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Biosorption of cadmium and chromium from water by endophytic *Kocuria rhizophila*: equilibrium and kinetic studies

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ABSTRACT

Heavy metal removal from water is an important issue of environmental concern. This study demonstrates the application of endophytic bacterium *Kocuria rhizophila* (gene bank No: KF875448) isolated from hyperaccumulator *Oxalis corniculata* for the adsorption of Cd(II) and Cr(III) from aqueous solution. Minimum inhibitory concentration of Cd and Cr for the selected strain was found to be 6 and 8 mM, respectively. The effects of certain parameters like pH, contact time, and initial metal concentration on biosorption were assessed at a temperature of 35 ± 2˚C. The optimum pH values for Cd and Cr biosorption were found to be 8 and 4, respectively. Maximum biosorption for both the metals was obtained after 60 min. Biosorption equilibrium was described by Langmuir and Freundlich isotherms. The Langmuir model showed maximum adsorption capacity (Q_{max}) for Cd and Cr as 9.07 and 14.4 mg g^{-1}, respectively. Kinetic data indicated that biosorption of the selected heavy metals on *K. rhizophila* follows pseudo-second-order rate equation. FTIR analysis showed that functional groups like OH, C=O, C=N, N–H, CH_2, PO_2, C–O, C–O–C and C–H on *K. rhizophila* surface might be responsible for heavy metals biosorption. Results suggested that *K. rhizophila* has potential for the removal of metal ions from aqueous solution. Therefore, current research presents novelty in terms of evaluating endophytic *K. rhizophila* as a biosorbent for Cd and Cr.

Keywords: Adsorption isotherm; Biosorption; Heavy metals; Kinetic models; *Kocuria rhizophila*

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1. Introduction

Water pollution is a major environmental problem that is the leading cause of disease and death worldwide [1]. The primary contaminants added to water due to human activities (industrialization and agriculture) include detergents, fertilizers, hazardous industrial chemicals, herbicides, heavy metals, pathogens, pesticides, and textile dyes [2]. Out of these contaminants, heavy metals affect the environment badly. Among heavy metals, cadmium (Cd) and chromium (Cr) are the most toxic and noxious water pollutants. Accumulation of these heavy metals has deleterious effects on living organisms threatening environment

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and public health. Level of these metals is elevating due to activities, such as mining and smelting operations, use of pesticides, refining and plastic industries [3], fertilizer applications, glass and steel manufacturing, leather tanning and wood preservation [4]. Varghese [5] reported the actual concentration of 0.0231–0.00896 mg L^{-1} for Cd in industrial water. In another study [6], the Cd concentration was recorded in the range of 0.001–0.02 mg L^{-1} in different samples of drinking water, while the actual concentration of Cr in different water samples was found to be in the range of 0.01–1.15 mg L^{-1} [7]. The permissible limits set by Environmental Protection Agency [8] for Cd and Cr are 0.005 and 0.1 mg L^{-1}, respectively.

Various conventional physicochemical methods employed to remove heavy metals from the environment include filtration, ion exchange, chemical oxidation/reduction, flocculation, reverse osmosis and the use of activated charcoal [9,10]. Since these methods are expensive as well as less practical and sometimes may generate toxic by-products, hence, an alternative emerging strategy is biosorption that involves use of microorganisms for metal extraction [11]. Microorganisms having capacity to survive under high concentrations of heavy metals are of great interest as biosorbents.

Endophytes are microorganisms that colonize internal plant parts without any visible infection or negative impact on its host. They promote plant growth through the production of various chemicals like hydrogen cyanide (HCN), indole acetic acid (IAA), and through phosphate solubilization and thus can be employed in agriculture, industry or medicine [12]. Besides these plant growth-promoting (PGP) traits, endophytes have potential to be used for soil and water reclamation through phytoremediation [13].

Various biosorbents, such as algae, fungi, bacteria, and agricultural, by-products have been used to remove heavy metals [14–18]. However, to the best of our knowledge, there are less data available for the use of endophytes in metal biosorption. Since endophytes isolated from hyperaccumulator plants can tolerate high concentrations of certain metals because of hyperaccumulation activity of their host, such endophytes from hyperaccumulators are expected to be novel and auspicious biosorbents for heavy metal treatment [19]. Most studied bacterial endophytes in this regard belong to the genera of *Pseudomonas*, *Enterobacter*, *Bacillus*, *Burkholderia*, *Methylobacterium*, and *Arthrobacter* [20].

In the present research work, endophytic bacterium *Kocuria rhizophila*, isolated from reported hyperaccumulator *Oxalis corniculata* [21] was for the first time utilized as a biosorbent to remove selected heavy metals (Cd and Cr). The optimum pH, contact time, and initial metal concentration were investigated. Adsorption equilibrium and kinetics models were examined systematically.

2. Materials and methods

2.1. Materials

2.1.1. Strain

The endophytic strain used in this study was 14ASP, which was isolated from the leaves of the hyperaccumulator *O. corniculata.* The isolation was done according to the procedure as described by Chaudhary et al. [22]. The strain was identified as *K. rhizophila* based on 16S rRNA gene sequencing analysis with gene bank no. KF875448.

2.1.2. Medium

The solid Luria–Bertani (LB) medium (containing 5 g yeast extract, 10 g tryptone, 10 g NaCl, and 15 g agar per liter) and liquid LB medium (containing 5 g yeast extract, 10 g tryptone, and 10 g NaCl per liter) were used for strain culturing. The optimum pH of 6.8 was used for the medium.

