Heavy Metals Biosorption in Liquid Solid Fluidized Bed by Immobilized Consortia in Alginate Beads

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Abstract

The adsorptive removal chromium, nickel, copper and cadmium by alginate beads containing a mixed consortium of Yeast, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli in batch and fluidized bed column reactor was investigated. Under optimized conditions (pH 4.5; contact time 3hrs; initial metal concentration of 150mg/L) batch experiments showed that the immobilized mixed culture was successfully used for the removal of these metal ions in waste water. Fluidized bed studies were carried out in an adsorbent dosage of 1g/L, a flow rate of 132 LPH, a bed height of length of the reactor. Efficiency of biosorption for copper, cadmium, chromium and nickel was found to be 84.62%, 67.17%, 49.25% and 61.02%. Desorption of the exhausted beads was found to be successful, however with a reduced biosorption capacity.

Keywords: Liquid solid fluidized bed; Immobilization; Heavy metals; Biosorption

Introduction

One of the most challenging environmental problems is the removal of heavy metals and other toxic contaminants from industrial wastewater of the important metals, Mercury, lead, cadmium, Arsenic and Chromium (VI) are regarded as toxic; whereas, others such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns [1,2,3]. Several methods are being used for the removal of heavy metals ions from aqueous wastes (Chemical Precipitation, Ion Exchange, Electrochemical Treatment, Membrane Technologies, adsorption on activated Carbon. etc. [4]. Each of these methods has its own merits and demerits. But the search for new eco-friendly and cost-effective technology for the removal of heavy metals from wastewaters has been directed towards biosorption.

Biosorption using potential metal biosorbents like algae, bacteria, fungi, and yeast can be an effective technique to decrease the concentration of heavy metal ions in solution [5]. Reduction of hexavalent chromium Cr(VI) to Cr(III) by bacteria such as Pseudomonas aeruginosa [6], Bacillus sp. [7] and Escherichia Coli [8] is already reported. However, application of free bacterial cells at industrial scale is disadvantageous due to the difficulty of biomass/effluent separation [9] etc., which may be overcome by using immobilized bacterial cells with the advantages of stability, regeneration, solid–liquid separation and minimal clogging in continuous systems [10]. Immobilization of microorganisms in a suitable matrix like polyvinyl alcohol, agar media and sol–gel materials has been proven to be an efficient solution to this problem [11,12].

Adsorption processes are traditionally carried out in fixed beds [13] due to the high concentration of solids and the obtainable uniform residence time. However since the wastewater to be treated often contains solid impurities leading to a plugging of the fixed bed, the liquid must be clear to avoid column blocking. Recently, many experimental studies have been conducted in fluidized beds, which allow treatment of turbid liquids while avoiding the channelling problems [14]. Fluidized beds are common and important reactors in process engineering because of the good mass and heat transfer rate between the fluid and the particles, and between the particles and the side wall of the column. The term fluidization is used to describe the condition of fully suspended particles. Liquids or gases are passed at certain velocity up through a bed of solid particles, at this velocity the pressure drop across the bed counter balances the force of gravity on the particles and further increase in velocity achieve fluidization at a minimum fluidization velocity. Fluidization quality is closely related to the intrinsic properties of particles, e.g. particle density, particle size and size distribution, and also their surface characteristics [15]. From the previous literature related to the biosorption of heavy metal using bacteria, it can be concluded that there is a lack in literature of using immobilized bacterial consortia in liquid solid fluidized bed to study the behavior of biosorbents and its efficiency in heavy metal adsorption. Hence, this work aims to study the adsorption of heavy metals like chromium, nickel, copper and cadmium in liquid solid fluidized bed using immobilized sol-gels as a solid catalyst containing mixed cultures of Yeast, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli.

Materials and Methods

Microorganisms: Pseudomonas aeruginosa, Bacillus subtilis, E.coli, Yeast obtained from the laboratory culture collection was maintained in the specific medium and appropriate proportions used for the experiment. Standard sterile techniques were used for inoculation of cultures. Medium used for the microorganism and all the glassware were properly sterilized autoclaved at 15 lb/in² pressure and 121°C for 30 minutes.

