

## Full Paper

# Bioaccumulation and biosorption of chromium by *Aspergillus niger* MTCC 2594

John Geraldine Sandana Mala,<sup>1</sup> Balachandran Unni Nair,<sup>2</sup> and Rengarajulu Puvanakrishnan<sup>1,\*</sup>

<sup>1</sup> Department of Biotechnology and <sup>2</sup> Chemical Laboratory, Central Leather Research Institute, Chennai-600 020, India

(Received August 26, 2005; Accepted April 17, 2006)

Chromium toxicity is of prime concern due to chrome tanning processes in the leather sector. Chrome tanning results in the discharge of toxic levels of chromium causing pollution hazards. Chromium levels of Cr(III) and Cr(VI) were high above permissible limits in chrome samples after chrome tanning. The potential of *Aspergillus niger* MTCC 2594 to accumulate chromium as well as its biosorption capacity is investigated in this study. Bioaccumulation of Cr(III) and Cr(VI) in the spent chrome liquor has resulted in a 75–78% reduction of the initial Cr content in 24–36 h. *A. niger* biomass is found to be very effective in the biosorption of Cr(III) and Cr(VI) in spent chrome liquor. Maximum adsorption of 83% for biosorption of Cr(III) at 48 h and 79% of Cr(VI) at 36 h in spent chrome liquor is observed. The biosorption characteristics fit well with Langmuir and Freundlich isotherms and the adsorption parameters are evaluated. The biosorption of Cr also follows Lagergren kinetics. *A. niger* biomass is effectively used for the biosorption of chromium with 79–83% Cr removal in 36–48 h.

**Key Words**—*Aspergillus niger*; bioaccumulation; biosorption; chrome liquor; chrome tanning; chromium

## Introduction

Chromium toxicity is one of the major causes of environmental pollution emanating from tannery effluents. Chromium is used in the tanning of hides and skins, as an alloy in the manufacture of stainless steel, in electroplating, in textile dyeing and as a biocide in the cooling waters of nuclear power plants, invariably resulting in chromium discharge causing environmental concerns (Bai and Abraham, 2001). Chromium toxicity has deleterious effects in vivo due to carcinogenic,

mutagenic and teratogenic potential and it also results in tissue damage (Dartsch et al., 1998). Chromium exists in several oxidation states (I–VI), more stable as Cr(III) and Cr(VI). Cr(VI) is the toxic form of the element (Baig et al., 2003). Cr(III) is also toxic at higher concentrations (Shrivastava and Nair, 2001). The permissible limits of total chromium in tannery effluents is between 1 and 2 mg/L according to USA, UK and Indian Standards (Buljan, 1996). Chromium removal, to reduce the toxicity, is generally by chromium recovery (Aravindhan et al., 2004; Money, 1999), bioaccumulation, chromate reduction, chromate efflux (Cervantes et al., 2001), ion-exchange, coordination, complexation (Bai and Abraham, 2001) and biosorption (Volesky and Schiewer, 1999). Spent chrome liquor is taken up for this study since onsite treatment is suggested for metal contaminants rather than the mainstream efflu-

\* Address reprint requests to: Dr. Rengarajulu Puvanakrishnan, Department of Biotechnology, Central Leather Research Institute, Adyar, Chennai-600 020, India.

Tel : 91–44–24430273 Fax : 91–44–2491 1589

E-mail: puvanakrishnan@yahoo.com

ents (Volesky and Schiewer, 1999).

Biosorption is widely used for metal removal from industrial effluents and subsequent recovery (Vieira and Volesky, 2000). The microbial biomass has an inherent capacity to adsorb metals due to the presence of functional groups such as  $-NH_2$ ,  $-COOH$ ,  $-SH$ , and  $-OH$  on microbial cell walls, which act as binding sites for interaction of metal ions (Kuyucak and Volesky, 1998). The microbial biosorbents can be specific for metal or have no specific priority. The microbial biomass can be from fungi, yeast, bacteria as by-product biomass or from marine algae and seaweeds (Volesky, 1994). In this report, a strain of *Aspergillus niger* MTCC 2594 isolated in our laboratory for lipase production (Geraldine, 1999) is tested for its ability to accumulate Cr and for the biosorption of chromium from spent chrome liquor after chrome tanning of hides and skins. The biosorption of spent chrome liquor by *A. niger* is also characterized for its adsorption isotherms.

