Bio sorption of Hexavalent Chromium from Aqueous Solution Using Immobilized Rhizopus Oryzae (MTCC 262)

Priyangi Chakrabarti[1], Prasanta Kumar Biswas[2], Debabrata Bera[3]
[1, 2, 3] *Department of Food Technology & Biochemical Engineering, Jadavpur University, Kolkata*

***Abstract*- Now a days environmental pollution is the major concern specialty heavy metals discharged from different industrial waste stream. There are different conventional methods for metal removal from aqueous stream like evaporation, ion-exchange, precipitation, solvent extraction etc. But due to some limitations of these methods currently biosorption methods are promising for metal removal. Application of immobilized biomass gaining milage emphasis to overcome limitations of free biomass. In this study CMC immobilized Rhizopus oryzae was used for optimization of different parameters like, cell mass concentration, matrix concentration, bead size, temperature, pH, time etc. Biosorption isotherm, metal removal kinetics were also studied. To establishe the role of different functional groups present on the cell surface FTIR and SEM study were done.**

***Index Terms*- immobilized fungus, Isotherm, Kinetic study, FTIR analysis**

I. INTRODUCTION

Hexavalent chromium is widely used in leather tanning, electroplating, chromate preparation and metal finishing. Extensive use of hexavalent chromium in various industrial activities has caused significant environmental contamination [1].

Among all the different oxidation states trivalent and hexavalent chromium exist as stable species. In aqueous solution, the hexavalent chromium species exists as oxyanionic entities which do not bind to the negatively charged mineral surfaces, e.g. silica or clay, become highly mobile in the environment and soluble in a solution of neutral pH. In contrast, trivalent chromium yields insoluble Cr $(OH)_3$ at neutral pH which is immobile in nature [2]. Cr (VI) is toxic, carcinogenic, and mutagenic to animals as well as humans and is associated with decreased plant growth and changes in plant morphology [3].

However, different conventional methods such as ion exchange, reverse osmosis, lime coagulation, chemical precipitation etc. are applied to reduce the toxicity level of hexavalent chromium into permissible limits (0.5 mg/L before discharging into water) [4]. But these methods have certain disadvantages such as incomplete precipitation, high operating cost, huge sludge generation and inefficient in low metal ion concentration. At this point, biosorption technology is gaining attention now a days for removal of toxic heavy metals from waste streams from the stand point of low cost, high efficiency, recovery of metal ions.

A variety of organisms such as bacteria [5], fungi, yeast [6], algae [7], husk [8] etc. have been reported as being capable of removing Cr (VI) from aqueous solutions.

Most of the literature lighted on the biosorption with non living biomaterials in powdered form. But this is not practicable in large scale operations in industries due to the difficulty in separation of biomass after biosorption, mass loss during separation, small particle size, and low mechanical strength [9]. These problems can be overcome using cell immobilized technology. Immobilisation can improve mechanical strength of the biosorbent, as well as increase biosorption potential in continuous stirred tank reactors and minimize clogging during continuous flow [10]. The selection of suitable matrix is a primary factor for the biomass immobilization. Among the various immobilization methods, physical entrapment of organisms within a polymeric matrix can yield beads with pores and optimum size [11-12] There are some suitable polymers, such as polysulfone [13], chitosan [14], agar [15], agarose

[16], acrylamide [17], alginate [18] etc. which have been reported in few research works. The present study deals with optimization of suitable carrier for immobilisation of *Rhizopus oryzae* as well as standardization of various physico- chemical properties using immobilized biomass.

II WORKING METHODOLOGY

Preparation of biomass

Fungi were grown in 50ml potato dextrose broth taken after inoculation with 0.1 ml spore suspension of the respective strain and incubated at 30°C for 72h in a BOD shaker (120rpm) incubator.

The mycelia were harvested by centrifugation; washed with double distilled water, dried by soaking in blotting paper, cut into small pieces and then air dried at room temperature [19]. The dry biomass was prepared by taking the washed biomass in previously weighed aluminium cup and dried at 70ºC till constant weight.

Chemicals and preparation of stock solution

All chemicals were of analytical grade and purchased from Merck, Germany and Sigma, USA. CMC-Na salt was supplied by Loba Chemie Pvt. Ltd. Potato dextrose was obtained from Hi Media, India.

The stock solution of Potassium dichromate ($K_2Cr_2O_7$) was prepared by dissolving 0.707g in double distilled water using a 500ml volumetric flask and diluted to get the desired concentrations of metal. Concentration of Cr (VI) ion was determined spectrophotometrically with HITACHI U-2000 UV-Vis spectrophotometer at 540nm after complexation with 1, 5 diphenyl carbazide and total chromium concentration was determined using Flame Atomic Absorption Spectrometer (Chemito AA203). Amount of Cr (VI) adsorbed by one gram of biomass was calculated using the mass balance equation:

$$q = \frac{(C_0 - C_f)V}{W \times 1000} \quad \text{(Eq: 1)}$$

% of Cr (VI) removal was calculated using the below equation:

$$= \frac{(C_0 - C_f) \times 100}{C_0} \quad \text{(Eq: 2)}$$

Where, q value is the amount of Cr (VI) uptake by the biomass in mg/g. C_0 and C_f are the initial and final Cr (VI) ion concentrations in mg/L, respectively. V and W represent the volume of solution (L) and weight of the biomass (g), respectively.

Immobilization of biomass

Immobilization of powdered biomass was carried out using various matrices viz. sodium alginate, agarose, polyvinyl alcohol and carboxymethyl cellulose (CMC).

Entrapment in calcium alginate

3% slurry of sodium alginate was prepared in distilled water in which 2% of biomass were added and stirred. The alginate- biomass suspension was then extruded into 0.5 M $CaCl_2$. $2H_2O$ solution by hypodermic syringe. The resultant beads were kept for 2 h at 4°C for hardening. After that, beads were separated from $CaCl_2$ solution by filtration and washed with distilled water thoroughly and then air dried. These beads were used for the experimental purpose [20]. Controlled beads were prepared by adding sodium alginate slurry without biomass onto $CaCl_2$ solution, rest of the process remaining the same.