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Methods

Preparation of metal solutions: Different metal concentrations were prepared by dissolving of CuCl₂, CdCl₂, NiSO₄, and K₂Cr₂O₇ salts in double distilled water in equal ratio to have metal concentrations of 50, 100, 150, 200, 250 and 300 mg/L. A stock solution of 1000 mg/L was prepared all other concentrations are obtained from it. All glassware washed with 0.1 M HCl before and after each experiment to avoid binding of the metal to it.

Preparation of biosorbent: The culture was transferred and grown on specific media (Bromfield medium-Bacillus subtilis; Cetrimide medium-Pseudomonas aeruginosa; YPD-Yeast; LB medium-E.coli) for subculture. 100 ml of sterilized culture media was transferred to 250 ml Erlenmeyer flask. The media was allowed to cool and then the 100µl microbial solution was inoculated into the medium in laminar air flow chamber. The inoculated flasks were incubated in an orbital shaker at 250 rpm at 32°C for 2 days to obtain the biomass. Mixed cultures were prepared by adding equal amounts of individual cultures. Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in double distilled water (MilliQ) for washing and again centrifuged above to make sure that no media remain on the cell surface. This biomass was used for sorption studies.

Immobilization of biosorbent: A 4% (w/v) solution of sodium alginate was prepared by continuous stirring in hot (60°C) distilled water. After cooling, 5% (w/v) of bacterial consortia was added and stirred. The mixture was extruded using a burette into 0.5 M CaCl₂·2H₂O. The resultant beads were kept in the polymerizing medium for 4 h. The beads were washed in saline and distilled deionized water. The beads were frozen at 20°C for 24 h, kept at 4°C for 12 h, and thawed at room temperature for further use.

Batch biosorption studies

Optimization of parameters: Batch studies were done using biomass as a function of various parameters such as pH, time, temperature, initial metal concentration and bed height. Biosorption experiments were conducted at an initial metal concentration of 100 mg/L and 100 mg sorbent in 100 ml of metal solution at 30°C for 3 hours at pH varying from 1.0 to 7.0 by adding 0.01 N HCl. Effect of contact time was studied at an initial metal concentration of 100 mg/L and 100 mg sorbent in 100 ml solution at 30°C and optimized pH. Samples were analyzed for the concentration of metal at regular interval of one hour for 24 hours. The effect of temperature on sorption was determined through batch experiments carried out at 10, 20, 30, 40 and 50°C. Sorption studies at optimized conditions were also carried out with initial metal concentrations in the range of 50–300 mg/L of metal solutions prepared as stated in section 2.2.1. Biosorbent dosage was optimized by using biosorbent amounts of 25, 50, 75, 100, 125 and 150 mg in100 ml of 100 mg/L of metals solutions. The optimization studies were carried out for mixed culture immobilized in alginate beads.

Experimental Protocol for LSFB

Experimental setup: Figure 1 shows the fabricated LSFB consists of a homogenizing section 14 cm long for the uniform mixing of the inlet wastewater before it enters the reactor. Above the homogenizing section is the distributor plate of diameter 5 cm, with pores of diameter 1.5 mm arranged in triangular pitch. Above the distributor plate is an acrylic riser of 88 cm, which functions as the LSFB. There is a solid disengaging section above the riser, from where the liquid effluent is withdrawn. Pressure tapings were made at different heights.
Determination of metal concentration in the supernatant:
The heavy metal concentration was determined by the use of atomic absorption spectrophotometer, Perkin Elmer Analyst 300. Determination of copper, chromium, cadmium and nickel was done by using its specific lamp for each metal and at a specific wavelength.

