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## Research Article

# Biosorption Potential of *Trichoderma gamsii* Biomass for Removal of Cr(VI) from Electroplating Industrial Effluent

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The potential use of acid-treated biomass of *Trichoderma gamsii* to remove hexavalent chromium ions from electroplating industrial effluent was evaluated. Electroplating industrial effluent contaminated with 5000 mg/L of Cr(VI) ions, collected from industrial estate of Gujarat, India, was mixed with acid-treated biomass of *T. gamsii* at biomass dose of 10 mg/mL. Effect of contact time and initial Cr(VI) ions was studied. The biosorption of Cr(VI) ions attained equilibrium at time interval of 240 minutes with maximum removal of 87% at preadjusted initial Cr(VI) concentration of 100 mg/L. The biosorption of Cr(VI) ions by biomass of *T. gamsii* increased as the initial Cr(VI) ion concentration of the effluent was adjusted in increasing range of 100–500 mg/L. At 500 mg/L, initial Cr(VI) concentration, acid-treated biomass of *T. gamsii* showed maximum biosorption capacity of 44.8 mg/g biomass from electroplating effluent. The Cr(VI) biosorption data were analysed using adsorption isotherms, that is, Freundlich and Langmuir isotherm. The correlation regression coefficients ( $R^2$ ) and isotherm constant values show that the biosorption process follows Freundlich isotherm ( $R^2 > 0.9$ ,  $n > 1$ , and  $K_f = 8.3$ ). The kinetic study shows that biosorption of Cr(VI) ions by acid-treated biomass of *T. gamsii* follows pseudo-second-order rate of reaction at increasing concentration of Cr(VI). In conclusion, acid-treated biomass of *T. gamsii* can be used as biosorbent for Cr(VI) ions removal from Cr(VI)-contaminated wastewater generated by industries.

## 1. Introduction

Variety of anthropogenic sources including leather tanning, electroplating, wood preservation, metal finishing, pigment, and dye industries contribute towards hexavalent chromium in the environment [1–3]. The hexavalent chromium is classified in group A of human carcinogens by United State Environmental Agency (USEPA). Therefore, USEPA has regulated/limited the industrial discharge of Cr(VI) to surface water up to <0.05 mg/L.

Many conventional methods including chemical precipitation, chemical coagulation, ion exchange, electrochemical methods, adsorption using activated carbon and natural zeolite, membrane process, and ultrafiltration have been employed by several industries to remove Cr(VI) from their effluent [4–6]. However, these methods suffer from several disadvantages which include high operating cost, excess production of sludge, decrease in removal efficiency in presence

of other metals, and large consumption of chemicals [7]. Hence, remediation of Cr(VI) demands some cost effective, economic, efficient, and eco-friendly methods.

In this context, biosorption is an emerging and attractive technology which is being worked out by many researchers since last two decades [8–10]. In general fungal cell walls are mainly 80–90% polysaccharides, with proteins, lipids, polyphosphates, and inorganic ions. Chitin is a common constituent of fungal cell walls. Chitin is a strong but flexible nitrogen containing polysaccharide consisting of N-acetyl-glucosamine residues. All these biopolymers offer many functional groups such as carboxyl, hydroxyl, sulphate, phosphate, and amino groups that can bind with several metal ions [11]. The ongoing research on Cr(VI) biosorption suggests that fungal biomass can passively bind metal ions via various physicochemical mechanisms or combination of several phenomena, namely, ion exchange, complexation, coordination, adsorption, electrostatic interaction, and

TABLE 1: Comparison of various fungal biomasses for Cr(VI) biosorption.

Fungal biosorbents	Maximum biosorption capacity (mg/g biomass)	Reference
<i>Aspergillus</i> sp.	1.56	[18]
<i>Rhizopus arrhizus</i>	8.40	[19]
<i>Termitomyces clypeatus</i>	11.1	[20]
<i>Aspergillus niger</i>	11.6	[21]
<i>Rhizopus nigricans</i>	12.70	[22]
<i>Neurospora crassa</i>	15.85	[23]
<i>Rhizopus arrhizus</i>	23.2	[24]
<i>Mucor hiemalis</i>	30.5	[25]

chelation [12, 13]. For example, biosorption of Cr(VI) by using *Rhizopus arrhizus* was found to be 23 mg/g biomass [14]. Similarly several other biomasses of fungal origin have been reported extensively for Cr(VI) biosorption (Table 1). The process of biosorption has gained importance over conventional methods due to several advantages like reusability of biomaterial, removal of heavy metal from effluent irrespective of toxicity, short operation time, and no secondary compound production [15]. However, reports on practical application of any of these biosorbents to Cr(VI) containing wastewater are sparse [13, 16].