Entrapment in agarose

A 3% agarose solution was prepared and cooled to 37^0 C. To this 2% dried and ground fungal biomass were added. The mixture was injected drop by drop by hypodermic syringe into edible oil kept in ice bucket. Controlled beads were prepared without adding biomass using same method. Phosphate buffer of pH 7.0 was then added to the edible oil. In presence of two phases, the beads moved to the aqueous portion [21]. Then beads were washed with distilled water and used for experimental purpose.

Immobilization using polyvinyl alcohol (PVA)

0.6g of PVA and 0.16g of sodium alginate were mixed in 20ml distilled water to bring the final concentration of PVA 3%. The solution was heated in water bath to dissolve PVA completely and cooled to room temperature. 2% of dried and ground fungal biomass was added to this solution. The PVA alginate cell suspension was extruded into 0.5M $CaCl_2$. $2H_2O$ solution using hypodermic syringe and then the beads were kept for 24 h at 4°C [22]. Beads were thoroughly washed with distilled water and air dried before use. Controlled beads were prepared by the same manner without adding the biomass.

Immobilization using carboxy methyl cellulose (CMC)

3% CMC solution was mixed with 2% biomass to form uniform suspension. The mixture was injected drop wise through hypodermic syringe to $FeCl_3$ solution (0.05 M) to form beads. The immobilized beads were cured in the $FeCl_3$ solution for 1h [23]. Beads were thoroughly washed with distilled water before use. Controlled beads were prepared using the same technique without adding the biomass.

Batch experiments

The batch biosorption experiments were conducted with definite amount of immobilized biomass in 50mL of $K_2Cr_2O_7$ solution containing 50 mg/L chromium taken in 250 mL Erlenmeyer flask, and incubated at 30°C for 24 h with shaking (120 rpm) unless stated otherwise. At the end of incubation, biomass was separated by centrifugation (6,000 rpm for 15 min) and the concentration of chromium in the supernatant was determined. Different immobilization matrices viz. Calcium alginate, agarose, polyvinyl alcohol and carboxy methyl cellulose were chosen to perform the experiment. To optimize the concentration of matrices, the experiment was conducted by varying the matrices concentration from 2% to 3.5%. Immobilization of biomass using CMC was done by varying the needle (no.16, 18, 20). The variation of needle changes the bead size (2.4mm, 2.3mm, and 2.2mm respectively).

To observe the effect of pH, temperature, initial concentration and kinetics of chromium (VI) adsorption on immobilised cell, the experiments were conducted over a pH range 2.0 to 6.0, temperature range 25^0C to 40^0C at pH 2.0. Initial concentration of chromium (VI) and biomass load were 50mg/L and 0.1 g respectively.

Equilibrium adsorption isotherm experiments were conducted using the chromium (VI) concentration range 25 to 225 mg/L. Other conditions remained same as above. Biomass was separated by centrifugation (6000rpm for 15 min), and concentration of chromium (VI) in the supernatant was determined.

The adsorption kinetics of chromium by immobilized beads of *R. oryzae* at pH 2.0 was observed at regular intervals of time up to 480 min with the metal ion concentration of 50 mg/L, keeping other experimental conditions same.

The effect of biomass dose was studied varying the concentration of *R. oryzae* (range 2 to 5 g/L dry biomass) at pH 2 and 30^0C other conditions remaining the same.

Surface morphology observation

The surface morphology of immobilized biomass was observed under scanning electron microscopy (SEM). The samples are prepared according to the method described by Micrograph of pristine beads, biomass loaded beads and chromium laden immobilized beads were recorded by FESEM (JEOL JSM- 6700F) instrument.

FTIR analysis

Fourier transform infrared (FTIR) spectra of pristine and chromium laden immobilized beads were recorded with Shimadzu IR Prestige- 21, Japan FTIR spectrometer (resolution 4 cm^{-1}). The sample was grounded with IR grade KBr in the ratio of 1:100 in an agate mortar and then pressed pellets were prepared to analyze under the region of 4000-400 cm^{-1}.

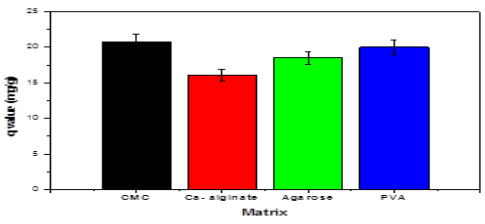
X- Ray diffraction studies

Micro structural characterization of pristine and metal adsorbed immobilized biomass was performed by X-ray diffraction (XRD) analysis using a Bruker, axs (D8 advance) diffractometer at room temperature (30°C). The scanning scope and scanning speed were 10–90° and 0.02 min^{-1}, respectively, using Cu Kα radiation operated at 40 kV and 40 mA.

III RESULTS

Screening of different matrices

To screen the different matrices on the basis of their Cr (VI) removal efficiency, dry *Rhizopus oryzae* biomass were immobilized in calcium alginate, agarose, polyvinyl alcohol (PVA), and carboxy methyl cellulose (CMC). Leaching of cells from immobilized matrices was also observed among these



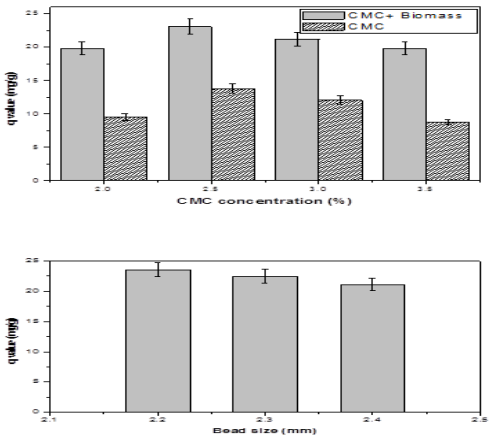


Figure 1 Removal of chromium (VI) by immobilized *R. oryzae* (MTCC 262) using (a) different matrices: carboxymethyl cellulose (CMC), calcium alginate, agarose and polyvinyl alcohol (PVA); (b) different CMC concentrations and (c) various bead sizes: 2.2, 2.3 and 2.4 mm.

four different matrices. The Experiments were carried out using 50 mg/L initial metal ion concentration at pH 5.0 and incubated at 30º C, 120 rpm for 24h. Among the four different carriers tested, CMC was found to be the most efficient carrier without any leakage of cells or visible disintegration of beads. PVA can also be chosen as one of the immobilization carrier but leakage of biomass was observed in this case. *R. oryzae* immobilized with CMC removed about 83% of 50 mg/L chromium (VI) from aqueous solution, while in other two carriers (calcium alginate and agarose) showed poor efficiencies for the removal of chromium (VI) (Fig.1a). The lower removal by calcium alginate and agarose may be attributed due to the fact that low porosity within the beads imparts higher mass transfer resistance to the overall adsorption process [24].

