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Research Article

Modeling of Biosorption of Chromium by Immobilized Whole Cells of *Aspergillus awamori* NRRL 3112

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Abstract

The presence of chromium in high concentrations in industrial effluents is of major concern, being extremely toxic and non-biodegradable, thus persistent and harmful to the ecosystem. Using microorganisms for adsorption of heavy metals has emerged as a potential alternative over the conventional methods and is gaining significance due to its incontestable merits. Since fungi amass metals more than their nutritional requirement, the present study in investigation of efficiency of *Aspergillus awamori* NRRL 3112 biomass for sorption of chromium by means of development of an immobilised whole cell system is significant. The effect of operational parameters like initial chromium concentration, time, temperature and pH on chromium removal was studied and the efficient conditions for the process were established. Kinetics studies revealed that, the pseudo second order model was found to best represent the process with higher R^2 values than others. The Langmuir and Freundlich isotherms were applied and the Langmuir isotherm was found to be the best fit. It was also established by means of thermodynamic analysis that the biosorption occurred chemically and the process was more feasible, spontaneous and efficient at lower temperatures.

Keywords: biosorption; chromium; tannery effluent; immobilisation; fungi

Abbreviations

- APHA - Standard methods for examination of water and waste water, 20th Edn. (Washington), 1998, p.145
- BOD - Biological oxygen demand
- COD - Chemical oxygen demand
- TDS - Total dissolved solids
- TSS - Total suspended solids

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Introduction

Rapid urbanisation, industrialisation and escalating population has led to increased disposal of toxic heavy metals into air, soil and water, and thus has resulted in cumulative hazardous effect on the environment due to their potential of bioaccumulation and biomagnification. Among all industrial wastes, tannery effluents are considered the highest pollutants, due to their enormous pollution load and toxic natures of waste water released, and are also the largest contributors of chromium (Cr) into our water ecosystem. The chrome tanning process produces spent liquors containing significant amounts of Cr because of poor or inefficient uptake; from the total Cr used for tanning only 60 to 70 percent is utilized, while the rest 30 to 40 percent remains in the spent tanning liquor unused (Belay 2010). For instance, in India alone about 2000–3000 tonne of Cr escapes into the environment annually from tannery processing industries, by means of aqueous effluent with chromium concentrations ranging between 2000 and 5000 mg.L⁻¹ (Altaf et al. 2008). Though, cleaner technologies like high exhaustion process, direct or indirect recycling, used to reduce Cr in waste water are implemented, they cannot eliminate it completely, 500-1000 mg.L⁻¹ of Cr still remains, in the effluent coming from post tanning section (Aravindhan et al. 2004). The maximum permissible levels of Cr in potable and industrial waste waters are 0.05 mg.L⁻¹ and 0.1 mg.L⁻¹ respectively (Srivastava and Thakur 2006) and even the slightest increase can cause health hazards to all forms of life.

This inefficient use of Cr and its release into the environment needs to be compensated by an efficient recovery and recycling scheme thereby providing a significant economic advantage in terms of its reuse and the simplification of the processing of wastewaters. The conventional methods for Cr removal include chemical precipitation, electrochemical treatment, evaporation, ion exchange, membrane processing and solvent extraction (Secil et al. 2007). The application of these treatment processes are limited as these technologies are

complicated, expensive, energy intensive, provide incomplete removal of chromium ions, offer low selectivity i.e. applicable only to a specific region and for a small range of initial metal ion concentrations and the potential for generation of secondary pollutants (Brown 1999; Kratovchil et al. 1998; Volesky and Holzen 1995). Thus, the search for new effective and economical technologies for the removal of toxic metals from waste waters has directed the attention towards adsorption and the most widely used adsorbent being activated carbon (Nadeem et al. 2006).

So, in the continuous exploration for economic and eco-friendly approach for heavy metal removal, biosorption, a biological method of environmental remediation, has emerged as a promising alternative to conventional waste treatment processes. Also, the extensive applicability of such a process depends upon the economics, that necessitates the biological material used to be naturally available, involve less processing and available abundant in nature (Niveditha et al. 2014) and thus, the biotechnological application of microorganisms was tested in this context. Biosorption is a physiochemical process that occurs naturally in certain microorganisms which allows it to passively concentrate and bind contaminants onto its cellular structure by the process of complexation, ion exchange and micro precipitation (Chhikara and Dhankhar 2008). Its advantages, such as reusability, low operation costs, improved selectivity for specific metals of interest and comparatively low production of secondary pollutants (Ahalya et al. 2007), serve as potential incentives for promoting biosorption as a viable clean up technology for heavy metal pollution. Microbial cell, live or dead can be highly efficient bioaccumulator of both soluble and particulate forms of metal as the cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures, which gives them the ability to effectively bind with metal cations (Srivastava and Thakur 2006).

