Research Article

Biosorption of copper(II) and cadmium(II) by *Bacillus cereus* sys1 isolated from oil-contaminated site

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Abstract
Biosorption of copper(II) and cadmium(II) was studied using *Bacillus cereus* sys1 cells. The biosorption capacity of the cells has been studied as a function of pH, initial metal concentration, cell concentration and contact time. Batch studies showed that the *B. cereus* sys1 cells could adsorb up to 90% of both metals. For copper, total contact time of 90 min was required with 100 ppm initial metal concentration at pH 5 using 10 mg/ml cells, whereas 180 min was required for 30 ppm cadmium at pH 7. The study demonstrated correlation with Langmuir model with different concentrations of both Cu(II) and Cd(II). The adsorption capacity was found to be 225 mg/g for copper and 34.67 mg/g for cadmium. As per the Freundlich isotherm, value of 1/*n*, which shows the strength of adsorption, was found to be 0.5114 for copper and 0.2492 for cadmium indicating a normal and favourable adsorption intensity. The surface adsorption of metals was confirmed using SEM with EDS. It revealed that the cells treated with oil could adsorb 7.55% copper as compared to untreated cells which showed 7.29% adsorption. Similarly, for cadmium oil-treated cells showed 9.44% adsorption compared to 2.63% of untreated cells. This indicated that the cells grown in the presence of oil have a better capacity for metal ion adsorption.

Keywords Heavy metal bioremediation · AAS · SEM with EDS · Cu(II) · Cd(II)

1 Introduction

Metal pollution is one of the major concerns of the current era [1, 2]. In recent years, mushrooming of several industries has intensified the problem of metal pollution [3]. There are several heavy metals that enter into the environment through different sources. Copper(II) and cadmium(II) are two of such metal contaminants [4]. Copper enters into the environment through natural and anthropogenic sources. The contamination of air and water by copper occurs due to mining, milling, concentrating, refining of copper ores, electroplating industries, petroleum and reefing melting plants and miscellaneous units from industries, whereas cadmium can be released to the atmosphere through metal production activities, fossil fuel combustion and waste incineration [5]. The presence of copper(II) ions causes serious toxicological concerns. It is usually known to deposit in brain, skin, liver, pancreas and myocardium [1, 6]. In high doses, it can cause anaemia, liver and kidney damage and sometimes stomach and intestinal irritation [7]. Cadmium is an extremely toxic metal commonly found in industrial workplaces. Cadmium is extensively used in electroplating, although the nature of the operation does not generally lead to over exposures. As per the different studies done over the years, only a small proportion (5–10%) of ingested cadmium is absorbed by humans [8, 9]. Cadmium is capable of causing severe renal dysfunction and damage to the bone structure [1, 10]. Currently, common methods in use for metal removal are physical, chemical or mechanical in nature [11]. Due to incomplete decomposition of pollutants and high cost of operation, there is a need for the better

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methods of pollution control. In view of this, biosorption is becoming an increasingly popular technique for cleaning up the metal-contaminated sites. Biosorption, which is the ability of certain microbial biomaterials to bind and concentrate heavy metals from even the most dilute aqueous solutions, offers a technically feasible alternative [12]. Algae, bacteria, fungi and yeasts have proved to be potential metal biosorbents [13]. It is considered an ideal alternative method for removing contaminants from effluents as well.

The *Bacillus cereus* sys1 cells used in this study were isolated from oil-contaminated sites. They were found to be exopolysaccharide producers and exhibited oil degrading potential [14]. The present work delineates the use of *B. cereus* sys1 cells as a potential biosorbent for copper(II) and cadmium(II) metal ion removal.

2 Materials and methods

2.1 Growth of *Bacillus cereus* sys1

The *B. cereus* sys1 cells were isolated from oil-contaminated soil in and around Thane District, Maharashtra, India. These cells were grown in sterile nutrient broth as described by Gupte and Sonawdekar [14]. All the studies during the present work were carried out using freshly harvested cells. Unless otherwise stated, the amount of cells used in these studies has been indicated in terms of wet weight of the packed cells obtained after centrifugation.