Effect of the concentration of carboxy methyl cellulose

To determine the optimum concentration of carboxy methyl cellulose, immobilization of biomass was carried out using different amount (2% to 3.5%) of CMC. After immobilization the washed beads were added to chromium (VI) solution for biosorption. The other conditions remained same. It has been found that (Fig.1b) the biosorption capacity was initially increased till 2.5% of CMC concentration and after that decreased with the increase of CMC concentration. This may be attributed due to the blockage of active sites of biomass by CMC and hence unavailable to bind the Cr (VI) ions.

Effect of bead size

Different bead sizes affect chromium uptake due to change in the surface area of beads. Therefore, 2.2, 2.3 and 2.4 mm sized beads were prepared using 18, 17 and 16 sized needles. It was observed that (Fig.1c) metal ions removal increased with the increase of beads size. This can be explained as the available surface area per unit amount of cell mass with increase with diameter of the bead [25].

Effect of biomass loading in CMC matrix

In order to optimize the biomass loading in CMC matrix immobilized beads were prepared with varying quantities (2-5 g/L) of biomass. Fig. 2 shows a gradual decrease in uptake capacity (q value) and a simultaneous increase in percentage removal of Cr (VI) by immobilized beads with increase in biomass loading in the matrix. From the present study, considering both q value and percentage of removal, 2g/L biomass load in CMC matrix was selected as optimum for removal of chromium by immobilized beads of *R. oryzae* [26].

Effects of initial pH on Cr (VI) biosorption

Initial pH is an important environmental parameter during biosorption process. It affects the protonation of functional groups of the biosorbent as well as metal speciation and mobility [27]. The experiment was carried out by varying the pH ranges from 2.0 – 6.0. The highest biosorption capacity of the

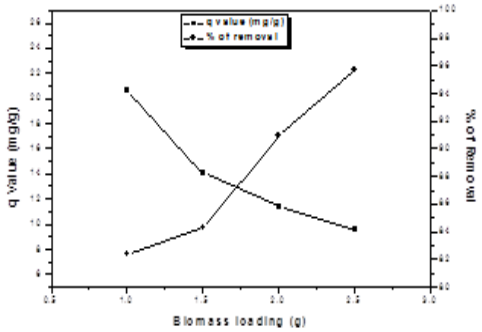


Figure 2 Effect of biomass loading in CMC matrix on removal of Cr (VI) using immobilized biomass of R. oryzae

biosorbent was found at pH 2.0 [28]. Biosorption capacity decreased with increasing pH values (Fig.3). Chromium ions generally exist in two stable oxidation states, trivalent and hexavalent in aqueous solution. The hydrolysis of Cr^{6+} produces only neutral and anionic species, predominantly CrO_4^{2-}, $HCrO_4^{2-}$, $Cr_2O_7^{2-}$ at pH 2-3. On the other hand, at low pH values, the hydronium ions surrounding the cell surface leads to a net positively charge to the CMC-*R. oryzae* bead surface [2]. Also, it is expected that positively charged functional groups such as, amines, imidazoles present on the cell surface can favour the attachment of oxyanionic species of Cr (VI) [26]. At higher pH, the reduction of adsorption may be due to the abundance of negatively charged functional groups on the cell surface which creates the hindrance to adsorption of dichromate ions. Hence, it can be concluded that in addition to the electrostatic interaction there are other factors which are also responsible for biosorption mechanism with respect to pH values.

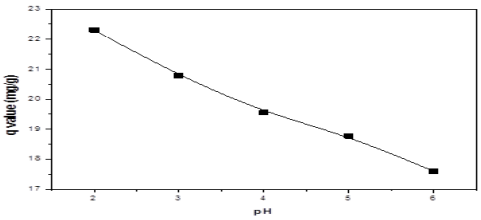


Figure 3 Effect of pH on removal of chromium (VI) by CMC immobilized R. oryzae (MTCC 262) beads.

Adsorption isotherm study

Adsorption isotherm studies were carried out using CMC immobilized *R. oryzae* beads. The relationship between the equilibrium concentrations of solute present in the solution and sorbent, at constant temperature, is governed by the adsorption isotherm [29].

Therefore three isotherms – Langmuir, Freundlich and Redlich- Peterson were used to analyze the experimental data using Eqs.(3-5) respectively.

$$q_e = q_0 K_L C_e / (1 + K_L C_e) \quad (3)$$

$$q_e = K_F C_e^{1/n} \quad (4)$$

$$q_e = A C_e / (1 + B C_e^{g}) \quad (5)$$

Where C_e is the equilibrium metal concentration and q_e is the amount of metal adsorbed per gram of biomass at equilibrium.q_0 indicates the monolayer sorption capacity of adsorbent (mg/g), K_L is the Langmuir constant related to the energy of adsorption (L/mg), K_F is the Freundlich constant related to adsorption capacity of adsorbent (mg/g), n is the Freundlich exponent related to adsorption intensity (dimensionless). If adsorption is unfavourable then n<1. A (L/g) and B (L/mg) are Redlich–Peterson isotherm constants, g is the exponent reflecting the heterogeneity of the sorbent.

The Langmuir equation [30] which is applicable for ideal monolayer sorption assumes that the binding sites on the surface of the biosorbent is homogeneously distributed and once adsorbate occupies a binding site, no further biosorption occurs at this site.

To determine if biosorption process is favourable or unfavourable, for the Langmuir type biosorption process, separation factor (R_L) is studied from following equation:

$R_L = 1/ 1+ K_L C_0$

Where, C_0 (mg/g) is the highest initial metal concentration, R_L is a dimensionless constant which indicates whether isotherm is unfavourable ($R_L > 1$) or linear ($R_L = 1$) or favourable ($0< R_L< 1$) or irreversible ($R_L = 0$).

