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Removal of Hexavalent Chromium from Synthetic Wastewater Using Alginate Immobilized Cyanobacteria: Experiment and Mathematical Modeling

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Abstract

The present study aims at removal of Cr(VI) using immobilized *Limnococcus limneticus* from synthetic wastewater. Four different methods were followed to immobilize test cyanobacterial strain onto calcium alginate bead and named as Cyanobacteria Immobilized Calcium alginate Bead (CICB). Based on the preparation method, they were termed as CICB1, CICB2, CICB3, and CICB4. Effect of input variables such as pH (5–11) and concentrations of sodium alginate (20–40 g/L), calcium chloride (14.7–44.1 g/L), and amount of algal dosage (0.957–2.871 g/L) on the efficacy of beads in removal of Cr(VI) were examined. Comparative study showed that CICB1 was least efficient and maximum removal was obtained with CICB2, in which maximum cyanobacterial biomass was used. The effect of initial concentration (IC) of Cr(VI) on removal was studied using both CICB3 and CICB4 individually. The removal gradually decreased with increase in IC of Cr(VI) for both the beads. Scanning electron microscopy, energy dispersive spectroscopy, and Fourier transform infrared studies of CICB3 and CICB4 were done. To understand the molecular transport mechanism of Cr(VI) within the solution, mesoscale simulations using computational fluid dynamics were used for predicting the removal of hexavalent chromium.

Keywords: computational fluid dynamics; cyanobacteria; hexavalent chromium; immobilization; mathematical modeling

AU7 ▶ Introduction

AU8 ▶ THE ADVANCEMENT IN industrialization, increase in human population, and practices of agricultural activities have intensified the environmental pollution, and affect mainly the ecosystem with the accumulation of different pollutants such as dyes, pesticides, nuclear waste, organic chemicals, and potentially toxic elements (Papageorgiou *et al.*, 2008). Water pollution in presence of potentially toxic elements causes a major threat to living organisms due to their recalcitrant and nonbiodegradable nature. After discharge to natural water bodies, potentially toxic elements directly affect the aquatic biota. The potentially toxic elements are present in different chemical states in the environment and eventually, accumulated in the food chain. Due to intensive industrial development and urbanization, the

noosphere receives high load of potentially toxic elements, which further results in the increase of contamination footprint (Baltrėnaitė *et al.*, 2018). Although trace amount of these metals is necessary for growth, depending on the different chemical forms and exposure level, potentially toxic elements cause serious health hazards to human beings due to their carcinogenic and mutagenic nature (Sen *et al.*, 2017). The presence of chromium in the water has been found as one of the major concerns due to its abundant use in the industrial sector. Chromium toxicity largely depends on its oxidation state, which varies in the range of –2 to +6, in which trivalent (III) and hexavalent (VI) forms are the most stable forms (Allway and Ayres, 1997; Prabhakaran *et al.*, 2019). Hexavalent chromium [Cr(VI)] is near about 300 times more toxic due to its mutagenic, teratogenic, and carcinogenic nature (Sen *et al.*, 2017). The details of the chromium compounds and their toxicity were described by Gad (1989). In a review article, Kumar *et al.* (2015) showed that most of the studies on removal of chromium were based on hexavalent chromium. While Cr(III) may be used as an essential nutrients for some animal and plants, Cr(VI) is

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carcinogenic in nature. Thus, most of the researchers are interested in removal of Cr(VI) or reduction of it into Cr(III) (Ma *et al.*, 2019). The sources of Cr include effluents from different industries such as dyeing, paint, wood processing, mining, metal cleaning, leather tanning, electroplating, and textile industries. As for example, Chhikara and Dhankhar (2008) reported the concentration of Cr(VI) in the electroplating industry effluent as 117 mg/L. According to WHO guidelines, the extreme allowable limit of Cr(VI) in drinking water is 0.05 mg/L, whereas EPA limits the total Cr level in drinking water to 100 μ g/L (Gupta and Rastogi, 2009). Thus, proper treatment of Cr laden wastewater is mandatory to reduce its concentration below the permissible limit.

Several research groups used several techniques for removal of Cr from wastewater over last few decades. Among them, the most popular and conventional techniques are membrane separation, solvent extraction, chemical precipitation, ion exchange (Ahalya *et al.*, 2003; Ahluwalia and Goyal, 2007; Mukherjee *et al.*, 2013). Usage of biochar in removal of pollutants may be an alternative method (Glaser *et al.*, 2017). However, the limitations of using these processes are high chemical or energy requirements, high cost of operation, and the formation of secondary pollutants. Further, the efficiency turns out to be very low when the Cr concentration in the solution is below 100 mg/L (Anjana *et al.*, 2007). As a polishing step, researches are now tending toward biological treatment process that has gained a great attention to treat the wastewater in an efficient manner due to its numerous benefits over traditional conventional techniques. Removal of pollutants through bioremediants is a two-step process, biosorption and bioaccumulation. In biosorption, the uptake of pollutants occurs only in the cell wall and considered as a metabolism independent process, whereas, the intracellular uptake of pollutants takes place in bioaccumulation, which is a metabolically active process and restricted to living cells only (Dutta *et al.*, 2016; Hu *et al.*, 2019). The main advantages of biological removal over any other methods are low cost of operation, environment friendly nature, and high effectiveness in low metal concentration solution. Microorganism such as bacteria, fungi, yeasts, and so on are capable of removing pollutants from wastewater and polluted air (Baltrėnas *et al.*, 2016), but cyanobacteria have some additional advantages over others due to their higher surface area and mucilage volume with better binding affinity and requirements of simple nutrients (Sen *et al.*, 2017). Several functional groups such as carboxyl, sulfate, amino, and amido present in algal or cyanobacterial cell attract the potentially toxic ions and quarantine within the cell (Bayramoğlu *et al.*, 2006). Living, dead, and immobilized biomass of cyanobacteria can effectively be used in remediation process (Kumar *et al.*, 2015).