2.2 Preparation of metal solution

Stock solutions of Cu(II) (2000 ppm) and Cd(II) (100 ppm) were prepared using copper sulphate ($CuSO_4 \cdot 5H_2O$) and cadmium nitrate [$Cd(NO_3)_2 \cdot 4H_2O$] in deionized water, respectively. The required concentrations of both the metal ions were prepared by appropriate dilution of corresponding metal stock solutions.

2.3 Uptake studies

Biosorption studies were carried out in a batch process. For this study, *B. cereus* sys1 cells (10 mg/ml) were inoculated in 50 ml of an aqueous solution of copper (100 ppm) and cadmium (30 ppm) separately. The pH was adjusted to 5 for Cu(II) and to 7 for Cd(II) metal solution. The adsorption studies were carried out at constant temperature (room temperature (30 ± 2 °C) [15]. Aliquots taken at a known interval of time were centrifuged at 8000 rpm for 5 min to remove cells, and the supernatant was used for the determination of residual metal concentration at 327.4 nm for copper and 228.8 nm for cadmium using atomic absorption spectroscopy (AAS) [15–20].

2.4 Effect of different pre-treatments on metal uptake

Different pre-treatments to remove exopolysaccharide from the cells were carried out as: (a) oven drying for 20 h at 60 °C, (b) autoclaving at 15 lbs, (c) 121 °C for 20 min, (d) incubating in a boiling water bath for 60 min and (e) acetone drying [18].

2.5 Characterization of biomass

The surface characteristics of *B. cereus* sys1 cells were analysed before and after metal biosorption using scanning electron microscope (SEM) with energy-dispersive X-ray spectroscopy (EDAX).

3 Results and discussion

3.1 Effect of cell concentration

The cell concentration influences metal uptake capacities. To study the effect of cell concentration on biosorption of copper and cadmium, the biomass of varying concentrations (1–30 mg/ml) was incubated with aqueous solution of respective metals for 24 h and sorption studies were carried out as described earlier. Figure 1 shows the effect of cell concentration on biosorption of Cu(II) and Cd(II). Copper biosorption was found to be maximum (73%) with cell concentration of 10 mg/ml. In the case of cadmium, 10 mg/ml and 30 mg/ml cells showed 86% and 88% sorption, respectively. This can be due to the increase in the number of binding sites at higher cell/biosorbent concentrations. At very low cell concentration, the cell surface becomes saturated with the metal ions and the large amounts of metal ions remain in the solution [19]. During the study, it was observed that Cu(II) uptake was found to be reduced with 30 mg/ml cells. At very high cell concentration, the competition between the binding sites increases which might have led to desorption of copper ions. This can also be attributed to availability of solute, electrostatic interactions, interference between binding sites and reduced mixing at high biomass densities [20].

3.2 Effect of pH

pH plays an important role in metal uptake. To study the effect of pH on biosorption of copper and cadmium, the biomass of varying concentrations (1–30 mg/ml) was incubated with aqueous solution of respective metals for