The Freundlich isotherm [31] assumes that the adsorption process takes place on heterogeneous surfaces and considers the multilayer adsorption phenomenon. It suggests that adsorption capacity is related to the concentration of metal at equilibrium.

The Redlich–Peterson isotherm [32] model describes the adsorption process satisfactorily suggesting that the adsorption mechanism is a hybrid one and does not follow ideal monolayer adsorption and the possibility of multilayer adsorption. It approaches the Freundlich model at high concentrations and also suits the low concentration limit of the Langmuir equation [33]. (Fig.5) shows the adsorption equilibrium plot of Chromium (VI).

Isotherm model parameters were obtained from the slope and intercept of their respective equilibrium plots. All the values of isotherm parameters, linear regression correlation coefficient (R^2) and non linear correlation coefficient (χ^2) for all three isotherm models have been represented in **Table 1** for comparison. The value of linear coefficient of

determination, R^2, represents the percentage of variability in the dependent variable that has been explained by the regression line and may vary from 0 to 1. If there is no relationship between the predicted values and actual values, the coefficient of determination is zero or very low, a perfect fit gives a coefficient of 1.0. On the other hand χ^2 will be small number if the experimental data are close to that obtained from model and will be bigger if they differ. Therefore, it is necessary to analyze the data sets using both linear coefficient of determination, and non-linear χ^2-square test to establish the best fit isotherm model for the adsorption system. The coefficient of determination, R^2, and Chi square test, χ^2, were determined in the range of the whole metal ion concentration [34].

Table 1 Isotherm constants and the values of linear and nonlinear coefficients of three isotherm models

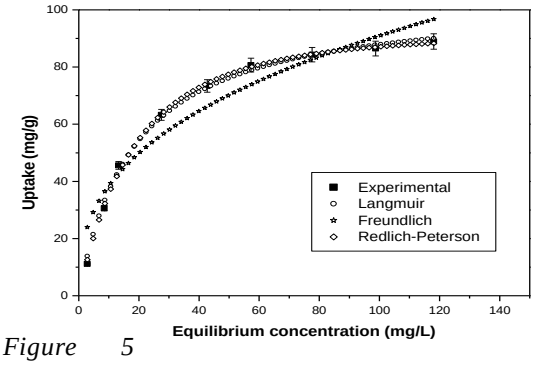


Figure 5

Adsorption isotherm using immobilized cell of R. oryzae (MTCC 262).

It was observed from the Table 1, that the adsorption data were very well represented by both Redlich–Peterson isotherm and Langmuir isotherm with correlation coefficient (R^2) value 0.998 and 0.996 respectively followed by Freundlich isotherm with correlation coefficient (R^2) of 0.929. But the nonlinear coefficient value (χ^2) 1.93227 of Redlich–Peterson isotherm model is lower than the χ^2 value 3.5139 of Langmuir isotherm model. The process of adsorption was considered favourable as the value of Langmuir separation factor R_L = 0.0752 lies between 0 and 1, also the Freundlich exponent, n = 2.6784 was in the range 1–10 and value of adsorption capacity (q_0) was also reasonably high, all of which indicated a strong electrostatic force of attraction between the adsorbate and adsorbent.

Hence we can conclude from the above observations that:

Based on linear regression correlation coefficient (R^2) and non linear coefficient (χ^2) values of all the three studied isotherm models, the present sorption data were found to be described best by Redlich–Peterson isotherm model.

These observations indicate that the adsorption mechanism is a hybrid one and does not follow the ideal monolayer adsorption behaviour and the presence of non-equivalent and multiple binding sites on the surface of immobilized *R. oryzae*.

Kinetic studies

Removal of chromium VI was comparatively rapid initially and gradually decreased with lapse of time until equilibrium. More than 70% of the adsorption was completed within first 45 min and reached equilibrium at about 300 min. initially the binding site on the surface of the biosorbent is abundant which attribute the initial fast rate.

LANGMUIR		FREUNDLICH		REDLICH-PETERSON	
q_0	104.044	K_F	16.29416	A	4.877
K_L	0.054	n	2.678	B	0.029
R^2	0.996	R^2	0.929	g	1.101
χ^2	3.514	χ^2	62.263	R^2	0.998
-	-	-	-	χ^2	1.932

There are several kinetic models exploited to discern the controlling mechanism. These are: Lagergren pseudo first order [35], pseudo second order [36] and Weber- Morris intra particle diffusion models [37]. The equations are shown below respectively:

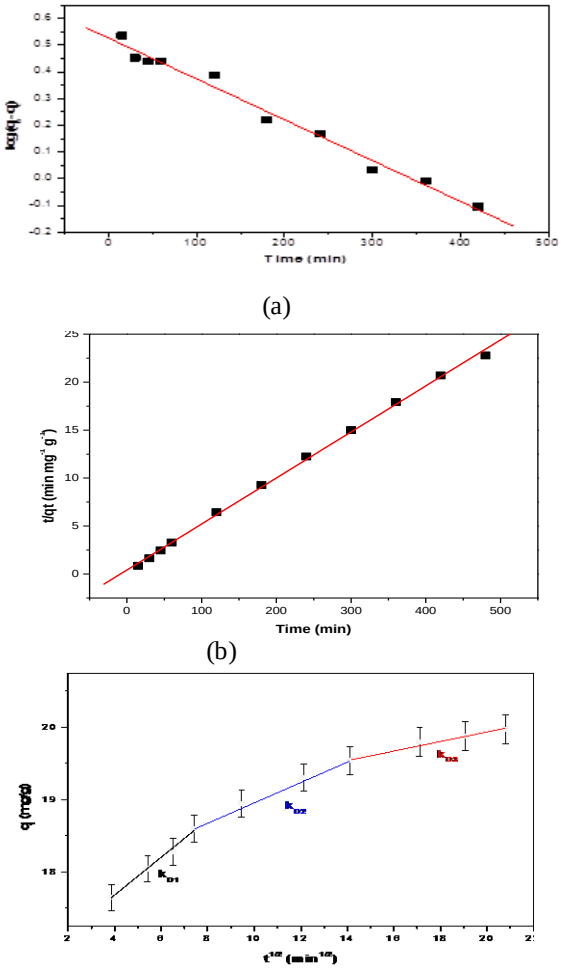
$$\log (q_e - q_t) = \log q_e - k_1 t / 2.303 \quad (6)$$

$$t/ q_t = 1/ k_2 q_e^2 + t/ q_e \quad (7)$$

$$q_t = k_D t^{1/2} \quad (8)$$

Where, q_e and q_t represent amount of dye adsorbed (mg/g) at equilibrium and time t (min), respectively. k_1, k_2 and k_D are the rate constants of first order (min^{-1}), pseudo second order sorption (g mg/min) and intraparticle diffusion rate constant, respectively.

