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An integrated approach to remove Cr(VI) using immobilized *Chlorella minutissima* grown in nutrient rich sewage wastewater

Shailendra Kumar Singh [a], Ajay Bansal [a,*], M.K. Jha [a], Apurba Dey [b]

[a] Department of Chemical Engineering, Dr. B.R. Ambedkar National Institute of Technology, Jalandhar 144011, India
[b] Department of Biotechnology, National Institute of Technology, Durgapur, West Bengal, India

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ABSTRACT

The potential of an integrated system for sewage wastewater treatment and biosorption of chromium(VI) was evaluated using immobilized *Chlorella minutissima* cells. Immobilized algal cells were grown in sewage wastewater in designed photobioreactor for 48 h and then subjected to removal of Cr(VI) from synthetic wastewater. The effect of pH, Cr(VI) concentration, biosorbent dose on Cr(VI) removal was investigated. *C. minutissima* showed a higher NH_4^+-N and PO_4^{3-}-P removal efficiency (above 99% removal) than the NO_3^{2-}-N (58% removal) in 48 h. Biosorption of Cr(VI) was found to be highly dependent on solution pH, biosorbent dose and initial Cr(VI) concentration. Maximum Cr(VI) uptake 57.33 mg Cr(VI)/g dry biosorbent/L of solution was observed at pH 2 with 20% (w/v) biosorbent. Further more than 90% of total Cr adsorbed could be recovered using 0.5 M NaOH as desorption medium.

1. Introduction

The toxic pollutants from the untreated sewage and industrial effluents find their way into surface water reservoirs and rivers. They also percolate into the ground to pollute ground water sources. The sewage discharges are characterized by excess amount of nutrients (NH_4^+, NO_3^{2-}, and PO_4^{3-}) which are responsible for eutrophication in surface waters (Alejandro et al., 2010). Whereas, effluents from industries like leather, mining, steel and electroplating, contain high levels of toxic heavy metals such as chromium, silver, cadmium, copper, cobalt, zinc, iron and lead. Conventional treatment methods of sewage and industrial wastewater are many times complicated and expensive in terms of the land requirement and the energy consumption. According to Asano et al. (2006) nitrification adds 20–30% additional electricity costs to an activated sludge system. Industrial processes for metal removal become ineffective and costly, especially when metal ion concentration in aqueous solution is lower than 100 mg/L (Liang et al., 2010). Furthermore, these wastewater treatment methods also generate huge amount of sludge to be treated with great difficulties. According to a WHO report (2000), only 35% of total sewage wastewater is actually undergoes treatment to secondary level in Asia and almost no sewage is treated in Africa. Biological methods such as bioremediation and biosorption could be attractive alternative technologies for sewage and industrial effluents treatment. Biosorption is considered as an impressive technique to remove metals from aqueous industrial wastewater because of obvious benefits of high efficiency and selectivity for absorbing metals in low concentrations, economically attractive due to the cheap raw materials, environment-friendly, wide operational range of pH and temperature, energy-saving, easy retrieval of metal, and easy recycling of the biosorbent (Naja et al., 2010). However availability and selection of cheap biosorbent material is a major challenge. Among easily available biomasses from all three major groups: algae, fungi and bacteria, algae hold better potential than others. Algal based wastewater treatment systems (phycoremediation) could provide a sustainable solution for the treatment of municipal and industrial wastewater. They are inexpensive and known for their ability to achieve good removal of sewage nutrients, pathogens and organic pollutants (Zimmo et al., 2000). Algae is able to accomplish nutrient removal with a net energy savings to the wastewater treatment system since they supply molecular oxygen to heterotrophic partners and support the initial steps of bio-degradation (Perez-Garcia et al., 2011). The nutrients in the wastewater, instead of being waste, become feed for the algae, which in turn may be utilized as a cheap biosorbent. Nutrient removal coupled with algal biomass production could be an efficient solution for availability and cost challenges of biosorbent. However, algal based treatment system has the difficulty of separating of algal biomass from treated effluent as the use of industrial filtration and centrifugation is not cost effective. Immobilization of the algal cells into a matrix, can offer a feasible alternative to filtration and centrifugation (Alejandro, 2010). Immobilized algae on an appropriate support increases the retention time of cell in aqueous medium. As such the cells become better adapted to the

* Corresponding author. Tel.: +91 9417223839; fax: +91 181 2690932.
E-mail address: bansala@nitj.ac.in (A. Bansal).

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substrate and the risk of washout of cells is diminished. The final separation of algal cells from liquid waste is not required. Their metabolic activity remains constant for long period (Lau and Tam, 1997). Calcium alginate, a very simple and cost-effective immobilizing matrix has been widely used to entrap microbial cells (Bhattacharyya et al., 2010).

Hexavalent chromium is well known for its negative health and environmental impact, and its extreme toxicity (Belay, 2010). The objective of the present investigation was to assess and develop an integrated approach for nutrient removal from sewage wastewater and biosorption of Cr(VI) using immobilized *Chlorella minutissima* cells. *C. minutissima* is already known for its plausible role in wastewater remediation (Bhatnagar et al., 2010). *C. minutissima* cells were first entrapped in calcium alginate matrix and then grown in nutrient rich sewage wastewater. The algal beads grown in sewage wastewater subjected to biosorb Cr(VI) ions aqueous solutions revealed encouraging results.

