International Biodeterioration & Biodegradation 65 (2011) 656–663

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Biosorption of chromium(VI) by spent cyanobacterial biomass from a hydrogen fermentor using Box-Behnken model

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ARTICLE INFO

Article history:
Received 18 March 2011
Received in revised form
4 April 2011
Accepted 6 April 2011
Available online 6 May 2011

Keywords:
Cyanobacterium
Biosorption
Process optimization
Waste biomass
Box-Behnken model
Chromium (VI)

ABSTRACT

The study explores utilization of waste cyanobacterial biomass of *Nostoc linckia* from a lab-scale hydrogen fermentor for the biosorption of Cr(VI) from aqueous solution. The biomass immobilized in alginate beads was used for removal of the metal in batch mode optimizing the process conditions adopting response surface methodology (RSM). Kinetic studies were done to get useful information on the rate of chromium adsorption onto the cyanobacterial biomass, which was found to follow pseudo second-order model. Four important process parameters including initial metal concentration (10–100 mg/L), pH (2–6), temperature (25–45 °C) and cyanobacterial dose (0.1–2.0 g) were optimized to obtain the best response of Cr(VI) removal using the statistical Box-Behnken design. The response surface data indicated maximum Cr(VI) biosorption at pH 2–4 with different initial concentrations of the metal in the aqueous solution. The biosorbent could remove 80–90% chromium from solutions with initial metal concentration of 10–55 mg/L. Involvement of the surface characteristics of the biomass was studied through its scanning electron micrographs and Fourier transform infrared (FTIR) analysis.

1. Introduction

Contamination of surface waters with heavy metals due to industrial discharge has emerged as a major problem in recent years that needs to be tackled in view of several health hazards associated with them. Once released into the environment, the heavy metals accumulate in living tissues through food chain that has humans at the top. The human beings have, therefore, to face the toxic effects of the pre-concentrated metals. Chromium is one of the most hazardous metals entering surface waters from the effluent of textile, tannery, electroplating, mining and metal cleaning industries and nuclear power plants (Arica et al., 2004). Toxicity of Cr(VI) ions is due to the negatively charged hexavalent Cr ion complexes that easily pass through cellular membranes by means of sulfate ionic channels and then undergo immediate reduction reactions leading to the formation of various harmful reactive intermediates (Wang and Shen, 1995). Chromium(VI) may not only cause allergies, eczema, and respiratory tract disorders, but is also known to be a powerful carcinogenic agent that may cause cancer in the digestive tract and lungs of human beings (Kaufman, 1970; Katz and Salem, 1993). It is therefore, very important to remove Cr(VI) from wastewaters before discharging into the environment.

The common techniques used for Cr(VI) removal from industrial effluents include chemical precipitation, ion exchange and membrane transfer methods; however, due to expensive nature their large scale application is hindered (Brasil et al., 2006). Amongst the commercial adsorbents activated carbon is most widely employed (Goel et al., 2005), but it is also expensive. Further, it requires the use of chelating agents for removal of inorganic species, making the process even more expensive for Cr(VI) bisorption (Cooney, 1999). Biosorbents are found to be more suitable for metal removal at relatively lower concentrations (10–100 mg/L), where other methods usually fail; and also, they are cost effective and environmentally benign (Tunali et al., 2006). Removal of Cr(VI) has been reported using several algal species (Travieso et al., 1999; Ayse et al., 2005) and cyanobacteria (Khattar et al., 2002; Anjana et al., 2007). Cyanobacterial cells in live as well as dead forms have been tried in recent years for removal of chromium ions from water, and more than 80% removal of Cr(VI) has been reported using these organisms at low metal concentrations (Kiran et al., 2007). Simple growth requirements, autotrophic mode of nutrition, fast growth and non-toxic nature of cyanobacteria favor their use as biosorbents.

In comparison to dry powdered biomass use of cell biomass in immobilized form has been found to be more useful as it not only avoids biomass-liquid separation, but also allows higher local cell

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doi:10.1016/j.ibiod.2011.04.002

density and retention of biomass within a definite working system that can be reused (Lu and Wilkins, 1995). Entrapment of microbial cells in some polymeric matrix, usually a gel, involving formation of beads provides mechanical strength and rigidity to the system thus making metal removal more efficient (Kuyucak and Volesky, 1989; Garrido et al., 2005) as compared to free cell system where separation of the biosorbent from the wastewaters after use becomes a major problem, while low strength and small particle size of the biosorbent also pose hinderance in its column applications (Vijayaraghavan et al., 2005).

Since microbial biomass is utilized in several fermentation industries, large quantity of biomass is generated, which instead of dumping as waste should be used as a commercial commodity. Cyanobacteria, which are known to produce hydrogen, and are being viewed by many as next generation hydrogen producers (Dutta et al., 2005) are under trial for commercial use. During microbial hydrogen production, large amount of waste biomass is generated, but till now very little attention has been paid to tap the potential of biomass waste of fermentors (Vijayaraghavan and Yun, 2007).