Since microbial biomass consists of small particles with low density and poor mechanical

strength, contacting large volumes of metal-bearing aqueous solution poses a certain impracticality that can be overcome by immobilising the biomass in a non-toxic solid structure (Veglio and Beolchini 1997) thus creating a material with physical properties desired for large scale operation. Among a variety of microorganisms, fungi accumulate metals into itself higher than their nutritional requirement (Bishnoi and Garima 2005) and hence the potential for usage of fungal biomass as biosorbent has been recognised for removal of heavy metals from industrial waste water. Although an array of fungal species have been tested for their potential to adsorb heavy metals, the testing conditions like the adsorption medium, which is expensive and thus not cost effective in the treatment of large waste water volumes, the initial metal concentrations and its range often have been laboratory based, and the results obtained cannot be expected to replicate itself when employed real time on industrial effluents, as there are many other influencing factors present. In light of these observations, the aim of the present work was to investigate the suitability and efficiency of the mould fungus, *Aspergillus awamori* NRRL 3112 for Cr removal from tannery wastewaters. Batch experiments were carried out and the effects of different parameters on the biosorption of chromium were studied. Equilibrium isotherms, kinetic studies and thermodynamic parameters were also investigated.

Materials and Methods

Microorganism and inoculum development. *Aspergillus awamori* NRRL 3112 donated by Northern Regional Research Laboratory, Illinois, USA was used throughout this work. It was grown on potato dextrose agar medium and sub cultured fortnightly. For growth in the liquid medium, a small amount of microorganism was scraped carefully from the slants and transferred aseptically to a 250 ml Erlenmeyer flask with 100 ml of the sterilised media containing (g.L⁻¹): maltose, 30; sodium nitrate, 2; potassium

phosphate, 1; potassium chloride, 5; magnesium sulphate heptahydrate, 0.5; ferrous sulphate heptahydrate, 0.01. It was then incubated for 72 hours at 120 rpm and 25°C. After 72 hours, it was refrigerated at 4±1°C for further use.

Immobilization procedure. The fully grown cells were separated from the growth medium by centrifuging at 500 rpm for 20 min and then suspended in sterile water and agitated using glass beads to achieve uniform cell distribution. Three percent (w/v) sodium alginate was dissolved in distilled water and then the cell suspension was added to it. The resulting mixture was then dropped into three percent (w/v) calcium chloride solution, and the beads thus formed were incubated at room temperature for 2 hours and then subjected to glutaraldehyde solution treatment, - 8 ml of 25 percent glutaraldehyde made up to 100 ml using distilled water, for a period of 24 hours at 25°C, after which they were thoroughly washed with 0.1 M phosphate buffer solution, and stored in calcium chloride at 4±1°C for further use. All solutions were sterilised by autoclaving at 121°C and 20 min before use, and all operations were performed in aseptic conditions.

Medium for biosorption studies. The effluent collected from a leather processing factory, in Ranipet, Tamil Nadu was used as the study medium throughout. The physiochemical properties of the obtained effluent such as its colour, odour, pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Chlorides, Sulphides, chromium and other heavy metals was determined as per procedure outlined by APHA (Eaton et al. 2005).

Estimation of chromium. Chromium analysis for each sample obtained was carried out by spectrophotometric method using 1,5-Di-phenyl carbazide according to the procedure outlined by APHA 2005 (Eaton et al. 2005). The Cr (VI) was determined colorimetrically by reaction

with diphenyl carbazide in acid solution. A red-violet colour was produced. The reaction was very sensitive and the absorptivity based on chromium being about $40000 \text{ lg}^{-1} \text{ cm}^{-1}$ at 540 nm wavelength was determined.

Batch adsorption studies. The effluent obtained from the industry was diluted with distilled water, to obtain solutions of desired initial chromium concentration ranging from 100 - 500 mg.L^{-1} . 100 ml of this solution was taken in 250 ml Erlenmeyer flasks and each was inoculated with 50 calcium alginate beads with immobilised whole cells of *Aspergillus awamori* NRRL 3112 and incubated at 25°C for 120 hours. The effect of time, temperature, initial concentration and pH and their inter-relations were studied, by altering one variable at a time. The extent of chromium adsorption by the immobilised beads was measured in terms of percentage biosorption and calculated by the following expression:

$$\% \text{ Biosorption} = (C_i - C_o) / C_o * 100 \quad (1)$$

where C_o is the initial concentration of chromium and C_i is the concentration of chromium at time t .

Adsorption isotherm studies. An adsorption isotherm describes the interactive relationship between the amount of adsorbate that is adsorbed on the adsorbent and the concentration of dissolved adsorbate in the liquid medium at equilibrium, by means of certain parameters whose values express the surface properties of the sorbent and its affinity to the sorbate. All experiments, for isotherm studies, were carried out in optimum conditions of temperature and pH ascertained by previous batch experiments. The experimental data thus obtained at equilibrium between the amount of chromium adsorbed by the adsorbent (Q_e) and the concentration of chromium left in the solution (C_e) at constant pH and temperature were used to describe the isotherm models - Langmuir and Freundlich, and the best fit was determined by comparing the correlation coefficients' (R^2)

value. The thermodynamic behaviour and spontaneity of biosorption process can be described by using parameters like free energy ΔG° (KJ.mol^{-1}), enthalpy ΔH° (KJ.mol^{-1}) and entropy ΔS° ($\text{KJ.mol}^\circ\text{K}^{-1}$), which were used to describe the thermodynamic behaviour of biosorption of chromium on *Aspergillus awamori* NRRL 3112. The values of thermodynamic parameters, the actual indicators of practical application of the process (Lin et al. 2009), are critical design variables in estimating the performance and predicting the mechanism of an adsorption separation process and are also one of the basic requirements for characterisation and optimisation of an adsorption process.