2.1.3. Preparation of solutions

The two tested heavy metals were prepared from their chloride salts: cadmium chloride ($CdCl_2·2½H_2O$) and chromium chloride ($CrCl_3·6H_2O$). For minimal inhibitory concentration (MIC) test, 0.5 M stock solutions of the respective metal salts were prepared using double-distilled water. From these stock solutions, further concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 mM were prepared by dilution using double-distilled water. For adsorption study, 1,000 mg L^{-1} stock solutions of the respective metals were prepared using double-distilled water. From these stock solutions, further concentrations of 10, 25, 50, 100, and 150 mg L^{-1} were prepared by dilution using double-distilled water. These solutions were kept at room temperature.

2.1.4. Bacterial biomass production

The selected strain was maintained on LB agar medium. For biomass production, the strain was inoculated into 100 mL LB broth (pH 6.8) in a 250-mL conical flask and incubated on a shaker at 150 rpm at 35 ± 2°C. Cells were harvested after 24-h incubation. The cells were then centrifuged in 50-mL falcon tubes at 4,000 rpm for 20 min at 4°C. Cell pellets obtained were first weighted for desired concentration in falcon tube and then directly used for further biosorption experiments.

2.2. Experiments

2.2.1. Heavy metals' resistance test

The MIC was determined in solid LB medium amended with a series of concentrations of Cd and Cr (1–10 mM) from stock solution. LB agar plates without metals were used as control. The strain was streaked on these agar plates. The cultures were incubated at 35 ± 2°C for 4 d. The minimum concentration of each metal at which the strain failed to give colonies on agar plates was considered as MIC. All experiments were performed in duplicate. MIC is the minimum concentration at which bacterial growth is completely inhibited [23].

2.2.2. Effect of heavy metals on bacterial growth

The growth of bacteria was also evaluated in LB broth in the absence and presence of metals. From an overnight grown pure culture, 1% inoculum was added to 100 mL LB broth in a working volume of 250-mL conical flask supplemented with fixed concentration (100 mg L^{-1}) of each metal and maintained at optimum pH (6.8). The culture was grown at optimum temperature (35 ± 2°C) with continuous shaking at 150 rpm in an orbital shaker. The bacterial growth was monitored after regular intervals of time for 5 d by measuring optical density at 600 nm using UV–vis Spectrophotometer (Smart Spec^{TM} Plus Spectrophotometer). The un-inoculated LB broth containing appropriate concentrations of each metal was used as a blank. All experiments were conducted in triplicate.

2.2.3. Adsorption studies

Adsorption studies of metal ions were conducted in batch experiments to examine the factors affecting adsorption and metal uptake capacity of the adsorbent. Batch experiments were conducted in Erlenmeyer flasks in incubator shaker operating at 150 rpm to evaluate optimum conditions, such as pH, contact time, and initial metal concentration.

Effect of pH on the adsorption capacity of *K. rhizophila* for Cd and Cr ions was evaluated at pH values of 2, 4, 6, 8, and 10 for 60 min. The pH adjustment was carried out using 0.1 M HNO_3 or 0.1 M NaOH. The bacterial biomass for this experiment was taken as 0.5 g in 250-mL volumetric flask containing 100 mL of metal solution at fixed concentration (50 mg L^{-1}) of each metal ion. After 1 h, samples were centrifuged at 4,000 rpm for 10 min. Supernatant was then used to determine residual metal concentration.

Effect of contact time was studied using batch experiments at optimum pH at different time intervals (15, 30, 60, 90, and 120 min) at fixed concentration (50 mg L^{-1}) of each metal ion. Bacterial biomass of 0.5 g in 250-mL conical flask containing 100 mL of each metal solution was used. At predetermined time intervals, samples were removed, centrifuged, and residual metal concentrations analyzed.

To determine the effect of initial metal concentration on biosorption, different metal concentrations of 10, 25, 50, 100, and 150 mg L^{-1} were prepared for each studied metal. For Cd optimum pH of 8 and for Cr 4 with contact time of 60 min was established. All experiments were carried out at 35 ± 2°C.

The metal sorption efficiency of biosorbent was calculated by using the following equation [24]:

$$q_e = \frac{C_o - C_e}{M} \times V \tag{1}$$

where q is the mass of metal ions in mg adsorbed per g of biosorbent, C_o (mg L^{-1}) is the initial metal ion concentration, C_e (mg L^{-1}) is the final (equilibrium) metal ion concentration, V (L) is the volume of metal ion solution in the flask, and M (g) is mass of biosorbent used [25,26].

2.2.4. FTIR analysis

The samples were assessed by FTIR spectroscopic analysis before and after metal loading. The infrared spectra were studied in the range of 4,000–400 cm^{-1} using Tensor27 spectrometer.

2.2.5. Analytical technique

The optical densities (OD) of bacterial cultures were measured by using UV–vis spectrophotometer (Smart Spec^{TM} Plus Spectrophotometer) at 600 nm. The concentration of metal ions was assessed by using 240FS Varian Atomic Absorption Spectrophotometer. The hollow cathode lamp was operated at 4 mA for Cd and 5 mA for Cr. The analytical wavelengths were set at 228.8 and 357.9 nm for the detection of Cd and Cr, respectively.