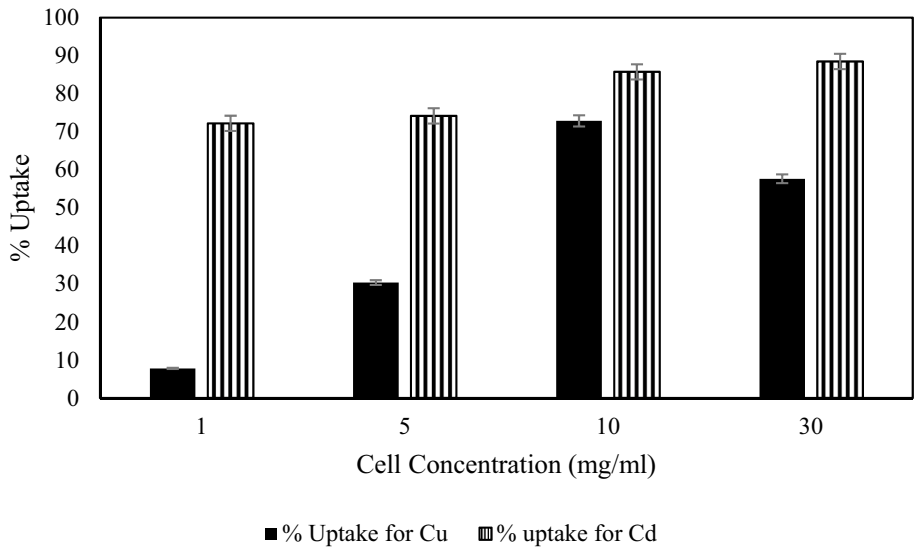

Fig. 1 Effect of cell concentration on biosorption using 100 ppm copper at pH 5 and 30 ppm cadmium at pH 7

24 h and sorption studies were carried out as described earlier. Due to precipitation of copper beyond pH 5, the study of effect of pH on copper sorption was conducted only till pH 5. Figure 2 shows the effect of pH on biosorption. Copper biosorption was found to be 73% at pH 5, whereas for cadmium it was found to be 88% at pH 7 with cell concentration of 10 mg/ml. It was seen that the cadmium sorption was not dependent on pH as no change in metal sorption was observed at different pH, whereas copper sorption was found to increase with increasing pH. At lower pH (< 6), concentration of proton is high, so metal binding sites become positively charged. Metal cations and protons compete for binding sites which results in lower metal uptake [21]. The studies conducted by Gupte and Nair [17] demonstrated that the removal efficiency of Cu(II) increases from the pH 3 to 5 with slight increase after pH 5. These results also suggest that ionic interaction is the main mechanism contributing to biosorption of metals. At low pH, hydrogen ions compete with metal ions, resulting in protonation of active sites. But as the pH increases, the negative charge density on the surface of adsorbent increases and the ionic point of ligands such as carboxyl, hydroxyl and amino groups is free so as to promote interaction with the metal cations [17]. Increased sorption with increasing pH can also be attributed to lower degree of hydration of hydrolysed species as it requires less energy for removal or reorientation of the hydrated water molecules upon binding.

3.3 Effect of metal ion concentration

The metal ion concentration can influence metal uptake capacity of the cells. To study the effect of copper and cadmium concentration on biosorption, 50–250 ppm and 10–50 ppm were used, respectively, and sorption studies were carried out as described earlier. Copper sorption

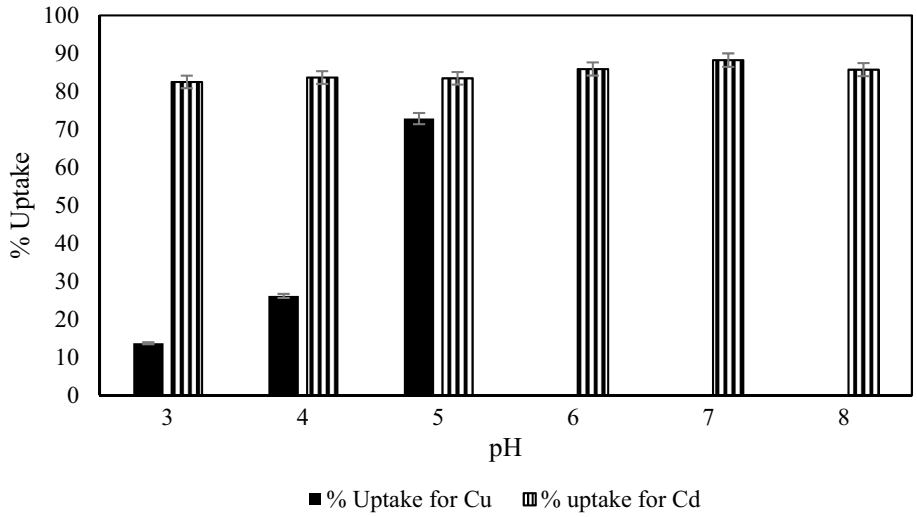

Fig. 2 Effect of pH on biosorption of 100 ppm copper and 30 ppm cadmium using 10 mg/ml cell concentration

