

Short communication

Studies on the applicability of alginate-entrapped *Chryseomonas luteola* TEM 05 for heavy metal biosorption

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Abstract

Chryseomonas luteola TEM 05 cells were entrapped both in alginate and chitosan coated alginate beads. Biosorption of metal ions on alginate beads was investigated by using a batch stirred system at pH 6.0, 25 °C, in initial metal concentration of 1.92 mM of Cr⁶⁺, 0.89 mM Cd²⁺ and 1.69 mM Co²⁺. Then, a process of competitive biosorption of these metal ions was described and compared to single metal ion adsorption in solution. The apparent equilibrium biosorption was reached within the 180 min of contact for all metals. Although the competitive biosorption capacities of the beads for all metal ions were lower than those of single conditions, Cd²⁺ biosorption on alginate and alginate–chitosan beads did not change significantly.

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1. Introduction

Removal and recovery of heavy metals are very important with respect to environmental and economical considerations [1–3]. Conventional methods for removing dissolved heavy metal ions include chemical precipitation, chemical oxidation or reduction, filtration, ion exchange, electrochemical treatment, application of membrane technology and evaporation recovery. New technologies require that we reduce heavy metal concentrations to environmentally acceptable levels at affordable costs [4–6]. The use of biological materials for removing and recovering heavy metals from contaminated industrial effluents has emerged as a potential alternative method to conventional techniques. The uptake of metals by microbial cells, known as biosorption, is passive in that no energy is required. It has advantages compared to other processes, which include cheap cost of materials, easiness of operation and selectivity over the alkaline metals [2,7–10].

Polysaccharide gel immobilized microorganisms can be used to remove heavy metal ions from aqueous solutions, providing

an alternative to physico-chemical technologies for wastewater treatment [11–13]. Alginic acid is a biopolymer carrying carboxyl groups capable of forming complexes with metal ions. It is a linear polysaccharide composed of (1 → 4) linked residues of α-L-gluronic acid (G) and β-D-mannuronic acid (M), and is found in many algal species especially in brown algae and is also produced by certain bacteria [14,15]. Chitosan, also named poly (β-1,4)-2-amino-2-deoxy-D-glucopyranose, is made from chitin by deacetylating its amido groups [16,17]. When alginate is mixed with chitosan, strong ionic interactions between the carboxyl residues of the alginate and the amino terminals of the chitosan occur to form a polyelectrolyte complex [18–20]. Most of the studies involve the removal of only one kind of metal ion by microorganisms from aqueous solutions. However, the presence of only one kind of heavy metal ion is a rare situation either in nature or in wastewaters. Simultaneous removal of metal ions by microorganisms and immobilized microorganisms from solution including two or more metal ions has gained importance recently. In the case of multi-metal ions, metal ion adsorption capacity of the microorganism may increase or decrease or may not change [3]. In this study, biosorption of each of the Cr⁶⁺, Cd²⁺ and Co²⁺ ions by a floc-forming bacteria, *Chryseomonas luteola* TEM 05, immobilized in alginate gel beads was investigated and partial competitive adsorptions of multi-metal ions

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were also studied. Further, no report is available on the use of this bacterium which is entrapped in gel beads for the adsorption of heavy metal ions.

2. Materials and methods

2.1. Materials

All chemicals used in this study were analytical grade and were purchased from Sigma Chem. Co. (St. Louis, MO) and Fluka Chemie AG (Buchs, Switzerland). Chitosan was provided from Marine Chemicals (India).

2.2. Bacterial strain

The floc-forming bacterium used in this work was *C. luteola*. This strain has been described previously [21] and deposited in the Microbial Culture Collection of the Basic and Industrial Microbiology Section, in the Biology Department of Ege University, Turkey (Izmir), with the code TEM 05.

The MICs of metal against this bacterium were determined with microtiter plates containing nutrient broth and increasing amounts (0–60 mM) of either metal [22].

The bacterium was cultivated aerobically in 500 ml conical flasks containing sterile nutrient broth (Difco) on a rotary shaker (100 rpm) at 30 °C. Cells were harvested at the end of exponential phase, i.e. after 48 h incubation. After cultivation, the cells were centrifuged at 10 000 × *g* for 20 min and then used for biosorption studies.