Biosorption kinetic studies. Kinetic models have been exploited to test the experimental data and to find the kinetic mechanism of adsorption and its potential rate-controlling step that include mass transport and chemical reaction. All experiments, for these studies, were carried out in optimum conditions ascertained by previous experiments and the data obtained were analysed for Langergren Pseudo-first-order, Ritchie Pseudo-second-order and Weber - Morris or intra particle diffusion kinetic models and the best fit was determined.

Results and Discussion

Characterisation of effluent. The physico-chemical properties of the effluent are listed in table 1 against their maximum permissible limits for industrial effluent discharge recommended by CPCB, 2005-06 (Central Pollution Control Board 2006).

Immobilization study. The potential and selectivity of immobilised systems are higher than the conventional bio-remediation treatment, since high densities of specialised microorganisms are brought in contact with the medium that is to be treated. In the present investigation, biomass was immobilised in a polymeric matrix, by cell entrapment, which was porous enough to allow the diffusion of substrate

into the cells and of cellular product away from the cells (Jianlong and Yi 1999). Alginate, a natural polymer containing mannuronic and glucuronic acid monomers, which have high selectivity for heavy metal ions, was converted into hydrogels via cross linking with divalent calcium cations (Secil et al. 2007).

Table 1. Physico-Chemical properties of the effluent obtained from leather industry

Properties	Type/Value	Permissible limits
Colour	Greenish black	-
Odour	Odourless	-
pH	9.5 - 11.5	5.5 - 9
BOD	120 - 140	30
COD	480 - 510	250
TDS	2260 - 2600	2100
TSS	500 - 550	600
Chlorides	13.2	1000
Sulphates	90 - 120	1000
Chromium	500 - 550	2
Other metals	Trace amounts	NA

All units are in mg/L except pH

Thus, immobilisation with calcium alginate not only created a structure with right physical properties, but also increased to the overall biosorption efficiency of the system. The other advantages of this system include biodegradability, hydrophilicity, presence of carboxylic groups and natural origin (Arica et al. 2003).

The whole cells of the fungus *Aspergillus awamori* NRRL 3112 were entrapped within the calcium alginate beads of diameter 6.5 mm. A cell loading of 0.025 g/bead and 0.0197 g of biomass.g⁻¹ of bead was maintained constant throughout the study. Further, to illustrate the role of biomass in the biosorption of Cr ions from the solution, a comparative biosorptive study involving alginate beads with and without biomass was conducted and found that, the beads with the fungal biomass adsorbed metal ions 52.88 % far more effectively than the ones without biomass, for all initial concentrations, similar to the results obtained in previous studies (Ozdemir et al. 2005; Prakashamet al. 1999; Veglio et al. 2002). The chromium biosorption percentage of the calcium alginate beads without

biomass varied from 45 to 15 percent, with the percentage of chromium ions adsorbed falling drastically for concentrations $\geq 300\text{mg.L}^{-1}$.

Batch adsorption studies

Effect of initial concentration and time.

Aspergillus awamori NRRL 3112 was immobilised in calcium alginate beads and tested for chromium biosorption. Fig.1 depicts the decrease in concentration of chromium in the medium, with time for different initial concentrations of 100, 200, 300, 400 and 500 mg.L⁻¹. Meanwhile, it was observed that the uptake capacity of the immobilised cells (mg.g⁻¹) increased with increase in initial concentration due to the availability of more Cr ions in the solution which aided to overcome all the mass transfer resistances thus increasing the rate at which adsorbate molecules passed from bulk solution to adsorbent (Alkan et al. 2007). A maximum uptake capacity of 399.13 mg.g⁻¹ for initial concentration of 500 mg.L⁻¹ was observed for chromium biosorption by immobilised whole cells of *Aspergillus awamori* NRRL 3112. Further analysis of the results revealed that, an increase in the metal ion concentration from 100 to 500 mg.L⁻¹ resulted in gradual decrease in percentage biosorption by 17 percent which may be due to reduction in ratio of sorptive surface to ion concentration (Chandra et al. 2005; Meena et al. 2004). That is, though the rate at which the ions passed from solution to adsorbent increased with increase in initial concentration, the amount of ions adsorbed with respect to initial concentration of the ions decreased. The maximum adsorptive capacity obtained for the present biosorption system was far superior to the ones investigated earlier (Aksu et al. 2002; Arica and Bayramoglu 2003; Elangovan et al. 2008; Javaid and Bajwa 2010; Malkoc et al. 2006; Mungasavalli et al. 2007; Onyanha et al. 2008; Preetha and Viruthagiri 2007; Sari and Tuzen 2008). Wherein, most of the earlier systems required specific pre-treatment for the microorganism, and were not tested on real time industrial effluents, the system used in this study

required no such pre-treatment and was tested directly on an industrial effluent.

It can be seen from Fig. 1 that, the rate of biosorption is higher at the initial stages and tends to slow down as time passed.

