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Research paper

Biosorption of chromium from electroplating and galvanizing industrial effluents under extreme conditions using *Chlorella vulgaris*

G. Sibi

Department of Biotechnology, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bengaluru 560 043, Karnataka, India

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Abstract

Hexavalent chromium [Cr(VI)] is a toxic oxidized form and an important metal pollutant in the water bodies. Biosorption of chromium(VI) offers a potential alternative to conventional metal removal methods. Dried biomass of *Chlorella vulgaris* was used as biosorbent for the removal of Cr(VI) from electroplating and galvanizing industry effluents as a function of biosorbent dosage, contact time, pH, salinity and initial metal ion concentration. Batch experiments were conducted for biosorption and the optimum conditions were 1 g/L biomass, 4 h contact time, pH 2 and 2.893 mS/cm of electrical conductivity. The chromium biosorption was strictly pH dependent with a maximum Cr removal of 63.2 mg/L at pH 2. Highest Cr removal at a concentration of 81.3 mg/L was observed at Electrical conductivity (EC) value of 2.893 mS/cm. A comparison of Langmuir and Freundlich isotherm models revealed that Freundlich isotherm model fitted the experimental data based on R^2, q_{max} and standard error values. The results suggest that *C. vulgaris* biomass could be considered a promising low-cost biosorbent for the removal of Cr(VI) from electroplating and galvanizing industry effluents.

Keywords: Biosorption; *Chlorella vulgaris*; Microalgae; Hexavalent chromium

1. Introduction

Chromium exists as hexavalent [Cr(VI)], trivalent [Cr(III)] or divalent [Cr(II)] forms and is an important metal pollutant released from leather tanning, textile, electroplating and metal finishing industries [23]. Trivalent chromium is relatively less toxic [4] whereas, Cr (VI) is highly soluble and toxic oxidized form to animals as well as humans [12,30]. Chromium(VI) toxicity tend to bioaccumulate and increasingly concentrated as they travel through the food chain [39]. Release of heavy metals from industries into water bodies implies a high risk to wildlife and humans and chromium is a vital heavy metal pollutant in the aquatic bodies [10].

The conventional metal removal technologies such as chemical precipitation and filtration, chemical oxidation or reduction, electrochemical treatment, reverse osmosis, ion exchange, adsorption and evaporation are employed for metal removal [40]. But constraints such as incomplete metal removal, high reagent or energy requirements, generation of toxic sludge and high cost associated with them made to focus on low cost methods [9,11]. Adsorption is the most versatile and widely used because of its initial cost, simplicity of design, facile operation and insensitivity to toxic substances [21]. Natural and synthetic adsorbents are being used for the removal of metal ions [14,27] but the cost and secondary product formation during absorption reduce the practices. Biosorption of chromium(VI) offers a potential alternative to existing methods for detoxification and recovery of chromium from industrial waste waters. Biosorption using biomaterials has advantages because of their high metal binding capacity [34]. Microorganisms decrease the heavy metal ion concentration by sequestration due to their cell wall constituents [13,19,43,44] and among them algae are proven to be one of the promising

E-mail address: gsibii@gmail.com.

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ones to remove metal ions through adsorption and biotransformation [13,36,41].

In this work, *Chlorella vulgaris* was used as biosorbent for removal of Cr(VI) from electroplating and galvanizing industry effluents as a function of biosorbent dosage, contact time, pH, salinity and initial metal ion concentration. The kinetics was obtained from batch experiments to fit the experimental data obtained at varying initial Cr(VI) concentrations.

2. Material and methods

2.1. Isolation and identification of algal strains

Effluents of electroplating and galvanizing unit (Raichur, 16°20′N, 77°34′E) were collected and poured into a closed 250 ml bottle and exposed in sunlight for 3 weeks. The upper layer of the water was inoculated in agar plates enriched with BG11 medium containing 200 μg ml^{-1} ampicillin to control the growth of bacteria. Agar plating technique was used to isolate the microalgae and the plates were incubated at 25 ± 2 °C under cool white fluorescent light (40 μmol photons m^{-2} s^{-1}; 15 h light/9 h dark) until algal growth was detected. The isolates were purified by streak plating and individual colonies were diluted in distilled water. Species of single cells were obtained using capillary pipette under microscope followed by inoculation into fresh media. After appropriate growth, cells were observed to confirm the single culture and the capillary method was repeated as many times as required to obtain axenic cultures. Standard protocols as described by Anderson [3], Stanier et al., [37] and the database http://web.biosci.utexas.edu/utex/default were used for identification of the algal isolates.