2.3. Immobilization procedure

Three percentage (w/v) of sodium alginate was dissolved in distilled water and sterilized by autoclaving (121 °C, 20 min). After cooling to room temperature, it was mixed with cell suspension. Cell–alginate mixture was then dropped into the 20% (w/v) CaCl₂ solution at sterile conditions. The beads were hardened in this solution for 2–6 h at 4 °C. The beads were washed with sterile physiological saline to remove excess calcium ion and untrapped cells. The second procedure is the same as described above except that 20% CaCl₂ solution was replaced by mixture solution of 20% (w/v) CaCl₂, 0.5% (w/v) chitosan and 1% acetic acid. The beads were stored in a CaCl₂ solution at 4 °C until use. The particle size of the beads was determined with the help of a magnifying glass. This was roughly checked by suspending the randomly chosen beads in water containing a 10 ml of graduated cylinder and by measuring the increase in volume. The procedure was repeated three times. The particle diameter was of 2.0 ± 0.3 mm (confidence limits of 95%).

2.4. Biosorption of heavy metal ions

The biosorption of Cr⁶⁺, Cd²⁺ and Co²⁺ on the immobilized live bacterium from artificial wastewaters containing single metal ions and multi-metal ions were investigated in batch biosorption experiments at pH 6.0 and 25 °C. Each heavy metal ion (in initial metal concentration of 1.92 mM of Cr⁶⁺, 0.89 mM

Cd²⁺ and 1.69 mM Co²⁺) was prepared in 50 ml growth medium and contacted with the beads (20 beads) in an erlenmeyer's flasks agitated by an orbital shaker at 200 rpm. All biosorption experiments were carried out at room temperature. Liquid samples (0.1 ml) were taken from the batch reactor at predetermined time intervals (every few minutes to every few hours) and were analyzed to determine the residual metal concentration in the aqueous solution. Blank experiments were run concurrently in all experiments. Each experiment was repeated three times and the results gave the average values. The amount of metal ions adsorbed per unit empty or organism immobilized alginate preparations were obtained by using the following expression:

$$q_{\text{eq}} = \frac{(C_0 - C_{\text{eq}})V}{m}$$

where C_0 and C_{eq} are the initial and final metal ion concentration (mmol l⁻¹), respectively. V is the volume of the medium (ml) and m is the amount of the biomass (g).

2.5. Analysis of metal ions

The concentration of unadsorbed metal ions of the biosorption medium were determined by using a ICP-AES (model DRE, Leeman Labs Inc.). Blank experiment was repeated three times and the results gave the average values.

3. Results and discussion

Immobilized cell systems have the potential to degrade toxic chemicals faster than conventional wastewater treatment systems since high densities of specialized microorganisms are used in immobilized cell systems. However, immobilization of the cells in solid structures creates a material with the right size, mechanical strength, rigidity and porosity necessary for use in unit operations typical of chemical engineering. Various techniques are used for the biomass immobilization. One of the most widely used methods is cell entrapment in a polymeric matrix, which is porous enough to allow the diffusion of substrate to cells and of product away from the cells [8,19]. In this study, alginate was used as a support material for immobilization of *C. luteola* TEM 05. It was used for the removal of Cr⁶⁺, Cd²⁺ and Co²⁺ ions from aqueous solution and also was used in partial competitive adsorption of these heavy metal mixtures. Alginate is a natural polymer that can be extracted mainly from brown seaweeds and various bacteria and has been used both as immobilization material and as biosorbent of several heavy metals. It consists of mannuronic and gluronic acid monomers. Oligomeric blocks of gluronic acid shows a high selectivity for heavy metal ions. Alginate could be converted into hydrogels via cross-linking with divalent calcium cations. It was preferred over other materials because of its various advantages such as biodegradability, hydrophilicity, presence of carboxylic groups and natural origin [6,11]. The alginate microcapsules with and without chitosan were used for metal biosorption.

