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ORIGINAL PAPER

Biosorption potential of *Bacillus anthracis* PM21 for the sequestration of cadmium, chromium, and nickel from contaminated water

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Abstract

Heavy metal pollution is a serious threat to the environment. The current study aims to assess the role of *Bacillus anthracis* PM21 for the adsorption of cadmium Cd(II), chromium Cr(VI), and nickel Ni(II) from contaminated water. Possible mechanisms and bacterial surface changes during the adsorption process were evaluated at constant temperature (35 ± 2 °C). The maximum biosorption (q) values 22, 57.04, and 5 mg/g for Cd, Cr, and Ni were observed at pH 8, 6, and 4, respectively. The optimum contact time for biosorption was 60 min at 50 mg L^{-1} concentration of heavy metals. Isotherm models were used to simulate the metals biosorption onto *Bacillus anthracis* PM21 surface at equilibrium. Langmuir's model gave the best fit with R^2 values for Cd = 0.997, Cr = 0.996, and Ni = 0.976. The adsorption kinetics followed the pseudo-second-order kinetic model. The Fourier-transform infrared spectroscopy analysis showed that different surface ligands: OH, C–O–C, C=O, S=O, N–O, CH_2, and C–H, could be involved in the biosorption of metals on to *Bacillus anthracis* PM21. The scanning electron microscope analysis showed that bacterial cells exposed to Cd, Cr, and Ni were rough and exhibited more cell size relative to the unexposed cells. There was adhesion of polymeric substances on the bacterial cell wall. Results revealed a variation in cell size, but cells maintained their shape. It is concluded that the *B. anthracis* PM21 is a promising biosorbent for the elimination of Cd(II), Cr(VI), and Ni(II) from aqueous solutions.

Keywords Biosorption · Fourier-transform infrared spectroscopy · Heavy metals · Isotherm models · Scanning electron microscope

Introduction

The wide exploitation of heavy metals (HMs) in different industrial processes has led to global issue of HMs pollution (Imran et al. 2021). Industrial wastewater is the main source of these toxic HMs in the ecosystem (Imran et al. 2021; Din et al. 2019). The bioaccumulation of HMs is the consequence of their persistence and non-degradable nature (Bhatnagar et al. 2010; Rajesh et al. 2014), thus threatening human health as well as ecosystem (Zhang et al. 2019). All the living organisms are at risk due to the toxic effects of HMs, i.e., cadmium (Cd), chromium (Cr), and nickel (Ni) (Tariq et al. 2020; Imran et al. 2021; Amjad et al. 2020; Elahi et al. 2020). The HMs adversely affect the plant germination, growth, and yield (Amjad et al. 2020; Mehmood et al. 2021). Not only plant health, HMs also have been linked to various human health issues including cancer and other problems (Din et al. 2019; Ahmad et al. 2018). Cadmium (Cd) is a highly toxic heavy metal that exists extensively in the environment and thus threatens human beings as well as the ecosystems (Tu et al. 2012; Hechmi et al. 2014; Peng et al. 2018a, b; Naeem et al. 2020). Furthermore, Cd reaches the human body via food chain (Koyuturk et al. 2007; Zhang et al. 2015; Huang and Keller 2015). Chromium and Ni are also highly concerned because of their vast utilization in

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developing countries and their non-degradable nature (Din et al. 2021). Hexavalent chromium is highly water soluble and carcinogenic for humans. In comparison with Ni(IV), Ni(II) is a more toxic and carcinogenic heavy metal (Amjad et al. 2020; Congeevaram et al. 2007). Conventional physicochemical techniques for instance electrochemical treatment, precipitation, ion exchange, chemical oxidation, reverse osmosis, evaporation, and sorption (Imran et al. 2020; Kadirvelu et al. 2002; Özdemir et al. 2009) are well known for the remediation of HMs. However, remediation with these techniques requires huge investment and is governed by various conditions for their operation (Vijayaraghavan et al. 2009; Lovley and Lloyd 2000; Vijayaraghavan and Balasubramanian 2013). Technical and economical constraints restricted utility of these remediation technologies (Xu et al. 2020), e.g., effectiveness of the ion exchange method is masked by expensiveness of adsorbent materials (Donmez and Aksu 2002).

Bioremediation is an innovative, efficient, and alternate approach to remediate heavy metal-contaminated sites to their actual state through physiological processes of the microbes (Akcil et al. 2015; Pena-Castro et al. 2004; Prasenjit and Sumathi 2005). Biosorption defines the uptake rate of sorbate, and this rate controls the residence time of sorbate at the solid–liquid interface (Akinyeye et al. 2020). Kinetic parameters are useful in the determination of rate-limiting step in the biosorption of heavy metal ions on the surface of bacteria (adsorbent). For solid–liquid biosorption, solute transfer process can take place by either external mass transfer process or particle diffusion (Aravindhan et al. 2009). Biosorption kinetics are described by various independent processes, e.g., bulk diffusion, film diffusion, chemisorption, and intra-particle diffusion. Different species of *Aspergillus*, *Bacillus*, *Pseudomonas*, *Phanerochaete*, *Saprophyticus*, etc., have shown their efficiency for reducing Cr and Ni (Yan and Viraraghavan 2003; Congeevaram et al. 2007). This process does not produce toxic sludge and reduces the concentration of heavy metal ions below permissible limit and may regenerate materials. Consequently, it provides an efficient, economic, and environmentally friendly way for the remediation of HMs (Imran et al. 2020; Gupta et al. 2006; Ren et al. 2015).