2.2. Culture conditions and biosorbent preparation

C. vulgaris was the major isolate identified and recultivated in Bold's medium at 24 ± 2 °C under continuous illumination. The cells were harvested after 14 days growth period by centrifugation at 4000*g* for 10 min and the biomass was washed with distilled water followed by drying in an oven at 40 °C until constant weight was obtained.

2.3. Metal solution standard

Stock solution of chromium was prepared by dissolving 100 mM $K_2Cr_2O_7$ in deionized water and working standard solutions were prepared by appropriate dilution of the stock solution for metal analyses.

2.4. Characteristics of effluent sample

Electroplating effluent was collected from an electroplating and galvanizing unit located in Raichur, Karnataka, India (16°20′N, 77°34′E). All chemicals used in this study were of analytical reagent grade and the physicochemical parameters of the effluent were analyzed using standard methods [5] (see Table 1).

2.5. Batch biosorption studies

The experiments were conducted at room temperature (25 ± 1 °C) to determine the effects of biosorbent dosage, contact time, pH, salinity and initial ions concentration on the biosorption of Cr(VI) ions. Each experiment was conducted in a mechanical shaker at 120 rpm. The samples were filtered through Whatman filter paper (No. 41) and the metal ions concentration was determined in the filtrate. Controls without biosorbent materials were used to distinguish between possible metal precipitation and actual metal sorption. All the experiments were carried out in triplicate and the mean of the quantitative results were used for further calculations.

2.6. Effect of algal dosage

The experiment was conducted with varying biomass from 0.2 to 2 g/L in diluted effluent (50 mg/L) for 2 h contact time at 25 °C. The optimum algal dosage of 1 g/L was used in further biosorption studies.

2.7. Effect of contact time

Batch biosorption experiments for optimum time was carried out at pH 4 with 100 ml of diluted effluent containing Cr(VI) in the concentration of 50 mg/L and 0.1 g biosorbent dosage under different time intervals (1, 2, 3, 4, 5 and 6 h).

2.8. Effect of pH

The batch experiments for Cr(VI) removal were determined under various initial pH (0.5–5) at an initial Cr(VI) concentration of 50 mg/L and 0.1 g algal biomass in 250 ml flasks containing 100 ml Cr(VI) solution. The pH of the effluent was adjusted using 0.1 N HCl or 1.0 M NaOH.

2.9. Effect of salinity

The effect of salinity on chromium biosorption was conducted by adjusting the electrical conductivity (EC) of the effluent between 1013 and 3796 μS/cm using NaCl solution. Algal biosorbent (0.1 g) was added to 100 ml of Cr(VI)

Table 1
Physical and chemical characteristics of effluent collected from electroplating and galvanizing unit.

Parameters	Findings
Colour	Yellowish brown
pH	2.6
Electrical conductivity	2.314 mS/cm
Temperature	31 °C
BOD	197 mg/L
COD	374 mg/L
Total solids	721.41 mg/L
Total dissolved solids	690 mg/L
Chlorides	237 mg/L
Sulphides	24 mg/L
Hexavalent chromium	227 mg/L

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solution (50 mg/L) with varying salinity and the biosorption was carried out for 4 h.

2.10. Effect of initial concentration

The effect of initial metal concentration was investigated by varying initial Cr(VI) concentrations in the range of 24.5–147 mg/L at different pH and salinity levels using same weight of algal biomass (1 g/L).

2.11. Chromium estimation

The concentration of Cr(VI) was analyzed using 1,5-diphenylcarbazide in UV/Vis spectrophotometer at 540 nm. Total Cr was estimated by converting Cr(III) to Cr(VI) at high temperature by the addition of excess potassium permanganate prior to using the 1,5-diphenylcarbazide method. Each experiment was repeated three times.

2.12. Equilibrium studies

The amount of Cr(VI) uptake by *C. vulgaris* in each flask was determined using the mass balance equation.

$$q = \frac{C_0/C_e}{W}$$

where q is the adsorption amount at equilibrium (mg/g), C_0 is the concentration of heavy metal (mg/L), C_e is the concentration remaining in solution at equilibrium (mg/L) and W is the biosorbent dosage (g/L).