The most exocellular polysaccharide (EPS) producing microorganism, *C. luteola* TEM 05 which was isolated from activated sludge flocs in this study was a gram negative, motile,

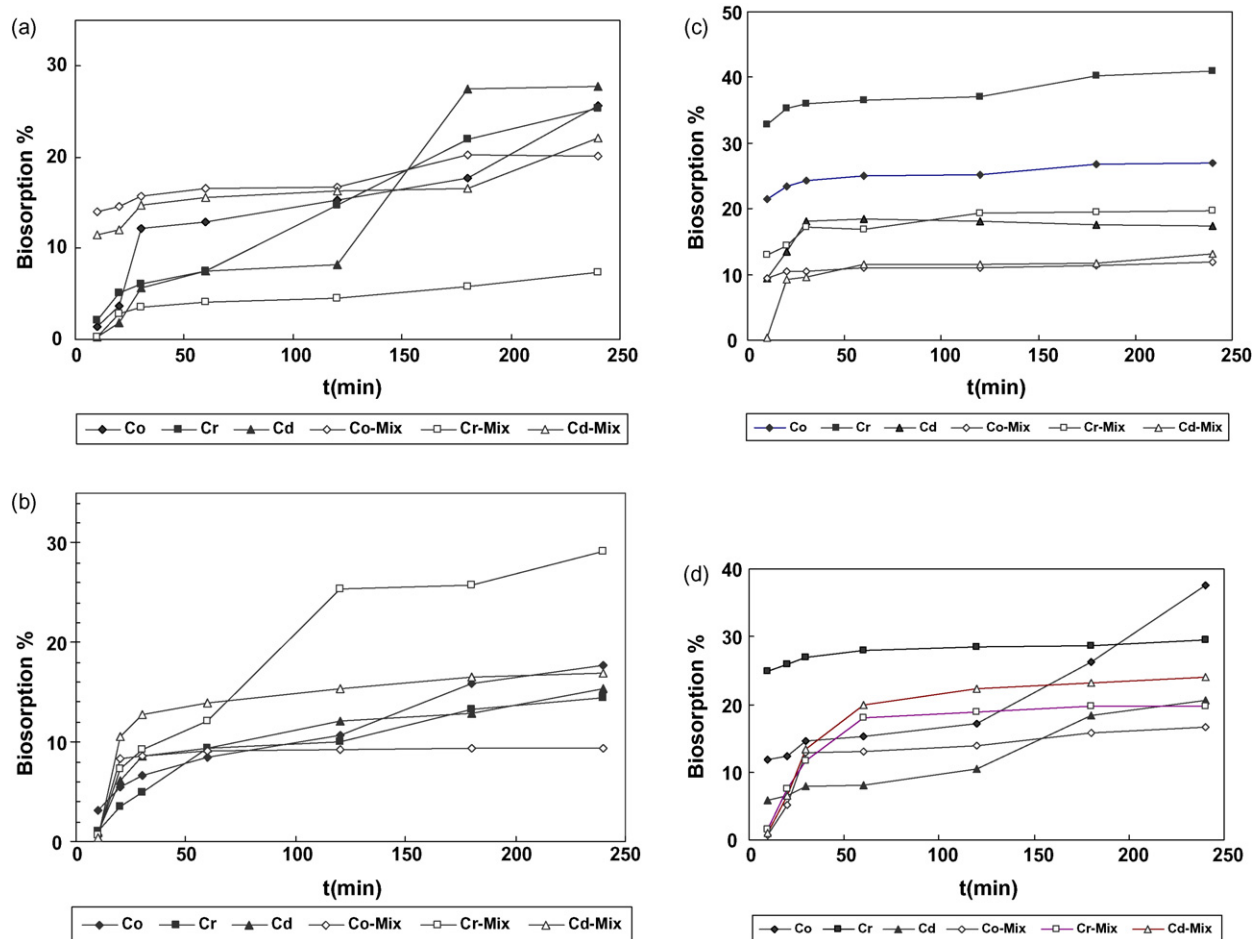


Fig. 1. Single and partial competitive adsorption of Cr^{6+} , Cd^{2+} and Co^{2+} on alginate (a), alginate–chitosan beads (b), *C. luteola* TEM 05 immobilized in alginate (c) and *C. luteola* TEM 05 immobilized in alginate–chitosan beads (d).

rod-shaped and 1.0–1.8 μm size. The characterization results of the isolated strain were given in previous work [21] and the strain TEM 05 was identified as *C. luteola* TEM 05. The cells were cultivated in nutrient broth media amended with various concentrations of each metal. The results showed that the cells were able to survive metal concentrations as high as 2.40 mM for Cr^{6+} , 0.22 mM for Cd^{2+} and 3.05 mM for Co^{2+} . Although MIC values of Cd^{2+} ions for *C. luteola* TEM 05 were very low concentrations, biosorption was determined at high values.

