Removal of Heavy Metals from Industrial Effluent Using Saccharomyces Cerevisiae (Baker’s Yeast) Immobilised in Chitosan/lignosulphonate Matrix

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Abstract: Bioremediation of heavy metal pollution remains a major challenge in environmental biotechnology. Many bacterial polysaccharides have been shown to bind heavy metals with varying degrees of specificity and affinity. This paper highlights the potential of yeast Saccharomyces cerevisiae immobilized in chitosan by forming beads of chitosan/lignosulphonate matrix encapsulating the microorganism for biosorbing chromium metal ion in aqueous solutions. Experiments were carried out as a function of pH, temperature, biosorbent concentration, chromium concentration, contact time, agitation speed, interference, and reusability. A removal of 95% was achieved under optimized conditions. The mechanism of metal sorption by yeast cells gave good fits for Freundlich and Langmuir models with q_m value of 86.95 mg/g. Characteristic of a good and useful biosorbent is its ability to be utilized as a fixed or expanded bed for continuous system. This immobilized yeast biomass in chitosan/lignosulphonate matrix was shown to be suitable for use in column reactor. The crosslinked matrix of chitosan/lignosulphonate shows superior properties as immobilizing support especially in maintaining high stability for extended period of time. After the biosorbent, the adsorbent was regenerated with 0.1 M Tris buffer pH 7.8. Maximum desorption of the metal takes place within 5 bed volumes while complete desorption occurred within 7 bed volumes.

Key words: Saccharomyces cerevisiae, chitosan/lignosulphonate, biosorbent, heavy metal adsorption, column chromatography, chromium (VI).

INTRODUCTION

Heavy metals released by a number of industrial processes are major pollutants in marine, ground, industrial and even treated wastewaters[20]. Lead is widely used in many industrial applications such as storage battery manufacturing, printing, pigments, fuels, photographic materials and explosive manufacturing[13]. Heavy metals can be extremely toxic as they damage nerves, liver, kidney and bones, and also block functional groups of vital enzymes. Two stable oxidation states of Chromium persist in the environment, Cr (III) and Cr (VI), which have contrasting toxicities, mobilities and bioavailabilities. Whereas Cr(III) is essential in human nutrition (especially in glucose metabolism), most of the hexavalent compounds are toxic, several can even cause lung cancer. Chromium and its compounds are widely used in electroplating, leather tanning, cement, dyeing, metal processing, wood preservatives, paint and pigments, textile, steel fabrication and canning industries. These industries produce large quantities of toxic wastewater effluents[24]. The maximum concentration limit for Cr (VI) for discharge into inland surface waters is 0.1 mg/l and in potable water is 0.05 mg/l. Procedures for the removal of toxic metal species from contaminated environments have been developed and most of them are based on ion-exchange technologies and/or precipitation of the cation in an inert form. Unfortunately, these methods are expensive and require the use of contaminating products for desorption of metals for cleaning up of the inorganic matrix. Physico-chemical methods presently in use have several disadvantages such as unpredictable metal ion removal, high reagent requirements and formation of sludge and its disposal, in addition to high installation and operational costs[5].

Natural materials that are available in large quantities or certain waste from agricultural operations may have potential to be used as low cost adsorbents, as they represent unused resources, widely available and are environmentally friendly[4]. The use of microbial cells as biosorbents for heavy metals offers a potentially inexpensive alternative compared to conventional methods of heavy metal decontamination from a variety of industrial aqueous process
The major advantages of biosorption over conventional treatment methods include low cost, high efficiency of metal removal from dilute solution, minimization of chemical and/or biological sludge, no additional nutrient requirement, regeneration of biosorbent and the possibility of metal recovery. Bacteria, fungi, marine algae, etc. have been studied for their heavy metal uptake capacities and suitability to be used as development of biosorbents. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups.

Application of immobilized enzymes or whole cells is advantageous, because such biocatalysts display better operational stability and higher efficiency of catalysis, and they are reusable. The use of immobilized whole microbial cells and/or organelles eliminates the often tedious, time consuming, and expensive steps involved in isolation and purification of intracellular enzymes. It also tends to enhance the stability of the enzyme by retaining its natural catalytic surroundings during immobilization and subsequent continuous operation. The metabolically active cell immobilization is particularly preferred where co-factors are necessary for the catalytic reactions. This novel process eliminates many constrains which are faced with the free-cell systems.