Electroplating is one of the metal finishing process which contributes in discharging toxic level of Cr(VI) in environment. Apart from Cr(VI), electroplating industrial effluent contains many other metal ions which may affect the Cr(VI) biosorption efficiency of biomass. Therefore, it is of key importance to investigate the biosorption efficiency of biomass in contaminated effluent and effect of other parameters of effluent on biosorption process.

We have isolated a hexavalent chromium tolerant fungal culture, identified as *T. gamsii*, from a chromium contaminated soil. This isolate exhibited a very efficient biosorption capacity for hexavalent chromium from pure solutions (50.6 mg/g biomass). In the present study we have investigated the utility of *T. gamsii* biomass for removal of Cr(VI) from electroplating industrial effluent.

## 2. Materials and Methods

**2.1. Chemicals and Fungal Strain.** All the chemicals used were of analytical grade (AR) and purchased from either Qualigens Fine Chemicals, India, or Hi-Media Laboratories, India or Ranbaxy Fine Chemicals Limited, India.

A laboratory Cr(VI) tolerant fungal strain designated as FCR16 was used for present studies. FCR16 was grown and maintained on Potato Dextrose liquid/solidified medium as per the requirement.

FCR16 was identified by 18s rDNA sequencing. The analysis of the nucleotide sequencing was carried out using Blast-n tool at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The phylogenetic tree was constructed by neighbour joining method using MEGA version 4.0 [17].

**2.2. Preparation of Fungal Biomass for Cr(VI) Ion Biosorption.** FCR16 was inoculated by transferring a block of fungal

TABLE 2: Physical and chemical characteristics waste water collected from effluent of electroplating industry.

Parameters	mg/L
COD	32
Ammonium nitrate	61.6
Total solids	10.30
Total dissolved salts	10.1
Chlorides	350
Sulphate	ND
Sulphides	ND
Nickel	32.8
Iron	10.31
Oil and grease	10.66
Hexavalent Cr(VI)	5,000
pH	1.5
Color	Yellow to brown

growth (16 mm diameter) on Potato Dextrose agar plates (grown for three days at 30°C) using sterile cup borer to 500 mL Erlenmeyer flasks filled with 200 mL of culture medium composed of the following (g/L): Potatoes infusion forms, 200; and Dextrose, 20 and incubated on to a rotary shaker at 150 rpm for five days at 30°C. Upon incubation, the biomass produced was separated by filtration and the resulting biomass was washed thoroughly for several times with distilled water. The biomass was treated with hydrochloric acid (6N), washed with distilled water to bring the pH of the biomass in neutral range, and then was used directly for Cr(VI) adsorption studies from electroplating industrial wastewater, considering the higher Cr(VI) biosorption capacity of acid-treated biomass of FCR16 in pure Cr(VI) solution.

**2.3. Characteristics of Effluent Sample.** Electroplating effluent was collected from an electroplating unit located in Industrial Estate of Vadodara, Makarpura, Gujarat, India. The characteristics of electroplating effluent are listed in Table 2. The major contaminants of wastewater were Cr(VI), Ni, and Fe. In addition, pH of the waste water was highly acidic that is, 1.5.