3. Results and discussion

3.1. Heavy metals resistance test

The isolate *K. rhizophila* showed a considerable resistance against Cd and Cr. The MIC values of the strain on solid LB-medium containing the heavy metal ions were 6 and 8 mM for Cd and Cr, respectively. The bacterial endophyte was isolated from leaf of *O. corniculata* which is a hyperaccumulator plant and has the capability of metal ion accumulation similar to the studies of Guo et al. [23]. In comparison to other metal-resistant strains [27–31], the endophytic *K. rhizophila* showed more resistance to Cd and Cr. The high metal-resistant ability of this endophytic bacterium may possibly have been resulted from the hyperaccumulation ability of its host plant. These results indicate promising application of *K. rhizophila* in the reclamation of heavy metal contaminated sites.

3.2. Effect of metals on bacterial growth profile

A relative growth pattern of the strain *K. rhizophila* was observed when it was grown in nutrient broth treated with fixed concentration (100 mg L^{-1}) of each metal at different time intervals as shown in Fig. 1. The growth of *K. rhizophila* in the presence of Cd showed a lag phase much longer (on average 6–9 h) than in the absence of metals, while in the presence of Cr it showed a short lag phase (about 1–3 h). The extended lag phase might be due to the acclimatization of bacterial cells with high concentration of Cd ions in the medium [32]. Bacterial growth inhibition was variable depending on the heavy metals present in the medium. Cd inhibited the bacterial growth by approximately 12% with regard to control. However, Cr did not show any significant effects on the growth of *K. rhizophila*. The bacterial growth inhibition in the presence of Cd might be due the toxic effects of Cd on bacterial cells as stated by Liao et al. [33].

For the application of biosorption of heavy metals by *K. rhizophila*, a growth profile study is important under metal stress condition. The growth rate of *K. rhizophila* in the presence of Cd is generally slower than that of control. Similar results have been reported by Hassen et al. [27] and Raja et al. [34]. This growth reduction is mainly due to interaction between the cell surface functional groups (phosphate, carboxyl, hydroxyl, amino) and metal ions [35].

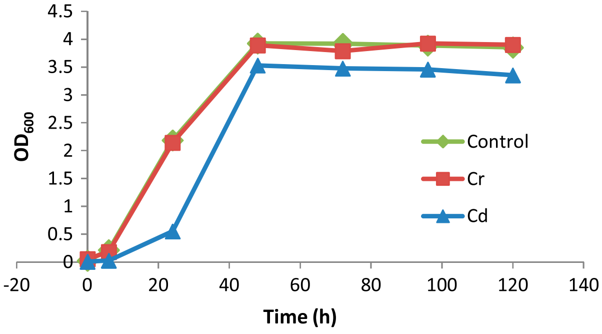

Fig. 1. Growth pattern of *K. rhizophila* grown in LB broth amended with 100 mg L^{-1} of Cd and Cr in comparison with control (LB broth without metals). OD = optical density.

3.3. Effect of solution pH

Initial solution pH is a crucial factor in adsorption studies, as it affects solubility and speciation of metal ions and more over controls/regulates charge distribution of sorbent [36]. It is observed from Fig. 2 that adsorption capacity of Cr at pH 4 is higher, while it decreases when the pH increases. It is because at acidic pH, the dominant cation is Cr^{3+}, while an increase in pH causes decease in Cr^{3+} ion concentration. At pH greater than 4, Cr^{3+} is precipitated as $Cr(OH)_2^+$ and $Cr(OH)^{2+}$ decreasing the concentration of Cr^{3+} for adsorption [37,38]. Similar results were also reported by Sala et al. [39] and Kanwal et al. [40].

In case of Cd, pH 8 favors adsorption process making the bacterial surface functional groups more negative which eventually enhances the biosorption of Cd. However, a further increase in the value of pH from 8 decreases the biosorption process possibly due to precipitation of Cd as cadmium hydroxide [41]. Our recent results for Cd are comparable with the previous studies [42–44], where the maximum Cd adsorption was observed at pH 8.0.

In the subsequent studies, pH 4 and 8 for Cr and Cd, respectively, were selected as the optimum pH values.

3.4. Effect of contact time

Contact time is an important factor affecting biosorption potential. The biosorption of Cd and Cr at

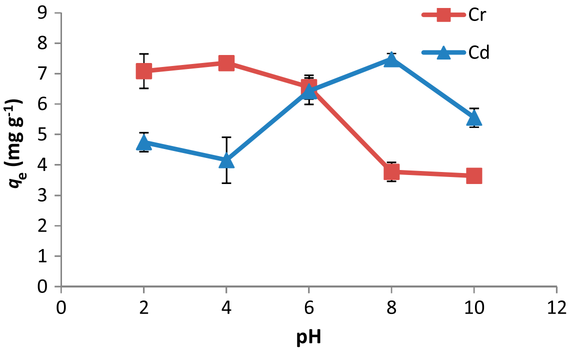

Fig. 2. Effect of pH on Cd and Cr biosorption by *K. rhizophila*. Metal ion concentration = 50 mg L^{-1}; Biomass (M) = 0.5 g; contact time = 1 h; *T* = 35 ± 2˚C. Results are mean of three independent experiments. Error bars show SEM.

different time intervals is shown in Fig. 3. The rate of metal ion biosorption was highest in the beginning due to high affinity of free metal ion binding sites on biosorbent but after few minutes the rate of biosorption slowed and reached to equilibrium in 60 min for both the metal ions. The attained equilibrium time for all biosorption studies ensures that no further metal ions can be adsorbed on the biomass surface, as the rate of metal adsorption and desorption are equal, ensuring maximum metal binding on biomass [45].

The effect of contact time on the sorption of heavy metal has been extensively studied for several sorbents, and variation in time required to attain equilibrium in solution was reported to be dependent on the sorbent. Equilibrium time for biosorption of Cd and Cr ions by *Bacillus thuringiensis* has been found to be 30 min [10], by *Pantoea* sp. TEM18 as 15 min [46], by *Rhodococcus opacus* as 60 min [47], by red algae as 60 min [48], and by *S. condensate* as 120 min [49].