The main requirement for a biosorption system to be commercialized is it’s ability to be used in column chromatography in a continuous process. This will necessitate some degree of pretreatment, sizing, pelleting, chemical modification or immobilization. These are aimed at obtaining a suitable structure for use in a bed reactor and may enhance metal-specific binding sites. The free cells generally have low mechanical strength and small particle size and excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass. The use of appropriate immobilization technique is crucial for the success of the biosorbent in industrial application. However, the cost of the matrix, the deleterious effects of various ions on the most commonly used polymers, and the difficulty in preserving the desired catalytic activity during immobilization have limited its application in wastewater biological treatment. Chitin and its deacetylated derivative, chitosan, have unique properties, which make them useful for a variety of applications. The traditional source of chitin is shellfish waste from shrimp, Antarctic Krill, crab and lobster processing. Chitosan is a natural, cationic, hydrophilic, nontoxic, biocompatible and biodegradable polysaccharide suitable for application in biosorption of heavy metals. Chitosan, is slightly soluble at low pHs and poses problems for developing commercial applications. It is also soft and has a tendency to agglomerate or form a gel in aqueous solutions. Crosslinking treatment to chitosan, will overcome the tendency to agglomerate or form a gel in aqueous solutions. Hence the chitosan modified matrix can be a good source of the biocompatible matrix for immobilization of bacterial whole cells.

In this study procedure for immobilization of microorganisms in a polymer matrix was developed. Immobilization is achieved by cross-linking a high molecular weight chitosan polymer with lignosulphonate under mild conditions to form beads encapsulating bacterial cells. Such a procedure allows reuse of the cells, high cell loading and continuous processing. This study will also highlight the ease of conversion of batch processes into a continuous mode and maintenance of high cell density. This is obviously the major advantage among others, for using immobilized cell systems.

**MATERIALS AND METHODS**

α-Chitosan (molecular mass 1.6×10⁶ Da, 85–89% deacetylation) was from Sigma Chemical Co. (St. Louis, MO, U.S.A.). The other current chemicals (Sigma–Aldrich Chemical Co., Milwaukee, WI, U.S.A.) were of reagent grade and used without further purification.

**Culture:** The saccharomyces cerevisiae biomass used was the commercial pressed baker’s yeast. S. cerevisiae, obtained from a local supplier, were routinely maintained on a solid medium YPD (standard yeast complete medium) agar and preserved at 4°C. The media was prepared as follows:

10 g of Baco Yeast Extract, 20 g of Bacto Peptone and 20 g of Bacto Agar were added to approximately 700 ml of distilled, deionized H₂O (DD H₂O) with constant stirring. Separately, 20 g of Glucose/Dextrose was dissolved in 100 ml of DD H₂O. Both were autoclaved separately. After allowing it to cool to about 50°C, both (media and glucose solution) were combined and the volume adjusted to 1 L with DD H₂O. The media was poured into sterile Petri dish.

For experimental purposes, cultures were grown by inoculating the liquid medium comprising the same substances except the agar with the bacterial cells obtained from the YPD agar medium. Cultures were grown at 37°C on an orbital shaker (200 rpm).

**Preparation of Biomass for Experimental Use:** The content of the flask from the late exponential growth phase (72 h) were harvested by centrifugation.
(2500 rpm, 10 min) at room temperature and the supernatant was decanted. The cell pellet was washed thoroughly with sterile 20.0 g/L potassium chloride solution, followed by sterile distilled water. Finally the cell mass was suspended in sterile distilled water and was freeze-dried. This freeze dried cells were used as biosorption biomass for immobilization in the chitosan/lignosulphonate matrix (as described below). The beads of chitosan/lignosulphonate with entrapped the yeast biomass were used for all batch biosorption and column biosorption studies.

Preparation of Chitosan Slurry: For chitosan slurry, 5 g of chitosan was dissolved in 950 ml of acetic acid solution (0.085 M) with stirring. The pH was adjusted to 6.0 with 0.5 M NaOH.