was found to be maximum (73%) for 100 ppm as shown in Fig. 3a. The sorption efficiency for copper was found to decrease with increase in the metal concentration. Figure 3b represents the effect of cadmium concentration. The maximum uptake (90%) was found to be at 20 ppm cadmium concentration. The efficiency of the cells to uptake cadmium was found to decrease at higher concentrations. It suggests that the biosorption is highly dependent on initial concentration of metal ion and at very high concentration of metal ions it reaches a saturation value [19].

This indicates that at higher metal concentrations, the available sites for adsorption become fewer and biosorbent sites take up the available metal more quickly at lower metal concentrations. The similar findings were reported by Khadivinia et al. [22] while studying cadmium sorption in *Ochrobactrum* spp.

3.4 Effect of different pre-treatments

The *B. cereus* sys1 cells used in the study were isolated from oil-contaminated sites and had exopolysaccharide (EPS) layer on the surface and were found to be capsular exopolysaccharides containing carboxyl group [14]. Different pre-treatments were used for removal of EPS, and the effect was studied on metal sorption. The untreated cells showed maximum metal adsorption for copper as well as cadmium (Fig. 4).

The similar study conducted by González et al. [18] using *Pseudomonas aureofaciens* showed that EPS-rich cells have better metal adsorption capacity than the EPS-poor cells. It was also observed that the pre-treatment of cells affected the copper sorption but did not have much effect on cadmium sorption. Similar pre-treatments were used in the metal uptake studies for *Aspergillus niger* [23].

3.5 Time kinetics

The kinetic studies were carried out to determine the rate of metal sorption by *B. cereus* sys1 cells. Rapid removal of both the metal ions was seen within first 5 min as shown in Fig. 5. After the initial rapid uptake, it continued with slow rate until the equilibrium was attained. Equilibrium for Cu uptake was achieved at 90 min, whereas for Cd it was achieved at 180 min.

Initial higher rate of metal uptake may be attributed to the initial high metal concentration and availability of a large number of active sites on adsorbent [24]. Similar

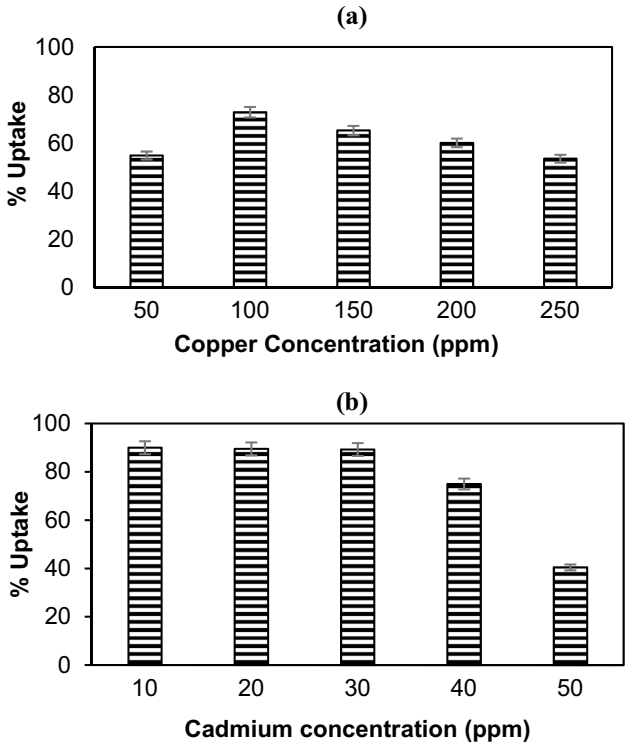

Fig. 3 Effect of **a** copper dosage on biosorption of copper at pH 5, **b** cadmium dosage on biosorption of cadmium at pH 7 using 10 mg/cell concentration