## Materials and Methods

**Preparation of biomass.** *Aspergillus niger* MTCC 2594 was used for biosorption of chromium. *Aspergillus niger* biomass was harvested at 72 h at room temperature (28–32°C) from Czapek yeast autolysate medium (Atlas, 1993) modified suitably as a Basal salt medium (BSM): 0.3%  $NaNO_3$ ; 0.05% KCl; 0.05%  $MgSO_4$ ; 1% yeast extract; 0.1%  $KH_2PO_4$ ; 0.3%  $Na_2HPO_4$  (w/v). Sucrose was eliminated from the medium composition due to overgrowth and lysis of the biomass. The biomass was washed twice with distilled water and used for biosorption studies.

**Estimation of chromium.** The Cr(III) content in spent chrome liquor was estimated according to Haupt (1952).

Twenty milliliters of chromium solution was neutralized using 1 M NaOH till alkaline pH ~9.0. One milliliter of 30% (v/v)  $H_2O_2$  was added and the mixture was digested over a hot plate until excess  $H_2O_2$  was removed. The solution was cooled and made up to a known volume. The absorbance of the solution was measured at 372 nm using a water blank. The initial concentration of the Cr(III) solution was calculated assuming a molar absorption coefficient ( $\epsilon$ ) of  $4.82 \times 10^3$  M/cm in 1 M NaOH and after providing due allowances for dilution factors.

$A = \epsilon lc$ , where,

$A$  = absorbance at 372 nm

$l$  = path length

$c$  = concentration of the sample (M)

The Cr(VI) content in spent chrome liquor was estimated according to Clesceri et al. (1998).

Nought point five–1.0 ml chromium solutions were made up to 1.0 ml with water. One hundred microliters of 0.2 N  $H_2SO_4$  was added to pH  $1.0 \pm 0.3$  followed by 2.0 ml of 0.5% (w/v) 1,5-diphenylcarbazide. The absorbance of the reaction mixture was measured at 540 nm using a reagent blank. Absorbance of samples made up to appropriately 1.0 ml was also measured at 540 nm and the difference in the readings was subsequently calculated for Cr(VI) using a  $K_2Cr_2O_7$  calibration graph.

**Estimation of chromium in chrome tanning process.** The Cr(III) and Cr(VI) levels were estimated in samples of spent chrome liquor and chrome effluent to assess the toxicity levels.

**Bioaccumulation of chromium.** One milliliter of spent chrome liquor was added to 50 ml of Basal salt medium (BSM) in 250 ml Erlenmeyer flasks. Before addition, spent chrome liquor was filter-sterilized and added to BSM due to precipitation of chromium hydroxide during autoclaving. Two hundred fifty microliters of spore inoculum (0.5%) was added to initiate growth and incubated at room temperature (28–32°C). The Cr(III) and Cr(VI) contents in the medium were estimated at 0, 12, 24, 36 h time periods. The experiments were carried out in triplicate and the results were expressed as the mean of best two data sets.

**Biosorption of chromium in spent chrome liquor.** Twenty milliliters of spent chrome liquor was taken and 2 g (10% w/v) of *A. niger* biomass was added and incubated at room temperature (28–32°C) for 48 h. The samples were estimated for Cr(III) and Cr(VI) at 12 h intervals. The experiments were carried out in triplicate and the results were expressed as the mean of best two data sets.

**Biosorption isotherms.** The biosorption of spent chrome liquor was characterized using Langmuir and Freundlich isotherm models (Bai and Abraham, 2001).

**Langmuir's isotherm.**

$$q = q_e \frac{bC_i}{(1 + bC_i)}$$

$q$ =metal uptake,  $\mu\text{g/g}$

$q_e$ =metal uptake at equilibrium concentration,  $\mu\text{g/g}$

$C_i$ =metal ion concentration at equilibrium,  $\mu\text{g/ml}$

$b$ =Langmuir's constant related to energy of adsorption

The linearized equation for the Langmuir's isotherm is

$$1/q = 1/q_e + 1/b q_e \times 1/C$$

The Langmuir's constant  $b$  was calculated from the initial slope of the linear plot of  $1/q$  vs.  $1/C$ . The plot was obtained using Origin Software V.7.0.