3.5. Effect of initial metal ion concentration

Adsorption isotherms for Cd and Cr were measured at varying initial metal ion concentrations (C_o) in the range of 10–150 mg L^{-1} for 60 min. Fig. 4 indicates an increase in q from 1.3 to 13 mg g^{-1} for Cr and from 1 to 17 mg g^{-1} for Cd with increasing C_o values. The results suggest that increase in C_o causes an increase in interaction between metal ions and adsorbent providing more binding sites for adsorption process [50]. The high initial metal concentration causes more availability of metal ions for adsorption. In addition, the increase in initial metal concentration causes increased driving force to overwhelm all mass transfer resistance of metal ions. This causes greater chances of collision between metal ions and sorbent [51,52]. Similar conclusions were drawn by other authors [47,53].

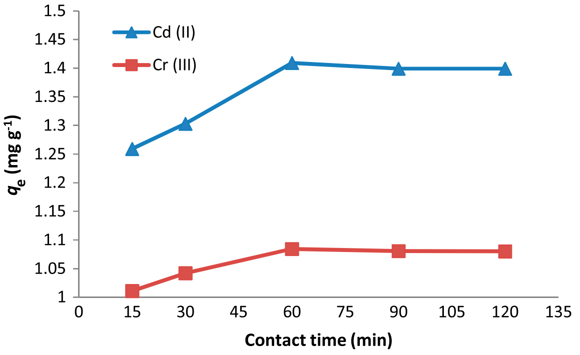

Fig. 3. Effect of contact time on Cd and Cr biosorption by *K. rhizophila*. Metal ion concentration = 50 mg L^{-1}; biomass (M) = 0.5 g; T = 35 ± 2˚C.

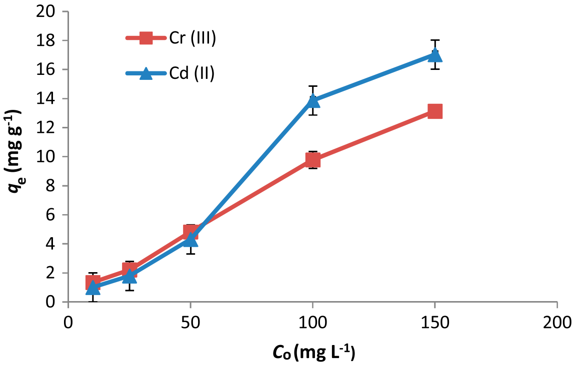

Fig. 4. Effect of initial metal ion concentration on Cd and Cr biosorption by *K. rhizophila*. Biomass (M) = 0.5 g; pH 4 for Cr(III) and 8 for Cd(II); contact time = 1 h; T = 35 ± 2˚C.

3.6. Equilibrium isotherms and models

Adsorption isotherms explain distribution of molecules between solid and liquid phases at equilibrium time. They provide some knowledge regarding surface properties, adsorbent affinities and possible adsorption mechanisms. Most accepted and widely used models are Langmuir and Freundlich models. Both models were used to fit the experimental data for Cd and Cr.

Langmuir model quantitatively describes monolayer adsorption on the surface of adsorbents having finite number of identical sites. This model assumes uniform energy distribution over all sites and no transmigration of adsorbate. Langmuir adsorption isotherm equation is given as follows [54]:

$$q_e = \frac{Q_{\max} b C_e}{1 + b C_e} \tag{2}$$

where q (mg g^{-1}) is the maximum mass of metal ion absorbed at equilibrium, $Q_{\max}$ (mg g^{-1}) is the capacity of adsorbent, b (L mg^{-1}) is the adsorption energy constant and C (mg L^{-1}) is the equilibrium metal ion concentration. The linear form of Eq. (2) can be written as:

$$\frac{1}{q_e} = \frac{1}{Q_{\max}} + \frac{1}{Q_{\max} b C_e} \tag{3}$$

Experimental results for Cd and Cr at varying concentrations in the range of 10–150 mg L^{-1} were fitted to Langmuir isotherm model. Graphs of $1/q$ vs. $1/C$ yielded straight lines for both metal ions (Fig. 5(a) and (b)), which demonstrate uniform adsorption. $Q_{\max}$, b and determination coefficient (R^2) were calculated from the graphs.

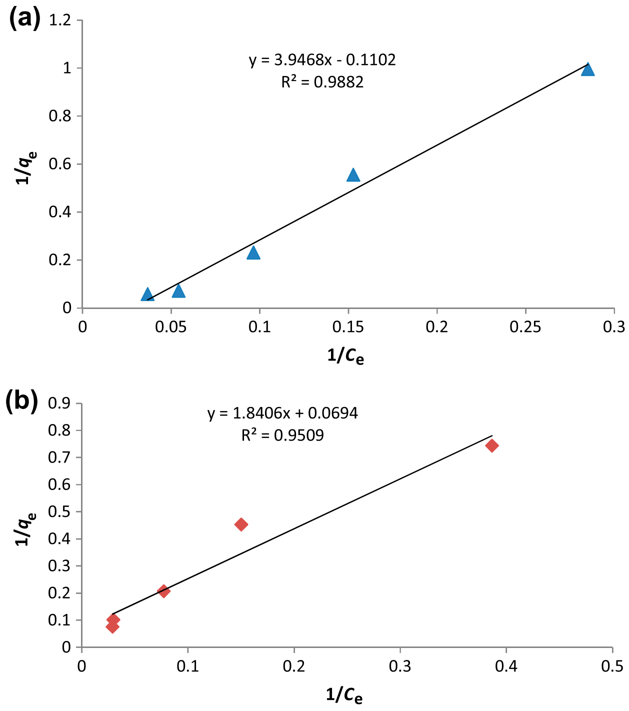

Fig. 5. Linearized Langmuir model for Cd (a) and Cr (b) adsorption on *K. rhizophila*.