Data evaluation: The amount of metal bound by the biosorbents was calculated as follows:

\[ Q = \frac{v(C_i-C_f)}{m} \]

Where \( Q \) is the metal uptake (mg metal per g biosorbent), \( v \) the liquid sample volume (ml), \( C_i \) the initial concentration of the metal in the solution (mg/L), \( C_f \) the final (equilibrium) concentration of the metal in the solution (mg/L) and \( m \) the amount of the added immobilized biosorbent on the dry basis (mg).

Desorption studies: The exhausted Alginate beads containing immobilized microorganisms after heavy metal biosorption were removed from the LSFB. The beads were then treated with Nitric Acid, and allowed to stay for an hour and loaded back into the LSFB. 10 ml of samples were withdrawn every half hour. The samples were then analyzed in the Atomic Absorption Spectrophotometer to determine the heavy metal concentration.

Sample analysis: Metal concentration at initial and at regular intervals was examined using atomic absorption spectrophotometer. 10ml of the samples were taken out for every 5 minutes in the first hour and continued by every 15 min in the subsequent hour. The metal concentrations were determined and the results are tabulated.

Results

Batch sorption studies

Effect of batch sorption parameters

Effect of pH: The experimental results of chromium, cadmium, nickel and copper using mixed cultures of Yeast, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* at varying pH was shown in the Figure 2(A). Effect of pH on biosorption has been studied over a range of 1 to 7. The highest removal of cadmium and copper was found at pH 4, while at pH 3 and 5 the highest removal of chromium and nickel obtained respectively. At pH<3, lowest biosorption was recorded and the experiments beyond pH 5 were hindered due to precipitation of metals during the biosorption process.

Effect of contact time: Before the starting of the batch experiments, it was very important to know the required time to reach equilibrium biosorption. In other words, for all batch experiments, the contact time should be sufficient to ensure reaching an equilibrium concentration. Figure 2(B) shows the results of biosorption removal of heavy metals concentration versus contact time for the four metal solutions. It can be seen that a 3 hour contact time is sufficient to reach equilibrium for all four heavy metals.

Effect of temperature: Biosorption studies of Metals solutions using immobilized mixed cultures were carried out at different temperatures ranging from 10°C to 50°C. The effect of temperature on metal sorption was presented in Figure 2(C). The percentage of metal sorption was increased from 10°C to 30°C and then showed decrease in sorption percentage with increase in temperature. Figure 3.4 shows a maximum percent of sorption around 5% achieved at 30°C at fixed pH 4.5 and biomass concentrations 2.0 mg/ml. This decrease in biosorption efficiency at temperatures 40-50°C may be attributed to: an increase in the relative desorption of the heavy metals from the solid phase to the liquid phase, deactivation of the biosorbent surface, destruction of active sites on the biosorbent surface due to bond disruption, or due to the weakness of the sorbent active site binding forces and the sorbate species and also between the adjacent molecules of the sorbed phase. From Figure 3 it was concluded that experiments can be carried out at room temperature.

Effect of initial metal concentration: Figure 2(D) shows the variation of percentage sorption of immobilized microbial culture at different initial metal concentrations. The results indicated that the percentage biosorption of metals was not altered greatly from concentrations between 50-150mg/L whereas still increasing in the initial metal concentration beyond 150mg/L shows a decrease in the sorption capacity, this may be due to the sorbent dosage depicted in Figure 2(E) which was found to be 1g/L of immobilized biosorbent containing enough biosorption surfaces and sites for this concentration.

LSFB studies for biosorption

Effect of pressure drop and flow rate: From Figure 3(A), it is evident that the pressure drop across the LSFB increases continuously till the flow rate of the liquid attains that of the minimum fluidization velocity, which is the velocity required to fluidize the particles, in this case, the Alginate beads. On further increase of the flow rate of the liquid beyond minimum fluidization velocity (43 LPH), the pressure drop across the LSFB is found to remain nearly constant, hence obtaining a straight line in the latter part of the graph. This signifies that once the fluidization starts, the pressure drop across the LSFB remains the same for different flow rate.