2.13. Kinetic studies

Evaluation of biosorption capacity by the microalgae under varying pH and salinity was performed by subjecting the data obtained from biosorption studies to the Freundlich and Langmuir isotherm models. Adsorption isotherms are used to express the equilibrium relationship between the concentrations of metal ions adsorbed per unit mass of original biosorbent.

Freundlich adsorption was employed to estimate the adsorption intensity of the adsorbent towards the sorbate and is given as:

$$q_e = K_f C_e^{\frac{1}{n}}$$

where, K_f and n are the distribution coefficient and a correction factor, respectively and C_e is equilibrium concentration of heavy metal (mg/L).

The Langmuir isotherm was used to correlate the equilibrium data and is given as:

$$q = \frac{q_{max} b C_e}{(1 + bC_e)}$$

where, q_{max} and b are maximum adsorbate loading (mg/L) and Langmuir adsorption constant (mg/L), respectively.

3. Results and discussion

The role of biosorbent dosage on Cr(VI) biosorption was studied by varying biomass ranged from 0.2 to 2.0 g/L. The data revealed that the biosorption efficiency of Cr(VI) was significantly affected by the dose of *C. vulgaris* and the removal percentage of Cr(VI) as a function of adsorbent dosage is shown in Fig. 1. The biosorption was almost constant at higher dosage and this could be explained by the decrease of surface area for biosorption due to formation of aggregates of biomass at higher doses and competition of the ions for the available sites.

Contact time is highly influencing the biosorption process. Fig. 2 shows the effect of contact time on the biosorption of Cr(VI) ions at 50 mg/L concentration using *C. vulgaris* at biomass dose of 1 g/L. The results indicated that biosorption was rapid in first 1 h and then was gradually increased till the equilibrium attained at 4 h. It was found that biosorption increased from 50.7% to 80.3% as the contact time was increased from 1hr to 4 h. Therefore contact time of 4 h was used as the optimum time for rest of the experiments. Metal biosorption is reported to be biphasic process, in which rapid sorption of metal ions to the surface groups of the biomass occurs at the first phase followed by diffusion of metal to internal binding sites on the biomass in the second phase [1] and [28].

Varying pH in the range between 0.5 and 5 were chosen to determine the biosorption of Cr(VI) and the biosorption was maximum at lower pH values. The increase in biosorption capacity by *C. vulgaris* from 54.4% to 81.6% was observed by increasing the pH from 0.5 to 2 (Fig. 3). This might be due to the protonation of the solution at lower pH levels. Maximum adsorption of Cr(VI) by *Scenedesmus quadricauda* was observed at pH 1 [24] whereas optimum pH was found to be 2 for macroalgae [26]. On the other hand, decrease in biosorption from 60.4% to 37.5% was observed by increased from pH 3 through 5 which might be attributed to precipitation of metal ions. One of the most important parameters that controls sorption process is pH of a solution [8,21,38]. pH can influence metal biosorption by affecting the configuration of

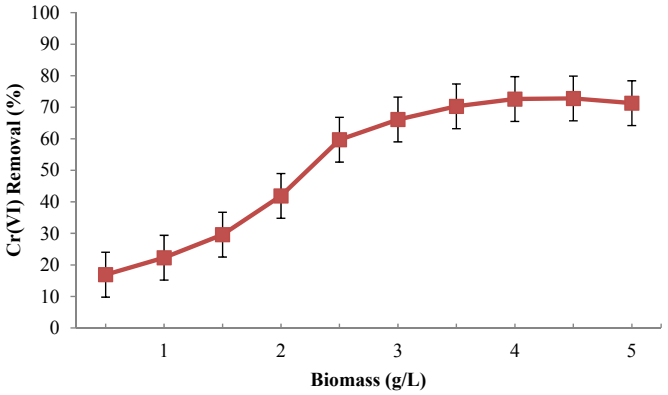

Fig. 1. Effect of biosorbent dosage on Cr(VI) biosorption at 50 mg/L concentration.

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the active ion-exchange sites, ionic state of the sorbate and damaging the structure of the biosorbent material [41].