2. Methods

2.1. Wastewater samples

For the nutrient removal analysis, sewage wastewater was collected from the sewage drain of Dr. B.R. Ambedkar National Institute of Technology, Jalandhar, Punjab, India. Standard methods (Clesceri et al., 1998) were followed for the characterization of the sewage wastewater (Table 1). Stock solution of hexavalent chromium (dissolving 0.2829 g of analytical grade $K_2Cr_2O_7$ in 1 L of double distilled water) was prepared to use further for desired initial hexavalent chromium concentrations of the synthetic wastewater.

2.2. Algal stock cultures and inoculum

The *C. minutissima* algal species used in this study was procured from Center for Blue Green Algae, Indian Agricultural Research Institute (IARI), New Delhi. An axenic culture was established in BG 11 medium (Rippka et al., 1979) at the Biochemical Engineering Laboratory, Department of Chemical Engineering, Dr. B.R. Ambedkar National Institute of Technology, Jalandhar, Punjab, India, under constant environmental conditions. The stock cultures were incubated at 27 ± 1 °C on a 12-h light, 12-h dark cycle on tissue culture rack (Vista Biocell Pvt. Ltd., India). Algal growth was measured at an optical density of 686 nm using a spectrophotometer (SMART Spectro LaMotte Company, USA) every 12 h. Cell numbers were counted under a microscope with a hemocytometer (Fein-optic, Germany).

2.3. Preparation of immobilized algal beads

After 10 days of incubation, growing *Chlorella* cells were taken out from the stock algal culture by centrifuging at 4000*g* force for 10 min at 25 °C. Algal cell residues were washed twice with double distilled water and resuspended in double distilled water to form a concentrated known algal suspension with a cell density 10×10^{14} cells mL^{-1}. Resuspended algal cells were gently homogenized in a hand-held glass tissue grinder with Teflon pestle to disaggregate cell clumps. The homogenized algal suspension of *C. minutissima* was then mixed with 4% Na-alginate (Sigma Chem. Co., USA) solution in 1:1 volume yield a mixture of 2% algal alginate suspension. This mixture was dropped into 0.5 M calcium chloride solution (Himedia Laboratories, India) using a peristaltic pump to form uniform algal beads (each of 4 mm in diameter). The solution was stirred to prevent aggregation of the algal cell entrapped Ca-alginate beads. The algal beads were left in calcium chloride solution for 12 h for proper hardening at 4 °C, then rinsed with sterile saline solution (0.85% NaCl) and subsequently with double distilled water. Blank alginate beads were also prepared in the same way as the algal beads except double distilled water was used instead of algal cell suspension.

Table 1
Characterization of sewage wastewater.

Parameters	Value
pH	7.6
Ammonia-N	37 mg L^{-1}
Phosphate	12.8 mg L^{-1}
Nitrate	350 mg L^{-1}
COD	175 mg O_2 L^{-1}

2.4. Photobioreactor

A photobioreactor was designed for the treatment of sewage wastewater in conjunction with growth algal cells in immobilized beads. The photobioreactor is comprised of four transparent cast acrylic tubes, four constant aeration pumps, four rotameters, silicon tubing, and six white fluorescent lights. Each tube has a length of 57 cm, external diameter of 7.5 cm and thickness of 3 mm. The volume of each reactor chamber was 2.5 L (Fig. 1).

2.4.1. Photobioreactor operation

Three chambers of photobioreactor were filled with immobilized algal beads (algal cell density 10×10^7 cells mL^{-1}) while one chamber was filled with blank alginate beads. The concentration of beads in the each chamber was 10 beads mL^{-1}. The 1.5 L of sewage wastewater was fed initially from the upper inlet of the each reactor. All the photobioreactor chambers were continuously aerated with atmospheric air provided from the bottom inlet of the reactor using aeration pump at a rate of 3 L/min. The continuous aeration was kept the immobilized beads in suspension and in good contact with wastewater. All the experiments in photobioreactor were run at a temperature of 25 ± 2 °C under a light intensity of 6480 ± 300 lux from six white fluorescent light-emitting diodes (LED) in complete cycle for a 12:12 h light and dark photoperiod.

2.5. Algal cell density measurement in immobilized algal beads

The cell density of immobilized algal beads in the photobioreactor was measured initially and at the regular interval of 8 h during the experiment. Ten algal beads were taken out from the each of three algal beads reactor chamber and dissolved in 1 mL sterilized 0.2 M sodium citrate (Himedia Laboratories, India) solution for 30 min at room temperature. Dissolve algal beads were diluted with double distilled water and algal cells were counted on neubauer haemocytometer under an inverted light microscope (Model MA 100, Nikon Instruments Inc., USA). The growth rate hour of algal cells in immobilized beads were calculated by the formula:

$$\text{Growth Rate } (k) = \frac{(\log_{10} X_t - \log_{10} X_0)}{\Delta t} \quad (1)$$

2.6. Nutrient removal analysis by immobilized algal cells

The sewage effluent collected from Institute sewage treatment facility and filtered to remove the large suspended solid particles. The effluents were not sterilized before addition to the photobioreactor. The pH of wastewater was adjusted initially 7.0 using 0.1 N HCl and 0.1 N NaOH. After characterization 1.5 L of sewage wastewater was fed from the upper inlet of the each reactor chamber containing immobilized algal beads and blank beads.