.63
	6.0	0.00	0.47	9.02	17.78	40.84	71.44
Inoculum concentration							
(spore/cm^3)	10^2	0.00	1.60	26.48	71.23	71.57	81.22
	10^3	0.00	3.03	3.39	10.41	29.98	37.64
	10^4	0.00	0.57	3.34	12.87	37.09	62.34
Temperature							
(°C)	25	0.00	1.43	2.34	5.05	20.14	37.47
	30	0.00	11.08	14.90	17.87	21.20	33.03
	37	0.00	8.65	26.80	32.41	46.32	58.45

experimental q_e values are not totally in agreement with the calculated ones, obtained from the linear plots (Table 2, Fig. 3). This shows that the biosorption process although fitted with first order kinetics, it is necessary to compare the data with second order equation and subject the models to validity test using sum of error square i.e., (SSE).

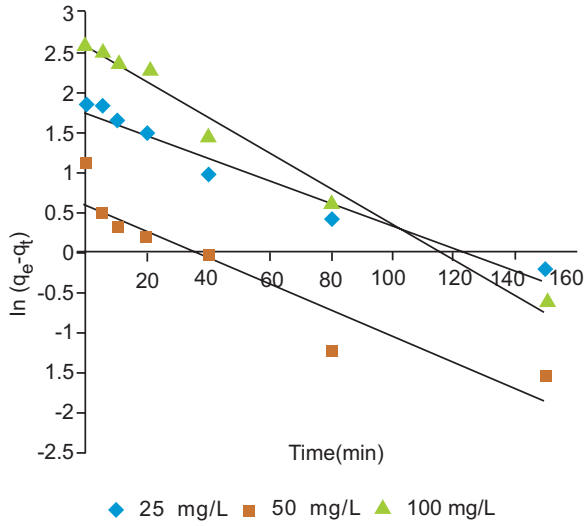

Fig. 3. First order kinetics for biosorption of Cr (VI) ion by *A. niger* at 25 °C.

Pseudo second order equation is based on equilibrium adsorption (Malik, 2004) and expressed as:

$$^t/q_t = {}^1/ k_2 q_e^2 + (^1/q_t)t \quad \text{.....(4)}$$

where:

k_2 (g/mg/s) is the rate constant of second-order adsorption. If second-order kinetics is applicable, the plot of t/q versus t should show a linear relationship. There is no need to know any parameter beforehand and q_e and k_2 can be determined from the slope and intercept of the plot. Also, this procedure is more likely to predict the behaviour over the whole range of adsorption. The linear plots of t/q versus t (Fig. 4) show a good agreement between experimental and calculated q_e values (Table 2). The correlation coefficients for the second-order kinetic model are about 0.9 indicating the applicability of this kinetic equation and the second-order nature of the biosorption process of Cr (VI) ion on the adsorbent.

Test of kinetic models. Besides the value of R^2, the applicability of both kinetic models are verified through the sum of error squares (SSE, %). The biosorption kinetics of Cr (VI) ion on adsorbent prepared from *A. niger* was tested at different initial concentrations. The validity of each model was determined by the sum of error squares (SSE, %) given by:

$$SSE(\%) = \sqrt{\frac{\Sigma(q_{e.exp} - q_{e.cal})^2}{N}} \quad \text{.....(5)}$$

where:

N is the number of data points. The higher is the value of R^2 and the lower is the value of SSE; the better will be the goodness of fit. Table 2 lists the calculated results.

Table 2. Comparison of the pseudo first- and second-order adsorption rate constants and calculated and experimental q_e values for different initial concentrations of Cr (VI) ions

Initial concentration (mg/L)	First order kinetic model					Second order kinetic model			
	$q_{e\ exp}$ (mg/g)	k_1 (1/min)	$q_{e\ cal}$ (mg/g)	R^2	SSE (%)	k_2 g (mg/min)	$q_{e\ cal}$ (mg/g)	R^2	SSE (%)
25	3.02	0.016	1.86	0.88	0.60	0.035	3.07	0.99	0.48
50	6.21	0.014	5.76	0.96	0.75	0.0035	7.19	0.92	0.35
100	13.68	0.02	13.13	0.99	0.37	0.0014	16.95	0.92	0.42