NaCl was used to obtain varying salinity of the effluent ranged between 0.5 and 2% and electrical conductivity (EC) was measured as it is the measure of salinity. The corresponding 0.5%, 1%, 1.5% and 2% salinity values for EC are 1.013, 1.968, 2.893 and 3.796 mS/cm. The measurement of EC of liquids is generally determined by the ionic compounds dissolved in water [2] and Cr(VI) biosorption under varying EC was measured using conductivity meter. Fig. 4 indicates that metal biosorption increased with salinity up to 1.5% and diminished considerably in the presence of 2% salinity (EC value of 2.893 mS/cm). Electrical conductivity is an indication of the level of inorganic constituents in water and the Cr(VI) biosorption by *C. vulgaris* at higher EC levels indicates the efficiency of microalgae in removing chromium under saline conditions.

3.1. Effect of initial Cr(VI) concentration at varying pH and salinity

pH in the range of 0.5–5 was chosen to determine the optimum level for the Cr removal for 4 h using 1 g/L biomass with series of dilutions to get final Cr(VI) concentrations in the range of 24.7–147 mg/L. The results showed that the removal efficiency of *C. vulgaris* increased with the increasing initial Cr(VI) concentration and the removal process was influenced significantly by variation of pH (Fig. 5). When the initial Cr(VI) concentration increased from 24.5 mg/L to 147 mg/L, the removal efficiency increased from 17.4 to 63.2 mg/g at pH 2 and from 16.9 to 58.1 mg/g at pH 1. Biosorption of metal ions from solutions is greatly affected by pH [17,25]. Removal of Cr(VI) from industrial water is higher at lower pH but the process requires large amounts of acid. Chromium exhibits different types of pH dependent equilibriums in aqueous solutions [6,31,32]. At neutral pH, surface charges of most microorganisms are negative whereas it becomes positive charge in acidic environments [29,33]. The negatively charged chromium species bind to positively charged functional groups of cell wall at lower pH through electrostatic attraction due to the exposure of more functional groups. At higher pH levels, the overall surface charge on cell walls become negative and biosorption decreases [7]. In this study, it was found that at pH 1 and pH 2 the removal rate was highest whereas the removal rate was lesser at pH < 1 and pH > 2. It can be seen that the percentage removal does not alter greatly if the concentration increases from 24.5 to 98 mg/L. This behavior is due to 1 g of algae that may contain enough exchangeable sites for this

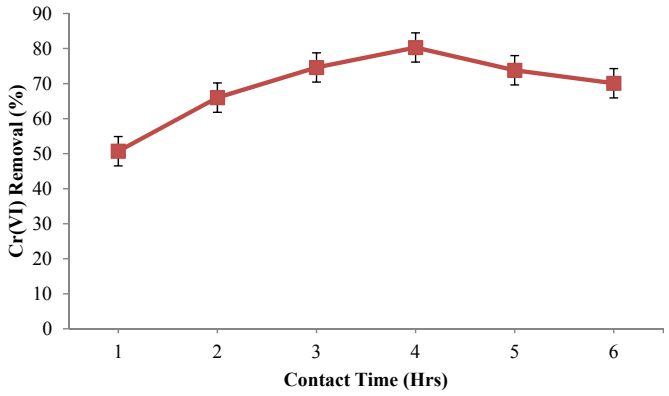

Fig. 2. Effect of contact time on Cr(VI) biosorption at 50 mg/L concentration and 1 g/L biosorbent dosage.

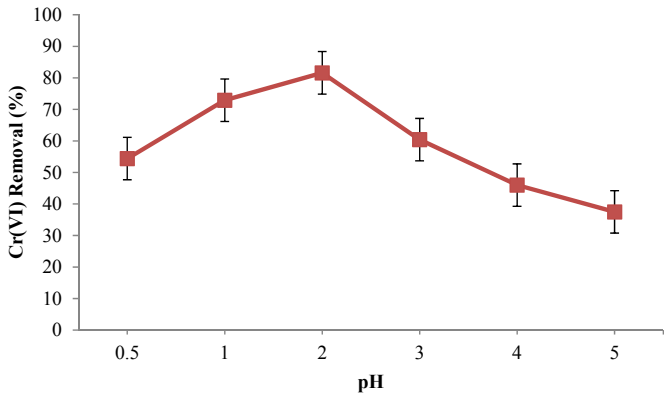

Fig. 3. Effect of initial pH on Cr(VI) biosorption at 50 mg/L concentration and 1 g/L biosorbent dosage.