*Freundlich's isotherm.*

$$q_e = K_F C_e^{1/n}$$

$q_e$ =metal uptake at equilibrium concentration,  $\mu\text{g/g}$

$C_e$ =equilibrium metal ion concentration,  $\mu\text{g/ml}$

$K_F$ =Freundlich's constant of adsorption capacity

$n$ =Freundlich's constant of adsorption intensity

The logarithmic equation for Freundlich's isotherm is

$$\ln q = \ln K + 1/n \ln C$$

The Freundlich's constants,  $K_F$  and  $n$  were calculated from the linear plot of  $\ln q$  vs.  $\ln C$ . The  $K_F$  was estimated from the  $Y$ -intercept and  $n$  was calculated from the slope. The plot was obtained using Origin Software V.7.0.

*Lagergren kinetics of biosorption.* The Lagergren plot for determining the kinetics of biosorption was evaluated according to Bai and Abraham (2001).

$$\log(q_e - qt) = \log q_e - \frac{k_{\text{ads}} t}{2.303}$$

$k_{\text{ads}}$ =rate constant of Lagergren kinetics,  $\text{g/h}$

$q_e$ =metal uptake at equilibrium concentration,  $\mu\text{g/g}$

$qt$ =metal uptake at time  $t$ ,  $\mu\text{g/g}$

The rate constant  $k_{\text{ads}}$  was calculated from slope of the

linear plot of  $-\log(q_e - qt)$  vs. time. The plot was obtained using Origin Software V.7.0.

## Results

### *Chromium levels in chrome tanning*

The Cr(III) and Cr(VI) levels in spent chrome liquor and chrome effluent are shown in Table 1. The amount of Cr(III) and Cr(VI) was found to be high above the permissible limits up to the order of 1.5 g/ml of Cr(III) and the spent chrome liquor showed the highest toxic levels of Cr(III) and Cr(VI).

### *Bioaccumulation of chromium*

Bioaccumulation of Cr by *Aspergillus niger* was found to be effective in the range of 500–800 mg Cr(III) at the end of 24 h and up to 2.8 mg Cr(VI) at 36 h. The *Aspergillus niger* strain was found to accumulate 180.3 mg Cr(III)/g and 0.6 mg Cr(VI)/g of biomass at 24 h and 36 h (Table 2). Bioaccumulation of Cr(III) and Cr(VI) in spent chrome liquor resulted in 75–78% of the initial Cr content in 24–36 h.

### *Biosorption of chromium in spent chrome liquor*

Maximum Cr(III) was adsorbed with 1.5 g of Cr(III)/ml in spent chrome liquor (Table 3) and 15.0 g of Cr(III)/g biomass at 48 h by *A. niger* biomass (Fig. 1). The biosorption parameters for Cr adsorption in spent chrome liquor indicated that a maximum Cr(III) adsorption of 83.3% was achieved at 48 h and Cr(VI) was adsorbed to the maximum (78.7%) at 36 h (Table 3).

### *Langmuir's adsorption isotherm*

The Langmuir's adsorption constant  $b$  was obtained from the slope of the linear graph (Fig. 2a, b) and it was calculated to be 3.97 and 0.04 for adsorption of Cr(III) and Cr(VI) in spent chrome liquor. The  $R^2$  value was calculated to be 0.89 for Cr(III) and Cr(VI) (Table 4).

Table 1. Chromium content in chrome samples.

Samples	pH	Cr <sup>a</sup> (III)	Cr <sup>a</sup> (VI)
Spent chrome liquor	3.8	1.5±0.83 <sup>b</sup> g/ml	383.7 $\mu\text{g/ml}$
Chrome effluent	4.3	127.0 mg/ml	64.7 $\mu\text{g/ml}$

<sup>a</sup> Chromium metal weight in valence states (III) and (VI) (atomic weight=51.9).

<sup>b</sup> Mean±SD of six different experiments.

Table 2. Bioaccumulation of chromium by *Aspergillus niger* MTCC 2594.

Chromium content	Initial chromium (mg)	Cr accumulated (mg)			Cr (mg/g biomass)		
		12 h	24 h	36 h	12 h	24 h	36 h
Cr <sup>a</sup> (III)	1,071.0	504.9	807.8	760.0	168.3	180.3	163.8
Cr <sup>a</sup> (VI)	3.6	0.1	0.9	2.8	0.03	0.2	0.6

<sup>a</sup>Chromium metal weight in valence states (III) and (VI) (atomic weight=51.9).