Both Lagergren first order and pseudo second order



(a)

(b)

(C)

Figure 6 Reaction kinetics for Cr (VI) biosorption by immobilized cell of R. oryzae (MTCC 262): (a) Pseudo first order kinetics; (b) Pseudo second order kinetics; (c) Intraparticle diffusion kinetics.

Pseudo first-orderkinetic			Pseudo second-order kinetic			Intraparticle diffusion kinetics				
k_1	q_e	R^2	k_2	q_e	R^2	k_{D1}	k_{D2}	k_{D3}	C	R^2
0.00035	3.367	0.984	0.00054	20.855	0.998	0.259	0.141	0.063	17.449	0.937

Table 2 Values of rate constant and linear regression constants for different kinetic models

models could explain the mechanism and rate of the adsorption phenomenon. However, contrary to Lagergren first order kinetic model, the pseudo second order model can predict the sorption behaviour over the whole time adsorption. Commonly, in most studied adsorption systems, the first order equation does not fit well over the entire adsorption period and is generally applicable for very initial stage of sorption process. The pseudo second order equation is based on the sorption capacity of the solid phase and confirms chemisorptions as rate control step in adsorption process.

The adsorption process is considered as a surface phenomenon where intraparticle diffusion plays a significant role. It is characterized by the relationship between the sorption capacities at any reaction time (q_t) and the square root of time ($t^{1/2}$). In the adsorption process, the pore size distribution plays a more significant role compared to that of surface area. The diffusion of solute particles within the pores resists the mass transfer for any adsorbent in an agitated system.

(Fig. 6 a,b,c) shows the Lagergren first order kinetic plot, pseudo second order kinetic plot and intraparticle diffusion kinetic plot for *R. oryzae* respectively. The values of rate constants (k_1, k_2 and k_D) and theoretical values of q_e were calculated from the slope and intercept of the respective plot and are shown in Table 2 Although correlation coefficient R^2 values are reasonably high in case of first order kinetic model, the calculated q_e values obtained from this model were too low compared with experimental q_e values. For pseudo second order kinetic model, the values of correlation coefficients were close to 1.0 and study almost same to theoretical q_e value. Based on larger correlation–coefficient value and lesser difference between experimental and theoretical q_e values, pseudo second order kinetic model was found to be the best fit for present study. Similar trends have been found in other studies. From the intraparticle diffusion kinetic plot it may be predicted that the process occurs in three steps. Sorption with fast kinetics initially may result from the outer-surface sorption followed by a relatively slow sorption may be due to the binding of the metal ions on the active sites of the adsorbent and the third plateau portion indicates final equilibrium stage where the intraparticle diffusion gets slow down due to decreasing amount of solute concentrations [38].

Thermodynamic study

The metal uptake capacity slightly increased with increase in temperature from 25°C to 40°C. This observation may be attributed due to higher affinity of the binding site of *R.oryzae* for the metal. At a higher temperature, the energy of the system is likely to facilitate the attachment of chromate ion on the cell surface (Fig.4) [23]. The change of thermodynamic parameters such as enthalpy (ΔH), the Gibbs free energy (ΔG) and the entropy (ΔS) for the adsorption process were calculated from the variation of the thermodynamic equilibrium constants (k_c) with temperature (T) using the following basic thermodynamic relations.

$$\Delta G = -RT\ln k_c \quad (9)$$

$$\Delta G = \Delta H - T\Delta S \quad (10)$$

$$k_c = C_{ad} / C_f \quad (11)$$

ΔG is the activation free energy change (KJ/mol), R is the universal gas constant (8.314 J/mol K), and T is temperature in Kelvin (K). ΔH is the enthalpy change (KJ/ mol). ΔS is the entropy change (J/ mole K). k_c is equilibrium constant. C_{ad} (mg/L) is adsorbed concentration.

Temperature (K)	ΔG0 (KJ/ mol)	ΔH0(KJ /mol)	ΔS0 (KJ /mol K)
298	-3.305	6.691	1.556
303	-4.073		
308	-4.590		
313	-4.669		

Table 3 Thermodynamic parameters on Cr (VI) biosorption by immobilized R. oryzae beads

The gradually increasing absolute value of ΔG^0 with temperature and negative value of ΔG^0 indicates feasibility of the process and spontaneous nature of chromium (VI) sorption, respectively. According to the Van't Hoff plot the relationship between the k_c and T can be expressed by the following equation:

$$\ln k_c = (\Delta S/ R) – (\Delta H/RT) \quad (12)$$

If the Van't Hoff plot of $\ln k_c$ against 1/T is a linear one then ΔH and ΔS can be obtained from the intercept and the slope respectively. Therefore, the values of the enthalpy and entropy are 6.691KJ/mole and 1.556KJ/mole/K, respectively Table 3. Positive value of ΔH indicating that sorption reaction is endothermic in nature. The positive value of ΔS reflects the increasing in randomness at the solute/solution interface during the sorption on the biomass [39].

SEM analysis

Scanning electron microscopy was used to characterize the surface morphology of blank beads, biomass loaded beads and beads after metal absorption, respectively. The pictures of the SEM shows that the surface morphology of the blank CMC beads (Fig.7a, b) have smooth surface which turned rough after biomass immobilization (Fig.7c, d). The Fig.(7e, f) is showing the binding of metals with light absorbing portion on the rough surface.