Yeast Biomass Immobilization in Chitosan/ Lignosulphonate Matrix: Both chitosan slurry (500 ml) and yeast biomass (1.0 g) were mixed and stirred for 10 minutes to get a uniform mixture. The slurry was taken into a sterile syringe and added dropwise into 1000 ml of 0.2 M lignosulphonate solution from 5-cm height to crosslink chitosan droplets and form beads of chitosan/lignosulphonate matrix immobilizing the yeast biomass. Beads were kept for curing at 4°C for 1 hour. The cured beads were washed with sterile physiological saline solution (0.85% NaCl) to remove excess lignosulphonate solution. They were then washed with sterile distilled water 3 to 4 times. When the beads were not being used, they were preserved in 0.9% sodium chloride solution in the refrigerator. All operations were carried out aseptically under laminar flow unit.

Metal Solutions: All the reagents were of Analytical Reagent Grade and were prepared in DDH2O. An aqueous stock solution (1000 mg/l) of Cr (VI) ions was prepared using potassium dichromate (K2Cr2O7) salt (Fluka, Switzerland). This was used as the source of Cr (VI) in the synthetic wastewater. pH of the solution was adjusted using 0.1 N HCl or NaOH. Fresh dilutions were used for each study.

Cr (VI) Ion Determination: The change in Cr (VI) concentration due to adsorption was determined colorimetrically (Spectronic Genesys UV-Vis spectrophotometer) according to Standard Methods[3]. A purple-violet colored complex was developed in the reaction between Cr (VI) and 1,5-diphenylcarbazide in acidic condition. Absorbance was measured at wavelength (l) 540 nm.

The percentage of Cr removal due to biosorption was calculated as

\[ \% \text{Cr removal} = \frac{(C_o - C_e)}{C_o} \times 100\% \]

where \( C_o \) and \( C_e \) are the initial and equilibrium concentration of Cr (VI) solution (mg/L), respectively.

Biosorption Experiments: Batch biosorption studies were carried out using a certain amount of beads of chitosan/lignosulphonate matrix encapsulating the yeast cells and 100 ml solution of Cr (VI) in a conical flask with constant shaking using a rotary shaker operated at 180 rpm. The following operating conditions such as pH, adsorbent amount, contact time and metal concentration were investigated. For each of the investigation, the mixture was shaken in a rotary shaker at 180 rpm followed by filtration using Whatman filter paper (No.1). The filtrate containing the residual concentration of Cr (VI) was determined spectrophotometrically at 540 nm using the method mentioned above. All biosorption experiments were performed in triplicate and mean values reported.

The effect of pH on Cr sorption by the immobilized yeast biomass was determined at pH values of 2, 3, 4, 5, 6, 7, 8 and 9. For the biosorption, 1 g of adsorbents beads (yeast cells encapsulated in chitosan/lignosulphonate beads) was added to 100 ml solution of Cr (at 50 mg/L) in eight different conical flasks at pH ranging from 2 to 9. All the flasks were shaken in a rotary shaker at 180 rpm for 45 min. The supernatant was analysed for residual Cr (VI) after the contact period.

The biosorption capacity of encapsulated yeast cells in chitosan/lignosulphonate matrix was determined by contacting 100 ml solution of Cr (VI) with concentration of 100 mg/L in 250 ml conical flasks, with various amounts of beads containing the immobilized yeast biomass (0.2 to 1.6 g of beads). All the flasks were shaken in a rotary shaker at 180 rpm for 60 min. The supernatant was analysed for residual Cr(VI) after the contact period.

For the determination of rate of metal biosorption by yeast cells in chitosan/lignosulphonate matrix (1 g) were mixed with Cr (VI) solution (100 ml) at concentration of 50 mg/L. The mixture was shaken in a rotary shaker at 180 rpm. The supernatant was analyzed for residual Cr(IV) after the contact period of 15, 30, 45, 60, 75, 90, 105 and 120 min.