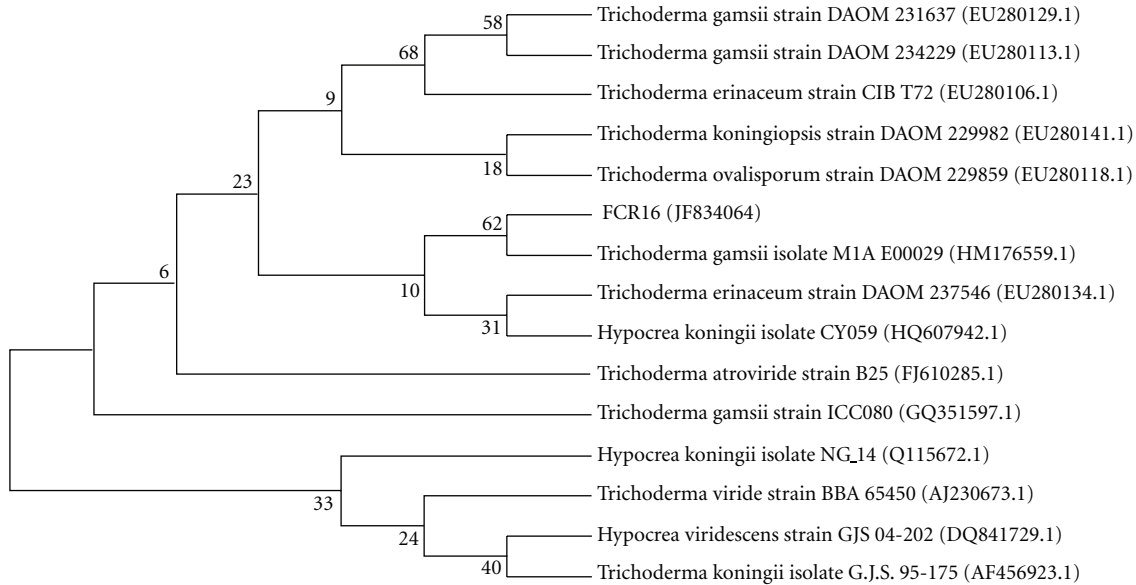


FIGURE 1: Phylogenetic affiliation based on 18 s rRNA gene sequence comparison over 561 nucleotides showing the relationship of FCR16 with other fungal strains. The value at node represents percentage of 1000 bootstrap replicates. Number in bracket represents GenBank accession numbers.

**2.4. Batch Experiment.** The biomass (at biomass dose of 10 mg/mL) of FCR16 was mixed with 100 mL of diluted effluent containing Cr(VI) in the range from 100 to 500 mg/L. Apart from Cr(VI) concentration adjustments, no other pretreatment was given to the effluent. After mixing, the experimental set was kept on shaker (150 rpm) at 30°C. 1 mL sample was withdrawn at regular time interval and residual Cr(VI) was measured.

**2.5. Kinetics of Cr(VI) Biosorption.** Pseudo-first-order and pseudo-second-order rate equations have been used for modelling the kinetics of Cr(VI) ion biosorption [26]. Linear form of pseudo-first-order rate equation is expressed as follows:

$$\log(q_{\text{eq}} - q_t) = \log q_{\text{eq}} - \frac{k_1 t}{2.303}, \quad (1)$$

where  $q_t$  and  $q_{\text{eq}}$  are sorption capacity at time  $t$  and at equilibrium, respectively, and  $k_1$  is pseudo-first-order rate constant.

In case the biosorption follows pseudo-first-order rate equation, a plot of  $\log(q_{\text{eq}} - q_t)$  versus  $t$  should generate straight line with intercept of  $\log q_{\text{eq}}$  and slope of  $-k_1/2.303$ .

Similarly, linear form of pseudo-second-order rate equation is expressed as

$$\frac{t}{q_t} = \frac{1}{k_2(q_{\text{eq}})^2} + \frac{t}{q_{\text{eq}}}, \quad (2)$$

where  $k_2$  is pseudo-second-order rate constant.

In case the biosorption follows pseudo-second-order rate equation, a plot of  $t/q_t$  versus  $t$  should generate a straight line with intercept of  $1/k_2 q_{\text{eq}}^2$  and slope of  $1/q_{\text{eq}}$ .

The shape (linearity) of graph and comparison of experimental and calculated  $q_{\text{eq}}$  values can help in deciding which kinetic model is followed by biosorption process. Another important factor which influences the kinetic model is the value of coefficient of determination:  $R^2$ . A value of  $R^2 > 0.9$  shows the suitability of model for describing the kinetics.