Table 3. Biosorption of chromium from spent chrome liquor by *Aspergillus niger* MTCC 2594.

Spent chrome liquor	Initial chromium $C_i$	Residual chromium				$C_f$	$q_e$	Time (h)	Adsorption (%)
		12 h	24 h	36 h	48 h				
Cr <sup>a</sup> (III) g/ml	1.8	1.4	1.1	1.1	0.3	0.3	15.0 g/g	48	83.3
Cr <sup>a</sup> (VI) $\mu$ g/ml	383.7	182.8	134.2	81.6	103.6	81.6	3.0 mg/g	36	78.7

<sup>a</sup>Chromium metal weight in valence states (III) and (VI) (atomic weight=51.9).

$$q_e = \frac{V(C_i - C_f)}{S}$$

$q_e$ =metal uptake at equilibrium, per g.  $V$ =volume of metal solution, ml.  $C_i$ =initial Cr concentration, per ml.  $C_f$ =final Cr concentration, per ml.  $S$ =biosorbent, g.

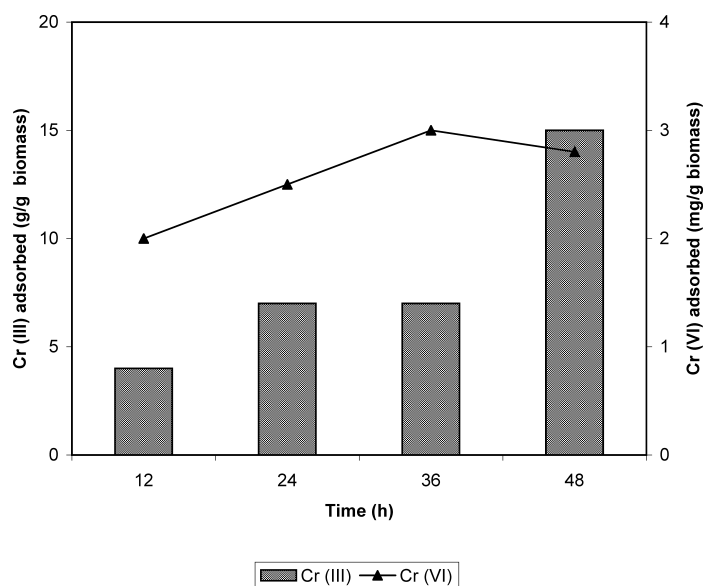


Fig. 1. Biosorption of Cr(III) and Cr(VI) in spent chrome liquor by *Aspergillus niger* MTCC 2594 as a function of time from 0–48 h at 12 h intervals.

#### Freundlich adsorption isotherm

The Freundlich's constants  $K_F$  and  $n$  were derived from the intercept and the slope respectively (Fig. 3a, b). The  $K_F$  values for Cr(III) and Cr(VI) adsorption were calculated to be 7.26 and 3.32 respectively. The inten-

sity of adsorption  $n$  was calculated to be 1.56 and 1.51 for adsorption of Cr(III) and Cr(VI) in spent chrome liquor. The  $R^2$  values were 0.99 and 0.97 for Cr(III) and Cr(VI) respectively (Table 4).