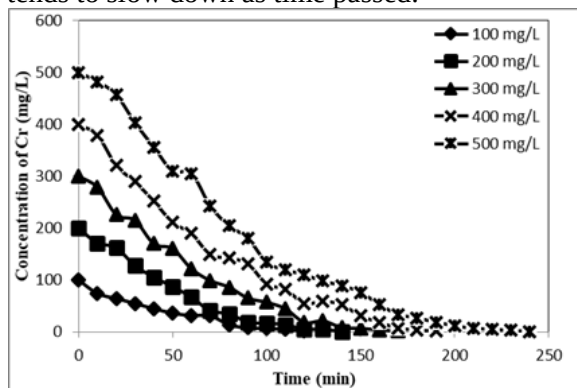


Figure 1. Decrease in Cr concentration with time for different initial concentrations

This trend can be attributed to the fact that, at later stages of the process, the adsorbent tends to get saturated with metal ions due to Vander Waal's forces of attraction (Yu et al. 2003). The chromium uptake by *Aspergillus awamori* NRRL 3112 can be divided into two phases. Firstly, a rapid phase from time 0-120 minutes, where the rate of adsorption of chromium was very high and almost 75-80 % of the biosorption process was over. Secondly a slow phase from time 120-250 minutes, where the rate of adsorption was comparatively low, during which almost all the chromium ions were adsorbed by the adsorbent (biosorption % $\geq 95 \pm 2$) and any further increase in contact time might not help more adsorption.

Effect of pH. The effect of pH is significant on biosorption as it affects the protonation of the functional groups on the adsorbent surface, the solubility of metal ions and also their interaction chemistry (Chandrakala et al. 2015). The influence of initial solution pH on the chromium ion uptake was investigated (Fig. 2) and found that, basic pH conditions were more favourable at all initial concentrations. This result was in accordance with the ones obtained for chromium removal by Elangovan et al. (2008). As, the surface charge on the fungal biomass was

predominantly negative at pH 3.0-10.0 (Rao and Viraraghavan 2002), acidic conditions did not support biosorption of chromium due to the competition extended by the H^+ ions for appropriate sites on the adsorbent surface (Mohanti et al. 2005); but at basic conditions, chromium cations like $Cr(OH)^{2+}$, $Cr(OH)^+$ and $Cr_3(OH)^{5+}$ were more effectively adsorbed.

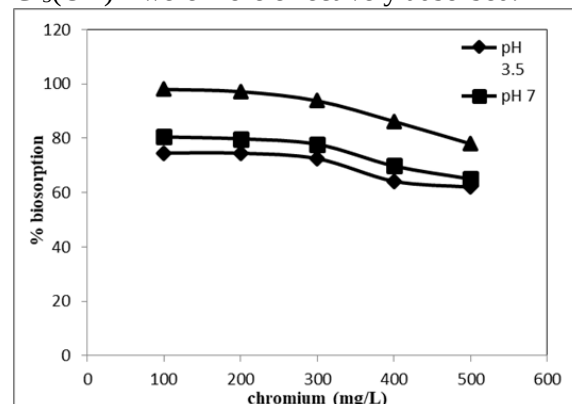


Figure 2. Variation of biosorption percentage with initial concentration at different pH

Effect of temperature. The effect of temperature on the metal uptake of the immobilised cells was conducted within the temperature range of 25-65°C. The results obtained are presented in Fig. 3 and it can be seen that the lower temperature conditions were more favourable for the process at all initial concentrations.

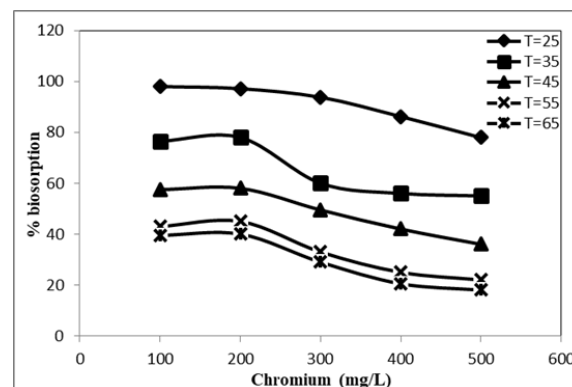


Figure 3. Variation of biosorption percentage with initial concentration at different temperatures

This decrease in biosorption efficiency with increase in temperature, may be due to an

increase in the available thermal energy which induces higher mobility of the adsorbate causing a relative increase in desorption of the metal ions from solid phase to liquid phase (Pandey et al. 2010). This may also lead to bond disruption and subsequent deactivation of biosorbent surface owing to the exothermic nature of biosorption reaction (Sari and Tuzen 2008).

Adsorption isotherm studies. The isotherm study was carried out for the removal of chromium by immobilised *Aspergillus awamori* NRRL 3112. Two isotherms namely Langmuir and Freundlich isotherm (Fig. 4 and 5) were tested in the present study and their respective constants along with R^2 value are tabulated in Table 2.