**2.6. Equilibrium Model for Cr(VI) Biosorption by FCR16 Biomass from Electroplating Wastewater.** Biosorption data were analyzed using Langmuir and Freundlich equilibrium isotherms to determine the feasibility of Cr(VI) ion biosorption. The Freundlich isotherm equation is an empirical equation based on the biosorption on a heterogeneous surface suggesting that the binding sites are not equivalent or dependent [27]. Langmuir isotherm equation is based on monolayer sorption onto a surface with finite number of identical sites, which are homogeneously distributed over the sorbent surface [28].

**2.7. Analysis of Cr(VI) Ions.** The concentration of the Cr(VI) ions was determined spectrophotometrically after complexation of the Cr(VI) with 1, 5-diphenylcarbazide [29]. The absorbance was recorded at 540 nm and concentration was determined from the calibration curve.

Characterization of effluent was done according to standard methods described by APHA [30].

### 3. Results and Discussion

The Cr(VI) tolerant fungal strain designated as FCR16 was identified as *Trichoderma gamsii* with 99% similarity (accession number: JF834064). The phylogenetic relationship of FCR16 with other related fungal species is presented in Figure 1.

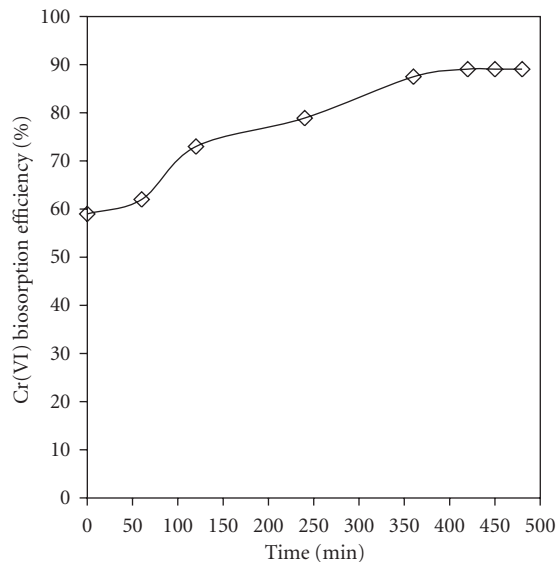


FIGURE 2: Time course for Cr(VI) removal by biosorption using *T. gamsii* biomass from electroplating industrial waste, diluted to a final Cr(VI) concentration of 100 mg/L.

**3.1. Effect of Contact Time on Cr(VI) Ion Biosorption from Electroplating Industrial Effluent by Acid-Treated Biomass of *T. gamsii*.** Electroplating industrial effluent (pH: 1.5) containing 5000 mg/L of Cr(VI) ions was diluted (without any pretreatment of effluent) 50 times with distilled water to get the final concentration of 100 mg Cr(VI)/L.

Figure 2 shows the role of contact time on Cr(VI) biosorption using acid-treated biomass of *T. gamsii* at biomass dose of 10 mg/mL under shaking condition of 150 rpm. It was found that biosorption increased from 50 to 89% as the contact time was increased from 0 to 420 minutes. As illustrated, one gram of *T. gamsii* biomass could remove 89% of Cr(VI) ions at equilibrium. Metal biosorption is reported to be biphasic process, with rapid sorption of metal ions to the surface groups of the biomass constituting the first phase followed by a second phase during which diffusion of metal to internal binding sites on the biomass limits the sorption rate [31, 32]. Furthermore, the Cr(VI) biosorption depends on protonation and deprotonation of the cell wall polymer functional group relative to their pKa. At low pH, the protonation of functional group gives an overall positive charge to the fungal biomass, thereby leading to enhanced Cr(VI) biosorption. In the present study the acidic nature of the electroplating effluent and acid pretreatment of biomass together led to the significant Cr(VI) biosorption as demonstrated by higher Cr(VI) removal (%) in Figure 2. This biosorption efficiency was slightly lower than Cr(VI) ion removal efficiency of *T. gamsii* (50.6 mg/g biomass) from pure solution of Cr(VI) (data not shown). This may be attributed to competition between Cr(VI) and other metal ions present in electroplating effluent for the functional groups on the surface of biomass. Similar reduced Cr(VI) biosorption efficiency from electroplating industrial waste by *A. niger* has been reported by Kumar et al. [33].