Langmuir constant, Q_{max} represents maximum adsorption capacity of *K. rhizophila* for Cd and Cr ions. As shown in Table 1, Q_{max} for *K. rhizophila* were obtained as 9.07 and 14.4 mg g^{-1} for Cd and Cr, respectively.

Weber and Morris [55] described a dimensionless parameter (R_L) which predicts the nature of adsorption process. R_L can be calculated from equation given below:

$$R_L = \frac{1}{1 + (b \times C_o)} \tag{4}$$

where C_o (mg L^{-1}) is initial metal concentration, b (mg L^{-1}) is Langmuir isotherm constant. R_L indicates unfavorable adsorption process when $R_L > 1$, favorable if $0 < R_L < 1$, linear when $R_L = 1$ and irreversible when $R_L = 0$. Table 1 shows that R_L values for both metal ions at different C_o values were all positive, greater than 0 and less than 1, indicating that Cd and Cr adsorption is favorable on *K. rhizophila* biomass.

Freundlich model describes adsorption characteristics assuming the heterogeneous nature of surface. The Freundlich adsorption isotherm is represented by the following equation [56]:

$$q = K_F C_e^{1/n} \tag{5}$$

where K_F is Freundlich binding constant related to the adsorption capacity of adsorbent and n is empirical parameter related to adsorption intensity. The linearized form of Eq. (5) can be written as follows:

$$\log q = \log K_F + \frac{1}{n} \log C_e \tag{6}$$

Adsorption data of Cd and Cr in the concentration range of 10–150 mg L^{-1} were also fitted to Freundlich isotherm model. Plots of $\log q$ vs. $\log C_e$ yielded straight lines for Cd and Cr adsorption on *K. rhizophila* (Fig. 6(a) and (b)). Freundlich constant K_F, n, and R^2 were evaluated from the graphs.

In case of Freundlich adsorption isotherm model, feasibility of adsorption process is indicated by values of n greater than 1. Table 1 shows "n" value greater than unity for Cr onto *K. rhizophila*, whereas, in case of Cd, n is close to unity, indicating that adsorption of Cr by *K. rhizophila* is far more favorable than Cd. Higher K_F value (Table 1) for Cr shows high adsorption capacity of *K. rhizophila* as compared to Cd.

Both models had a good agreement with the data for Cd and Cr biosorption, because R^2 values were close to unity. However, in case of Cr, R^2 values for Freundlich isotherm model was higher than Langmuir isotherm model with n values greater than unity illustrating that

Table 1
Isotherm coefficients of Langmuir and Freundlich models

	Langmuir model				Freundlich model		
Adsorbate	Q_{max} (mg g^{-1})	b (mg L^{-1})	R_L	R^2	K_F	n	R^2
Cd(II)	9.070	0.028	0.241–0.807	0.988	0.130	0.670	0.975
Cr(III)	14.400	0.038	0.209–0.740	0.950	0.524	1.160	0.974

Notes: Q_{max} = maximum adsorption capacity of adsorbent, b = adsorption energy constant, R_L indicates the nature of adsorption process, K_F = Freundlich binding constant, n = adsorption intensity, R^2 = determination coefficient.

Freundlich isotherm model better fits the experimental data for Cr. This suggests that Cr biosorption by *K. rhizophila* was heterogeneous surface adsorption instead of monolayer adsorption. While for Cd, Langmuir isotherm model better fits the experimental data than Freundlich isotherm model as evident from determination coefficient (R^2) values, suggesting monolayer coverage of Cd ions on the surface of *K. rhizophila*.

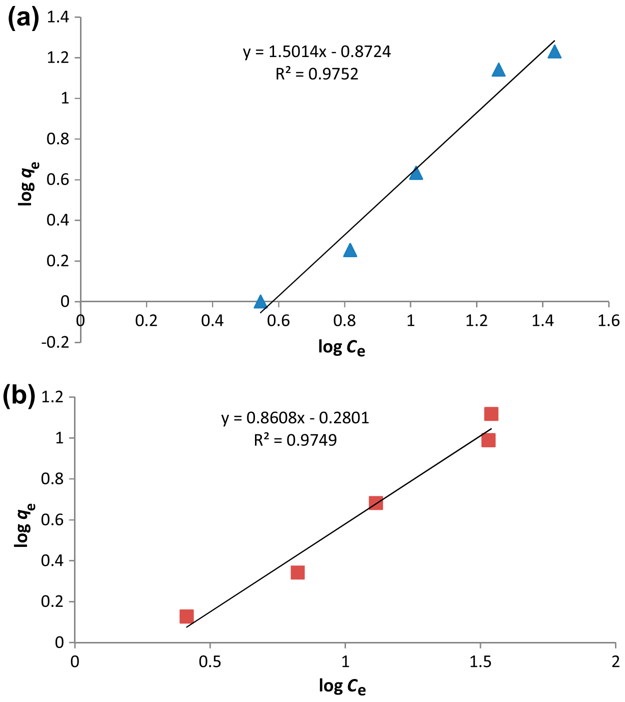

Fig. 6. Linearized Freundlich adsorption isotherm for biosorption of Cd (a) and Cr (b) onto *K. rhizophila*.