Table 2. Values of isotherm constants for chromium biosorption on *A.awamori* NRRL 3112

Isotherm	Parameter	Value
Langmuir	Q_0 (m.g ⁻¹)	333.33
	B (L.mg ⁻¹)	0.167
	R^2	0.998
Freundlich	K (mg.g ⁻¹)	63.307
	N (g.L ⁻¹)	2.941
	R^2	0.925

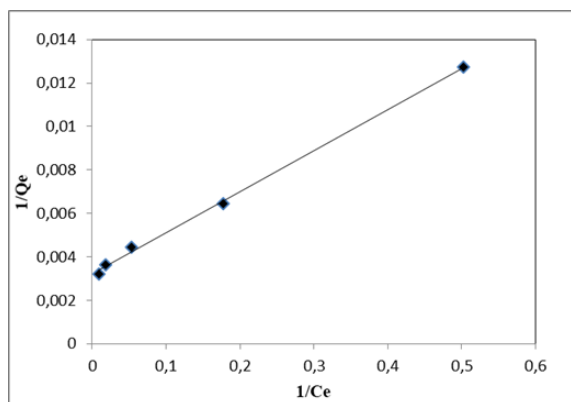


Figure 4. Langmuir isotherm for chromium adsorption on to immobilised *Aspergillus awamori* NRRL 3112 whole cells

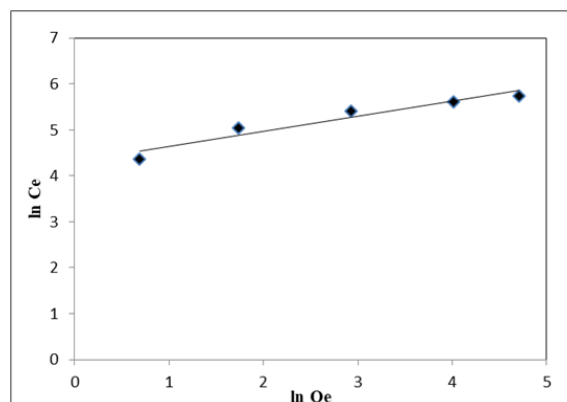


Figure 5. Freundlich isotherm for chromium adsorption onto immobilised *Aspergillus awamori* NRRL 3112 whole cells

Langmuir isotherm. The Langmuir isotherm is the simplest quantitative description of the Fig. 4. Variation of biosorption percentage with initial concentration at different pH maximum uptake values of the adsorbent. It is based on the following assumptions, the surface of the adsorbent is uniform, that is, all adsorption sites are equivalent with no variation in adsorption energy, adsorbed molecules do not interact, all adsorption occurs through the same mechanism and at maximum adsorption only a monolayer is formed (Hall et al. 1966). Langmuir model can be described by the equation:

$$C_e/Q_e = (1/(Q_0 b)) + (1/Q_0) C_e \quad (2)$$

Q_0 and b , the constants related to the maximum adsorption capacity (mg.g⁻¹) and adsorption energy (L.mg⁻¹) respectively, are obtained by plotting $1/Q_e$ versus $1/C_e$. R_L , separation factor or equilibrium parameter, a dimensionless constant is an essential feature of the Langmuir isotherm may be expressed as follows:

$$R_L = 1/(1 + bC_e) \quad (3)$$

R_L value indicates the adsorption nature to be either unfavourable when $R_L > 1$ and favourable when $0 < R_L < 1$, linear when $R_L = 1$ and irreversible when $R_L = 0$. It can be seen from Table 2 that the R^2 value for Langmuir isotherm was higher than that obtained for Freundlich and hence it is concluded that the Langmuir isotherm was the best fit for the obtained experimental

data. The regression correlation coefficient generated was 0.998, exhibiting excellent linearity, indicating good binding of chromium ions to the surface of *Aspergillus awamori* NRRL 3112. A slight variation was observed in the experimental maximum adsorptive capacity of 399 mg.g⁻¹ and the theoretical one obtained by means of Langmuir isotherm (333.33 mg.g⁻¹). A low value of adsorption energy 'b' indicates low surface energy and favourable bonding of adsorbent and adsorbate (Srihari and Das 2008). The value of R_L, the dimensionless constant varied from 0.056 to 0.700 for all initial concentrations of chromium between 100 mg.L⁻¹ and 500 mg.L⁻¹, indicating favourable adsorption.

Freundlich isotherm. The Freundlich isotherm accounts for several kinds of adsorption sites on the solid adsorbent and assumes that the ratio of the amount of solute adsorbed onto a given mass of an adsorbent to the concentration of the solute in the solution is not constant at different solution concentrations. It can be represented by the equation:

$$\ln(Q_e) = \ln(K_f) + 1/n (\ln C_e) \quad (4)$$

where K_f (mg.g⁻¹) is a relative measurement of the maximum adsorptive capacity and n (g.L⁻¹) is related to the intensity of adsorption and indicates both the relative distribution of energy and the heterogeneity of the adsorbent sites. Values of n>1 represent favourable adsorption conditions. The value of 'n' obtained from the Freundlich isotherm for the chromium biosorption on to *Aspergillus awamori* NRRL 3112 cells was 3.067, 1/n being 0.326, indicated favourable adsorption of the Cr with strong interaction between immobilised cells and metal ions and that the process was good over the entire range of concentration studied. The slope range between 0 and 1 was a measure of surface heterogeneity and implied varying adsorption energy for all sites (Senturk and Buyukgungor 2013). Also, a value of 1/n below unity, was an indicator of chemical adsorption (Foo and Hameed 2010). Further, to confirm the above

obtained result, D-R isotherm equation was employed. A value of 12.5KJ.mol⁻¹ for the energy of adsorption was obtained, implying the process was chemisorption (Hong et al. 2009).