Literature review shows that immobilized cell systems have several advantages such as easy separation of cells, provision of getting high cell concentration, suitable micro-environmental conditions, and elimination of cell washout problem at high dilution rates and improve chemical stability (Hu *et al.*, 2016, 2017). Immobilization on some suitable matrix such as a form of bead has been proved to be useful to combat pollution due to its porous characteristics, rigidity, and higher mechanical strength. Further, through desorption, the metal can be recovered from the loaded beads which minimizes the potential of environmental contamination

(Bayramoğlu *et al.*, 2006). Alginate, being commercially available as sodium salt of alginic acid at low cost, may be converted into a hydrogel via cross-linking with divalent calcium ions, where each divalent metal ion binds to two carboxyl groups as adjacent alginate molecules (Szekalska *et al.*, 2016). As a skeletal component of marine algae, it has the property of being strong and at the same time flexible (Chatterjee *et al.*, 2015). Some advantages of alginate beads are biodegradability, hydrophilicity, presence of carboxyl groups, and natural origin (Eroglu *et al.*, 2015). Alginate has also been used for many biomedical applications due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by the addition of divalent cations such as Ca^{2+} . Due to such advantages, Ca-alginate is used as matrix for immobilization of bacterial cells, enzymes, and so on (Chatterjee *et al.*, 2015; Ontañón *et al.*, 2017). Again, the removal operation in a continuous packed bed reactor using microorganism immobilized calcium alginate bead as packing material would be a better approach to treat the wastewater effectively (Migahed *et al.*, 2017). Several researchers used microorganism immobilized calcium alginate bead to remove heavy metals from wastewater (Cataldo *et al.*, 2016; Wang *et al.*, 2016, 2017). In a review article, De-Bashan and Bashan (2010) illustrated the synergistic effect of microalgae and calcium alginate. They showed that immobilization of microalgae in alginate beads had significantly increased the chlorophyll, carotenoids, dry weight, and lipid contents during the stationary and resting growth phases, compared to free-living cells. Further, photosynthesis had also been enhanced compared to free cells (De-Basan and Basan, 2010). Thus, the practical application of cyanobacteria immobilized calcium alginate bead (CICB) for the removal of Cr(VI) can be considered as a promising technique. Although there are several advantages of using Ca-alginate as immobilization matrix for microorganisms or enzymes, the usage of immobilized beads instead of free-living strain increases the cost of chemical for immobilization. It is found from the literature that under metal stressed condition, algae and/or cyanobacteria can accumulate higher lipid content and from the extracted lipid, biodiesel can be produced through transesterification (Markou and Nerantzis, 2013). Apart from lipid, other biomolecules such as protein and carbohydrate of biomass can be used for production of other value added products (Upendar *et al.*, 2018). For free cyanobacterial cell, these value added products can be extracted easily from the biomass after the removal of pollutants, while the extraction of such products from immobilized cells is a technically challenged task. Further, the increase in mass transfer resistance and thereby, reduction in rate of metal uptake owing to immobilization of cells is an added disadvantage of such process. Keeping all these aspects in consideration, a comprehensive study comprising of preparation of immobilized cell system, characterization, kinetic study, and state-space representation using computational fluid dynamics (CFD) modeling to predict removal of Cr(VI), which has hardly been seen in literature, has been reported in the present article. Many researchers have studied the removal of Cr(VI) using algal biomass, but the implementation of COMSOL Multiphysics to predict the concentration at different time is quite terse. The present study highlights the removal of Cr(VI) from synthetic wastewater by *Limnospira limneticus*, a cyanobacterial species, immobilized on calcium alginate

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following the four different methods. The effects of initial concentration (IC) of calcium chloride, sodium alginate, pH, and biomass on removal of Cr(VI) were studied and the optimum values of these parameters were determined. Finally, state-space modeling has been used to predict the removal of Cr(VI).

Experimental Protocols

All the chemicals used in the present study were of Analytical Reagent grade and purchased from Merck, India. All the experiments were performed in triplicate and the mean data with standard deviation values were reported.

Collection, identification, and culture of cyanobacterial strain

The test strain was collected from the East Kolkata Wetland (EKW), a Ramsar Site as per 2002 convention, consisting of multifunctional wetland ecosystem. It comprises of sewage fed fisheries and small agricultural plots. The EKW nurtures the world's largest wastewater fed aquaculture system. Thus the microalgal strains obtained from such wastewater are resistant to pollutants (Sen *et al.*, 2018). The details of collection, separation, and identification were reported by Sen *et al.* (2018). The strain was identified as *L. limneticus*. Finally, the identified strain was grown in pure sterile BG-11 media for laboratory culture inside an algal incubator maintained at temperature of $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with light/dark cycle (16 h/8 h) with light intensity of 2,500 Lux. Subcultures were maintained for every 15 days.

Immobilization of cyanobacteria in calcium alginate bead

Calcium alginate was selected as organic matrix for immobilization of cyanobacterial biomass. When sodium alginate is added to the aqueous solution of calcium salt, the calcium ions replace sodium ions and form insoluble calcium alginate compound. Four different methods were followed to immobilize test cyanobacterial strain onto calcium alginate bead.

Without BG 11 medium

The well-grown culture obtained after 15 days was used for the immobilization on calcium alginate bead. Specific volume (100 mL) of cyanobacterial culture was harvested by centrifugation at 5,000 rpm for 15 min. Sodium alginate solution was prepared by dissolving definite amount of sodium alginate in 100 mL of distilled water. Cell residue was mixed with 20 mL of autoclaved solution of sodium alginate. Later on, calcium chloride solution was prepared by dissolving requisite amount of $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ in 1,000 mL of distilled water. The solution was then autoclaved. The cyanobacterial mixture was added drop by drop into $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution using micro pipette at 4°C to form uniform size spherical beads (~ 3 mm diameter) in the laminar chamber. The cyanobacterial beads were rinsed by sterile distilled water.

To get optimum condition for immobilization, the following experiment was carried out. First, the concentrations of sodium alginate (20, 30, and 40 g/L), calcium chloride (14.7, 29.4, and 44.1 g/L), algal biomass (0.957, 1.914, and 2.871 g/L), and pH (5, 7, 9, and 11) were varied individually. The ability to remove Cr(VI) was tested for beads prepared at

each experimental condition and the corresponding condition at which maximum removal was obtained was considered as optimum. The beads produced at optimum condition following such method were termed as *CICB1*.