Plant growth-promoting microbes are usually regarded as collaborative remediators having the potential to enhance plant growth and adsorb metals, thereby remediating as well as inhibiting the metal uptake by plants (Chi et al. 2020). In a previous study, *Bacillus anthracis* PM21 was screened and found that it demonstrated growth-promoting activities in plants including indole-3-acetic acid (IAA) formation and solubilization of phosphorus (Ali et al. 2021). This bacterial strain showed resistant against Cd, Cr, and Ni and helped the plant to cope with these stresses (Chi et al. 2020). *Bacillus anthracis* has been reported multiple times for its versatile functions including plant growth promotion, heavy metal tolerance, and biocontrol potential (Govindasamy et al. 2010; Ali et al. 2021). Microorganisms remove metals using two major physiochemical processes; an active (energy-dependent) process also known as bioaccumulation and a passive (energy independent) process known as biosorption (Wang and Chen 2009; Limcharoensuk et al. 2015). Mechanisms involved in bioaccumulation are intracellular accumulation, precipitation, and oxidation–reduction. These processes often involve slow transport and accumulation of metals within the cytoplasm after a quick cell surface binding that leads to longer response time and are associated with an active defense system (Rajesh et al. 2014; Yuan et al. 2019). Physicochemical interactions between the cell surface and surrounding metals are involved in the passive biosorption process (Vijayaraghavan and Yun 2008). It undergoes complex formation with the chelates/ligands present outside cell with the help of carboxyl, hydroxyl, and amine functional groups (Li et al. 2018) along with sorption onto the cell surfaces (Alam and Ahmad 2011). Hence, considerable attention has been paid to the remediation of HMs polluted effluents through utilization of metal-resistant microorganisms (Nourbakhsh et al. 2002; Rajesh et al. 2014). Additionally, different parameters affecting the metals alleviation potential of microbes are pH, contact time, and metal concentration (Yuan et al. 2019). Furthermore, variation in strategies being adopted by different microbial species for metal adsorption leads to difference in efficiencies as well as their practical utilization (Li et al. 2019).

The thickness of the polysaccharide layer and negative charges possessed by the phospho-wall acids are involved in the adsorption of HMs in many *Bacillus* species. In a *Bacillus* sp. L14, inhibition of ATPase function enhanced the adsorption rate (Guo et al. 2010). Scholars performed genetic-based studies to recognize processes adopted by microbes to remediate HMs. For instance, gene *cadA* is responsible for Cd resistance regulation, commonly observed in Gram-positive bacterial species (Icgen and Yilmaz 2016; Guffanti et al. 2002; Fang and Dos Santos 2015). This study was carried out at Plant Microbe Interactions Laboratory, Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan, and it was completed at the end of 2019. Therefore, the present study aims to assess the biosorption capability of *B. anthracis* PM21, for the sorption of Cd, Cr, and Ni, under different operational environments including temperature, pH, and inoculum concentrations. The experimental adsorption data were validated with equilibrium sorption models (Freundlich and Langmuir), and adsorption kinetics were described with pseudo-second-order and intra-particle diffusion models. The Fourier-transform infrared spectroscopy (FTIR) and scanning electron microscope (SEM) were used to analyze the functional groups and surface morphology of the adsorbent.

Materials and methods

Microorganism and its tolerance to Cd, Cr, and Ni

Currently studied bacterial strain *Bacillus anthracis* PM21 (NCBI No OK255528) was isolated from the rhizospheric soil of tomato and was found with growth-promoting capacity of plants. The PM21 was cultured and checked regarding its growth under different HMs up to 7 days. The highest growth for the strain PM21 was examined on the 6th day of culturing (Ali et al. 2021).

Batch adsorption studies

Based on the previous evaluation, adsorption studies of three heavy metals (Cd, Cr, and Ni) were performed in batch mode to evaluate the impact of different factors affecting efficiency of an adsorbent. Experiments were performed in Erlenmeyer flasks at a rotation of 150 rpm for optimizing different factors including pH, interaction time with the biosorbent, and initial concentration of heavy metals.

Influence of pH on adsorption study

The impact of different pH (2–10) was studied by adjusting initial pH with 1 N HNO_3 or NaOH. The experiment was conducted in a 250 ml flask, each containing 50 mg L^{-1} of metal solution and 0.5 g of bacterial biomass at 35 ± 2 °C. After 60 min, samples were centrifuged for 600 rpm. The resulting supernatant was utilized for the evaluation of residual Cd, Cr, and Ni in water.

Influence of contact time on adsorption study

The impact of different contact times (0–120 min), optimum pH (Cd: 8.0, Cr: 6.0, and Ni: 4.0), and fixed values (50 mg L^{-1}) for Cd, Cr, and Ni removal was conducted at batch scale. The experiment was carried out in a 250 mL of working volume. At different time intervals, samples were taken out and centrifuged for further analysis of the supernatant (Gillania et al. 2017).

Impact of initial metal concentration on adsorption study

The effect of initial concentration of Cd, Cr, and Ni was evaluated by changing concentration from 25 to 200 mg L^{-1} at optimum pH (Cd: 8.0, Cr: 6.0, and Ni: 4.0) for 60 min of contact time. After biosorption, the solution was centrifuged and resulting supernatant was analyzed (Chi et al. 2020).

All experiments were conducted at a constant temperature of 35 ± 2 °C. Equation was used to calculate metal sorption efficiency of the biosorbent (Ali et al. 2008):

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

where q_e (mg g^{-1}) gives the mass of metal ions adsorbed per mass of the adsorbent, C_o is the concentration of metal at the initial stage (mg L^{-1}), C_e represents the final concentration of metal (mg L^{-1}) after interaction with the adsorbent, V is the volume of heavy metal containing solution (L), and M shows the biosorbent mass (g) (Liu et al. 2006; Tunali et al. 2006).