Effect of bed height and flow rate: Figure 3(B) shows that the bed height of the constructed Liquid Solid Fluidized Bed (LSFB) remains constant till the flow rate reaches the minimum fluidization velocity, which is 43 LPH for the above mentioned bed. Since the beads are supposed to fill 25% of the entire volume of the reactor, the static bed height is 22cm. Once the flow rate of the liquid exceeds the minimum fluidization velocity, the fluidization of the beads starts and the bed expands. The bed height was found to increase more or less linearly with the liquid flow rate. This indicates the proper construction of the LSFB.

The optimum flow rate was determined as 132 LPH since it was found that at this flow rate, the bed height is the length of the reactor. In other words, at a liquid flow rate of 132 LPH, the beads filled the entire length of the column, without any overflow.

LSFB biosorption studies under optimized conditions: Biosorption studies in LSFB were conducted, which was packed with the known quantity of immobilized alginate beads. The synthetic metals solution was pumped at desired flow rate. Optimized parameters were used and the metal concentrations were determined by atomic absorption spectrophotometer at an interval of 15 min from the sampling port. Figure 3(D) shows the plot of metals concentration in the LSFB against the residence time in the fluidized bed. The maximum amount of copper was adsorbed in the first 170 minutes. Nickel, Chromium and cadmium were adsorbed at 70 min. The maximum efficiency of biosorption of Cadmium in the LSFB was found to be 67.17%. Biosorption of Chromium using in the LSFB was also found to increase up to 90 minutes, after which the biosorbed metal leached back into the solution. The maximum biosorption efficiency for uptake of Chromium (VI) metal was calculated to be 49.25%. Biosorption of copper in the LSFB were found to be high than other metals this may be due to the high suitability of optimized parameters and enough surfaces...
Figure 2: (A) Effect of pH. (B) Contact time (C) Temperature (D) Initial Metal Concentration (E) Biosorbent Concentration of the bacterial consortium immobilized in alginate beads.
Figure 3: (A) Effect of Pressure drop and flow rate (B) Bed height on flow rate (C) Efficiency of Fluidized bed sorption studies (D) Removal of metal concentration in LSFB on time (E) Desorption studies on removal of copper.
and sites for this concentration. The optimum time for the biosorption was determined to be around nearly 3 hours, after which the biosorption efficiency remains more or less constant which is same as that of the batch studies. Similar to the batch studies performed, the concentration of Copper in the solution was found to continuously decrease in the first 3 hours. Figure 3(C) gives the maximum biosorption efficiency of metals in which the copper biosorption efficiency in the LSFB was determined to be high as 84.62%. Biosorption efficiency of the other metals may increase by optimizing the biomass concentration of each microorganism with respect to the metals solution before immobilizing in alginate beads.

Desorption and column regeneration: The desorption of exhausted Alginate beads containing mixed culture using 0.1 N Nitric Acid. Figure 3(E) shows the concentration of was found to decrease from 40 ppm to 27.12 ppm for copper. The efficiency was found to decrease from 82.4% to 33.46%. Reusability of biosorbent was studied by three cycles of alternating sorption/desorption experiments with the supplement of 100 mg/L of metals solution at the beginning of each cycle. The sorption capacities for each cycle were shown to be decreasing by 5%.

Conclusion

The present study evaluated the removal of copper, chromium, nickel and cadmium from synthetic metals solutions using immobilized consortium of Yeast, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli in alginate beads in fluidized bed reactor. Batch experiments showed that the mixed culture immobilized in alginate beads can be successfully used for the removal of these metal ions. LSFB studies showed that the removal of copper and nickel was high compared to chromium and cadmium. This concluded that biosorption was found to depend significantly on pH of the solution. Optimum flow rate 132 LPH obtained from batch studies results in good contact time between the adsorbent and adsorbate in LSFB also. It can thus be concluded from this project work that biosorption of heavy metals in an LSFB is not only a cost effective method, but also a highly efficient alternative to chemical and physical treatments employed for the removal of heavy metal pollutants from contaminated water. Further work will be carried out in developing a model with higher fluidization velocity with different particle size and with different biomass concentration.

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