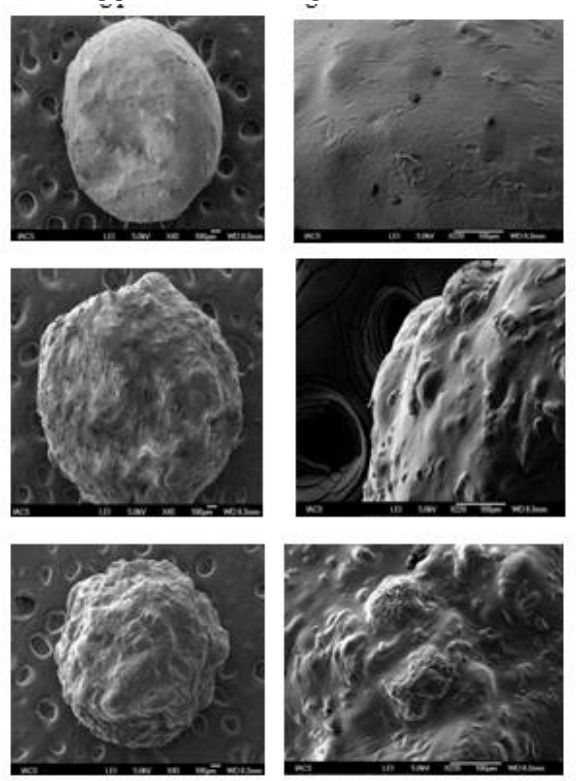


Figure 7 Scanning Electron micrograph of blank CMC bead (a) low magnification, (b) high magnification; R. oryzae immobilized in CMC beads (c) low magnification, (d) high magnification; metal loaded CMC immobilized R. oryzae beads (e) low magnification, (f) high magnification.

FTIR analysis

In order to identify the existence of functional groups such as amino, hydroxyl, carboxyl etc. on the surface of the adsorbent (which may be involved in the surface binding mechanism), FTIR spectra study

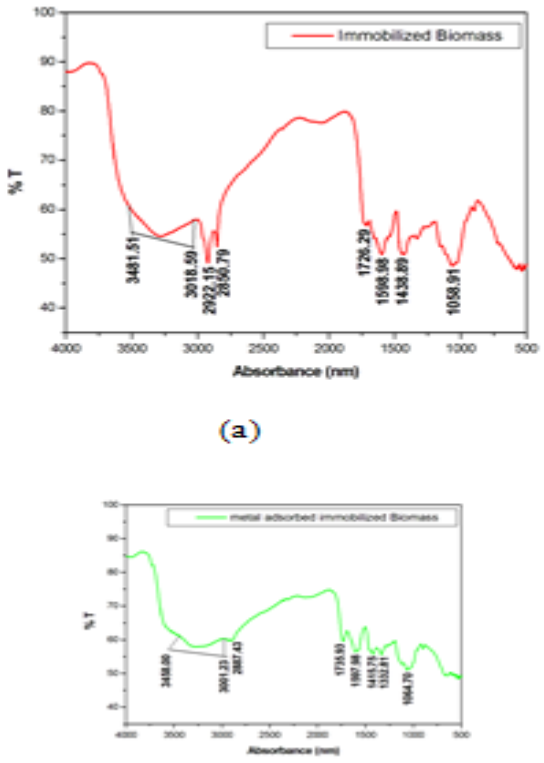


Figure 8 FTIR spectra of R. oryzae immobilized in CMC beads (a) before and (b) after chromium (VI)

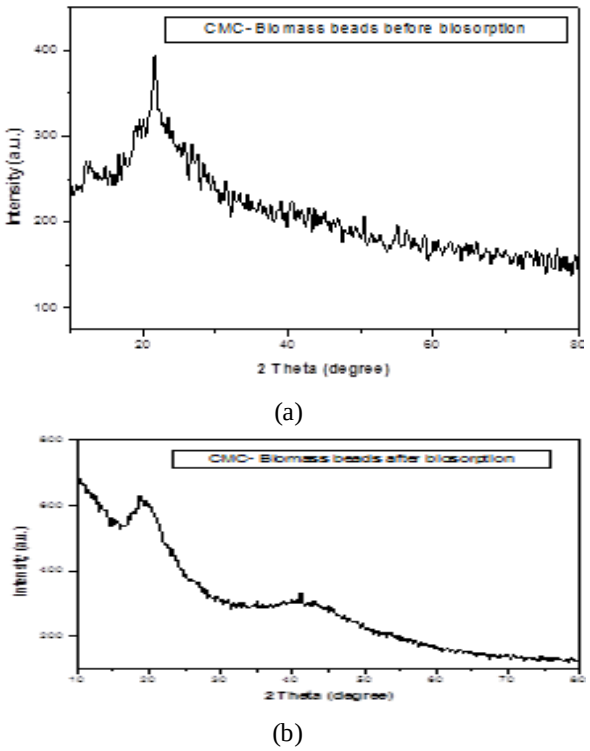


Figure 9 XRD plots of R. oryzae immobilized in CMC beads (a) before and (b) after chromium (VI) biosorption. were obtained [40].

FTIR spectrum of *R. oryzae* immobilized in CMC beads exhibits distinct peaks suggesting the presence of various functional groups on the surface. The characteristic strong bands of N-H and O-H stretching vibrations were appeared in the region 3481.51 – 3018.59 cm^{-1} and sp^3 CH_2 hydrogen configurations are observed around 2922.15- 2850.79 cm^{-1}. A distinct peak at 1726.29 cm^{-1} can be attributed due to the C=O stretching band from the protonated carboxylic groups of amino acids. The Peak position at 1598 cm^{-1} is due to the primary amine bending of the biomass. The wave number 1438.89 cm^{-1} may be caused by the CH_2 deformation. The peak position of 1058.91 cm^{-1} is due to the presence of P-O-C stretch of the organophosphorus groups on the biomass (Fig.8a).

In FTIR spectrum of the *R. oryzae* immobilized in CMC beads after chromium adsorption (Fig. 8b), shift of the stretching vibration from 3481.51 cm^{-1} to 3458.00 cm^{-1} and 3018.59 cm^{-1} to 3001.23 cm^{-1} indicate the involvement of N-H and O-H groups. The transmittance of the band 1726.29 cm^{-1} is shifted to 1735.93 cm^{-1} and 1438.89 cm^{-1} to 1415.75 cm^{-1} on adsorption. A new band formation at 1332.81 cm^{-1} is observed due to complex amide III band formation. The shift of peak from 1058.91 cm^{-1} to 1064.70 cm^{-1} indicates phosphate bond intervention on chromium adsorption.

At low pH value carboxyl and phosphate groups were protonated, thus suggesting the electrostatic interaction of carboxyl and phosphate groups with chromium in the present study.