Equilibrium isotherms and models

At the state of equilibrium, the distribution of molecules among different phases was studied by adsorption isotherms. They describe surface properties, affinities of adsorbent, and possible mechanisms of adsorption. Most frequently applied models are Langmuir and Freundlich. So, both models were lamented for this research data of Cd, Cr, and Ni. Monolayer adsorption is quantitatively determined by the Langmuir model considering the uniform distribution of energy over all the sites (Eq.) (Imran et al. 2021; Tariq et al. 2020; Langmuir 1918):

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

where Q_{max} gives the maximum adsorption amount (mg g^{-1}) of metal ions being absorbed at equilibrium stage, q_e represents the equilibrium adsorption (mg g^{-1}) of the adsorbent, b is the constant for adsorption energy (mg L^{-1}), and C_e represents the residual heavy metal at equilibrium (mg L^{-1}). The linear form of Langmuir model has been given in Eq. to find the values of model parameters from the slope and intercept of the curve.

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

The process of adsorption, in terms of its nature, was predicted by R_L that is dimensionless separation constant (Eq.) (Weber and Morris 1963).

$$R_L = \frac{1}{1 + (b \times Co)}$$

In this equation, initial concentration of metals is represented by C_o (mg L^{-1}), and constant b (mg L^{-1}) is Langmuir model constant. The negative process of adsorption is given by $R_L > 1$: when $0 < R_L < 1$, it gives a favorable process; if $R_L = 1$, then the process is linear; while $R_L = 0$, it gives the irreversible process. Freundlich model explains heterogeneous adsorption on the adsorbent surface. The q value of the Freundlich model was calculated using Eq. (Freundlich 1906):

$$q = K_F C_e^{1/n}$$

here K_F represents the constant for Freundlich binding that is linked with the efficiency of adsorbent and empirical factor of adsorption intensity/heterogeneity is n. The values of Freundlich model parameters were determined from the slope and intercept of the curve using linear form of Freundlich model (Eq.).

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

Kinetic study of adsorption

Biosorption kinetics describes the biosorption rates and their parameters that affect in maintenance of equilibrium within a particular period (Imran et al. 2021; Tariq et al. 2020). For the prediction of biosorption kinetics, both pseudo-second-order and intra-particle diffusion models were applied in the study. The linear pseudo-second-order kinetic model has been shown in Eq. (McKay et al. 1999; Ho and McKay 1999; Aly et al. 2014):

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

In this equation, q_t and q_e indicate the mass of metal ions (mg) adsorbed per gram of the adsorbent at equilibrium and at time t, correspondingly, and k_1 shows the pseudo-second-order rate constant in g mg^{-1} (Hussain et al. 2020; Imran et al. 2020; Din et al. 2021).

The intra-particle diffusion model was applied to the obtained data. The value of q_t for the intra-particle diffusion model is calculated as follows (Imran et al. 2020; Weber and Morris 1963):

$$q_t = k_{int} t^{1/2} + C_i$$

In Eq. k_{int} is the rate constant for intra-particle diffusion mg g^{-1} min$^{-1/2}$, and thickness of the layer is explained by the intercept, C_i (Din et al. 2021, 2019; Shah et al. 2019). When the adsorption undergoes process of intra-particle diffusion, it results in a straight line by plotting a graph between q_t and $t^{1/2}$ (Hussain et al. 2020).

Fourier-transform infrared spectrometer study

The samples were assessed by Fourier-transform infrared spectrometric (FTIR) analysis to find the changes in functional groups on the adsorbent surface before and after the attachment of heavy metals (Murtaza et al. 2019). The infrared spectrum used for analysis was 4000–400 cm^{-1} and a resolution of 4 cm^{-1} using FTIR, Nicolet 6700, USA (Gillania et al. 2017). Sample disks were prepared from KBr (200 mg) enclosed cells (2 mg) (Li et al. 2018).

Scanning electron microscopy

The morphology of cells was examined through a scanning electron microscope (NOVA Nano SEM 230, USA) (Li et al. 2019) to find changes in surface morphology before and after metal attachment on the surface of cells (Iqbal et al. 2019; Murtaza et al. 2019).

Statistics analysis

The experimental data were analyzed with MATLAB and SPSS 17.0 software (SPSS, Inc., Chicago, IL). Statistical analysis was performed by one-way ANOVA. All data were significantly different ($p < 0.05$).

Results and discussion

Influence of solution pH on metal adsorption

The solution pH influences the biosorption of metals onto the adsorbent surface. The biosorption of Cd, Cr, and Ni ions by *B. anthracis* PM21 with respect to pH was studied in the range of 2–10 at 50 mg L^{-1} metal concentrations, 0.5 g *B. anthracis* PM21 L^{-1}, temperature 35 ± 2 °C, time 12 h and 150 rpm. The rate of *B. anthracis* PM21 adsorption for Cd was highest at pH 8.0, for Cr at pH 6.0, while for Ni the adsorption rate was highest at pH 4, respectively (Fig. 1). The minimum efficiency bacterial biosorption was observed at pH 2.0 for Cd and Cr, while at pH 10 for Ni.

Influence of contact time on adsorption study

Effect of contact time on biosorption rate at different time intervals is shown in Fig. 2 Biosorption of metals onto Bacillus anthracis PM21 surface at various incubation time and contact time intervals ranging from 0 to 120 min was studied at 50 mg L^{-1} metals concentration, 0.5 g L^{-1} biosorbent, temperature 35 ± 2 °C, pH 8 for Cd, pH 6 for Cr, and pH 4 for Ni and 150 rpm. The results reveal that as the contact time increased, the biosorption also enhanced up to 60 min, achieving 76.6%, and subsequently, no significant rise in biosorption efficacy was observed. The *B. anthracis* PM21 quickly achieved maximum rate of adsorption for Cd, Cr, and Ni.