In order to investigate the effect of different initial metal concentration on the uptake of Cr (VI) ions from aqueous solution, 1 g of beads containing the yeast cell in chitosan/lignosulphonate matrix were mixed with 100 ml of chromium solution at concentration of 10, 20, 40, 60, 80, 100 and 120 mg/L. The mixture was shaken in a rotary shaker at 180 rpm for 45 min. Each of the supernatant were analyzed for residual Cr (VI) after the contact period.
Adsorption Isotherms: The sorption of chromium ions was carried out at different initial chromium ion concentrations ranging from 10 to 250 mg/L, at optimum pH, at 185 rpm with the optimum agitation period (optimum conditions of all pertinent factors were used) while maintaining the adsorbent dosage at 1g of beads. Langmuir and Freundlich models were applied to the adsorption isotherm and different constants were generated[18,19].

Biosorption Column Experiments: Continuous biosorption experiments were carried out using jacketed pyrex column reactor packed with chitosan/lignosulphonate beads immobilized with yeast cells. The inner diameter, height and bed volume of column were 1.2 cm, 27.0 cm and 30.5 ml, respectively. The column was equilibrated with 10 bed volumes (305 ml) of 0.01 M sodium acetate buffer at pH 4.0 and effluent pH was monitored to ensure that the column was continuously at the optimal binding pH (pH 4). A flow rate of 2.5 ml per minute was used to pass the buffer. Solutions containing 50 mg/l (pH = 4.0) of Cr (VI) ions were pumped upward with a peristaltic pump through the column packed with chitosan/lignosulphonate beads immobilized with yeast cells. The flow rate of the metal solution was 2.5 ml/min. Effluent liquid overflowed from an outlet port at the top of the bioreactor, maintaining a constant level inside the column. The temperature of the bioreactor system was maintained at 30°C by circulating constant temperature water from a circulator bath through the jacket of the bioreactor. The residence time of metal ions through the column was 12.4 min. Samples fractions of 25 ml each of the effluent were collected from the outlet port and analyzed for Cr (IV) ions using the method mentioned above. The experiment was continued until a constant Cr (VI) ion concentration was obtained.

Recovery of Metal from the Column: To remove the bound metal, 0.1 M Tris-HCl buffer pH 7.8 was passed through the column at a flow rate of 2.5 ml per minute. Each effluent fraction of volume 30 ml was collected and analyzed for metal content using the previously mentioned method.

RESULTS AND DISCUSSIONS

Yeast biomass immobilization in chitosan/lignosulphonate matrix: The practical problems of chitosan solubility at low pH aqueous systems, gel forming behavior and mass transfer limitations were overcome by coating it on other adsorbents like alumina, charcoal or interacting it with other adsorbents like alginate to form a rigid matrix structure of better mechanical strength[2,11,27]. In this study these problems were overcome by cross-linking chitosan with lignosulphonate under mild conditions to form beads immobilizing bacterial cells. The process yielded a stable composite adsorbent that allows the reuse of the cells, provides high cell loading and continuous processing using column chromatography. This immobilization also protects the cells against harsh external conditions, while maintaining high stability of bioparticles for extended period of time.

Effect of pH: pH is an important parameter for adsorption of metal ions from aqueous solution because it affects the solubility of the metal ions, concentration of the counter ions on the functional groups of the adsorbent and the degree of ionization of the of the adsorbate during reaction. All the metal ions before gaining access to the plasma membrane and cell cytoplasm come across the cell wall. The cell wall consists of a variety of polysaccharides and proteins and hence offers a number of active sites capable of binding metal ions. Thus it is regarded as a complex ion exchanger similar to a commercial resin. The potential metal binding groups in this class of microbes are carboxylate, amine, imidazole, phosphate, sulphhydryl, sulfate and hydroxyl. Of these, amine and imidazoles are positively charged when protonated and may build negatively charged metal complexes. The amino and carboxyl groups, and nitrogen and oxygen of the peptide bonds are also available for coordination bonding with metal ions such as lead (II), copper (II) or chromium (IV). Such bond formation could be accompanied by displacement of protons and is dependent in part on the extent of protonation which is determined by the pH[33].