X- Ray diffraction studies

The X- ray diffraction analysis (XRD) gives the information about the changes in crystallographic structure, chemical composition and physical properties of the adsorbent due to biosorption. For a crystalline material a well- defined peaks are observed, whereas amorphous materials show a hallow peak. Fig. (9 a, b) is showing the XRD patterns of the pristine and chromium loaded immobilized biomass. The 2θ values for pristine immobilized biomass obtains at 21.65° and for chromium laden immobilized biomass 18.72° and 41.11° respectively. Occurrences of crystalline peaks

at 21.65° and 18.72° for pristine and metal loaded immobilized cell signifies the existence of cellulosic materials [41]. The small peak around 41.11° of metal loaded biomass may be accounted for binding of chromium on the biomass. This implies that chromium molecules diffuse into macro pores as well as micro pores by chemisorptions process thereby altering the cell wall structure [42].

Desorption of immobilized biomass

For any successful biosorption process desorption of metal from the adsorped immobilized biomass by suitable eluent is necessary [43]. In the present study, HCl, Na_2CO_3 and NaOH with 0.1M concentration were used for desorption purpose. It was observed that ~86% desorption is possible using 0.1(N) NaOH (Fig.10).

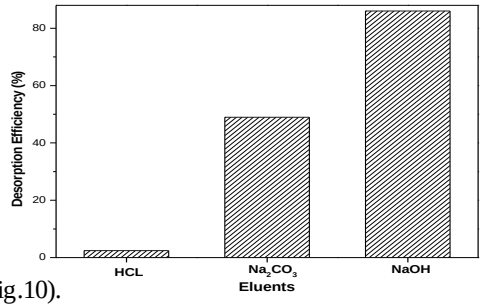


Figure 10 Desorption efficiency of different eluants for desorption of Cr (VI) from immobilized R. oryzae beads using 0.1 M concentration of each.

IV CONCLUSION

From the present study it can be concluded that:

The CMC immobilized biomass has been found to be most effective for removal of hexavalent chromium.

Physico-chemical properties indicate that maximum adsorption achieved at pH 2.0.

Our result indicates the possibility to presence of non-equivalent and multiple binding sites on the surface of immobilized *R. oryzae* biomass controlled by chemisorptions. Intraparticle diffusion of metal ions was the important rate limiting process.

The sorption process was found to be spontaneous and endothermic with increasing randomness according to the thermodynamic study.

Characterization of surface morphology using SEM, FTIR and XRD analysis suggest the involvement of cell surface during the biosorption process.

The present study offers a possibility for considerable technological improvement towards the removal of chromium from wastewater.

V ACKNOWLEDGEMENT

The authors gratefully acknowledge University Grant Commission, Government of India for financial support to pursue the research work. The authors also gratefully acknowledge the guidance and support given by Late Prof. Lalitagauri Ray, Department of Food Technology and Biochemical Engineering, J.U.

REFERENCES

[1] E. Parameswari, A. Lakshmanan and T. Thilagavathi,- Chromate Resistance and Reduction by Bacterial Isolates, AJBAS, vol 3(2), pp.1363-1368, 2009.

[2] S.K.Das and A.K. Guha, - Biosorption of chromium by Termitomyces clypeatus, Colloids and Surfaces B, vol 60, pp.46–54, 2007

[3] B.R.James and R.J. Bartlett,- Plant–soil interactions of chromium. J. Environ.Qual., vol 13, pp. 67–70, 1984.

[4] D. Park, Y.S. Yun and J.M. Park,- Reduction of hexavalent chromium with the brown seaweed Ecklonia biomass, Environ. Sci. Technol. Vol 38, pp. 4860–4864, 2004

[5] S.Y. Kanga, J.Lee and K.W. Kima,-Biosorption of Cr(III) and Cr(VI) onto the cell surface of Pseudomonas aeruginosa. Biochem. E. J., vol 36, pp.54–58, 2007.

[6] N. Lokeshwari and K. Joshi,- Biosorption of Heavy Metal (Chromium) Using Biomass, Global J. Environ. Res., vol 3(1), pp.29-35, 2009.

[7] K.K. Kumar, M.K. Prasad, G.V.S. Sarma and Ch.V.R. Murthy,- Biosorption studies for removal of chromium using immobized marine alga Isocrysis galbana, Indian j. Mar. Sci., vol 35 (3), pp.263-267, 2006.

[8] S. Sharma and P. Malaviya, - Bioremediation of Tannery Wastewater by Chromium Resistant Fungal Isolate Fusarium chlamydosporium SPFT2, Curr. World environ., vol 9 (3), pp. 721-727, 2014.

[9] F. Beolchini, F. Pagnanelli, L.Toro and F. Veglio, - Biosorption of copper by Sphaerotilus

natans immobilised in polysulfone matrix: equilibrium and kinetic Analysis, Hydrometallurgy, vol 70, pp. 101- 112, 2003.

[10] J. Wu and Q. H. Yu, -. Biosorption of 2, 4-dichlorophenol by immobilized white- rot fungus Phanerochaete chrysosporium from aqueous solutions. Bioresour. Technol., vol 98, pp. 253-259, 2007.

[11] Y.P. Ting, G. Sun, - Comparative study on polyvinyl alcohol and alginate for cell immobilization in biosorption. Water Sci. Technol., vol 42, pp. 85-90, 2000(a).

[12] Y.P. Ting and G. Sun, - Use of polyvinyl alcohol as a cell immobilization matrix for copper biosorption by yeast cells. j chem., Technol biotechnol., vol 75, pp. 541-546, 2000(b).

[13] R.S. Bai and T. E, Abraham,- Studies on chromium (VI) adsorption- desorption using immobilized fungal biomass. Bioresour. Technol., vol 87, pp.17-26, 2003.

[14] V. M. Kaya and G. Picard, - Stability of chitosan gel as entrapment matrix of viable Scenedesmus bicellularis cells immobilized on screens for tertiary treatment of wastewater. Bioresour. Technol., vol 102, pp. 2394- 2399, 1996.

[15] D. Bera, P. Chattopadhyay and L. Ray, - Chromium (VI) biosorption by immobilized biomass of Bacillus cereus M^1_{16}. J. Hazard. Subst. Res., vol 7, pp. 1- 19, 2007.