With BG-11 medium

In the next study, a new approach was tested for immobilization of cyanobacterial strain in calcium alginate bead. Two different weights of biomass such as 2.87 and 7 g/L were used separately. Initially, to confirm higher removal of Cr(VI), higher algal biomass (7 g/L) was used. Later, the concentration of algal biomass (2.87 g/L), which was found to be optimum during preparation of *CICB1*, was chosen for preparation of immobilized cyanobacterial strain having lower concentration of biomass. Sodium alginate solution (20 g/L) was prepared by dissolving 2 g sodium alginate in 100 mL of distilled water. Cell residue was mixed with 20 mL of autoclaved solution of 20 g/L sodium alginate. Later, 40 mL sterile BG 11 medium was added to this mixture to yield a mixture of cyanobacterial suspension, sodium alginate, and BG-11 media. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (29.4 g/L) was dissolved in 1,000 mL of distilled water and the solution was then autoclaved. The cyanobacterial cells in the mixture of sodium alginate and BG-11 was then dropped into 29.4 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution aseptically using micro pipette at 4°C to form uniform-sized spherical beads (~ 3 mm diameter). The algal beads were rinsed using sterile distilled water. The beads with higher algal biomass (7 g/L) were termed as *CICB2* and the beads with lower algal biomass (2.87 g/L) were termed as *CICB3*. They were tested for Cr(VI) removal independently. In the fourth method, 2 g sodium alginate solution was dissolved in 100 mL of BG-11 media. The concentration of cyanobacterial biomass (2.87 g/L), which was found to be optimum during preparation of *CICB1* was chosen to be used in such method. Cell residue was mixed with 20 mL of autoclaved solution of 20 g/L sodium alginate. Later, 40 mL sterile BG 11 medium was added in this mixture to yield a mixture of cyanobacterial cells, sodium-alginate and BG-11 media. The cyanobacterial mixture was then dropped into 29.4 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ solution aseptically using micro pipette at 4°C to form uniform-sized spherical beads (~ 3 mm of diameter). The cyanobacterial beads were rinsed using autoclaved distilled water. The beads produced following such method were termed as *CICB4*. To assess the role of cyanobacterial strain, control experiments were performed with the calcium alginate bead without cyanobacteria. The beads were prepared under identical conditions as mentioned above without immobilizing cyanobacteria. The glimpse of methods to prepare the alginate beads using different methods are shown in Table 1.

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Investigation of potential of beads for Cr(VI) removal

Stock solution of Cr(VI) (100 mg/L) was prepared by dissolving 0.1414 g of $\text{K}_2\text{Cr}_2\text{O}_7$ in 500 mL distilled water. It was then diluted to 10 mg/L solution by adding requisite amount of distilled water. To study the efficacy of prepared beads, 50 mL of 10 mg/L Cr(VI) solution was contacted with 5 g of beads, prepared by any of the above four methods, in 100 mL of Erlenmeyer flask. The flasks were kept in an algal incubator under specified input conditions and were occasionally shaken manually. For *CICB1*, the removal of

TABLE 1. METHODS OF PREPARATION OF DIFFERENT BEADS

Types of bead	Medium to prepare sodium alginate solution	Medium to prepare mixture of cell suspension and sodium alginate	Cyanobacterial dose (g/L)
<i>CICB 1</i>	Distilled water	Distilled water	2.87
<i>CICB 2</i>	Distilled water	BG-11	7
<i>CICB 3</i>	Distilled water	BG-11	2.87
<i>CICB 4</i>	BG-11	BG-11	2.87

CICB, Cyanobacteria Immobilized Calcium alginate Bead.

Cr(VI) was achieved within 1 h. Thus for *CICB1*, samples were collected after 1 h. However, a distinct increase in removal was observed with *CICB2*, *CICB3*, and *CICB4*. Experiments were run for 20 days and the samples were collected. From the collected samples, the beads were separated from solution by filtration and the solution was analyzed for residual Cr(VI) concentration using Atomic Absorption Spectrophotometer (Perkin-Elmer PinAAcle 900T). To examine the efficacy of *CICB*, removal of Cr(VI) using only alginate beads (abiotic control) and free cyanobacterial strain without immobilization (biotic control) were investigated under identical removal conditions.

Characterization of immobilized bead

SEM (scanning electron microscopy), EDS (energy dispersive spectroscopy), and FTIR (Fourier transform infrared) spectroscopy studies were performed to characterize the biosorbent with and without Cr loading conditions. SEM images of biosorbents, which were prepared following the same condition as that of *CICB3* and *CICB4* without using cyanobacterial cells, were taken using Scanning Electron Microscope (ZEISS) to acquire their topographical characteristics at desired magnification. Further, SEM images of *CICB3* and *CICB4* with and without Cr(VI) loading, were also taken. Analysis was done at working distance of 6 mm and acceleration voltage of 5 kV. EDS study (ZEISS) was performed to get elemental analysis of the *CICB3* and *CICB4* before and after treatment with Cr(VI). FTIR analyses of the samples were obtained using a Perkin-Elmer FTIR Spectrometer (IACS, Kolkata, India) for determination of functional groups with their stretching frequencies over the range of 400–4,000 cm⁻¹ wavenumber.

The efficiency of the beads was estimated in terms of its stability and strength. To study the stability of the beads, they were kept under distilled water taken in a beaker, and the beaker was kept inside a refrigerator. Beads were collected after 1 week interval and the efficacies of beads in removal of Cr(VI) were judged. The strength of the beads was measured in terms of its hardness. To measure the hardness of the beads, compression test was done with increasing load over a bead sample and measured the stress of sample.

Variation of removal of Cr(VI) with time for different IC of Cr(VI)

Cr(VI) solution (10 mg/L) was contacted with 5 g of each of *CICB2*, *CICB3*, and *CICB4* individually for 14 days to compare the efficiency of these beads. The volume of solution was kept 50 mL. After 14 days, the solution was analyzed for residual Cr(VI) concentrations.