Impact of initial metal concentration on adsorption study

Biosorption experiments at different initial metal concentrations (25–200 mg L^{-1} range) were conducted with 0.5 g L^{-1} of biosorbent at constant temperature 35 ± 2 °C,

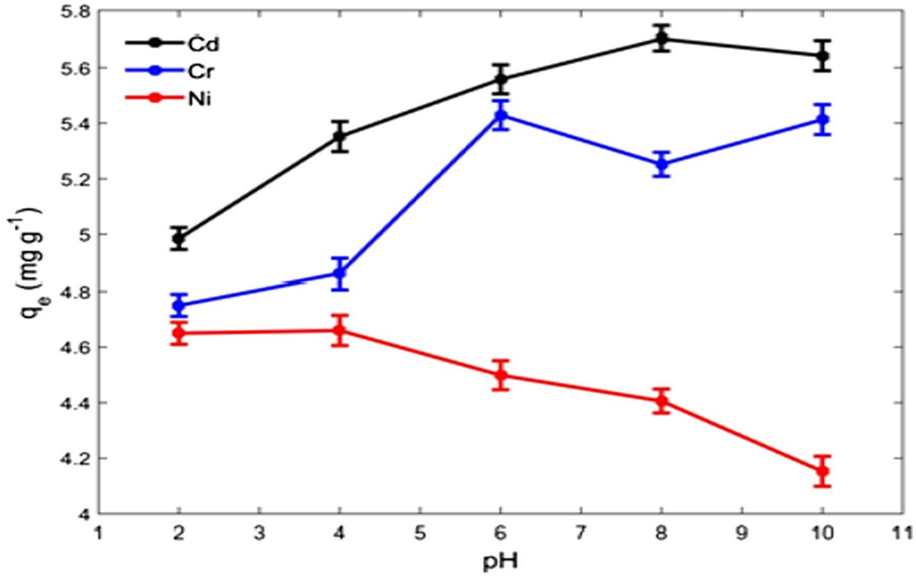


Fig. 1 Influence of pH on biosorption of Cd, Cr, and Ni by *B. anthracis* PM21 at constant temperature (35 ± 2 °C), contact time (60 min), and initial metal concentration (50 mg L^{-1}). Data are presented as means ± SE ($n = 3$)

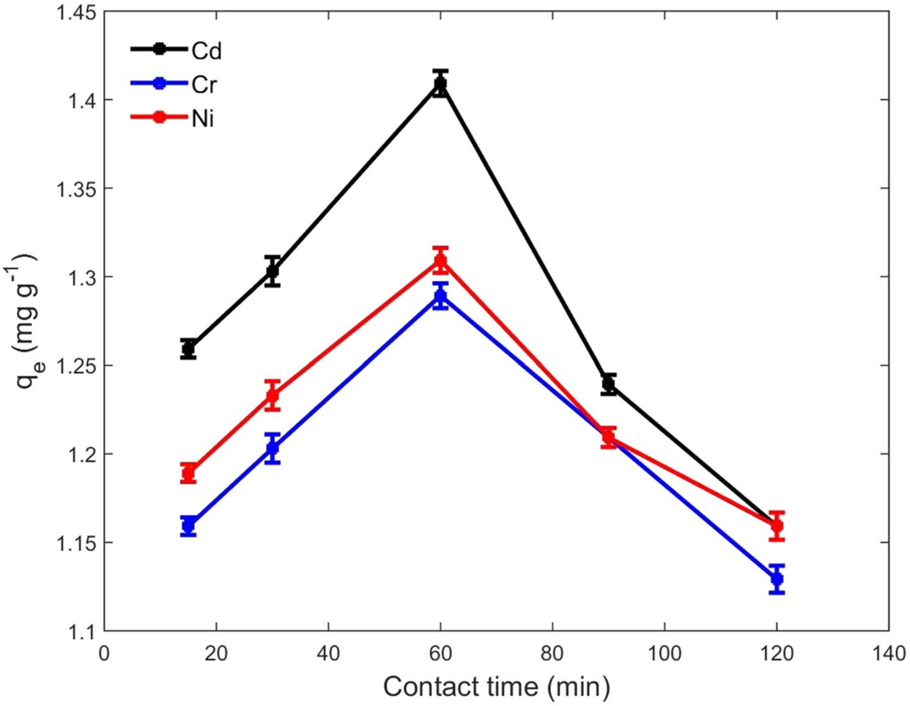


Fig. 2 Influence of contact time on biosorption of Cd, Cr, and Ni by *B. anthracis* PM21 at constant temperature (35 ± 2 °C), pH (8 for Cd, 6 for Cr, and 4 for Ni), and initial metal concentration (50 mg L^{-1}). Data are presented as means ± SE ($n = 3$)

time 12 h, pH 8 and 150 rpm. The effectiveness of biosorption improved from 34.13% as the metal concentration (Cd) enhanced. Maximum efficiency was achieved with Cd concentration of 200 mg L^{-1}. An increase in the metal concentration, Cr, and Ni beyond this stage indicated a decrease in biosorption efficiency to 24.13% and 22.9% with metal concentration of 200 mg L^{-1}. To evaluate the optimum metal concentration for adsorption, Cd, Cr, and Ni were applied at 25–200 mg L^{-1} for 60 min. An increase in q with increasing C_o values with maximum biosorption at 200 mg L^{-1} for Cd, Cr as well as Ni is shown in Fig. 3.