To examine the effect of pH on the Cr removal efficiency, the pH was varied from 1.0 to 10.0. As shown in Fig 1. the uptake of free ionic Cr depends on pH, where optimal metal removal efficiency occurs at pH 4 and then declining at higher pH. Removal efficiency for S. cerevisiae immobilized on chitosan increased from 27% to 96% over pH range from 1.0 to 4.0. Chromium (VI) and some other metals such as arsenic, depending on the pH, are known to exist as anions. The pH plays a vital role in biosorption of Cr (VI) due to the nature of chemical interactions of each metal with the functional groups present on the microbial cell surface. At pH values above the isoelectric point, there is a net negative charge on the cell wall components and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such as to repel a reaction with negatively charged ions. At low pH, overall surface charge on the cell is positive and facilitates biosorption of negatively
charged \( \text{Cr}_2\text{O}_7^{2-} \). At pH 4.0, the negatively charged dichromate ions would interact more strongly with the positively charged functional groups of \( S. \text{cerevisiae} \) biomass resulting in high Cr (VI) uptake. It is known that the dominant form of Cr(VI) at pH 1.0 is the acid-chromate ion species (HCrO\(_4^-\)), and any increase in pH shifts the concentration of HCrO\(_4^-\) to other forms, CrO\(_2^-\) and CrO\(_2^{2-}\). Since there is an increase in sorption of Cr (VI) as pH is increased up to 4.0, it may be suggested that CrO\(_2^-\) and CrO\(_2^{2-}\) are active forms of Cr (VI), which are being sorbed by the biomass. Reduction in biosorption of Cr (VI) at pH values lower than 4.0 is probably due to the change in the chemical nature of the \( S. \text{cerevisiae} \) and chitosan biomass, due to hydrolytic activity of the acid at high concentrations. This might change the surface characteristics of the the adsorbent, including surface area availability\(^{29}\). With increase in pH from 5 to 10, the degree of protonation of the adsorbent functional group decreased gradually and hence removal was decreased. At pH greater than 9.0, insoluble chromium hydroxide starts precipitating from the solution, making true sorption studies impossible. A close relationship between the surface basicity of the adsorbents and the anions is evident. This is similar to the findings of others, where the interaction between oxygen-free Lewis basic sites and the free electrons of the anions, as well as the electrostatic interactions between the anions and the protonated sites of the adsorbent are the main adsorption mechanism\(^{19,23,24}\). For all other studies pH 4.0 was selected as optimum pH for biosorption of Cr(VI) ion.

**Effect of Amount of Biosorbent:** The concentration of both the metal ions and the biosorbent is a significant factor to be considered for effective biosorption. It determines the sorbent/sorbate equilibrium of the system. Biosorption of chromium with varying biosorbent concentration is shown in Fig. 2. Chromium uptake rose with increase in biosorbent concentration from 32.5% at 0.2 g biomass to 91% at 1.0 g biomass. This appears to be due to the increase in the available binding sites in the biomass for the complexation of chromium. However, the chromium uptake decreased gradually when the biosorbent concentration exceeded 1.0 g. A similar trend in metal uptake with variations in biosorbent concentration has been reported for lead biosorption from its synthetic aqueous solutions by *Spirulina maxima* by Gong et al.\(^{19}\). High biosorbent concentrations are known to cause cell agglomeration and consequent reduction in inter-cellular distance. This is reported to produce a 'screen effect' among the dense layer of cells, leading to 'protection' of the binding sites from metal ions\(^{25}\). In other words, metal uptake is higher when the inter-cellular distance is more (at low biosorbent concentration), as this condition ensures optimal electrostatic interaction between cells, a significant factor for biosorption.

**Effect of Contact Time:** Shaking time was varied from one minute to 24 hours. As can be seen in Fig. 3, the percentage removal of iron increased with the time of shaking. A sharp increase was observed at around the time of 30 min. and attained an optimum at the time of 45 min. Hence the contact time of 45 minutes was set for all other experiments. Greater availability of various functional groups on the surface of yeast cell, which are required for interaction with anions and cations, significantly improved the binding capacity and the process proceeded rapidly. This result is important, as equilibrium time is one of the important parameters for an economical wastewater treatment system.

**Effect of Initial Metal Concentration:** Biosorption experiments with beads of chitosan/lignosulphonate immobilized with yeast cells were conducted for solutions containing 10-120 mg/l Cr (VI). As seen in Fig. 4, at lower concentrations of Cr (VI) (10-50 mg/l) biosorption was complete in about 5 min but at higher concentrations it took 30-60 min. At lower concentrations...
concentrations, all metal ions present in the solution would interact with the binding sites and thus facilitated 100% adsorption. At higher concentrations, more Cr ions are left unabsorbed in solution due to the saturation of binding sites. This appears to be due to the increase in the number of ions competing for the available binding sites in the biomass.