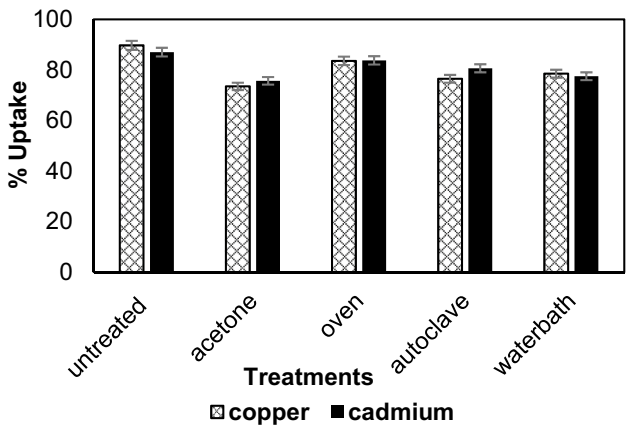

Fig. 4 Effect of different pre-treatments on copper and cadmium uptake at 2 h

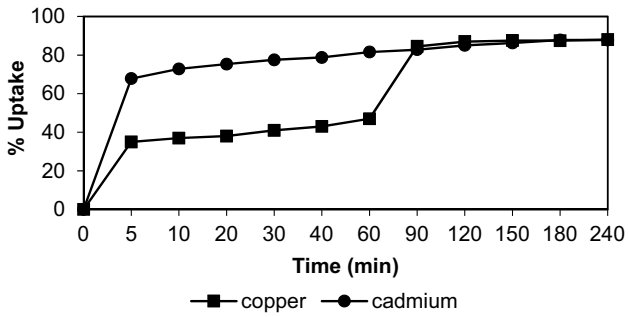

Fig. 5 Time kinetics for normal cells using copper and cadmium

trend was observed in study conducted by Rajendran et al. [25] and showed that copper-resistant *B. cereus* spp. isolated from industrially polluted areas could adsorb copper in the range of 84% within 24 h. Several other studies carried out for copper adsorption [20, 26, 27] also reported maximum uptake within 4–24 h. Kulkarni et al. [28] studied the cadmium sorption capacity of *Bacillus laterosporus*, and the similar phenomenon of rapid adsorption in initial phase followed by slow chemical sorption phase was observed.

During the present study, it was also observed that the *B. cereus* sys1 cells could adsorb cadmium faster than copper. The adsorption order of the biosorbent for metal adsorbate may be related to the properties of the metal adsorbate like ionic radius and electronegativity. The ionic radius of Cd(II) is 0.98 Å, while that of Cu(II) is 0.69 Å. The smaller the ionic radius, the greater is tendency to be hydrolysed, leading to reduced biosorption of Cu(II), and the electronegativity of Cd(II) (1.69 Pauling) is lower value than that of Cu(II) (1.9 Pauling). The sequence of selectivity followed the order of decreasing electronegativity and increasing ionic radius. A similar observation was reported on biosorption of Cd(II) and Ni(II) by *B. laterosporus* by Kulkarni et al. [28].

3.6 Surface characterization

The SEM with EDX analysis confirmed the metal sorption on the cell surface. The cells grown in the presence and absence of oil were compared for their sorption efficiency. The comparative analysis of the surface elements is shown in Table 1. The study carried out on different *Bacillus* strains for metal biosorption showed similar results [29, 30].

3.7 Adsorption isotherm

Equilibrium adsorption isotherm studies were carried out with 10 mg/ml of biomass with copper concentration in the range of 25–1000 ppm and cadmium concentration in the rage of 10–250 ppm for 4 h (Figs. 6, 7). The uptake for construction of isotherm was calculated using the equation $(C_i - C_{eq}) \times \frac{V}{M}$, where C_i is the initial metal concentration, C_{eq} is the equilibrium metal concentration, M is the mass of biomass in grams, and V is the working volume of the metal solution in litre.