Equilibrium isotherms and models

Biosorption isotherms are necessary for evaluation of equilibrium among the biosorbent and biosorbate in solution. In the present study, we used to model including Langmuir and Freundlich isotherm models to examine the experimental adsorption data. Table 1 shows the values of model's parameters acquired from their respective linear plots. The regression coefficient value (R^2) presented excellent fit with Langmuir isotherm model (0.997) as compared with Freundlich (0.919). Best fit with Langmuir model exhibited

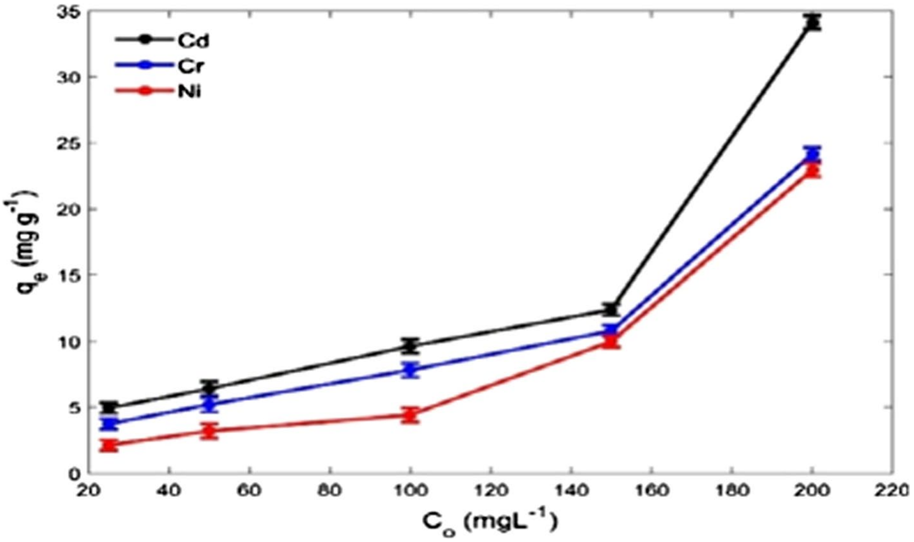


Fig. 3 Influence of initial metal concentration on biosorption of Cd, Cr, and Ni by *B. anthracis* PM21 at constant temperature (35 ± 2 °C), contact time (60 min), and pH (8 for Cd, 6 for Cr, and 4 for Ni). Data are presented as means ± SE ($n = 3$)

the occurrence of monolayer Cd biosorption onto *Bacillus anthracis* PM21 with uniform binding energy. Biosorption is considered as favorable from the values of R_L and n which are 0 and greater than unity, respectively.

Data for Cd, Cr, and Ni with different amounts of metals (25–200 mg L^{-1}) were suitable for the Langmuir model. Adsorption was uniform as revealed by straight lines of graphs plotting between $1/q$ versus $1/C$. Other parameters (Q_{max}, b, and R^2) were also measured from these graphs. As presented in Table 1, *B. anthracis* PM21 biomass showed maximum sorption for Cd $Q_{max} = 22.07$ mg g^{-1}, Cr $Q_{max} = 57.04$ mg g^{-1}, and least for Ni $Q_{max} = 5.34$ mg g^{-1}. Similarly, R^2 values were calculated to be 0.997, 0.996, and 0.976 for Cd, Cr, and Ni. Favorable adsorption was indicated by the positive R_L values for Cd, Cr, and Ni. Current findings for Cd, Cr, and Ni at different amounts of metals (25–200 mg L^{-1}) were suitable for the Freundlich model. Straight lines were obtained by plotting a graph between log q and log C_e. Other parameters (K_F, n, and R^2) were measured from these graphs. The value of $n > 1$ shows that the process of adsorption was feasible. Data show that $n > 1$ in the case of Cr as well as Ni onto *B. anthracis*, while, for Cd, n is approximately equal to1. High values of K_F for Cd and Cr indicated a higher adsorption capacity by *B. anthracis* (Table 1). Both R^2 and n values for Cd, Cr, and Ni biosorption suggest the Langmuir isotherm model as more suitable in comparison with the Freundlich isotherm model. This indicates that monolayer surface adsorption was involved in the biosorption process by *B. anthracis* PM21 as compared to heterogeneous adsorption.

Adsorption kinetics

The adsorption kinetics of experimental data were validated with pseudo-second-order and intra-particle diffusion models. The values of model's parameters were estimated from the linear form of the respective model as shown in Fig. 4. Other values (k_1 and q) were calculated from the graph.

Different values such as k_1, q, h, and the pseudo-second-order factors are presented in Table 2. In this kinetics, the value of R^2 was 0.96–0.97, thus revealing the applicability of this kinetics for adsorption of Cd, Cr, and Ni on *B. anthracis* PM21. Furthermore, the value of q for both data (experimental and pseudo-second-order kinetic) was found closer, favoring the pseudo-second-order kinetic model for Cd, Cr, and Ni. By comparing the h values, it is indicated that the rate of Cd and Cr adsorption is faster than Ni. For modeling results, the values of k_{int} were higher for Cr and Cd than for Ni. Good sorbate, adsorbent attachment, and a faster adsorption process are depicted by high k_{int} values. These two models were compared in terms of their constant rate values, and the pseudo-second-order model was more reliable.

Table 1 The values of parameters for Langmuir and Freundlich isotherms models used for the biosorption of Cd, Cr, and Ni ions onto the *Bacillus anthracis* PM21

Langmuir model						Freundlich model		
Strain name	Adsorbate	Q_{max}	b (mg L^{-1})	R_L	R^2	K_f (mg g^{-1})	N	R^2
PM21	Cd	22.07	0.043	0.317	0.997	1.7	0.97	0.919
	Cr	57.04	0.017	0.541	0.996	1	1.58	0.964
	Ni	5.34	0.001	0.952	0.976	1.1	1.14	0.96

Q_{max}, adsorption capacity of the adsorbent; b, adsorption energy constant; R_L, separation factor indicating the nature of adsorption process; K_F, Freundlich binding constant; n, adsorption intensity/heterogeneity; and R^2 = correlation coefficient