C. luteola TEM 05 has been immobilized into alginate beads via entrapment and then tested for heavy metal biosorption. The biosorption time is an important parameter that affects the partial competitive biosorption. The role of contact time on biosorption of metal ions was studied at pH 6.0, 150 rpm, 25 °C using initial metal concentration of 1.92 mM of Cr^{6+} , 0.89 mM Cd^{2+} and 1.69 mM Co^{2+} . Samples were collected at regular intervals and analyzed for metal ion concentration. For comparative study, alginate and alginate–chitosan beads with and without biomass in metal solutions were added. Results of this research indicate that alginate and alginate–chitosan beads without biomass adsorbed the metal ions far less than that of alginate and alginate–chitosan beads with biomass, suggesting the role of biomass in the biosorption of metal ions from the solution.

Similar results were obtained in previous studies [6,23,24]. The biosorption occurred in two steps; an initial fast step which lasted for about 60 min followed by a slower second phase which continued until the end of experimental period. Pseudo-equilibrium was achieved in about 180 min. Further increase in contact time did not show an increase in biosorption. The effect of contact time on role of metal biosorption indicated that Cr^{6+} and Co^{2+} are the most adsorbed metals on alginate and alginate–chitosan beads (Fig. 1). These results are well agreed with the literatures [23–25].

The alginate beads were also used in partial competitive adsorption of heavy metal mixtures of 1.92, 0.89, 1.69 mM for Cr^{6+} , Cd^{2+} and Co^{2+} , respectively. The medium containing of each metal ion was incubated with beads in batch system. Fig. 1 shows the biosorption % of each metal ion. As shown in figure, the competitive biosorption capacities of the beads for all metal ions were lower than single conditions. The presence of other metal ions slightly decreased the total biosorption capacity. According to these results, there is approximately 2.5 times and 2.0 times decrease for partial competitive biosorption of Cr^{6+} on alginate and alginate–biomass respectively and also, 1.4 times decrease for Co^{2+} and Cd^{2+} was observed. Besides, about 3.0 times decrease for Co^{2+} was observed in the partial

competitive biosorption on alginate-biomass in comparison with single metal ion adsorption. Cd^{2+} biosorption on alginate did not change significantly.

As shown in Fig. 1, approximately 2.0 times decrease for Cr^{6+} biosorption and 2.0 times decrease for Co^{2+} biosorption on alginate–chitosan and approximately 1.4 times decrease for Cr^{6+} biosorption and 3.0 times decrease for Co^{2+} biosorption on alginate–chitosan-biomass were observed in the partial competitive adsorption in comparison with single metal ion adsorption. Besides, the biosorption of Cd^{2+} on alginate–chitosan and alginate–chitosan-biomass did not change.

4. Conclusion

The aim of this work was to find the biosorption characteristics of selected a new biomaterial against to heavy metals for the removal of chromium, cadmium and cobalt ions. The obtained results showed that, the alginate entrapped microorganism (*C. luteola* TEM 05) is found as a good adsorbing medium for these metal ions and has high adsorption yields for the treatment of wastewater containing chromium, cadmium and cobalt ions as compared to beads without biomass. Most of the work done to this day has been oriented to the evaluation of the adsorbent of interest. However, considering the knowledge that has been obtained to this day in regards to capacity, selectivity, efficiency, easiness of metal uptake and possibilities for biological adsorbent reuse, the alternative discussed in this work presents itself as very promising. Consequently, the use of biomaterial biosorption then may provide an attractive alternative to use of conventional ion exchange resins. However, biomaterial biosorption technologies are still being developed and much more work is required.

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