3.7.1 Langmuir isotherm

The Langmuir adsorption isotherm experimental data were plotted as $1/q_e$ on Y-axis versus $1/C_{eq}$ on X-axis, and its constants q_m and K_L were obtained by linear regression method as shown in Table 2 [20, 31–33]. The exponential data fit the Langmuir isotherm model. The maximum

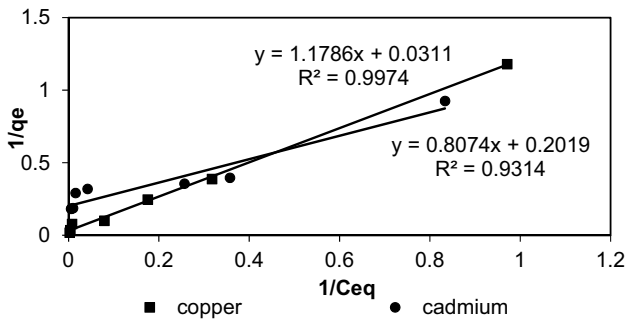

Fig. 6 Langmuir isotherm for copper and cadmium uptake by *B. cereus* cells

Table 1 Comparative analysis of weight% of elements present on cell surface

Elements	Cells grown in NB	Cells grown in NB + oil	Cells grown in NB and inoculated in Cu (100 ppm)	Cells grown in NB + oil and inoculated in Cu (100 ppm)	Cells grown in NB and inoculated in Cd (30 ppm)	Cells grown in NB + oil and inoculated in Cd (30 ppm)
C	48.42	53.70	43.59	44.0	55.86	46.51
N	6.55	5.97	9.52	8.98	0.00	0.00
O	48.42	35.46	33.69	30.34	37.35	37.66
Na	6.55	0.58	1.06	1.28	0.66	0.64
Mg	40.84	ND	ND	ND	ND	ND
P	0.72	2.03	3.18	4.67	1.85	3.35
S	0.25	0.76	0.49	0.65	0.75	0.94
K	1.44	1.18	0.90	1.91	0.90	1.17
Ca	0.49	0.33	ND	ND	ND	ND
Cl	ND	ND	0.28	0.61	ND	0.30
Cu	ND	ND	7.29	7.55	ND	ND
Cd	ND	ND	ND	ND	2.63	9.44

NB nutrient broth, *ND* not detected

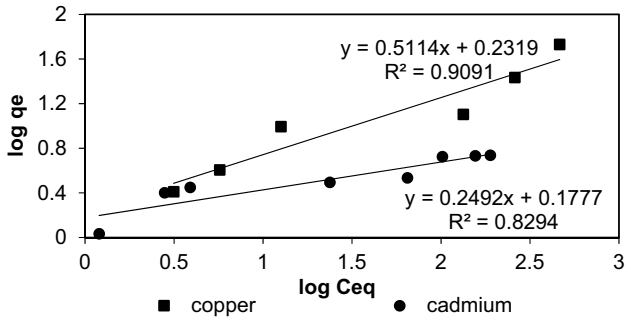

Fig. 7 Freundlich isotherm for copper and cadmium uptake by *B. cereus* cells

Table 2 Langmuir constants for copper and cadmium

Metal	q_m (mg/g)	K_L (l/mg)	R_L
Copper	225.07	3.76×10^{-3}	0.7049
Cadmium	34.67	3.57×10^{-2}	0.4660

Table 3 Comparative data for copper and cadmium biosorption capacity of different microorganisms