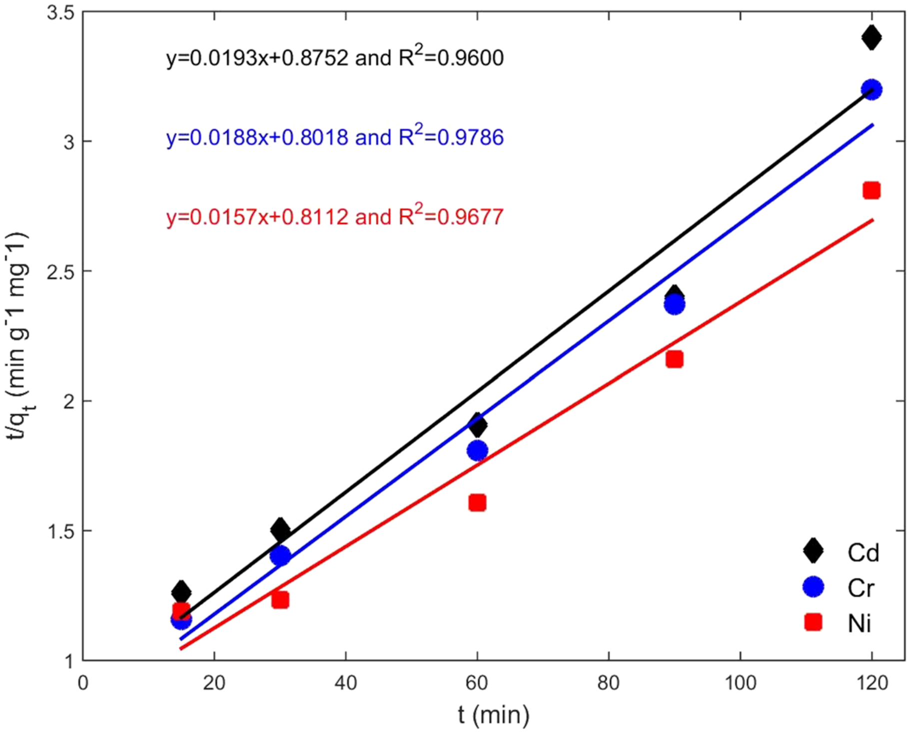


Fig. 4 Fitting behavior of pseudo-second-order kinetic adsorption model with the biosorption of Cd, Cr, Ni ions onto *Bacillus anthracis* PM21

Table 2 Kinetic parameters of pseudo-second-order and intra-particle diffusion models for the biosorption of Cd, Cr, and Ni ions onto the Bacillus anthracis PM21

Strain name	Metals	Exp. value	Pseudo-second order				Intra-particle diffusion		
	Adsorbate	q_e (mg g^{-1})	k_2	q_e (mg g^{-1})	h	R^2	k_{int}	C_i	R^2
PM21	Cd	1.2	0.013	1.41	0.019	0.96	0.018	1.193	0.993
	Cr	1.3	0.011	1.27	0.018	0.978	0.021	1.079	0.895
	Ni	1.2	0.011	1.3	0.016	0.967	0.019	1.084	0.878

q_e, adsorption capacity at equilibrium; k_2, pseudo-second-order rate constant; H, initial adsorption rate; k_{int}, intra-particle diffusion rate constant; Ci, boundary layer thickness; and R^2, correlation coefficient

Fourier-transform infrared spectroscopy

The FTIR study of bacterial biomass exposed to Cd, Cr, and Ni revealed a variety of absorption peaks suggesting the presence of various functional groups. Absorption peaks showed on bacterial cell surface grown under absence and presence of HMs Cd, Cr, and Cd 100 mgL^{-1} stress at different wavelengths (Fig. 5a–d).