In the present study waste biomass of *Nostoc linckia* from a lab-scale hydrogen fermentor was utilized for removal of Cr(VI) from aqueous solution. The authors have already reported on the hydrogen production potential of *N. linckia* under different physico-chemical conditions (Mona et al., 2011a). The waste biomass has been found to remove upto 72% of crystal violet dye, a common triphenyl methane dye found in textile effluent (Mona et al., 2011b). The present study was conducted to examine the biosorption potential of the waste biomass for a carcinogenic heavy metal, Cr(VI) found in the textile wastewaters.

The study was aimed at achieving maximum sequestration of Cr(VI) by the waste biomass immobilized in calcium alginate matrix, for which kinetic parameters and adsorption capacity (q_e) of the biosorbent were studied followed by RSM based optimization of parameters. Box-Behnken model was used to analyze the effectivity of the system under different combinations of four important operating parameters. There are several advantages of using statistical models like RSM over classical models particularly in terms of rapid and more reliable information on interacting factors important in the process. Four factorial Box-Behnken experimental design was applied to investigate and validate pH, temperature, algal dose and initial metal concentration of the aqueous solution influencing the removal of chromium by *N. linckia*. The data was analyzed by fitting to a second-order polynomial model, which was statistically validated by performing Analysis of Variance (ANOVA) and lack-of-fit test to evaluate the significance of the model. Surface characteristics of the biosorbents were studied before and after Cr biosorption using scanning electron microscope (SEM), while Fourier transform infrared (FTIR) analysis was done to understand the involvement of various functional groups present on the surface of the cyanobacterium in Cr(VI) biosorption.

2. Materials and methods

2.1. The biosorbent and the sorbate

Biosorbent from the spent biomass of *N. linckia* collected from a lab-scale hydrogen fermentor was prepared by immobilizing in calcium alginate matrix as describe in detail elsewhere (Mona et al., 2011b). A stock solution (1000 mg L^{-1}) of the sorbate i.e. Cr(VI) was prepared using AR grade $K_2Cr_2O_7$ and desired concentrations of the metal were obtained by further dilutions.

2.2. Kinetic studies

Batch studies were performed to determine the equilibrium time required for the maximum adsorption of chromium onto the immobilized cyanobacterial biosorbent (biomass 0.1 g dry wt). Experiments were conducted in 250-mL Erlenmeyer flasks containing 100 mL of Cr(VI) solutions (20 and 50 mg/L initial concentration). The flasks were agitated at a 120 rpm constant shaking rate for 3 h on an orbital shaker (Orbitek LT-IL) and samples were withdrawn in triplicates at different time intervals. The studies were performed at 25 °C, the representative ambient temperature and at desired pH 2, based on our earlier reports (Kiran et al., 2007). The samples taken at different time intervals were analyzed on atomic absorption spectrophotometer (Shimadzu AA 6300) for residual metal concentration in the aqueous solution. The data were the mean values of three replicate determinations. Adsorption of Cr(VI) by blank alginate beads was also recorded to see their role in the biosorption of the metal.

$$\text{Removal of chromium}(\%)\text{was determined as}(C_0 - C_e)/C_0 * 100 \quad (1)$$

where C_0 is the initial metal concentration (mg/L) and C_e is the residual concentration of the metal (mg/L) at different time intervals.

2.3. Optimization of process parameters using Box-Behnken model

Four operational parameters (initial chromium concentration, pH, cyanobacterial dose and temperature) important in the biosorption process were studied using Box-Behnken model of RSM with two levels (the minimum and the maximum). RSM represents independent process variables in the following quantitative form:

$$Y = f(X_1, X_2, X_3, X_4, \ldots, X_n) \pm \varepsilon \quad (2)$$

where Y is the percent of Cr (VI) removed, f is response function, X_1, X_2, X_3, X_4,…, X_n are independent variables and ϵ is the experimental error.

Response surface is obtained by plotting the expected response but the value of f is unknown and may be very complicated. So RSM approximates its value by using a suitable lower order polynomial. If the response varies in a linear manner, it can be represented by this linear function equation

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \cdots + \beta_n x_n \quad (3)$$

But if curvature is there in the system, a higher order polynomial like quadratic model is used which can be stated in the form of the following equation:

$$Y = \beta_o + \sum \beta_i x_i + \sum \beta_{ii} x_{ii}^2 + \sum \beta_{ij} x_i x_j + \varepsilon \quad (4)$$

where Y, is the response (dependant variables), β_o, the constant coefficient, β_i the slope or linear effect of the input factor x_i, and β_{ij} the linear by linear interaction effect between the input factor x_i and x_j and β_{ii} is the quadratic effect of input factor x_i (Benyounis et al., 2005). Thus, RSM is a sequential procedure of performing experiments rapidly in various combinations to get optimal set of conditions for improved response.

For calculation of 10 coefficients of a second-order polynomial equation, 29 experiments were performed (Raikumar et al., 2005). In the experimental design model, Cr(VI) concentration (10–100 mg/L), pH (2–6), temperature (25–45 °C) and cyanobacterial dose (0.1–2.0 g), were taken as input variables, while percent Cr(VI) removal by the biosorbent was taken as the response of the system. The experimental design matrix derived from the Box-Behnken model may be seen in Table 1 along with the data on Cr(VI) adsorbed (%) by the biosorbent in different experimental conditions.

The biosorption data were subjected to analysis of variance to test the significance of the model at 98% confidence limits and the