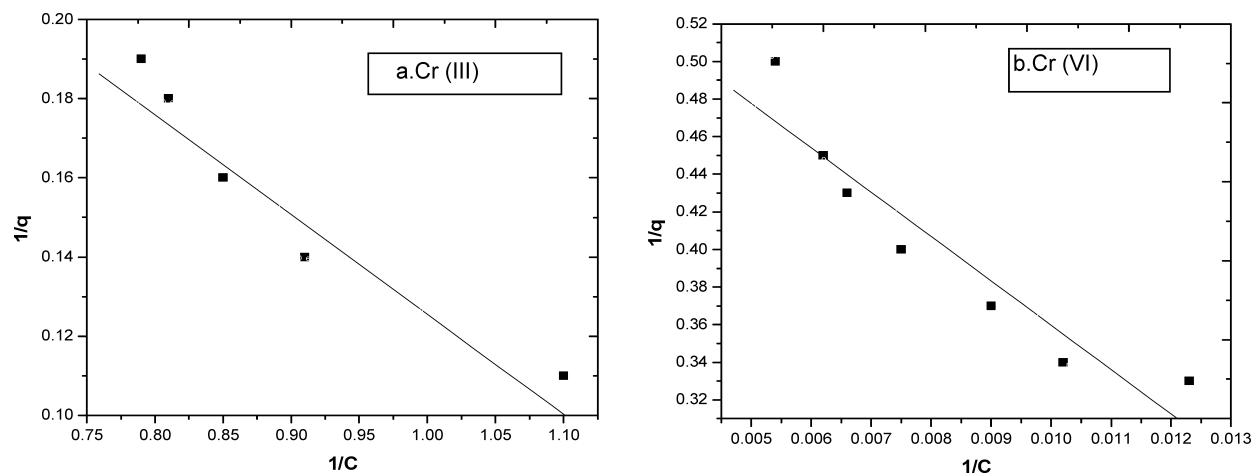


Fig. 2. Langmuir isotherm for chromium biosorption in spent chrome liquor.

a, Cr(III); b, Cr(VI). The Langmuir isotherm model was obtained by the linear plot of  $1/C$  vs.  $1/q$  of the respective chromium concentrations and metal uptake. The Langmuir constant  $b$  was calculated from the initial slope.

Table 4. Langmuir and Freundlich adsorption isotherm parameters for biosorption of spent chrome liquor by *Aspergillus niger* MTCC 2594.

Chromium samples	Correlation of deviation ( $R^2$ )		Langmuir constant	Freundlich constants	
	Langmuir isotherm	Freundlich isotherm	$b^a$	$K_F^b$	$n^c$
Chrome liquor Cr (III)	0.89	0.99	3.97	7.26	1.56
Chrome liquor Cr (VI)	0.89	0.97	0.04	3.32	1.51

<sup>a</sup>  $b$ =energy of adsorption, calculated from slope of  $1/q$  vs.  $1/C$ .

<sup>b</sup>  $K_F$ =adsorption capacity, calculated from Y-intercept of  $\ln q$  vs.  $\ln C$ .

<sup>c</sup>  $n$ =adsorption intensity, calculated from slope of  $\ln q$  vs.  $\ln C$ .

### Lagergren kinetics

The rate constant  $k_{ads}$  was calculated from the slope of the linear graph (Fig. 4a, b). The  $k_{ads}$  values were determined to be 0.01 g/h and  $0.03 \times 10^{-3}$  g/h for the adsorption of Cr(III) and Cr(VI) in spent chrome liquor.

### Discussion

Metals are indispensable in electroplating and alloys and as mineral ores in metallurgy. Metals such as Cr, Mn, Fe, Cu, Mo and Zn are also required as trace elements in biological systems (Nies, 1999). All microbes whether prokaryotic or eukaryotic use metals for structural and/or catalytic functions (Ehrlich, 1997). Increased metal levels require proper treatment to reduce the pollution risks (Gadd and White, 1993). Microbes are recognized as potent sources for pollution abatement (Sullia, 2005). Varghese and Lesitha (2004)

have reported a biotechnological method of using the metal munching capacities of microbes in arsenic heavy metal pollution.

Bioaccumulation of Cr by *A. niger* is studied for effective utilization of Cr as trace element. *A. niger* is found to accumulate Cr(III) and Cr(VI) to an appreciably higher extent at 24 h for Cr(III) and 36 h for Cr(VI). It is possible that chromate reduction could occur (Aoyama, 2003). However, the rate of chromate reduction strongly depends on the pH of the solution. Significant chromate reduction takes place only at acidic pH. At  $pH > 5$ , the rate of chromate reduction is very slow and negligible. The observation of this study showing an uptake of chromium levels with maximum bioaccumulation of 17.5 mmol Cr(III)/g and 60.0  $\mu$ mol Cr(VI)/g of dry cell weight (Table 2) is found to be higher than that reported for a recombinant *E.coli* sp. with 15  $\mu$ mol/g dry weight for Ni(II) bioaccumulation (Krish-