Biosorption thermodynamics.

The thermodynamic parameters like free energy, enthalpy and entropy were calculated by the following equations:

$$\Delta G^\circ = -RT \ln(K) \quad (5)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (6)$$

$$\ln(K) = ((\Delta S^\circ)/R) - ((\Delta H^\circ)/RT) \quad (7)$$

where ΔG° (KJ.mol⁻¹), ΔH° (KJ.mol⁻¹) and ΔS° (KJ.mol⁻¹) represent the free energy, enthalpy and entropy of the system respectively and K, the equilibrium constant, is the ratio of the equilibrium concentration of the adsorbate attached to adsorbent (Q_e) to equilibrium concentration of the adsorbate in solution C_e. The values of ΔH° and ΔS° were obtained from the slope and intercept of a linear plot between ln K and 1/T. Fig. 6 indicates the variation of distribution coefficient with temperature and the values of free energy change, enthalpy and entropy for different initial concentrations are shown in Tables 3 and 4.

Analysing the values of the parameters in Tables 3 and 4, the following was inferred. The negative values of ΔG° indicated that the biosorption of chromium on to immobilised *Aspergillus awamori* NRRL 3112 was thermodynamically feasible and the process is spontaneous. Higher negative values of free energy change reflected a more energetically favourable adsorption process (Tan et al. 2008). Also, the value of ΔG° changed from negative to positive with increase in temperature, indicating that biosorption was less favoured at higher temperatures. Further, the values of change in enthalpy obtained, indicated the exothermic nature of biosorption and its magnitude being above 84 KJ.mol⁻¹ for T=25°C and T=35°C suggested that chemisorption was the phenomena occurring at these temperatures.

This further confirmed the results obtained from D-R isotherm studies for $T=25^{\circ}\text{C}$. It should also be noted that the decrease in value of the rate constant K with increase in temperature signifies that the adsorption process in study is exothermic in nature, wherein an exothermic reaction, the total energy adsorbed in bond making is less than the total energy released in bond making, resulting in release of extra energy as heat.

Furthermore, it can be inferred from the negative ΔS° values that the adsorption process involves an associate mechanism and that the adsorption leads to order by formation of an activated complex between the adsorbent and adsorbate. Also, these values reflected that no significant change occurred in the internal structures of the adsorbent during adsorption (Saha and Chowdhury 2011).

Table 3. Values of ΔH° and ΔS° for various temperatures for chromium biosorption onto immobilised *Aspergillus awamori* NRRL 3112

Temperature ($^{\circ}\text{C}$)	ΔH° (J.mol^{-1})	ΔS° ($\text{J.mol}^{-1} \cdot \text{K}^{-1}$)
25	-86033.272	-263.304
35	-89325.616	-272.949
45	-61365.634	-191072
55	-56485.13	-179.66
65	-60584.118	-193.88

A decrease in ΔS° values and its negativity suggests that the adsorption process is enthalpy driven and a decrease in randomness, thus a decrease in the degree of freedom at the solid liquid interface due to association, fixation and immobilisation of Cr ions during biosorption (Pandey et al. 2010). Also, with increase in initial concentration of chromium, the degree of randomness increased suggesting that the sorbate ions may be less stable on the solid surface at higher concentrations. It can also be seen from table 3 that, ΔS° and ΔH° were negative and the magnitude of ΔH° was greater than the value of $T\Delta S^{\circ}$ ($\Delta H^{\circ} > T\Delta S^{\circ}$) for all temperatures.

Table 4. Gibbs free energy values at varying temperatures for chromium biosorption onto immobilised whole cells of *Aspergillus awamori* NRRL 3112

Concentration of chromium (mg/L)	ΔG° (J.mol^{-1})				
	$T=25^{\circ}\text{C}$	$T=35^{\circ}\text{C}$	$T=45^{\circ}\text{C}$	$T=55^{\circ}\text{C}$	$T=65^{\circ}\text{C}$
100	-9655.19	-302238	-799.186	768.6058	1189.079
200	-8762.73	-3241.01	-853.365	547.2274	1139.41
300	-6723.24	-1038.28	52.8788	1931.215	2516.147
400	-4536.89	-617.547	853.365	2995.607	3809.662
500	-3126.55	0513.86	1521.178	3451.462	4261.137

Kinetic studies. The biosorption kinetics of chromium removal was evaluated by fitting the obtained experimental data with the existing kinetic models - Pseudo first order (Fig. 7), Pseudo second order (Fig. 8) and Weber Morris model (Fig. 9).