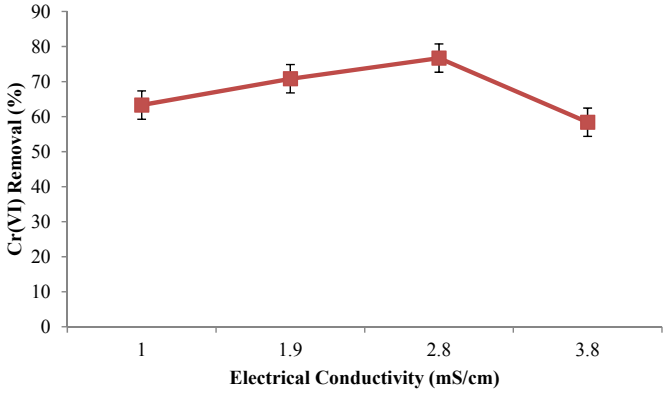

Fig. 4. Effect of initial electrical conductivity on Cr(VI) at 50 mg/L concentration and 1 g/L biosorbent dosage.

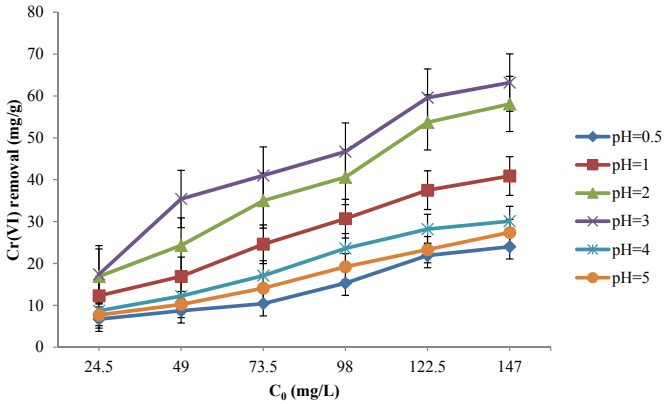

Fig. 5. Removal efficiency of *C. vulgaris* vs initial concentration of Cr(VI) at various pH units.

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concentration range, but when the concentrations increase to 122.5 and 147 mg/L, the exchangeable sites in 1 g will not be enough to cover these concentrations so that the depletion in percentage removal will be obvious.

When electrical conductivity increased from 1.013 to 2.893 mS/cm, Cr(VI) uptake increased from 43.1% to 81.3% at 147 mg/L initial concentration (Fig. 6). At the same time, biosorption decreased sharply when the EC or salinity was increased to 3.796 mS/cm or 2% which may be due to the inhibition effect of salt on the permeability of cell membrane for Cr(VI) ions and relative competition between chloride and chromate anions on the active centers of algae [35]. Electrical conductivity is the measurement of salinity of a solution and the results clearly demonstrate that *C. vulgaris* was effectively removing the metal from the effluent having high salinity.

3.2. Biosorption equilibrium isotherm

The experimental data were fitted to Langmuir and Freundlich isotherm models to examine the relationship between sorption and aqueous concentrations of metal ions. Both isotherms are widely used to analyze data for effluent treatment application to characterize the interaction of metal ions with biomass preparations [42]. The adsorption plots of Langmuir and Freundlich isotherm model for biosorption of Cr(VI) ions from electroplating effluent by *C. vulgaris* are presented in Fig. 7a–b. Cr(VI) removal abilities of *Chlorella* sp was studied by earlier and the equilibrium time for metal removal was dependent on initial pH, biomass and metal concentration [18,22]. Biosorption of Cr(VI) and Cr(III) by other microalgae also reported [15,20]. It was observed that a rapid equilibrium is established between adsorbed metal ions on the algal cell and unadsorbed metal ions in solution during the biosorption [16].

The adsorption constants evaluated from the isotherms with the correlation coefficients are given in Table 2. It can be seen that R^2 value for the Freundlich isotherm is 0.9465 against the Langmuir isotherm R^2 value of 0.5471. Analysis of correlation regression coefficient shows that biosorption process fits better into Freundlich isotherm.