**Biosorption Equilibria Studies:** Analysis of the isotherm data is important in order to develop an equation which accurately represents the results of the column and which could be used for column design purposes. Adsorption isotherm also describes how solutes interact with adsorbent and so is critical in optimizing the use of adsorbent. The chromium uptake capacity of yeast biomass immobilized in chitosan/lingosulphonate matrix was evaluated using the Langmuir\(^{[18]}\) and Freundlich\(^{[1]}\) adsorption isotherms. The Langmuir equation which is valid for monolayer sorption onto a surface with a finite number of identical sites which are homogeneously distributed over the adsorbent surface is given by Equation:

\[ q_{eq} = \frac{q_{max}bC_{eq}}{1+bC_{eq}} \]

where \(q_{eq}\) is the amount of metal ion bound to per gram of the dried biomass at equilibrium and \(C_{eq}\) is the residual (equilibrium) metal ion concentration left in solution after binding, respectively. \(q_{max}\) is the maximum amount of metal ion per unit weight of sorbent to form a complete monolayer on the surface, and \(b\) is a constant related to the affinity of the binding sites. \(q_{max}\) and \(b\) can be determined from \(C_{eq}/q_{eq}\) versus \(C_{eq}\) plot which gives a straight line of slope \(1/q_{max}\) and intercept \(1/bq_{max}\)\(^{[1]}\).

On the other hand, the Freundlich equation is an empirical equation based on adsorption on a heterogeneous surface is given by Equation

\[ q_{eq} = K F C_{eq}^{1/n} \]

where \(C_{eq}\) is the equilibrium concentration (mg/l), \(q_{eq}\) is the amount of metal ion bound to per gram of the dried biomass at equilibrium (mg/g) and \(K\) and \(n\) are the Freundlich constants related to the sorption capacity and sorption intensity of the sorbent, respectively. The equation can be linearized in logarithmic form and Freundlich constants can be determined.

The Langmuir and Freundlich equations were used to describe the data derived from the adsorption of Cr (VI) by the biosorbent over the entire concentration range studied (20 to 600 mg/L Cr). The linear plot of the Langmuir and Freundlich isotherm models for sorption of Cr (VI) on yeast cells immobilized in chitosan/lingosulphonate matrix are presented in Fig. 5 and 6. The plot of \(C_{eq}/q_{eq}\) versus \(C_{eq}\) (Fig 5) showed that the experimental data fitted reasonably well to the linearized equation of the Langmuir isotherm over the whole Cr (VI) concentration range studied. The correlation coefficient was 0.9643. \(q_{max}\) and \(b\) were determined from the slope and intercept of the plot and were found to be 86.95 mg/g and 0.010 L/mg respectively. The essential characteristics of Langmuir isotherm can be explained in terms of a dimensionless constant separation factor \((R_L)\), defined by:

\[ R_L = \frac{1}{1+bc_{eq}} \]

where \(b\) is the Langmuir constant and \(c_{eq}\) is the initial concentration of metal ion. The value of \(R_L\) indicated the type of Langmuir isotherm to be irreversible \((R_L = 0)\), favorable \((0 < R_L < 1)\), linear \((R_L = 1)\), or unfavorable \((R_L > 1)\)\(^{[21]}\). The \(R_L\) were found to be 0.8786 to 0.1431 for concentrations of 20-600 mg/L Cr (VI).

Linear plots of ln \(q_{eq}\) versus ln \(C_{eq}\) showed that the Freundlich isotherm was also representative for the Cr adsorption by the biosorbent tested. The correlation coefficient was 0.9869. \(K_f\) and \(n\) were calculated from the slopes of the Freundlich plots (Fig 6) and were found to be 3.922 and 1.980 respectively. The magnitude of \(K_f\) and \(n\) shows easy separation of heavy metal ion from wastewater and high adsorption
Fig. 5: Langmuir isotherm plot of Cr(VI) adsorption on yeast biomass immobilized in chitosan/lignosulphonate matrix.