[16] A. Lopez, N. Lazaro and A.M. Marques, - The interphase technique: a simple method of cell immobilization in gel beads, J. Microbial. Meth., vol 30, pp. 231-234, 1997.

[17] G.A. Codd, - Immobilized micro- algae and cyanobacteria. Phycol. Soc. Newslett., vol 24, pp. 1-5, 1987.

[18] Y. N. Mata, M. L. Blazquez, A. Ballester, F. Gonalez and J. A. Munoz, - Biosorption of cadmium, lead and copper with calcium alginate xerogels and immobilized Fucus vesiculosus, J. Hazard. Mater., vol 163, pp. 555-562, 2009.

[19] S. S. Majumdar, S. K. Das, R. Chakravarty, T. Saha, T. S. Bandyopadhyay and A. K. Guha, - A study on lead adsorption by Mucor rouxii biomass, Desalination, vol 251, pp. 91- 102, 2009.

[20] M.N. Kathiravan, R. K. Rani, R. Karthick and K. Muthukumar, - Mass transfer studies on the reduction of Cr (VI) using calcium alginate immobilized bacillus sp. In packed bed reactor, Bioresour. Technol., vol 101, pp. 853-858, 2010.

[21] Z. Aksu, G. Egratli and, T. Kutsal,- A comparative study of copper (II) biosorption on ca- alginate, agarose and immobilized C. Vulgaris in a packed bed column. Process Biochem., vol 33, pp. 393- 400,1998

[22] S.K. Das, P. Ghosh, I. Ghosh, A.K. Guha, - Adsorption of rhodamin B on Rhizopus oryzae: Role of functional groups and cell wall components, Colloids and Surfaces B, vol 65, pp. 30-34, 2008.

[23] B.E. Wang, Y.Y.Hu, L. Xie, K. Peng,- Biosorption behaviour of azo dye by inactive CMC immobilized Aspergillus fumigatus beads, Bioresour. Technol., vol 99, pp. 794-800, 2008.

[24] H. Bairagi,-Studies on biosorption of heavy metal and textile dye from their aqueous solution by microbial biomass, Thesis chapter vol 2, pp. 27-60, 2013.

[25] J. Nath, A. Das and L.Ray, - Biosorption of Malachite Green from Aqueous Solution Using Resting and Immobilised Biomass of Bacillus cereus M^1_{16} (MTCC 5521), Ind. chem engg.,pp. 1–19, 2015.

[26] Y.G. Liu, T.Liao, Z.B.He, T.T. Li, H. Wang, X.J.Hu, Y.M.Guo and Y. He, - Biosorption of copper (II) from aqueous solution by Bacillus subtilis cells immobilized into chitosan beads, Trans. Nonferrous Met. Soc. China, vol 23, pp. 1804-1814, 2013.

[27] N. Ahalya, R.D. Kanamadi and T.V. Ramachandra, -Biosorption of chromium (VI) by Tamarindus indica pod shells, Journal of Environmental Science Research International, vol 1 (2), pp. 77-81, 2008.

[28] Z. Aksu and S.S. Cagatay,-Investigation of biosorption of Gemazol Turquise Blue-G reactive dye by dried Rhizopus arrhizus in batch and continuous systems, Sep. Purif. Technol., vol 48, pp. 24–35, 2006.

[29] R. Chakravarty, M.M.R. Khan, A.R. Das and A.K.Guha, - Biosorptive removal of chromium by huskof Lathyrus sativus: Evaluation of the binding mechanism, kinetic and equilibrium study, Eng. Life Sci., vol 13 (3), pp.312–322, 2013.

[30] I. Langmuir, -The adsorption of gases on plane surfaces of glass, mica and platinum, J. Am. Chem. Soc. Vol 40 (9), pp. 1361–1403, 1918.
[31] H.M.F. Freundlich, -Uber dye adsorption in losungen. Z. Physiol. Chem., Vol 57, pp. 385-470, 1906.
[32] O. Redlich and D.L. Peterson, - A useful adsorption isotherm. J. Phys. Chem.,Vol 63(6), pp.1024–1026, 1959.
[33] J. Nath, A. Das and L.Ray, -Biosorption of Malachite Green from Aqueous Solution by dry cells of Bacillus cereus M^{1}_{16} (MTCC 5521), JECE, pp. 386-394, 2015
[34] H. Bairagi, M.M.R. Khan, L. Ray and A.K. Guha, -Adsorption profile of lead on Aspergillus versicolor: A mechanistic probing, J. Hazard. Mater. 186, 756-764, 2011.
[35] S. Lagergren, - Zur theorie der sogenannten adsorption geloster stoffe. about the theory of so-called adsorption of soluble substances, Handlinger, 24, 1-39, 1998.
[36] Y.S. Ho and G. McKay,- Pseudo- second order model for sorption processes, Process. Biochem., vol 34, pp.451- 465, 1999.
[37] W.J. Weber and J.C. Moris, - Kinetics of adsorption on carbon from solution, J. Saint. Eng. Div. Am. Soc. Civ. Eng., vol 89, pp. 31-60, 1963.
[38] F.C.Wu, R.L. Tseng and R.S. Juang, - Kinetic modelling of liguid- phase adsorption of reactive dyes and metal ions on chitosan, Water Res., vol 35, pp. 613- 618, 2001.
[39] A.S. Ahmed, M.Ridha and N.N. Raoof,- Kinetic, thermodynamic, and equilibrium biosorption of Pb (II0, Cu (II0, and Ni (II) using dead mushroom biomass under batch experiment, Bioremediat. J., vol 20 (3), pp. 252-261, 2016.
[40] S. Madala, S.K. Nadavala, S. Vudagandla, V.M. Boddu and K. Abburi,- Equilibrium, kinetics and thermodynamics of Cadmium (II) biosorption on to composite chitosan biosorbent, Arabian J. Chem., vol 10, pp. 1883- 1893,, 2017.
[41] M. Basu, A.K. Guha and L. Ray, - Adsorption of lead on cucumber peel. J. Cleaner Prod., vol 151, pp. 603-615, 2017.
[42] V.K.Gupta, A. Rastogi, -Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium Nostoc muscorum biomass, J. Hazard. Mater., vol 154, pp. 347–354, 2008.