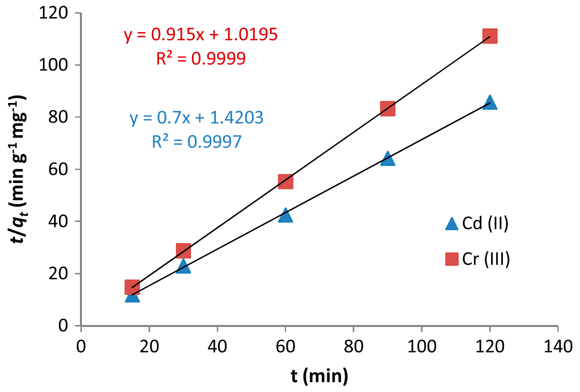

Fig. 7. Pseudo-second-order kinetic model for the adsorption of Cd and Cr on *K. rhizophila*.

3.7. Adsorption kinetic study

Biosorption kinetics is the study of sorption rates and the factors influencing them in the attainment of equilibrium in a reasonable amount of time. It also shows us the mechanism of adsorption process and estimation of the adsorbent loading at equilibrium (capacity). In this study, we applied the pseudo-second order and the intraparticle diffusion models for the experimental data to predict the kinetics of biosorption. The linearized pseudo-second-order kinetic equation is given by the following equation [57–59].

$$\frac{t}{q_t} = \frac{1}{k_1 q_e^2} + \frac{1}{q_e} \times t \tag{7}$$

where q_t and q (mg g^{-1}) is the mass of metal ions adsorbed at time t and at equilibrium respectively, t is the contact time (min) and k_1 (g mg^{-1} min^{-1}) is the pseudo-second-order rate constant.

Using the linearized form of pseudo-second-order equation, plotting t/q_t vs. t gives a straight line (Fig. 7). The values of k_1 and q are calculated from the intercept and gradient of the graph, respectively. The initial adsorption rate, h (mg g^{-1} min^{-1}), is calculated from the following equation:

$$h = k_1 q_e^2 \tag{8}$$

The values of k_1, q, h and determination coefficient R^2 of the pseudo-second-order kinetics are listed in

Table 2
Kinetic parameters of the pseudo-second-order and intraparticle diffusion models for Cd and Cr adsorption on *K. rhizophila*

	Exp. value	Pseudo-second-order				Intraparticle diffusion		
Metals	q_e^{exp} (mg g^{-1})	k_1 (g mg^{-1} min^{-1})	q_e (mg g^{-1})	h (mg^{-1} g^{-1} min)	R^2	k_{int} (mg g^{-1} min$^{-1/2}$)	C_i (mg g^{-1})	R^2
Cd(II)	1.400	0.345	1.430	0.700	0.999	0.021	1.194	0.803
Cr(III)	1.084	1.217	1.093	1.453	0.999	0.009	0.985	0.794

Notes: q_e = adsorption capacity at equilibrium, k_1 = pseudo-second-order rate constant, h = initial adsorption rate, k_{int} = intraparticle diffusion rate constant, C_i = thickness of boundary layer, R^2 = determination coefficient.