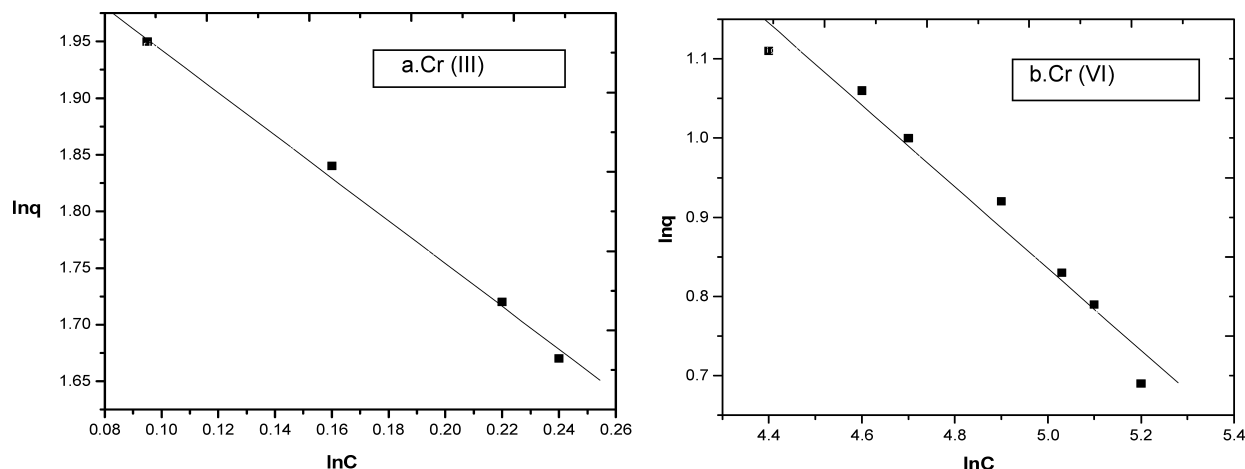


Fig. 3. Freundlich isotherm for chromium biosorption in spent chrome liquor.

a, Cr(III); b, Cr(VI). The Freundlich isotherm model was obtained by the linear plot of  $\ln C$  vs.  $\ln q$  of the respective chromium concentrations and metal uptake. The Freundlich constants  $K_F$  and  $n$  were calculated from the Y-intercept and the initial slope respectively.

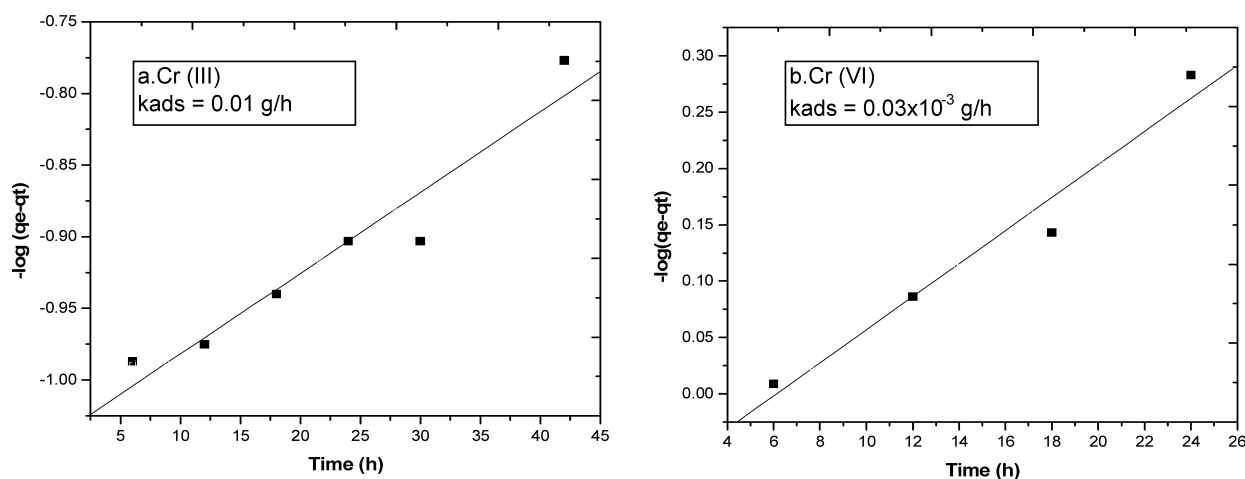


Fig. 4. Lagergren plot for chromium biosorption in spent chrome liquor.

a, Cr(III); b, Cr(VI). The Lagergren plot for the kinetics of biosorption was obtained by the linear plot of  $-\log(q_e - q_t)$  vs. time. The rate constant  $k_{ads}$  was calculated from the initial slope and multiplied by 2.303.

naswamy and Wilson, 2000).