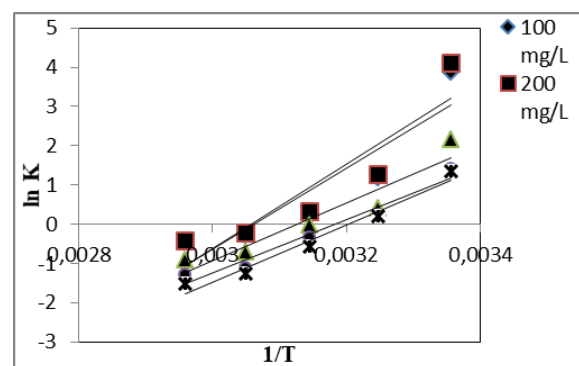


Figure 6. Variation of distribution coefficient with temperature for chromium biosorption onto immobilised whole cells of *Aspergillus awamori* NRRL 3112

Table 5. Values of various kinetic model parameters for chromium biosorption on *A. awamori* NRRL 3112

Kinetic model	Parameter	Concentration of Chromium mg.L^{-1}				
		100	200	300	400	500
Pseudo first order	$K, \text{L.min}^{-1}$	0.03	0.03	0.04	0.02	0.02
	R^2	0.92	0.92	0.84	0.86	0.91
Pseudo second order	$K \times 10^5, \text{g.mg}^{-1} \cdot \text{min}^{-1}$	30.93	10.09	5.71	3.76	2.68
	$V_0, \text{mg.g}^{-1} \cdot \text{min}^{-1}$	1.90	2.55	3.26	3.82	4.27
	R^2	0.97	0.93	0.94	0.97	0.94
Weber Morris Model	$K, \text{mg.g}^{-1} \cdot \text{min}^{-0.5}$	8.21	20.73	25.20	30.96	35.91
	C	-7.71	-53.25	-62.72	-74.12	-106.00
	R^2	0.97	0.98	0.99	0.99	0.98

Lagergren Pseudo-first-order model. The linearized form of the Pseudo-first order equation of Lagergren is generally expressed as follows:

$$\ln(Q_e - Q_t) = \ln(Q_e) - kt \quad (8)$$

where Q_t (mg/g) and Q_e (mg/g) are the amounts of chromium adsorbed at time t and at equilibrium time respectively. The plot of the $\ln(Q_e - Q_t)$ as a function of t provides the k (min^{-1}), the first order rate constant and Q_e value.

Ritchie Pseudo-second-order model.

This model is represented by the following equation:

$$t/Q_t = 1/(k(Q_e)^2) + t/Q_e \quad (9)$$

where k ($\text{g.mg}^{-1}.\text{min}^{-1}$), the second order rate constant, obtained by plotting t/Q_t upon time (h). The values of the rate constants and the regression coefficient are tabulated in table 5 and it was found that, the R^2 values for the pseudo second order model was much higher, in comparison with Pseudo-first order for all initial concentrations, which suggested that the biosorption of Cr on *Aspergillus awamori* NRRL 3112 was best described by Pseudo-second order kinetics. Thus, it was inferred that the rate limiting step was not the boundary layer resistance but most likely chemical adsorption involving sharing of electrons between metal ions and the biomass adsorbent.

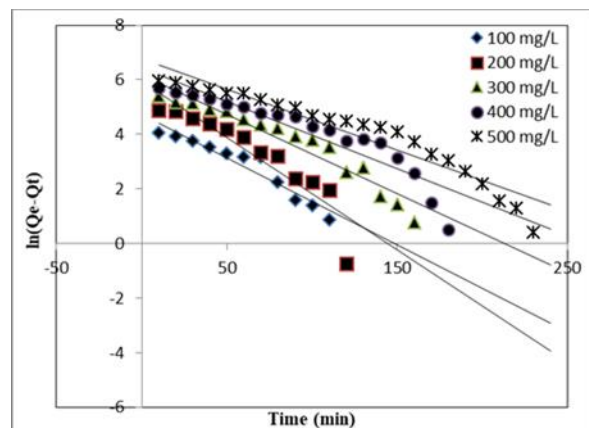


Figure 7. Pseudo-first order plot for Cr biosorption on to immobilised *Aspergillus awamori* NRRL 3112 cells at different initial concentration

Thus, the external resistance model cannot sufficiently explain the mechanism (Hameed et al. 2008; Al-Ghouti et al. 2005). Also, it was seen from table 5 that, with increase in the initial concentration of Cr from 100 to 500 mg.L^{-1} , the initial biosorption rate (V_0) increased from 1.90 to 4.27 $\text{mg.g}^{-1}.\text{min}^{-1}$. This is attributed to the increase in driving force between the solid and the liquid phases, with increase in initial concentration. On the contrary, an opposite trend was observed with the Pseudo-second order rate constants. Similar results were reported previously by other researchers (Allen et al. 2005; Papandreou et al. 2007; Li-e-Liu et al. 2012). This opposing trends of increasing driving force and decreasing rate constant, is in accordance with the ones obtained for biosorption capacity and percentage biosorption respectively.