To observe the variation of Cr(VI) removal with time, *CICB3* and *CICB4* were contacted with simulated solution of Cr(VI) for 20 days in a batch contactor. One input parameter such as IC (5, 10, and 15 mg/L) was varied. All the experiments were done in 250 mL of conical flask. The volume of solution and pH were kept 50 mL and 7, respectively. Samples were collected after 2 days interval and analyzed for residual Cr(VI) concentrations. The algal incubator was maintained at temperature of 25°C ± 2°C with light/dark cycle (16 h/8 h) with light intensity of 2,500 Lux.

Mathematical modeling

In the present study, a mathematical modeling using CFD has been implemented to assess the molecular transport mechanism of Cr(VI) within the solution. Model can predict the concentration profile of Cr(VI) with time, which can be validated with the experimental results. Normally, three basic conservation equations of mass, momentum, and energy conversation laws are considered for solving any practical problem. As the system is isothermal, no motion in the system makes the momentum and energy conversation laws undisturbed. So, a standard transient convective–diffusion mass transport equation is considered to study the experimental phenomena on removal of Cr(VI), and to solve such a phenomenon, the approach of CFD was used.

As the concentration changes with time, the unsteady state mass balance has been done here. The equation is represented as follows:

$$\frac{\partial C^*}{\partial t} + \nabla \cdot (U^* C^*) = \nabla \cdot (D^* \nabla C^*) + S^* \quad (1)$$

$$S^* = -k_{ad}(C^* - C_e) \quad (2)$$

Accumulation term and convective mass transport term are the first and second terms, respectively, in the left hand side of Equation (1) while diffusive term and source term are in right hand side of Equation (1).

As, $U^* = 0$ due to absence of agitation, Equation (1) reduces to:

$$\frac{\partial C^*}{\partial t} = \nabla \cdot (D^* \nabla C^*) - k_{ad}(C^* - C_e) \quad (3)$$

The suggested model was based on some assumptions which are given below:

- (1) The adsorbent bed was considered as a bunch of particles having equal size with uniform porosity.

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- (2) An adsorbent bed was considered settled at the bottom and the system was stationary throughout the process.
- (3) Initially the adsorbent bed was free of Cr.
- (4) No convective mass transfer present in the system. Only mass transport resistance and biosorption resistance are present. Biosorption resistance was the dominant factor in this model.
- (5) The entire system was temperature and pressure independent, that is, isothermal and isobaric.
- (6) As convective velocity has been assumed as zero, there is no turbulence

First order bio-adsorption rate kinetics, boundary conditions, and fine mesh size were considered for developing and solving the model.

The values of D^* and k_{ad} were found by adjusting parameter method. Using the values of D^* and k_{ad} , concentration profiles with respect to time would be obtained from the nonlinear Equation (3). Thus, minimum number of model parameters (D^* and k_{ad}), which are reliable and capable to explain the removal of Cr(VI) from the contaminant solution over a wide range of IC of Cr, was used to verify experimental results. For the model implementation, the finite difference commercial solver COMSOL Multiphysics (version 4.3a; COMSOL, Inc., Burlington, MA) solves the partial differential equations.

Statistical analysis

To analyze the difference between the equality of variance and mean values for the removal of Cr(VI), a paired-samples F -test and t -test (two-tailed) was performed. Statistical analysis was done by varying the IC of Cr(VI) from 5 to 15 mg/L, and the removal of Cr(VI) was analyzed for every 2 days. For statistical analysis, the confidence level was set as 0.05 and IBM SPSS Statistics 20.0 (IBM, Armonk, NY) was used.

Results and Discussion

Growth study in normal BG-11 media

F1 ► The variation of dry biomass content with time is shown in Fig. 1 when *L. limneticus* has been grown in BG-11 medium. From Fig. 1, it is seen that the lag phase exists for the duration of 0–2 days. During lag phase, cyanobacteria synthesize new enzymes and orienting the metabolite pathway for adaptation to new environment. Thus, growth during such period is very less (Sen *et al.*, 2017). Stationary phase starts after 14 days. In between, the log phase or exponential phase exists. Biomass has increased from 0.0408 ± 0.002 g/L to 0.4272 ± 0.024 g/L during log phase in the time course of 2–14 days. For the growth of cyanobacteria, some essential nutrients play a very important role. During log phase, the abundance of such essential nutrients facilitates the active growth of the strain, whereas after the active growth is over, lack of those nutrients causes no further growth of the strain (Sen *et al.*, 2017).

Characterization of immobilized bead

SEM of blank Ca-alginate bead, cyanobacteria immobilized alginate beads, and cyanobacteria immobilized bead after the treatment with Cr(VI) for both *CICB3* and *CICB4*

FIG. 1. Growth study of the cyanobacterial strain in normal BG-11 media.

have been done and the micrograms are shown in Fig. 2a–f, respectively. From Fig. 2a–f distinct differences in surface structure have been seen. SEM image of Ca-alginate bead without cyanobacterial biomass shows polymeric bead-like structure, whereas after algal immobilization, a smoothening effect is seen and size has also been enlarged. After treatment with Cr(VI), the smoothening effect is even more, which indicates the binding of Cr(VI) onto the treated alginate beads. Such smoothness of the surface may be due to the binding of Cr(VI) onto the alginate beads that makes the surface less coarser than its original form. SEM studies were performed by several researchers during the removal of pollutants using alginate immobilized beads (Cataldo *et al.*, 2016; Wang *et al.*, 2016, 2017). Cataldo *et al.* (2016) showed that before treatment, the surface of the beads were smooth, whereas a shrinkage of the beads was observed at high metal ion concentration for the treated bead. Wang *et al.* (2016) observed from the SEM study that for the treated beads, the metal ions distributed homogeneously over the entire surface. In another study Wang *et al.* (2017) observed a pore-like structure of the beads after being contacted with metal ions. The EDS spectra of the four samples have been done. EDS spectra of untreated and treated *CICB3* and *CICB4* have been shown in Fig. 2g–j, respectively. Tables 2 and 3 give a comparative elemental analysis of the same samples. From Fig. 2g–j and Tables 2 and 3, it is seen that the presence of Cr is found only in the treated algae immobilized alginate bead, whereas no trace of Cr has been found in the other two beads. The presence of Cr in the treated algae immobilized alginate bead confirms the binding of Cr(VI) onto the treated bead. Tables 4 and 5 represent the FTIR data of both *CICB3* and *CICB4* for blank beads, cyanobacteria immobilized beads, and immobilized beads after treatment with Cr(VI). Supplementary Figure S1a–f representing the FTIR spectra of both *CICB3* and *CICB4* for blank beads, cyanobacteria immobilized beads, and immobilized beads after treatment with Cr(VI) are incorporated as “Supplementary Data.” In case of blank *CICB3*, the main characteristics bonds are C-S and C-O-C, C-C and C-N, N-H (Bend), -PH, and -OH at the corresponding wave number of 667, 1,047, 1,605, 2,367, and 3,742 cm^{-1} . For immobilized *CICB3* before treatment, the responsible bonds are C-S and C-OH, C=O and C=C, and