Biosorption is an inexpensive process for sequestering toxic metals by passive adsorption using microbial biosorbents from algae, moss, fungi, or bacteria (Kratohvil and Volesky, 1998). The phenomenon of metal biosorption by microorganisms has been thoroughly documented (McHale and McHale, 1994). Biosorption using microbial biomass has been reported for different metal ions such as Pb (Lo et al., 1999), Zn (Taniguchi et al., 2000), Cd and Cu (Ozdemir et al., 2003) and Ni (Mogollon et al., 1998). Chromium toxicity in the tanning industries is well-documented and requires intensive studies to overcome this pollution

load. The risk assessments of Cr in health and environmental effects is also described by Money (1999). Biosorption of chromium has earlier been reported using bacteria (Srinath et al., 2002), fungi (Dais et al., 2002) and algae (Hamdy, 2000), but with lesser impact in chrome tanning. Here, we report the biosorption of chromium with maximum biosorption of 1.5 g/ml Cr(III) at 48 h and 302.0  $\mu\text{g/ml}$  Cr(VI) at 36 h (Table 3) up to 79–83% with spent chrome liquor using *Aspergillus niger*.

*A. niger* is reported to be a safe organism by Occupational Health and Safety Organizations (Schuster et al., 2002). The Cr(III) and Cr(VI) in chrome samples

are estimated to be high above permissible limits. The biosorption capacity of *A. niger* is tested for Cr adsorption from spent chrome liquor. The results obtained are favorable with 83% biosorption of Cr(III) at 48 h and 79% biosorption of Cr(VI) at 36 h. The biosorption level of Cr(III) has reached equilibrium at 48 h with rapid biosorption between 36 h and 48 h, and no further biosorption of Cr(III) is observed. This explains an initial lag phase till 24 h followed by complexation of all binding sites with Cr(III) between 36–48 h. Cr(VI) is adsorbed to a lesser extent, probably due to the biosorption of Cr(III) complexed with the binding sites in the biomass. The rate of adsorption is a function of initial concentration of the metal ion. At lower concentrations, all metal ions interact with the binding sites on the biomass cell surface. Higher initial Cr concentration is adsorbed to maximum extent up to saturation of the available binding sites (Bai and Abraham, 2001).

Rao et al. (2005) have reported the biosorption of Cu, Cd, Co and Ni using *Aspergillus fumigatus* biomass from fermentative waste, showing 72% sequestration with  $\text{Cu}^{2+}$ , 61%  $\text{Cd}^{2+}$ , 49%  $\text{Co}^{2+}$  and 37%  $\text{Ni}^{2+}$ . We report 79–83% efficiency of biosorption of chromium with *Aspergillus niger* biomass. The Freundlich isotherm has resulted in a better fit than the Langmuir model, with  $R^2$  values of 0.99 for Cr(III) and 0.97 for Cr(VI). The Freundlich and Langmuir isotherm models implicate heterogeneous and homogeneous processes of biosorption respectively. The Freundlich isotherm of heterogeneous process depends upon two or more factors, the sorptivity of the biomass and the concentration of chromium, while the Langmuir homogeneous adsorption depends only on the sorptivity of biomass. Since, in the present case, both physical adsorption of Cr to the surface of *Aspergillus niger* as well as complexation of Cr to the functional groups of the cell wall are taking place simultaneously, the process of binding of Cr to *Aspergillus niger* is a heterogeneous process. Hence, it is not surprising that the process is better described by the Freundlich model. The biosorption of Cr also follows Lagergren kinetics. However, as the rate of adsorption of Cr(VI) has increased with time up to 24 h and later, this has resulted in deviation of the Lagergren plot.

It is suggested that on-site treatment of effluents would be more efficient for elimination of metals than treating them in a general sewage plant. Large-scale processes require a continuous flow column for biosorption of metals, regeneration of biosorbent, recy-

cling of the metal solution and subsequent metal recovery (Volesky and Schiewer, 1999). In conclusion, *Aspergillus niger* MTCC 2594 is effectively used in the removal of up to 79–83% of chromium from spent chrome liquor at 48 h at room temperature (28–32 °C), by a simple, economic biosorption process.

### Acknowledgments

The authors thank Dr. T. Ramasami, Director, CLRI, Chennai, India for his kind permission to publish this work. The financial assistance extended by the Council of Scientific and Industrial Research, New Delhi, India, to J. Geraldine Sandana Mala is gratefully acknowledged.

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