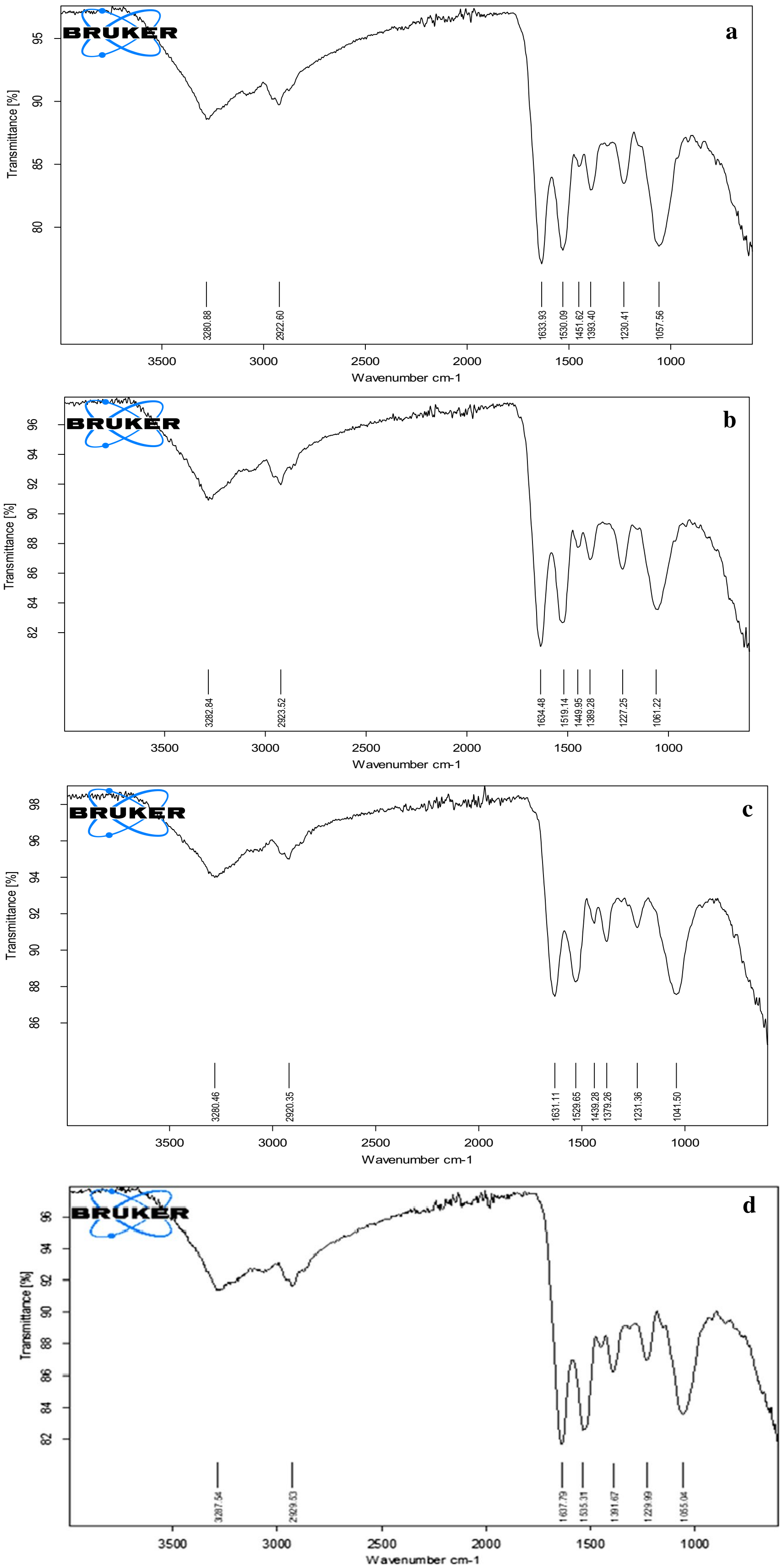
Scanning electron microscopy

The SEM analysis showed that bacterial cells exposed to Cd, Cr, and Ni were rough and showed increased cell size relative to unexposed cells with a smooth cell surface (Fig. 6a–d). The Cd-treated cells retained the rod shape, and there was adhesion of polymeric substances on the bacterial cell wall. Furthermore, there was a difference in cell size, but cells maintained their shape.

Discussion

Plant growth-promoting rhizospheric bacterial strains showed a high level of tolerance to heavy metals. The growth curve analysis of selected bacterial strains in previous study demonstrated their capacity to tolerate high levels of Cr, Cd, and Ni metals (Ali et al. 2021). The high resistance potential of bacterial strain PM21 to several metal ions and its growth profile study against Cd, Cr, and Ni recommend its potential to be exploited in the biosorption of heavy metals. In this experiment, some of the important parameters, for instance, effect of solution pH, contact time, and initial metal concentrations, affecting biosorption ability of different heavy metals, i.e., Cd, Cr, and Ni ions by PM21, were studied. Among all major factors, pH was an important environmental factor affecting the adsorption mechanism of heavy metals. It affected the dissolution and speciation of metals and action of the biosorbent surface ligands (Ren

Fig. 5 FTIR analysis of *Bacillus anthracis* PM21 (**a**), cadmium (**b**), chromium (**c**), and nickel (**d**) treated 200 mg L^{-1} cells of *Bacillus anthracis* PM21



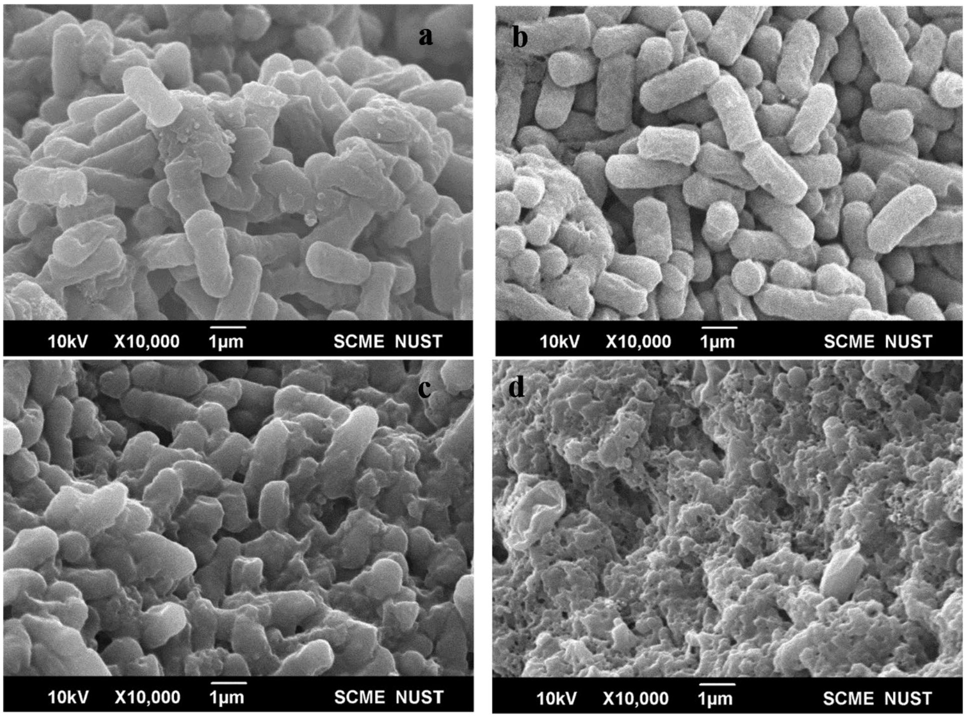


Fig. 6 SEM analysis of *Bacillus anthracis* PM21 (**a**), cadmium (**b**), chromium (**c**), and nickel (**d**) treated 100 mg L^{-1} cells of *Bacillus anthracis* PM21