FIG. 2. (a–c) SEM images of blank Ca-alginate bead for *CICB3*, *CICB3* before treatment with Cr(VI), *CICB3* after treatment with Cr(VI); (d–f) SEM images of blank Ca-alginate bead for *CICB4*, *CICB4* before treatment with Cr(VI), *CICB4* after treatment with Cr(VI); (g, h) EDS spectra *CICB3* before and after the treatment with Cr(VI); (i, j) EDS spectra *CICB4* before and after the treatment with Cr(VI). *CICB*, cyanobacteria immobilized calcium alginate bead; EDS, energy dispersive spectroscopy; SEM, scanning electron microscopy.

-PH at wavenumbers 667, 1,633, and 2,358 cm^{-1} , respectively. While for treated bead, the bonds present are C-N-C, P-O-C, and C-C, C-N and -OH, COO-, and -NH₂ at 416, 1,038, 1,420, 1,596, and 3,399 cm^{-1} wavenumbers, respectively. Similarly for blank *CICB4*, the responsible functional groups are C-C, C-N and -OH, NH₂, -PH, and N-H at wavenumbers 1,020, 1,420, 1,596, 2,367, and 3,296 cm^{-1} , respectively. For untreated *CICB4*, C-N-C, P-O-C, -OH, COO-, -CH₃ and -CH₂, and -NH₂ bonds are responsible at wavenumbers 462, 1,038, 1,438, 1,605, 2,925, and 3,389 cm^{-1} . While for treated bead, the peak of 1,438 and

3,389 cm^{-1} are shifted to 1,429 and 3,436 cm^{-1} . Further, at wavenumbers 667, 1,652, and 2,367 cm^{-1} , additional functional groups of C-N-C, COO-, -CH₃, and -CH₂ are appeared in treated bead. In a study of Cd(II) removal using immobilized *Rhizomucor tauricus*, Kumar *et al.* (2009) found that the responsible groups for cadmium removal are -OH and -NH stretching, -CH stretching, and C=O groups. In another study on removal of heavy metal ions from drinking water by alginate-immobilized *Chlorella sorokiniana*, Petrovič and Simonič (2015) found the strongest absorption peak at 3,402 cm^{-1} , which corresponds to the -OH stretching of

FIG. 2. (continued)

TABLE 2. ENERGY DISPERSIVE SPECTROSCOPY ANALYSIS OF (A) CYANOBACTERIA IMMOBILIZED ALGINATE BEADS AND (B) CYANOBACTERIA IMMOBILIZED BEAD OF *CICB3* AFTER THE TREATMENT WITH Cr(VI)

<i>CICB3 before experiment (a)</i>		<i>CICB3 after experiment (b)</i>	
Elemental line	Wt %	Elemental line	Wt %
C K	0.00	C K	0.59
N K	1.95	N K	3.51
O K	52.14	O K	65.14
F K	0.27	F K	3.51
Na K	0.46	Na K	0.00
P K	0.00	P K	2.91
P L	—	P L	—
Cl K	8.50	Cl K	3.79
Cl L	—	Cl L	—
Ca K	23.29	Ca K	20.02
Ca L	—	Ca L	—
Zr L	13.40	Cr K	0.53
Zr M	—	Cr L	—
Total	100.00	Total	100.00

TABLE 3. ENERGY DISPERSIVE SPECTROSCOPY ANALYSIS OF (A) CYANOBACTERIA IMMOBILIZED ALGINATE BEADS AND (B) CYANOBACTERIA IMMOBILIZED BEAD OF *CICB4* AFTER THE TREATMENT WITH Cr(VI)

<i>CICB4 before experiment (a)</i>		<i>CICB4 after experiment (b)</i>	
Elemental line	Wt %	Elemental line	Wt %
C K	0.00	C K	0.73
N K	0.00	N K	3.99
O K	59.88	O K	61.04
Na K	0.45	F K	0.00
P K	3.39	Na K	0.86
P L	—	Si K	4.75
Cl K	13.91	Si L	—
Cl L	—	P K	0.00
Ca K	22.19	P L	—
Ca L	—	K K	0.88
Fe K	0.18	K L	—
Fe L	—	Ca K	17.16
Total	100.00	Ca L	—
		Cr K	0.60
		Cr L	—
		Zr L	10.00
		Zr M	—
		Total	100.00

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TABLE 4. FOURIER TRANSFORM INFRARED ANALYSIS OF (A) BLANK CA-ALGINATE BEAD, (B) ALGAE IMMOBILIZED ALGINATE BEADS, AND (C) ALGAE IMMOBILIZED BEAD OF *CICB3* AFTER THE TREATMENT WITH Cr(VI)