Organism	Biosorption capacity (mg/g)	References
Copper		
Bacillus cereus sys1	225	Present study
Bacillus cereus M_{16}^{1}	104–714	[20]
Bacillus sp. (ATS-1)	16.3	[34]
Bacillus subtilis	100	[26]
Aspergillus niger	23.62	[19]
Micrococcus luteus	33.33	[35]
Pseudomonas aeruginosa	70.4	[30]
Kluyveromyces marxianus	344.8	[17]
Cadmium		
Bacillus cereus sys1	34.67	Present study
Bacillus cereus M_{16}^{1}	15.38	[36]
Bacillus circulans strain EB1	26.5	[37]
Bacillus laterosporus	85.47	[28]
Bacillus licheniformis	142.7	[38]
Aspergillus niger	1.31	[23]
Halomonas spp.	12.023	[16]
Pseudomonas aeruginosa	58.5	[30]
Ochrobactrum sp. *GDOS*	83.33	[22]

sorption was found to be 225 mg/g for copper and 34.67 mg/g for cadmium. Comparative data for copper and cadmium biosorption capacity of different microorganisms are shown in Table 3.

The results of this study also support the assumptions of Langmuir model that the presence of a limited number of binding sites distributes over the biosorbent surface homogeneously, thus presenting the same affinity for biosorption of a single molecular layer [20]. The separation factor, R_L obtained in the current study, is 0.7049 for copper and 0.4660 for cadmium, which indicates that the adsorption process is favourable using *B. cereus* sys1 *cereus*.

Table 4 Freundlich constants for copper and cadmium

Metal	K_f (l/mg)$^{1/n}$	*n*	*1/n*
Copper	1.7051	1.7325	0.5114
Cadmium	1.5055	4.0128	0.2492

3.7.2 Freundlich isotherm

The Freundlich adsorption isotherm was plotted as logq_e on *Y*-axis versus log C_{eq} on *X*-axis. The calculated Freundlich constants were found to be as given in Table 4. The constant K_f is an approximate indicator of adsorption capacity, while 1/*n* is a function of the strength of adsorption in the adsorption process. As the value of 1/*n* reduces, heterogeneity increases. This study showed the 1/*n* value as 0.5114 for copper and 0.2492 for cadmium which indicates a normal adsorption. A favourable sorption process is indicated by the value of '*n*' lying between one and ten [39]. As the value of *n* in the present study for both the metals was found to be higher than 1 and lesser than 10, it can be said that the adsorption intensity is favourable. Chatterjee and Ray [20] compared the copper sorption capacity of immobilized *B. cereus* M_{16}^{1}cells. Their study showed that the *B. cereus* cells show normal adsorption with 1/*n* being lesser than 1 as also observed in the present study. Liu et al. [26] reported their findings for immobilized *Bacillus subtilis* cells which confirmed that *Bacillus spp.* usually show normal and favourable sorption at higher concentration of copper.

3.8 Kinetic models

To determine the controlling mechanism of biosorption process, two kinetic models were used to interpret the experimental data using 100 ppm copper concentration and 30 ppm cadmium concentration.

3.8.1 Lagergren's first-order kinetic model

The correlation coefficient (R^2) for the first-order kinetic plot of 100 ppm concentration for copper was found to be 0.7. It suggests that the Lagergren's first-order plot does not adequately describe the adsorption having a low correlation coefficient (Fig. 8). Generally, the first-order kinetic

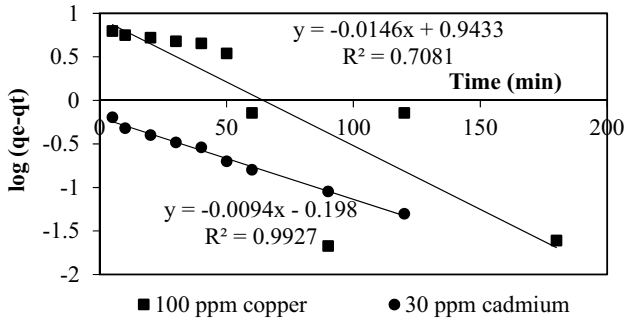

Fig. 8 Lagergren's first-order kinetic plot for copper and cadmium sorption

model is applicable for initial stage of the biosorption processes and does not fit well with the whole range of contact time [40]. In the current study, similar findings were observed with the lower correlation coefficient. Liu et al. [26] and Chatterjee and Ray [20] also have reported that the first-order kinetic model does not fit well for copper using *Bacillus* cells.