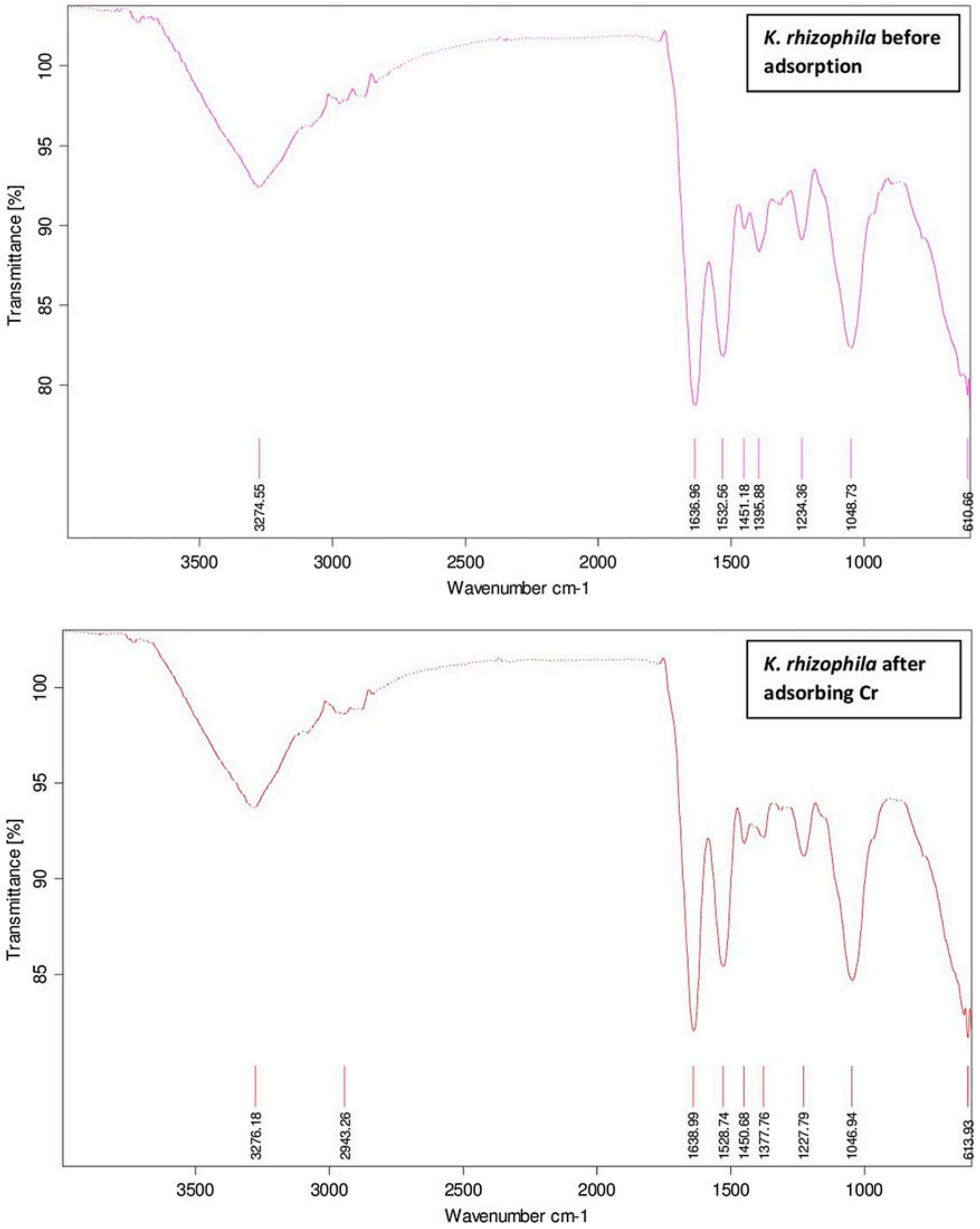

Fig. 8. FTIR spectra of *K. rhizophila* before and after metal adsorption.

Table 2. Fig. 7 indicates that the pseudo-second-order kinetic model is applicable for the adsorption of Cd and Cr on *K. rhizophila* as the determination coefficient is equal to 0.99 for both metals. In addition, the experimental value of q and pseudo-second-order kinetic value q were close to each other, indicating that the Cd and Cr adsorption follows pseudo-second-order kinetic model. By considering initial adsorption rate (h), the result indicates that Cr adsorption rate is faster than Cd.

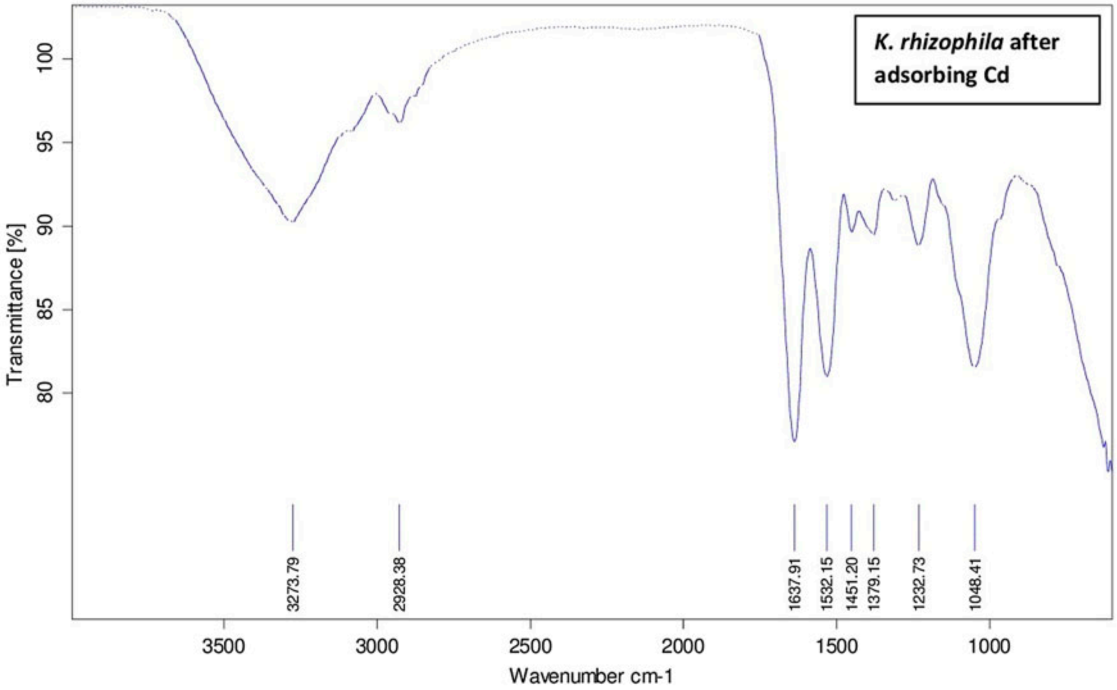

Fig. 8. (*Continued*).

Table 3
Comparison of *K. rhizophila* for Cd and Cr biosorption with other sorbents

Biosorbent	Metal	$Q_{\max}$ (mg g^{-1})	Refs.
Aspergillus niger with kaolinite	Cr(III)	4.33	[68]
Penicillium chrysogenum	Cr(III)	18.6	[69]
Pichia guilliermondii	Cr(III)	4.0 to13	[70]
Ganoderma lucidum	Cr(III)	2.16	[71]
Scenedesmus quadricauda	Cr(III)	12	[72]
Kocuria rhizophila	Cr(III)	14.4	This study
Saccharomyces cerevisiae	Cd(II)	8.17	[73]
Rhodococcus opacus	Cd(II)	1.55	[74]
Mucor rouxii (live biomass)	Cd(II)	8.46	[75]
Caulerpa lentillifera	Cd(II)	4.69	[76]
Pseudomonas aeruginosa (wild type)	Cd(II)	3.01	[77]
Anoxybacillus amylolyticus	Cd(II)	18.72	[78]
Spirogyra hyalina	Cd(II)	18.18	[79]
Chlorella vulgaris	Cd(II)	6.79	[80]
Kocuria rhizophila	Cd(II)	9.07	This study