<i>CICB3 blank beads (a)</i>		<i>CICB3 before experiment (b)</i>		<i>CICB3 after experiment (c)</i>	
Frequency range	Functional groups	Frequency range	Functional groups	Frequency range	Functional groups
667	C-S, C-O-C	667	C-S, C-OH	416	C-N-C
1,047	C-C, C-N	1,633	C=O, C=C	1,038	P-O-C, C-C
1,605	N-H (Bend)	2,358	-PH	1,420	C-N, -OH
2,367	-PH	—	—	1,596	COO ⁻
3,742	-OH	—	—	3,399	-NH ₂

hydroxyls, indicating the presence of these groups on the biosorbent. The region 3,600–3,300 cm⁻¹, next to the O–H vibrations, showed the characteristic bands for N–H stretching vibrations, present in algal biomass. Besides, several other functional groups were identified, such as C–H bending of the aliphatic functional group (2,924–2,854 cm⁻¹) and C–C stretching vibrations of the aromatic ring (1,419 cm⁻¹), while C–N stretching of aromatics and C–O stretching of alcohols and carboxylic acids were found in the region of 1,265–1,319 cm⁻¹. In the region between 1,800 and 1,500 cm⁻¹, the characteristic bands for proteins were seen, more specifically C=O stretching vibrations of peptide bonds typical of amide-I bands and N–H bending vibrations characteristic of amide-II bands. The strongest peak in this area was found at 1,604 cm⁻¹, reflecting the amines and amides of algal biomass. On the contrary, the C–O stretching vibrations of alcohols, carboxylic acids, and carbohydrate of polysaccharides of both algal biomass and alginate were noticed in the region between 1,026 and 1,087 cm⁻¹. From the stability analysis, it has been found that the efficacies of the beads in removal of Cr(VI) has remained same up to 7 weeks. To measure the strength, five similar compression tests have been carried out and the average value of the breaking stress has been found as 0.2197 Mpa.

Immobilization of *L. limneticus*

Effect of input factors on immobilization of cyanobacterial biomass onto calcium alginate bead

F3 ► *Effect of pH on immobilization.* While concentrations of sodium alginate (20 g/L), calcium chloride (29.4 g/L), and algal biomass (0.957 g/L) have been kept constant during immobilization of test strain, pH has been varied as 5, 7, 9, and 11 separately. The variation of removal of Cr(VI) using beads prepared at various pH condition is shown in Fig. 3. Removal of Cr(VI) has increased from 4.52% ± 0.14% to

18.52% ± 0.57% with increase in pH from 5 to 9. However, with further increase in pH, there is a decrease in removal of Cr(VI). At pH 11, removal obtained is only 13.35% ± 0.41%. Therefore, optimum pH for immobilization has been found to be 9. With the increase in pH from 5 to 9, there is increase in adsorption of metal ions per unit weight of biosorbent. When the pH of the medium is in acidic range, there is an adverse effect on biosorption capacity. For further increase in pH up to 11, the removal is comparatively low. The reason behind the phenomena may be less availability of Cr(VI) in the solution due to the formation of complex compound in presence of other anion group (Sen *et al.*, 2017).

Effect of pH on removal of mercury, cadmium, and lead was studied by Bayramoğlu *et al.* (2006). According to their study, the dependency of metal biosorption on pH is associated with both the functional groups present in the cell wall of the biosorbent and the chemistry of the metals in solution. The ionization state of the different functional group and the solubility of the metals strongly depend on the solution pH. The negatively charged carboxylate and phosphate ions allow the microbial cells to bind cations. The maximum biosorption capacity was found in between the pH of 5 and 6 (Bayramoğlu *et al.*, 2006). In another study Feng and Aldrich (2004) showed that the optimum pH for the removal of Cu(II) and Pb(II) is in the range of 5.8–8.5.

Effect of concentration of sodium alginate on immobilization. Concentration of sodium alginate was maintained at 20, 30, and 40 g/L, keeping other immobilization parameters such as concentrations of calcium chloride, algal biomass, and pH constant at 29.4 g/L, 0.957 g/L, and 9, respectively. The beads have been tested for Cr(VI) removal. The removal is strongly dependent on sodium-alginate concentration. The removal decreases from 18.52% ± 0.57% to 2.25% ± 0.11%, when sodium-alginate concentration increases from 20 to 40 g/L. At 30 g/L sodium alginate concentration, the removal is minimum (1.98% ± 0.06%). Therefore, 20 g/L has been

TABLE 5. FOURIER TRANSFORM INFRARED ANALYSIS OF (A) BLANK CA-ALGINATE BEAD, (B) ALGAE IMMOBILIZED ALGINATE BEADS, AND (C) ALGAE IMMOBILIZED BEAD OF *CICB4* AFTER THE TREATMENT WITH Cr(VI)

<i>CICB4 blank beads (a)</i>		<i>CICB4 before experiment (b)</i>		<i>CICB4 after experiment (c)</i>	
Frequency range	Functional groups	Frequency range	Functional groups	Frequency range	Functional groups
1,020	C-C	462	C-N-C	667	C-S
1,420	C-N, -OH	1,038	P-O-C	1,038	P-O-P, C-N
1,596	NH ₂	1,438	-OH	1,429	-OH
2,367	-PH	1,605	COO ⁻	1,652	C=O
3,296	N-H	2,925	-CH ₃ , -CH ₂	2,367	-PH
—	—	3,389	-NH ₂	3,436	-NH ₂

FIG. 3. The variation of removal of Cr(VI) for different pH.

taken as optimum concentration of sodium alginate. This may be due to the adverse effect of higher concentration of sodium alginate during preparation of calcium alginate bead.

Effect of concentration of calcium chloride on immobilization. Concentration of calcium chloride was varied as 14.7, 29.4, and 44.1 g/L, keeping other immobilization parameters such as concentrations of sodium alginate, algal biomass, and pH constant at 20 g/L, 0.957 g/L, and 9, respectively. To examine Cr(VI) removal efficacy, the same method has been followed as described above and it is seen that calcium chloride concentration has prominent effect on the removal of Cr(VI). The removal increases from $14.15\% \pm 0.44\%$ to $18.52\% \pm 0.57\%$ when calcium chloride concentration increases from 14.7 to 29.4 g/L. However, further increase in calcium chloride concentration results in decrease in removal of Cr(VI). At 44.1 g/L calcium chloride, removal obtained is only $7.36\% \pm 0.23\%$. Therefore, 29.4 g/L has been taken as optimum concentration of calcium chloride.