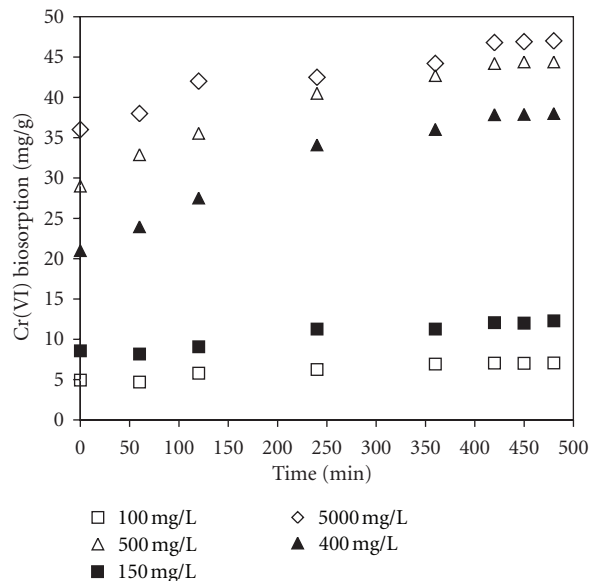


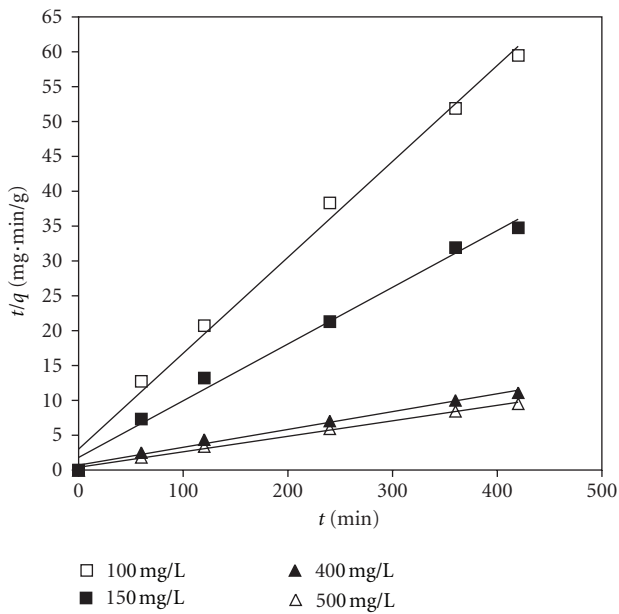
FIGURE 3: Cr(VI) biosorption (mg/g) by *T. gamsii* biomass from electroplating industrial waste diluted to final Cr(VI) concentration in the range from 100 to 500 mg/L.

**3.2. Effect of Initial Cr(VI) Ion Concentration of Effluent on Biosorption.** The biosorption of Cr(VI) ions from electroplating effluent was carried out for 420 minutes at 150 rpm using acid-treated biomass (10 mg/mL) of *T. gamsii* with series of dilutions of effluent to get final Cr(VI) concentration in the range of 100 to 500 mg/L. It can be demonstrated from the experimental results that uptake capacity ( $q_{eq}$ , mg/g biomass) increased from 7.06 mg to 42.71 mg Cr(VI)/g acid-treated biomass of *T. gamsii* (Figure 3) when initial Cr(VI) concentration was increased from 100 to 500 mg/L, suggesting the increased propelling force provided by higher initial Cr(VI) ion concentration to overcome all mass transfer resistance of metal ions between the aqueous and solid phases, consequently, resulting in higher probability of collision between Cr(VI) ions and biosorbents [25, 34]. The Cr(VI) biosorption capacity of acid-treated *T. gamsii* biomass (42.71 mg/g) was comparable or better than other biosorbents reported for removal of Cr(VI) from electroplating effluent, namely, *Padina boergesenii* (49 mg/g), *Lentinus edodes* (21.5 mg/g), *C. lipolytica* (10 mg/L), and *A. niger* (65% from electroplating effluent contaminated with 47 mg/L Cr(VI)) [34–36].