et al. 2015; Yuan et al. 2019). The implication of pH was studied during the removal of heavy metals (Cd, Cr, and Ni). The structure of bacterial cell wall was studied to investigate various functional groups including carboxylic group, amino group, imidazole, organic acid, and phosphate. A minor fluctuation in pH range may alter the chemistry of the solution and can influence the degree of ionization up to a certain limit of the said functional groups (Gillania et al. 2017; Peng et al. 2018a, b). The current investigation revealed maximum adsorption at optimum pH of 8 (Cd), 6 (Cr), and 4 (Ni) for PM21 (Fig. 1). Generally, it has been observed that maximum biosorption of heavy metal ions occurred at pH 4.0–8.0 by bacteria (Vijayaraghavan and Yun 2008). The current results are in line with the findings of Gillania et al. (2017) in which *Agrobacterium tumefaciens* showed adsorption of Cr and Pb at pH of 4 and 6, respectively. A minor fluctuation in pH affects the positive charges on the cell surface, which retard the attachment of metal ions. It was observed that the capacity for the adsorption of Cr was maximum at pH 6 and decreased by a further increase in pH value and this may be attributed to the precipitation of Cr as Cr $(OH)_2^+$ and Cr $(OH)^{2+}$ (Bla´zquez et al. 2009; Anwar et al. 2011). Similar findings were also proposed by Sala et al. (2002) and Kanwal et al. (2012). An increase in pH up to 8 for Cd increased the negative charge of the functional groups and thus enhanced the process of adsorption on the surface, while, above pH 8, cadmium hydroxide was precipitated leading to a decrease in the process of biosorption (Lodeiro et al. 2006). The current results in case of Cd were in line with the earlier research (Anwar et al. 2009).

Among factors affecting the process of biosorption, the contact time of metal with biomass was another important factor that influenced the biosorption potential (Imran et al. 2020; Tariq et al. 2020; Haq et al. 2016). A biosorption experiment was carried out at various ranges of contact time (0–120 min) for all studied heavy metals at 35 ± 2 °C with a fixed concentration of heavy metals (50 mg L^{-1}). The results indicated that *B. anthracis* exhibited maximum adsorption capacity after 60 min (Fig. 2). The results were strongly supported by the research work of Gillania et al. (2017) as they also reported maximum adsorption capacity by bacterial strains at 60 min. In the initial time, adsorption of target heavy metals was increased quickly because of abundant accessibility of active binding sites of bacterial strains and surface adsorption mechanism of the cell wall; however, with steady possession of active sites, the biosorption appeared less efficient in the latter periods (Liu et al. 2018; Mohapatra et al. 2019). Similar results were obtained previously using different sorbents (Kumar et al. 2006). The Cd, Cr, and Ni in range of 25–200 mg L^{-1} were used to evaluate their adsorption kinetic under constant conditions of temperature (35 ± 2 °C). The adsorption capacity was dramatically increased with increasing initial concentrations of metals providing additional sites for binding, more availability of metal ions, and more driving force to overcome metals resistance (Overah 2011). Maximum biosorption was observed at 200 mg L^{-1} (Fig. 3). As a result, a high probability exists for interaction between metal ions and the sorbent (Tewari et al. 2005; Bueno et al. 2008; Vimala and Das 2009; Aly and Luca 2013). An increase in adsorption might be due to electrostatic interactions between metal ions and surface sites of sorbent (Al-gami 2005; Bueno et al. 2008).

Adsorption is regarded as one of the most widely applied techniques for pollutant removal from contaminated water. The Langmuir and Freundlich isotherm constant and the correlation coefficient (R^2) must be greater than zero and less than unity, predicting that the adsorption of heavy metals is favorable for PM21. Langmuir, Freundlich linearized, and pseudo-second-order models were graphically represented between metal uptake (q_e) and final metal concentration (C_e). The application of different models to the biosorption of Cd, Cr, and Ni by PM21 with R^2 values for Cd = 0.997, Cr = 0.996, and Ni = 0.976 showed the biosorption process to be monolayer, under the experimental conditions employed (Chi et al. 2020). The correlation coefficients (R^2) calculated from the Freundlich model were 0.919 (Cd), 0.964 (Cr), and 0.96 (Ni) for PM21. In the Freundlich model, it is considered that the binding sites affinity on the biomass surface vary with the interaction between the adsorbed molecules (Davis et al. 2003). The maximum adsorption capacity Q_{max} defines the total capacity of the biosorbent for different heavy metal ions. In the present study, maximum biosorption capacity (Q_{max}) was observed in PM21 and found for Cd, Cr, and Ni, respectively. In contrast, the Q_{max} value found in this study was comparable with values found in previous literature using different biosorbents for the removal of different heavy metals (Haq et al. 2016). Good sorbate and adsorbent attachment and a faster adsorption process are depicted by higher k_{int} values (John et al. 2011).

The biosorption data of this strain were best in the case of pseudo-second-order model, and values of R^2 were Cd = 0.96, Cr = 0.978, and Ni = 0.967. The results indicated that the rate-limiting step in Cd, Cr, and Ni biosorption mechanism is the chemical connections between surface ligands and heavy metals (Li et al. 2017). Generally, metal ions may enter the cell through the cell membrane (Sinha et al. 2018; Mohapatra et al. 2019). These results suggested that during Cd, Cr, and Ni biosorption, bacterial strain PM21 revealed mostly surface adsorption.

Conclusion

The current study is the first scientific evidence on strain *B. anthracis* PM21 for the biosorption of heavy metals including Cd, Cr, and Ni. In this biosorption study, a PGPR, *Bacillus anthracis* PM21, showed resistance to Cd, Cr, and Ni, thus evaluated as a potential biosorbent here. This process was largely affected by pH, initial metal concentration, and time for interaction with the biosorbent. *Bacillus anthracis* PM21 was highly efficient for remediating metals at pH 8 for Cd, pH 6 for Cr, pH 4 for Ni, contact time of 60 min, and metal concentration of 200 mg L^{-1}. The intra-particle diffusion model and pseudo-second-order kinetic model well fitted with the experimental data obtained from *B. anthracis* PM21. Using SEM and FTIR analysis, surface ligands were explored for their role in metal adsorption by *B. anthracis* PM21. It is concluded that the heavy metal-resistant *Bacillus anthracis* PM21 is a promising biosorbent for the detoxification of Cd, Cr, and Ni. The present findings may help in evaluating the potential of *Bacillus anthracis* PM21 for the sequestration of other contaminants (dyes and other metals).

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