Effect of concentration of cyanobacterial biomass on immobilization. Concentration of algal biomass has been varied as 0.957, 1.914, and 2.871 g/L, keeping other immobilization parameters such as concentrations of sodium alginate, calcium chloride, and pH constant at 20 g/L, 29.4 g/L, and 9, respectively. It is observed that algal biomass concentration has pronounced effect on removal of Cr(VI). The removal increases from $18.52\% \pm 0.57\%$ to $19.43\% \pm 0.53\%$ and then to $20.52\% \pm 0.64\%$ when algal biomass concentration varies as 0.957, 1.914, and 2.871 g/L. More the cyanobacterial biomass, the more will be the functional groups as present in cell wall, and hence, more will be the removal of Cr(VI). Therefore, the optimum condition for immobilization by varying one parameter at a time has been found to be $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 29.4 g/L, sodium alginate: 20 g/L, cyanobacterial biomass: 2.871 g/L, and pH: 9. Sample obtained at optimum condition is termed as *CICB1*. The removal of Cr(VI) obtained using *CICB1* is $20.92 \pm 0.72\%$. Similar experiment on increasing metal removal process with the increase in biomass dosage was reported by Chhikara and Dhankhar (2008). They studied biosorption of Cr(VI) using immobilized *Aspergillus niger* for different amount of biomass dosage ranging from 0.1 to 1.2 g. The study revealed

that up to 1 g biomass dosage, the uptake of Cr(VI) increased significantly and beyond that, the removal became stationary. Thus, 1 g was chosen as optimum dosage.

Variation of removal of Cr(VI) with time for different IC

The comparative study on removal of Cr(VI) between *CICB2*, *CICB3*, and *CICB4* indicates that the removal is maximum ($89.8\% \pm 2.81\%$) for *CICB2* in comparison with *CICB3* ($44.82\% \pm 1.40\%$) and *CICB4* ($48.89\% \pm 1.52\%$). This is because in the case of *CICB2*, higher amount of algal biomass (7 g/L) was used in comparison with the other two beads. More the algal biomass, the more is the availability of binding site and possibility of binding. However, between *CICB3* and *CICB4*, *CICB4* gives more removal. This may be due to the presence of more amount of BG-11 medium in *CICB4*. Although *CICB2* shows higher removal in comparison to the other two beads, from the economic point of view, it is better to use *CICB3* and *CICB4*, as these two beads require lower cyanobacterial dosage. Thus, in the present study, these two beads (*CICB3* and *CICB4*) have been chosen for further analysis.

Further, to examine the efficacy of *CICB*, removal of Cr(VI) using only alginate beads (abiotic control) and free cyanobacterial strain without immobilization (biotic control) have been investigated. From the experiment, it has been observed that $0.53\% \pm 0.002\%$, $0.72\% \pm 0.002\%$, and $0.73\% \pm 0.001\%$ Cr(VI) have been removed using blank alginate beads with 5 mg/L initial Cr(VI) concentration after 4, 8, and 12 days, respectively, whereas under identical operating conditions, free cyanobacterial strain without immobilization can remove $21.02\% \pm 0.032\%$, $37.23\% \pm 0.43\%$, and $43.11\% \pm 0.083\%$ Cr(VI) from the solution for same period of time. However, in the current study, $39.22\% \pm 1.063\%$, $56.03\% \pm 2.085\%$, and $60.77\% \pm 2.082\%$ Cr(VI) were removed using *CICB3* and $44.76\% \pm 1.013\%$, $64.35\% \pm 2.023\%$, and $82.81\% \pm 3.051\%$ Cr(VI) were removed using *CICB4* under the same operating condition.

The variation of removal with time for different ICs using *CICB3* has been shown in Fig. 4a. From Fig. 4a, it is seen that with increase in IC from 5 to 15 mg/L, the removal decreases from $75.63\% \pm 2.36\%$ to $41.78\% \pm 1.30\%$ for 20 days of operation. This suggests that removal process is not diffusion controlled, but kinetically controlled. Maximum removal of $75.63\% \pm 2.36\%$ is obtained with 5 mg/L IC. The rate of removal is more for initial 4 days and after that, the removal becomes almost constant. The variation of removal with time for different ICs for *CICB4* has been shown in Fig. 4b. From Fig. 4b, it is seen that removal increases with decrease in concentration. When IC increases from 5 to 15 mg/L, removal of Cr(VI) decreases from $85.78\% \pm 2.66\%$ to $55.98\% \pm 1.13\%$ for 20 days of operation. This suggests that bulk diffusion resistance is negligible for the system. As the removal of Cr(VI) is significantly affected with the varying IC, it can be stated that, for this system, the resistance due to kinetic parameter plays the dominant role, and by altering such kinetic parameter, the rate of reaction can be controlled. Thus, the removal process is not diffusion controlled, but kinetically controlled (Treybal, 1980). However, higher removal at lower concentration of Cr(VI) may be due to the presence of sufficient amount of immobilized bioremediant (100 g/L in each case) and when the IC increases, the ratio of the sorptive surface to the Cr(VI) ion concentration becomes

FIG. 4. (a) The variation of removal with time for different initial concentration of Cr(VI) using *CICB3*. (b) The variation of removal with time for different initial concentration of Cr(VI) using *CICB4*. (c) The experimental and model predicted results of removal of Cr(VI) for different initial concentration of Cr(VI) for *CICB3*. (d) The experimental and model predicted results of removal of Cr(VI) for different initial concentration of Cr(VI) for *CICB4*.

less, which results in a decrease in removal (Chhikara and Dhankhar, 2008). Chhikara and Dhankhar (2008) showed that with the increase in Cr(VI) concentration up to 100 mg/L, the removal gradually decreased, and after that, there was a sharp decrease in removal. Further, it was seen that the initial rate of Cr(VI) removal is high up to 4 days of operation. The rate became slow after 4 days and the removal became almost invariant with time. The biosorption is a fast process, and thus, initially biosorption is the only mechanism for metal removal. For biosorption, surface molecules can have a saturation level, after which no more metallic ions can be adsorbed. During bioaccumulation, the metal ions get inside the cell cytoplasm by some metabolism leaving biosorption site vacant. For accumulation of metal, an efflux mechanism can be functioning at a certain metal concentration preventing accumulation of more metal (Velasquez and Dussan, 2009). For lower IC, biosorption as well as bioaccumulation may be taking place, whereas for higher IC of 10 and 15 mg/L, the active sites of cell have been occupied by metal ions through biosorption, and since bioaccumulation is slow process, it may not happen so quickly, and thus, removal becomes invariant with time. Therefore, the constant removal after 12

days may be attributed to the continuous biosorption and desorption processes by immobilized microalgae with Cr(VI).