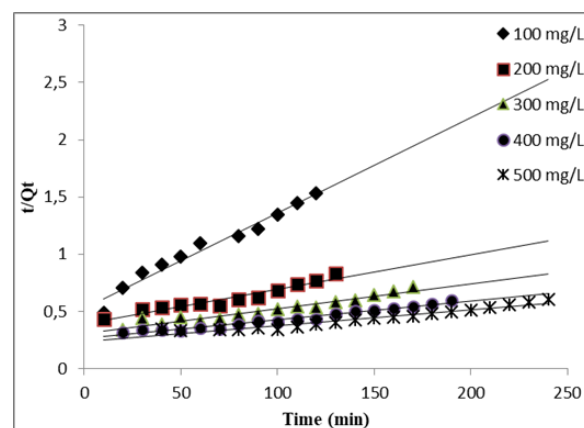


Figure 8. Pseudo-second order plot for Cr biosorption on to immobilised *Aspergillus awamori* NRRL 3112 cells at different initial concentration

Intra particle diffusion model. In order to investigate the mechanism of adsorption, the intra-particle diffusion based mechanism was studied, which can be described as:

$$Q = Kt^{0.5} + C \quad (10)$$

where Q (mg.g^{-1}) is the amount of adsorbate adsorbed and K ($\text{mg.g}^{-1}.\text{min}^{-0.5}$) represents intra particle diffusion rate constant and t denotes the contact time in hours. Thus, a plot of Q versus $t^{0.5}$ yielded K as slope and Q as intercept. Owing to the insufficiency of external resistances

model, the intra-particle diffusion model by Weber-Morris was tested for chromium adsorption on *Aspergillus awamori* NRRL 3112 (Fig. 9). The plot was curved at the initial section which is attributed to bulk diffusion, followed by a linear portion that corresponded to intra particle diffusion. In Fig. 9 it was seen that the data points in the linear section exhibited good linearity with a R^2 value of ≥ 0.97 for all the concentrations tested. When these lines were extrapolated, all of them seemed to not pass through the origin but give an intercept.

Such a deviation from the origin indicated that intra particle pore diffusion was not the only rate controlling step but other mechanisms like ion exchange and complexation reaction may also be involved in the biosorption process (Wu and Yu 2006). Further, it was observed that with increase in initial metal ion concentration from 100 to 500 mg.L⁻¹, the magnitude of the intercept increased from 7.713 to 106, indicating an increase in the thickness of boundary layer and thus an increasing boundary layer effect on the adsorption process (Hashem and El-Khiraigy 2013).

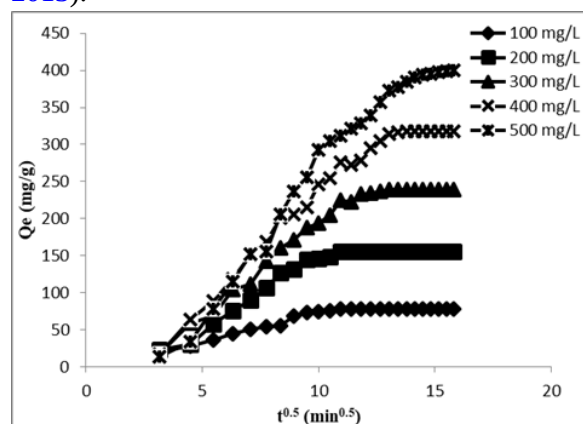


Figure 9. Weber - Morris plot for Cr biosorption onto immobilised *Aspergillus awamori* NRRL 3112 cells at different initial concentration

The biosorbent used in the present study consists of *Aspergillus awamori* NRRL 3112 whole cells entrapped in calcium alginate gel. The calcium alginate is a highly porous structure made of acid monomers that have high selectivity for metal ions. The fungal biomass on the other hand also contains organic compounds like

lignin, chitin, cellulose, hemi cellulose etc. that is useful for binding metal ions. Taking into account the data obtained from the intra particle diffusion model, the mechanism of adsorption of Cr ions tend to follow the following steps:

- 1) diffusion of Cr ions to the external surface of the adsorbent-influenced by initial concentration of metal ions and thus the boundary layer effect
- 2) Diffusion of the ions into the adsorbent via the alginate hydrogel-intra particle diffusion and
- 3) adsorption of the ions into the internal surface of the biomass followed by complex formation between the electron donating nature of functional groups in *Aspergillus awamori* NRRL 3112 and electron accepting nature of Cr cation-chemisorption following Pseudo-second order kinetics, and exothermic reaction.

Conclusions

A novel immobilised system, *Aspergillus awamori* NRRL 3112 whole cells entrapped in calcium alginate membrane was obtained for the purpose of chromium biosorption and was successfully tested. It was established that the immobilised system is a good absorbing medium for the chromium ions with a biosorptive capacity of 399 mg.g⁻¹ at 25°C and pH of 11.5 for an initial concentration of 500 mg.L⁻¹. The isotherm and kinetic studies revealed that the process is well described by Langmuir isotherm and Pseudo-second order kinetics. The values of the parameters obtained from these studies reveal that the rate controlling step is chemisorption along with intra particle diffusion and boundary layer phenomena. The thermodynamic parameter analysis revealed the spontaneity and degree of freedom with which the process occurred. Most of the work done to this day has been oriented to evaluate the biosorbent of interest in the lab environment contrary to the real time scenario. However, considering the knowledge that has been obtained from this study, in regards to the capacity, selectivity, efficiency, easiness of metal uptake and scale up, the alternative discussed in this work presents itself as very promising. Though the use of immobilised whole systems provide an attractive alternative

to the use of conventional methods, these technologies need to be developed and fine tuned and much more work is required in this area. Since *Aspergillus awamori* NRRL 3112 is previously proven to be successful in degrading organic chemicals, the present study of its absorptive capacity, opens up a plethora of opportunities for further research on its environmental remediation capability.

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