Fig. 6: Freundlich isotherm plot of Cr(VI) adsorption on yeast biomass immobilized in chitosan/lignosulphonate matrix.

capacity. The value of $n$, which is related to the distribution of bonded ions on the sorbent surface, represent beneficial adsorption if is between 1 and 10 \[1^{[14]}\]. The $n$ value for the biosorbent used was found to be greater than one, indicating that adsorption of Cr (VI) is favourable.

Table 1 gives the isotherm parameters for both Langmuir and Freundlich isotherms. From linear correlation coefficients of the adsorption isotherm, it is noted that the Freundlich isotherm model exhibits better fit to the sorption data of Cr (VI) than the Langmuir isotherm model. This phenomenon suggests that multilayer sorption takes place on the surface of bacteria.

**Biosorption of metal by biomass column chromatography:** Essentially, the main requirement of an industrial sorption system is that the sorbent can be utilized as a fixed or expanded bed and it should not cause much pressure drop across the bed. In order to retain the ability of microbial biomass to absorb metal(s) during the continuous industrial process, it is important to utilize an appropriate technique since the free cells are not suited for column packing in industrial applications. The free cells generally have low mechanical strength and small particle size and

**Table 1:** Isotherm parameters for Cr(VI) adsorption on yeast biomass immobilized in chitosan/lignosulphonate matrix.

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<th>Langmuir isotherm</th>
<th>Freundlich isotherm</th>
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<td>$b$ (L/mg)</td>
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<td>86.95</td>
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<td>$q_{max}$ (mg/g)</td>
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<td>$R^2$</td>
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Fig. 7: Breakthrough Curve for Chromium Binding Under Flow Conditions. A solution of 50 mg/L Chromium (VI) in 0.01 M sodium acetate at pH 4 was passed through a column containing immobilized biomass (30.5 ml bed volume). The flow rate was at 2.5 ml per min.

Fig. 8: Desorption curve for chromium after binding under flow conditions. Using the same column (as in Fig 7), ten bed volumes (one bed vol = 30.5 ml) of 0.1 M Tris-HCl buffer pH 7.8 were passed at a flow rate of 2.5 ml per min.

excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass.

Immobilized biomass offers many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems. Immobilization in a support matrix is an effective strategy to enhance the stability and ease of use of whole cell systems. In this study immobilization of microorganisms in a polymer matrix of cross-linked chitosan and lignosulphonate under mild conditions was performed. Besides the obvious advantages of immobilization using this matrix such as inexpensive and biodegradable polymers, ability to maintain high
stability of the bacterial cell for extended period of time and reusability, the most important feature is in the development of continues column chromatography bioprocesses. Hence it is very useful for environmental applications where large volumes of immobilized microorganisms are required. Using this matrix, column experiments were conducted to study the effects of metal binding by the yeast biomass under flow conditions. Fig. 7 represents the breakthrough curve for chromium (VI) passed through the column. The curve shows the amount of metal remaining after solutions at pH 4 were passed through the column. In this experiment, 50 mg/L of Cr (VI) solution prepared in 0.01 M sodium acetate pH 4 was continuously applied to the column which was prepared by packing about 30.5 ml of the beads as described previously. Overloading of the biomass occurred after application of approximately 370 ml of the metal solution. Chromium in the solution passed through the column without additional retention after approximately 1200 ml had been applied.

After elution with 0.1 M Tris-HCl buffer pH 7.8, the bound metal ions were recovered with most of the metal retrieved in bed volumes between 2 to 6. Fig. 8 represents the curve showing this recovery.

**Biomass Reuse:** The stability and potential recyclability of the biomass were assessed by monitoring the change in recoveries through adsorption-desorption cycles. It was seen that the immobilized biomass in the matrix has good stability for extended period of time. There was no significant decrease in recoveries even after eight runs.

**Conclusion:** From an overview of microbial sorbents and biowaste as sorbent candidate, it can be concluded that laboratory trials do show their potential for commercialization since it is technically feasible, eco-friendly with good metal-binding capacity. Besides that, being composed entirely of agricultural and fishing industry waste, it helps in reduction of waste generation. The adsorbent can be regenerated using higher pH buffer and reused up to 8 times without any loss in metals binding capacity. This adsorbent can be a good candidate for adsorption of not only chromium ions but also other heavy metal ions in wastewater stream.

**REFERENCES**