It has been observed from the statistical analysis that for *CICB3*, there is no significant difference between the mean values of percentage removal with increase in time since $p > 0.05$, and there is a difference between the values of percentage removal with increase in concentration of chromium from 5 to 15 mg/L ($p < 0.05$). However, for *CICB4*, statistical analysis results reveal that there is no significant difference between the values of percentage removal from days 0 to 10; but there is a significant difference between days 0 and 12 and later on ($p < 0.05$). Also, it has been seen that there is a significant difference between the mean values of percentage removal with increase in IC of chromium from 5 to 15 mg/L.

Mathematical modeling

Optimization of model parameters. In this study, only one equation of transient diffusive mass transport equation is considered. The equation is nonlinear with two unknown parameters, diffusivity (D^*), and biosorption capacity (k_{ad}) of Cr(VI). So, the practical situation cannot be solved

easily. Regression method is considered with practical knowledge of diffusivity of the component in liquid medium in the range of 10^{-9} m²/s. During the process of solving, transport of diluted species is considered, within which the backward differentiation formula (BDF) solver, an implicit solver, is used with an absolute concentration tolerance 10^{-5} mg/L to study the concentration profile of Cr(VI). After so many iterations by changing the values of D^* and k_{ad} , the reasonable values have come out and these are 3×10^{-9} m²/s and 1.494 m/s, respectively.

Model validation. The values of D^* (3×10^{-9} m²/s) and k_{ad} (1.494 m/s), obtained through a trial and error method, were used to solve Equation (3) to get concentration profile with time for both *CICB3* and *CICB4*. The experimentally observed and model predicted concentration-time histories of Cr(VI) for solution of different IC in the range of 5–15 mg/L are represented in Fig. 4c and d, when treated with *CICB3* and *CICB4*, respectively. From Fig. 4c, it is seen that the simulated data match well with experimental ones with average relative deviation of 3.54%, 2.97%, and 3.19% for solution of 5, 10, and 15 mg/L, respectively. Similarly, from Fig. 4d, average relative deviation of 3.84%, 3.01%, and 3.22% reconfirms the suitability of the model to predict the concentration profile with time for Cr(VI) solution of 5, 10, and 15 mg/L, respectively.

For developing the model, few assumptions were considered. These assumptions, on the contrary, can be thought as limitations of the modeling. They are listed as follows:

- The experimental results are validated with the model prediction at different ICs, while the concentration distribution of Cr(VI) at different space of the biosorption reactor has not been addressed.
- The *L. limneticus* strain was immobilized in calcium alginate bead (polymeric matrix) and was kept at the bottom inside the conical flask, considered as batch reactor for removal of Cr(VI). The bed size may not remain constant throughout the reaction time due to the minute enlargement of the alginate beads. However, to simplify the model, the adsorbent bed was assumed to remain same as a bunch of particles having equal size with uniform porosity during the reaction time.
- Hydrodynamic model prediction makes an important role for optimization of process parameter and designing a biosorption reactor in cost-effective way. This study was not incorporated here.
- Further, no convective mass transfer has been assumed in the system. Only mass transport resistance and biosorption resistance are present. Biosorption resistance was the dominant factor in this model.

Conclusion

The effectiveness of alginate immobilized *L. limneticus* in remediation of Cr(VI) has been assessed. Four different methods were followed for immobilization. Input variables such as pH, concentrations of sodium alginate, calcium chloride, and cyanobacterial dose have significant effect on the efficacy of bead in removal of Cr(VI). The maximum dose of cyanobacterial biomass was used in preparation of *CICB2* and it showed maximum removal efficacy. EDS study con-

firms the existence of Cr(VI) in the alginate immobilized biomass after treatment with Cr(VI) laden wastewater. Removal of Cr(VI) was seen inversely related with IC when *CICB3* and *CICB4* were used as bioremediant. Two types of mass transport, molecular or diffusive transport and convective transport, were discussed using CFD model. The predominance of molecular or diffusive transport over convective transport was observed. The simulated data matched quite well with experimental ones. Thus, it can be stated that alginate immobilized *L. limneticus* can effectively be used for Cr(VI) removal. However, based on the cited work in the literature and recent articles, the present study has envisaged the following scope for future work in the context of abatement of Cr(VI), such as (1) finding out suitability and feasibility of using the present microalgal strains for removal of Cr(VI) from real industrial wastewater; (2) implementation of the test strains for removal of Cr(VI) in a continuous flow reactor; (3) scaling-up of reactor suitable for industrial use; (4) prediction of the concentration distribution of Cr(VI) at different space of the biosorption reactor using mathematical model; and finally, (5) exploration of different immobilization matrix in place of alginate only.

Nomenclature

- C^* = concentration at any time (mg/L)
 C_e = equilibrium concentration (mg/L)
 D^* = diffusivity of chromium element within the solution (m²/s)
 k_{ad} = rate constant (second⁻¹)
 S = generation term
 t = contact time (days)
 U^* = convective velocity (m/s)

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

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Supplementary Data

SUPPLEMENTARY FIG. S1. (a) FTIR image of blank Ca-alginate bead for *CICB3*. (b) FTIR image of *CICB3* before treatment with Cr(VI). (c) FTIR image of *CICB3* after treatment with Cr(VI). (d) FTIR image of blank Ca-alginate bead for *CICB4*. (e) FTIR image of *CICB4* before treatment with Cr(VI). (f) FTIR image of *CICB4* after treatment with Cr(VI). *CICB*, cyanobacteria immobilized calcium alginate bead; FTIR, Fourier transform infrared.

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