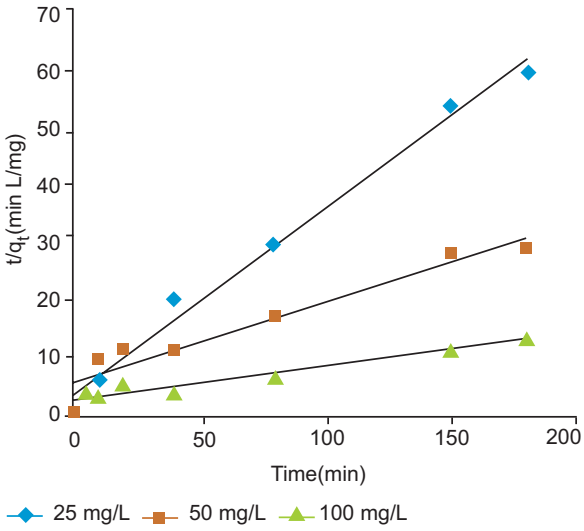

Fig. 4. Pseudo second order kinetics for the biosorption of Cr (VI) ion by *A. niger* at 25 °C.

It is found that the biosorption kinetics of Cr (VI) ion on adsorbent prepared from *A. niger* can be best described by the pseudo second order kinetic model.

Adsorption isotherms. The adsorption isotherm indicates how the biosorbed molecules are distributed between the liquid phase and the biosorbent phase when the biosorption process is at equilibrium state. The analysis of equilibrium adsorption data by fitting them to different isotherm models is an important step to find the suitable model that can be used for design purpose (Haghseresht and Lu, 1998). Biosorption isotherm study is carried out on two well-known isotherms, Langmuir and Freundlich. Langmuir isotherm assumes monolayer adsorption onto a surface containing a finite number of adsorption sites of uniform strategies with no transmigration of adsorbate in the plane of surface (Fytianos *et al.,* 2003). While, Freundlich isotherm model assumes heterogeneous surface energies, in which the energy term in Langmuir equation varies as a function of the surface coverage (Fytianos *et al.,* 2003). The applicability of the isotherm equation is compared by judging the correlation coefficients R^2.

Langmuir isotherm. The linear form of Langmuir's isotherm model is given by the following equation:

$$C_e/q_e = {}^1/_{Q_o b} + ({}^1/_{Q_o})C_e \quad \text{.....(6)}$$

where:

C_e is the equilibrium concentration of the adsorbate (Cr (VI) ion) (mg/L), q_e, the amount of adsorbate adsorbed per unit mass of adsorbate (mg/g), and Q_o and b are Langmuir constants related to monolayer adsorption capacity and affinity of adsorbent towards adsorbate, respectively. When C_e/q_e was plotted against C_e, straight line with slope $1/Q_o$ was obtained (Fig. 5), indicating that the biosorption of the Cr (VI) ion by *A. niger* follows the Langmuir isotherm. The Langmuir constants 'b' and 'Q_o' were calculated from this isotherm and their values are given in Table 3.

Confirmation of the experimental data into Langmuir isotherm model indicates the homogeneous nature of *A. niger* surface, i.e., each Cr (VI) ion molecule / *A. niger* biosorption has equal adsorption activation energy.

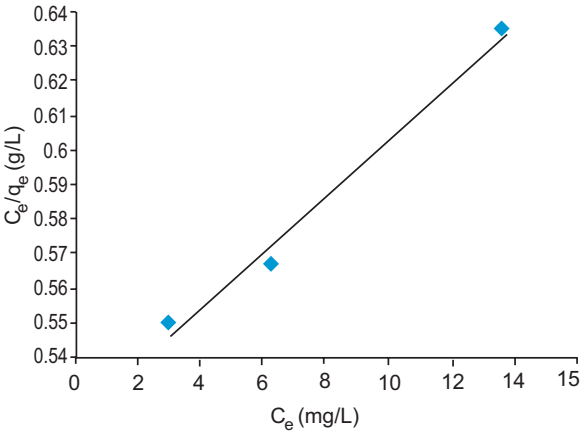

Fig. 5. Langmuir adsorption for the biosorption of Cr (VI) ion on *A. niger* at 25 °C.