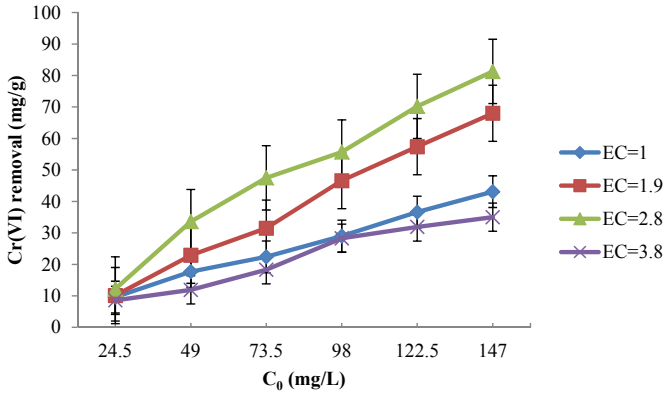

Fig. 6. Removal efficiency of *C. vulgaris* vs initial concentration at various electrical conductivity (mS/cm).

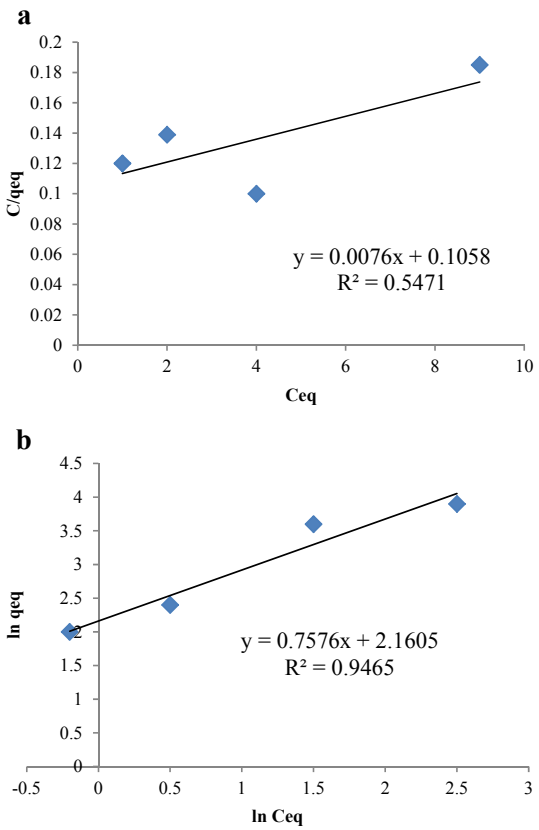

Fig. 7. Biosorption isotherm (a) Langmuir and (b) Freundlich isotherm for Cr(VI) biosorption by *C. vulgaris* biomass.

Table 2
Adsorption constants obtained from isotherms for Cr(VI) biosorption and *C. vulgaris* biomass at 1 g/L biosorbent, 4 h contact time, pH 2 and 1.5% salinity.

Langmuir isotherm constants			Freundlich isotherm constants		
b	Q, mg/g	R^2	*n*	K_f, mg/g	R^2
0.072	161.41	0.5471	1.20	8.5	0.9465

4. Conclusion

The removal percentage and biosorption capacity for Cr(VI) ions as function of biosorbent dosage, contact time, pH, salinity and initial metal ion concentration were studied. Algal species, metal ion charges and chemical composition of the metal ion solution influences the mechanism of binding metal ions by algal biomass. In this study, varying degree of metal biosorption was observed under different dosage levels, contact time, pH and salinity. In order to determine the efficiency *C. vulgaris* on Cr(VI) biosorption, dried algal biomass was used on electroplating and galvanizing industry effluent and the results revealed that biosorption was highest in lower pH and high salinity conditions. Equilibrium uptake capacity, correlation regression coefficient and rate constants were used to develop the kinetic model which has illustrated that the biosorption follows second order rate of reaction. The Freundlich adsorption model was found to better describe the phenomenon of Cr(VI) biosorption onto dried biomass of *C. vulgaris*. Thus

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the results suggest the reasonable potential of *C. vulgaris* as biosorbent for removal of Cr(VI) from electroplating and galvanizing unit effluents with varying pH and salinity.

Conflict of interest

The author declares that there is no conflict of interest.

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