Unlike copper, first-order kinetic model fits well for all tested concentrations of cadmium with correlation coefficient values ranging from 0.98 to 0.99. The same is shown in Fig. 8 for 30 ppm cadmium concentration. Similar results were observed by Kulkarni et al. [28] while studying the adsorption capacity of *B. laterosporus*.

3.8.2 Ho's second-order kinetic model

Second-order plots for all the concentrations of copper as well as cadmium were found to be linear with correlation coefficients higher than 0.9, which indicates that the second-order kinetic model fits well with the experimental data. Figure 9 shows the second-order plot for 100 ppm copper and 30 ppm cadmium. Table 5 represents the rate constants (K_1, K_2) and adsorption capacity (q_e) correlation coefficient for the same.

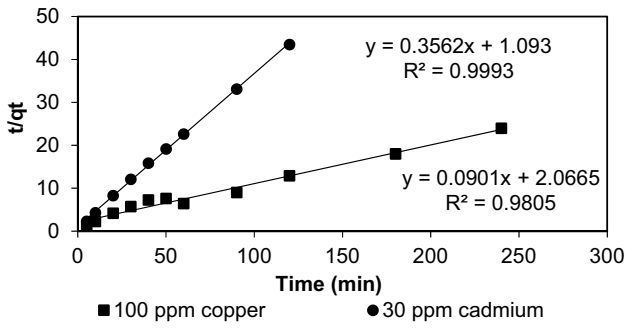

Fig. 9 Ho's second-order kinetic plot for copper and cadmium sorption

Table 5 Pseudo-first-order and second-order kinetics constants and correlation coefficients for copper (100 ppm) and cadmium (30 ppm)

	Metal	K (l/min)	q_e (mg/g)	R^2
Pseudo-first-order kinetics	Copper	$K_1 = 3.3 \times 10^{-2}$	61.4362	0.7081
	Cadmium	$K_1 = 2.2 \times 10^{-2}$	11.0432	0.9927
Pseudo-second-order kinetics	Copper	$K_2 = 3.9 \times 10^{-3}$	77.6914	0.9805
	Cadmium	$K_2 = 1.2 \times 10^{-1}$	19.6518	0.9993

The second-order kinetic model involves valency forces through sharing or exchange of electrons between adsorbent and adsorbate [41]. In chemisorption, the metal ions form a chemical bond and stick to the adsorbent surface. They usually tend to form a covalent bond and find sites that help them enhance the affinity towards absorbent surface by increasing their coordination number with the surface [42]. The pseudo-second-order kinetic analysis reveals that value of initial adsorption rates increases with increase in the initial metal ion concentration.

In the present study, it was observed that the metal adsorption rate increases from 100 to 1000 ppm for copper and 10 to 30 ppm for cadmium. The coefficient of correlation for pseudo-second-order kinetic model for different copper and cadmium concentrations was found to be 0.98 to 1.0. Both factors suggest that the biosorption of copper and cadmium ions followed the pseudo-second-order kinetic model, indicating that the rate-limiting step was a chemical biosorption process between metal ions and biosorbent through the exchange of electrons between the particles involved [43–46].

4 Conclusion

The present study evaluates possibility of using *B. cereus* sys1 cells for biosorption of copper and cadmium. Effect of various parameters like pH, cell concentration and metal concentration on biosorption was studied for process optimization. The comparison between different pre-treatments revealed that the adsorption capacity is dependent on the functional groups present on the surface and they might be affected due to the pre-treatments. Freundlich and Langmuir models as well as pseudo-second-order plot exhibited good fit to Cu(II) and Cd(II) sorption which indicated a favourable and normal adsorption. The results from the study would contribute to understanding the potential of oil degrading strain of *B. cereus* sys1 as a biosorbent.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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