Table 3. Langmuir and Freundlich isotherm constants for Cr (VI) ions at 25 °C

Langmuir isotherm	
Q_o (mg/g)	125
b (L/mg)	0.004
R^2	0.99
RL	0.24
Freundlich isotherm	
1/n	1.08
K_F [(mg/g) (1/mg)1/n]	0.42
R^2	0.9

The essential characteristics of the Langmuir isotherm can be expressed in terms of a dimensionless equilibrium parameter (R_L) (Weber and Chakkravorti, 1974), which is defined by:

$$R_L = {}^1/(1 + b\ C_o) \quad \text{.....(7)}$$

where, b is the Langmuir constant and C_o the highest metal concentration (mg/L). The value of R_L indicates the type of the isotherm to be either unfavourable ($R_L > 1$), linear ($R_L = 1$), favourable ($0 < R_L < 1$) or irreversible ($R_L = 0$). Value of R_L was found to be 0.24 and confirmed that the biosorbent prepared from *A. niger* is favourable for biosorption of Cr (VI) ion under conditions used in this study.

Freundlich isotherm. The well-known logarithmic form of Freundlich model is given by the following equation:

$$\text{Log } q_e = \log K_F + ({}^1/n) \log C_e \quad \text{.....(8)}$$

where, q_e is the amount adsorbed at equilibrium (mg/g), C_e the equilibrium concentration of the adsorbate Cr (VI) ion and K_F and n are Freundlich constants, n giving an indication of how favourable the adsorption process and K_F ($mg/g(L/mg)^n$) is the adsorption capacity of the adsorbent. K_F can be defined as the adsorption or distribution coefficient and represents the quantity of Cr (VI) ion adsorbed onto biosorbent (*A. niger*) for a unit equilibrium concentration.

The slope 1/n ranging between 0 and 1 is a measure of adsorption intensity or surface heterogeneity, becoming more heterogeneous as its value gets closer to zero (Haghseresht and Lu, 1998). A value for 1/n below one indicates a normal Langmuir isotherm while 1/n above one is indicative of cooperative adsorption (Fytianos *et al.,* 2003). The plot of log q_e versus log C_e gives straight lines with slope '1/n' (Fig. 6), which shows that the biosorption of Cr (VI) ion does not fitted well with the Freundlich isotherm. Accordingly, Freundlich constants (K_F and n) were calculated and presented in Table 3.

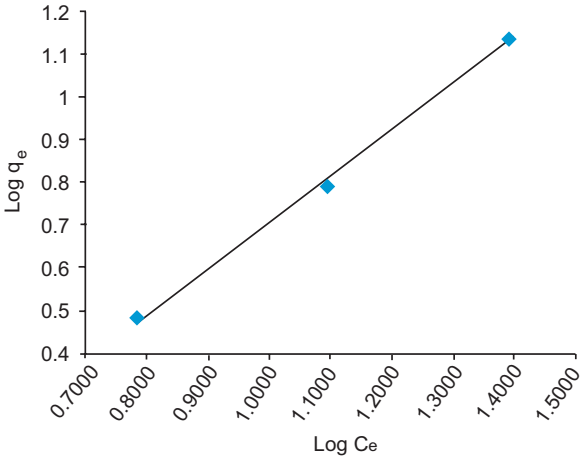

Fig. 6. Freundlich adsorption isotherm for the biosorption of Cr (VI) ion at 25 °C.

Table 3 shows the values of the parameters of the two isotherms and related correlation coefficients. As seen from Table 3, the Langmuir model yields a somewhat better fit ($R^2 = 0.99$) than the Freundlich model ($R^2 = 0.9$). As also illustrated in Table 3, the value of 1/n is 1.08, which indicates favourable adsorption (Adamson, 1990).

Conclusion

The present investigation shows that *A. niger* can be effectively used as a material for biological removal of Cr (VI) ion from aqueous solution over a wide range of concentration and pH. Biosorption behaviour is described by a monolayer Langmuir type isotherm. Kinetic data follows pseudo second order kinetic model. The value of the maximum adsorption capacity, Q_o, (125 mg/g) is comparable with the values observed for other biosorbent reported in earlier studies.

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