Kinetic studies based on pseudo-second-order plot of  $t/q_t$  versus  $t$  (2) indicated that the biosorption of Cr(VI) ion followed pseudo-second-order rate of reaction in the Cr(VI) concentration range of 100 to 500 mg/L (Figure 4). The values of experimental/calculated equilibrium uptake capacities ( $q_{eqexp}$  and  $q_{eqcal}$ ), correlation regression coefficient ( $R^2$ ), and second-order rate constants ( $k_2$ ) are presented in Table 3. The values of equilibrium uptake capacity increased (from 7.26 to 44.8 mg/g biomass) whereas second-order rate constant ( $k_2$ ) was found to decrease (from 0.376 to 0.075) with increasing concentration of Cr(VI) ions (from 100 to

TABLE 3: Second-order kinetic parameters for biosorption of Cr(VI) by *T. gamsii* biomass at various dilutions of electroplating industrial wastewater.

Cr(VI), mg/L	$q_{eqexp}$ , mg/g (experimental)	$q_{eqcal}$ , mg/g (calculated)	$k_2$	$R^2$
100	7.064	7.26	0.376	0.992
150	12.09	12.28	0.218	0.991
400	37.85	39.06	0.054	0.988
500	42.71	44.8	0.075	0.995

FIGURE 4: Linearized second-order kinetic plot of Cr(VI) biosorption by *T. gamsii* biomass at varying initial concentrations of Cr(VI).

500 mg/L). This shows that the chromium sorption kinetics is strongly dependent on mass transfer phenomenon [25]. The rate of biosorption increases at slower rate compared to the increase in concentration due to sorption site saturation, which thus leads to the decrease in rate constant. The calculated uptake capacity values estimated from second-order kinetic model were in agreement to the experimental values. Additionally, correlation regression coefficients of pseudo-second-order model are quite high ( $R^2 > 0.98$ ), very close to unity. Therefore, Cr(VI) ion biosorption by acid-treated biomass followed pseudo-second-order model. These observations are in agreement with the observations made by Ye et al. [37] where they have used *Candida lipolytica* and dewatered sewage sludge for biosorption of Cr(VI) ions from electroplating wastewater.

**3.3. Adsorption Isotherms for Cr(VI) Ion Biosorption.** The experimental values of equilibrium uptake capacities of Cr(VI) ions from electroplating effluent (Table 3) by acid-treated biomass of *T. gamsii* were analyzed by Freundlich and Langmuir isotherm models. Langmuir and Freundlich isotherms are single-solute adsorption isotherm models, which are widely used to analyze data for effluent treatment

TABLE 4: Isotherm parameters for Cr(VI) biosorption by *T. gamsii* biomass at various dilutions of electroplating industrial wastewater.

Freundlich isotherm constants			Langmuir isotherm constant		
$n$	$K_f$ , mg/g	$R^2$	$b$	$Q$ , mg/g	$R^2$
1.13	8.3	0.9423	0.068	133.33	0.5046

application to characterize the interaction of metal ions with biomass preparations [13]. The linearized plots of Freundlich and Langmuir isotherm model for biosorption of Cr(VI) ions from electroplating effluent by acid-treated biomass of *T. gamsii* are presented in Figure 5. It can be seen that  $R^2$  value for the Freundlich isotherm is 0.9423 against the Langmuir isotherm  $R^2$  value of 0.5046. Analysis of correlation regression coefficient shows that biosorption process fits better into Freundlich isotherm (Figure 5). The Langmuir and Freundlich adsorption constants calculated from the corresponding isotherms are presented in Table 4. The Freundlich isotherm constants  $k_f$  and  $n$  were calculated as 8.3 and 1.13, respectively. The high magnitude of  $k_f$  and  $n$  illustrates high adsorption capacity of biomass.

All these results showed that Freundlich isotherm model fitted the results quite well which are in agreement with the heterogeneity of sorbent (*T. gamsii* biomass) surface. Binding sites are not independent and adsorption energy of a metal binding site depends on whether or not the adjacent sites are already occupied. Thus, the adsorption of Cr(VI) ions by *T. gamsii* seems to be a complex process involving multilayer, interactive, or multiple-site type binding.