The intraparticle diffusion model was also applied to the experimental data. The simplified equation of the intraparticle diffusion model is as follows [55]:

$$q_t = k_{\text{int}} t^{1/2} + C_{\text{i}} \tag{9}$$

where k_{int} (mg g^{-1} min$^{-1/2}$) is intraparticle diffusion rate constant and C_{i} is intercept which describes the thickness of the boundary layer. If the adsorption mechanism follows the intra-particle diffusion process, then a plot of q_t vs. $t^{1/2}$ will give a straight line. Moreover, if the graph passes through the origin then the intraparticle diffusion process would be a rate limiting step. Otherwise, if the graph does not pass through the origin, then the intraparticle diffusion process will not be the sole rate determining step [59].

Here, in this study, the plot of q_t vs. $t^{1/2}$ is linear (graphs not shown) and does not pass through the origin indicating that the intraparticle diffusion process is involved but is not the sole rate-limiting step. The values of k_{int}, C_{i}, and R^2 are shown in Table 2. The values of k_{int} are two times higher for Cd than for Cr. The high value of k_{int} shows an increase in the rate of adsorption and better adsorption mechanism which is related to better bonding between the sorbate and adsorbent [60]. By comparing the rate constant values of both the kinetic models, it was observed that pseudo-second-order model shows best fit for the experimental data.

3.8. FTIR analysis

FTIR analysis is a significant technique used for knowing the mechanisms involved in Cr and Cd adsorption on the surface of *K. rhizophila* by detecting the functional groups of *K. rhizophila* before and after biosorption [61].

FTIR spectra of *K. rhizophila* biomass before and after metal loading are shown in Fig. 8. Several peaks can be observed in the spectrum of raw *K. rhizophila*. The peak at 3,274.55 cm^{-1} is assigned to stretching vibration of –OH of carboxylic acid [62], peak at 1,636.96 cm^{-1} is attributed to amide I, (C=O) different conformation [63], peak at 1,532.56 cm^{-1} is characteristic of amide II band (–N–H deformation coupled to –C=N– deformation) [64], peak at 1,451.18 cm^{-1} is assigned to CH_2 bending of lipids [65,66], peak at 1,234.36 cm^{-1} is characteristic of PO_2^- asymmetric stretching: mainly nucleic acids with the little contribution from phospholipid [65], peak at 1,048.73 cm^{-1} corresponds to the C–O and/or C–O–C from polysaccharides [67] and the peak at 610.66 cm^{-1} is associated to C–H of alkynes.

After adsorption of Cr and Cd, the band at 3,274.55 cm^{-1} is shifted to 3,276.18 and 3,273.13 cm^{-1}. The peak at 1,532.56 cm^{-1} is shifted to 1,528.74 and 1,533.17 cm^{-1}. The peak at 1,451.18 cm^{-1} is shifted to 1,450 and 1,455 cm^{-1}. The peak at 1,234.36 cm^{-1} is shifted to 1,227.79 and 1,232.92 cm^{-1}. The peak at 610.66 cm^{-1} is shifted to 613.93 cm^{-1} and 613.34 cm^{-1}. After Cr adsorption, the peak at 1,636.96 cm^{-1} is shifted to 1,638.99 cm^{-1}, and the peak at 1,048.73 cm^{-1} is shifted to 1,046.94 cm^{-1}. All these changes suggest that Cr and Cd might react with hydroxyl, amide I, amide II, phosphate, methyl, and alkyne groups in the adsorption process.

Extra peaks were also observed in metal-loaded spectrum, indicating metal-binding mechanism taking place on the surface of *K. rhizophila*.

3.9. Comparison with other biosorbents

The adsorption capacity of *K. rhizophila* was compared with other biosorbents. The comparison indicates that *K. rhizophila* has good adsorption capacity compared to other biosorbents (Table 3). Thus, it can be concluded that *K. rhizophila* is very effective in binding Cd and Cr on its surface.

4. Conclusions

The present study found that the metal tolerant endophytic *K. rhizophila* isolated from hyperaccumulator *O. corniculata* was effective for the adsorption of Cd and Cr ions from water. Parameters, such as pH, contact time, and initial heavy metal concentration, affect biosorption process. The maximum sorption of Cd and Cr on *K. rhizophila* occurred at pH 8 and 4, respectively, within 60 min at 35 ± 2°C. The most important equilibrium isotherm models Langmuir and Freundlich were applied well to the experimental data. By using Langmuir model, the maximum adsorption capacity (Q_{max}) values of *K. rhizophila* KF875448 for Cd and Cr were 9.07 and 14.4 mg g^{-1}, respectively. The R_{L} values ranged from 0 to 1 demonstrating that adsorption of Cd and Cr from the solution on *K. rhizophila* is favorable. Kinetic studies showed that data were best fitted to pseudo-second-order model, whereas intraparticle diffusion was also involved in the adsorption of Cd and Cr on *K. rhizophila* but is not a rate-limiting step. FTIR analysis revealed that functional groups like –OH, C=O, −C=N, −N−H, $-CH_2$, $-PO_2$, C−O, C−O−C, and C−H on *K. rhizophila* might be responsible for heavy metals' biosorption. The study confirms our hypothesis that endophytic bacteria of metal hyperaccumulator may be potential biosorbent for heavy metals.

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