#### 4. Conclusion

In conclusion, the present study provides the practical application of the *T. gamsii* biomass. Acid-treated biomass of *T. gamsii* is effective in removing Cr(VI) ions from acidic (pH 1.5) electroplating effluent contaminated with 5000 mg/L of Cr(VI) and other coexisting metal ions. At initial pH of electroplating effluent and biomass dose of 10 mg/mL, 89% of Cr(VI) ions were removed within 420 minutes of contact time. The biosorption of Cr(VI) ions increased with increasing contact time and initial Cr(VI) ion concentration. Kinetic model developed based on the values of equilibrium uptake capacity, correlation regression coefficient, and rate constants 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 acid-treated biomass of *T. gamsii*. Thus,

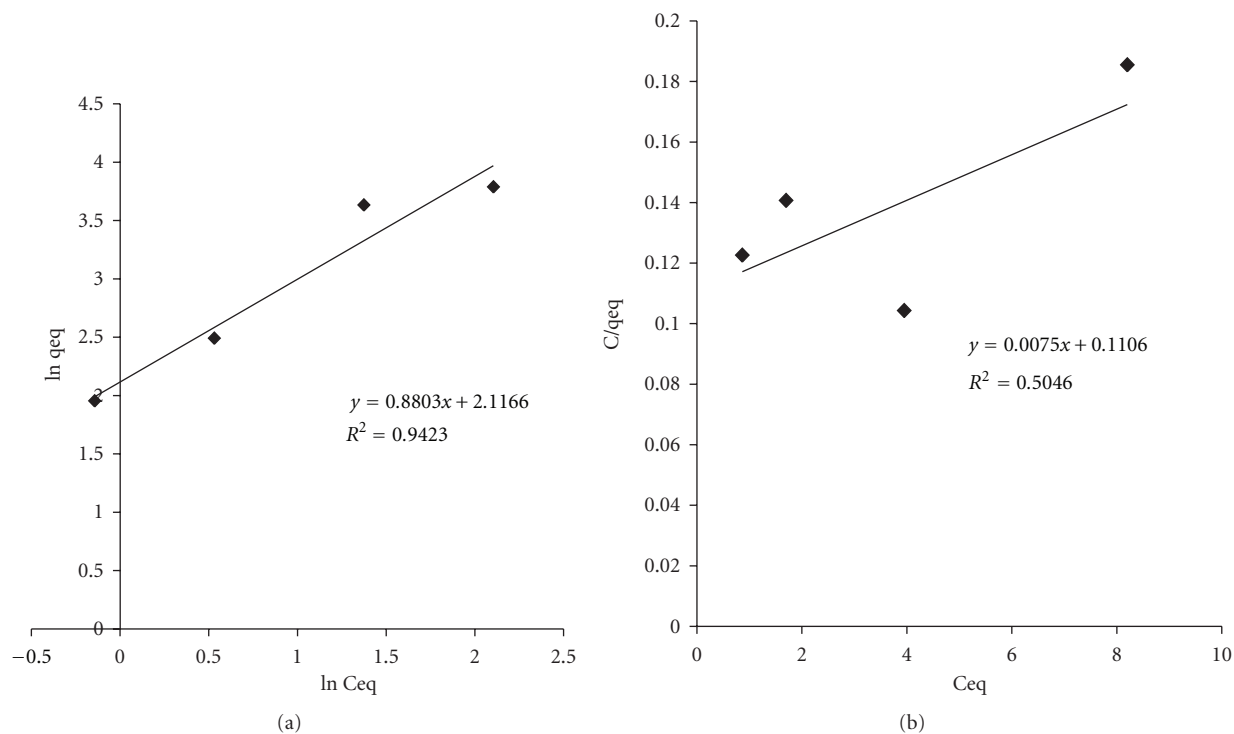


FIGURE 5: Biosorption isotherm: (a) Freundlich and (b) Langmuir isotherm for Cr(VI) biosorption by *T. gamsii* biomass at various dilutions of electroplating industrial waste water.

the results suggest the reasonable potential of acid-treated biomass of *T. gamsii* as sorbent for removal of Cr(VI) from electroplating effluents.

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