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ORIGINAL ARTICLE

Biosorption of heavy metals by *Bacillus thuringiensis* strain OSM29 originating from industrial effluent contaminated north Indian soil

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KEYWORDS

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Abstract The study was navigated to examine the metal biosorbing ability of bacterial strain OSM29 recovered from rhizosphere of cauliflower grown in soil irrigated consistently with industrial effluents. The metal tolerant bacterial strain OSM29 was identified as *Bacillus thuringiensis* following 16S rRNA gene sequence analysis. In the presence of the varying concentrations (25–150 mgl^{-1}) of heavy metals, such as cadmium, chromium, copper, lead and nickel, the *B. thuringiensis* strain OSM29 showed an obvious metal removing potential. The effect of certain physico-chemical factors such as pH, initial metal concentration, and contact time on biosorption was also assessed. The optimum pH for nickel and chromium removal was 7, while for cadmium, copper and lead, it was 6. The optimal contact time was 30 min. for each metal at 32 ± 2 °C by strain OSM29. The biosorption capacity of the strain OSM29 for the metallic ions was highest for Ni (94%) which was followed by Cu (91.8%), while the lowest sorption by bacterial biomass was recorded for Cd (87%) at 25 mgl^{-1} initial metal ion concentration. The regression coefficients obtained for heavy metals from the Freundlich and Langmuir models were significant. The surface chemical functional groups of *B. thuringiensis* biomass identified by Fourier transform infrared (FTIR) were amino, carboxyl, hydroxyl, and carbonyl groups, which may be involved in the biosorption of heavy metals. The biosorption ability of *B. thuringiensis* OSM29 varied with metals and was pH and metal concentration dependent. The biosorption of each metal was fairly rapid which could be an advantage for large scale treatment of contaminated sites.

1. Introduction

Industrial operations such as electroplating, steel manufacturing, leather tanning, wood preservation, ceramics, glass manufacturing and chemical processing and fertiliser applications release alarmingly higher amounts of heavy metals into the natural environment (Zoubolis et al., 2004; Khan et al., 2009; Oliveira et al., 2011; Tian et al., 2012). The accumulation

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of these heavy metals in soils may cause reduction in soil fertility through their adverse impact on heterogeneous microbial communities inhabiting soils. Also, via food chains, heavy metals are known to cause toxic effects on both plant and human health (Dutton and Fisher, 2011; Takahashi et al., 2012). Therefore, due to the fact that heavy metals persist in the environment and that they are biologically non destructive, it has become extremely important to find an eco-friendly option to cleanup metal contaminated environment and consequently to preserve the health of the deteriorating environment (Sekhar et al., 2004). In this regard, several approaches have however, been attempted focusing especially on heavy metals removal from derelict environment. Of the various options, conventional strategies like, filtration, flocculation, activated charcoal, and ion exchange resins have been found effective in some cases but are expensive, disruptive and less practical under natural environmental conditions. In contrast, bioremediation, a relatively young, inexpensive and socially acceptable technology involves the use of renewable resources like microbes and plants (bioremediation) to tackle heavy metal problems and subsequently to restore the lost fertility of soils (Nies, 1999). Among the various bioremediation options, many scientists spread over different countries have used live or dead culture of bacteria (Gutnick and Bach, 2000), fungi (Dhankhar and Hooda, 2011), yeast (Ruta et al., 2010) and algae (Poole and Gadd, 1989) to biosorb heavy metals. The speciation, behaviour, transport, and decisive fate of heavy metals in instinctive ecosystems depend mainly on the sorption with surface functional groups of microbial communities (Pabst et al., 2010). Volesky and Holan (1995) reported that different types of biomass were capable of efficiently accumulating heavy metal ions. Of the different microbial populations, the bacterial biomass has been found to be one of the most important biosorbents used for metal removal/detoxification, and hence, the adsorption of heavy metals onto bacterial cell walls has received considerable attention in recent experimental and modelling studies (Daughney and Fein, 1998b; Naik et al., 2011; Feng et al., 2012). In order to better understand the complex process of biosorption, the studies targeting the cell surface architecture, chemical functional group identification, and acid–base characteristics of the biomass are necessary for predicting metal biosorption behaviour and modifying metal biosorption property.

Considering the metal threat to microbes, plants and consequently to human health and realising the biosorbing ability of microbes, experiments were carried out to characterise the bacterial strain and to evaluate the metal biosorption ability of the strain *B. thuringiensis* OSM29 (Acc. No. HM222647) isolated from rhizospheric soils of cauliflower (*Brassica oleracea*) grown in metal contaminated soil. In addition, effects of various factors such as, metal concentrations, contact time, biomass, and pH on bacteria mediated biosorption were investigated. To complete the study, an attempt was also made to determine the functional groups of bacterial surface using Fourier transform infrared (FTIR) analytical technique.

2. Materials and methods

2.1. Isolation of bacteria

The soil samples were collected from rhizosphere of cauliflower grown in soils irrigated by metal contaminated water, near Aligarh, North India, located at 27°53′N 78°05′E and 584 ft elevated level from ocean. One gram of wet soil sample was serially diluted using 25 mM phosphate buffer and spread over nutrient agar solid plates. The plates were incubated at 30 ± 2 °C for 24 h.

2.2. Selection of heavy metal tolerant bacterial strains

The ability of the bacterial strains to grow under increasing concentrations of heavy metals such as copper, cadmium, chromium, nickel and lead, was tested by solid agar plate and liquid culture methods. To determine tolerance, bacterial strains were aseptically streaked on nutrient agar plates supplemented with 100–2000 μg ml^{-1} of cadmium sulphate ($CdSO_4$), potassium dichromate ($K_2Cr_2O_7$), copper sulphate ($CuSO_4.5H_2O$), nickel chloride ($NiCl_2.6H_2O$) and lead chloride ($PbCl_2$) and checked for growth after incubation at 30 ± 2 °C for 48 h. The culture was incubated for 48 h with shaking (160 r/min.) at 30 ± 2 °C and growth was detected by measuring the optical density at 600 nm. Each experiment was replicated three times. The highest concentration of individual metal supporting bacterial growth was defined as the maximum tolerance level (MTL).

2.3. Bacterial characterisation

Among 22 bacteria, strain OSM29 showing the highest MTL values was selected and characterised morphologically, and biochemically. Properties of the strain OSM29 that included Gram reaction, citrate utilisation test, indole production test, methyl red test, nitrate reduction, Voges Proskauer, catalase test, carbohydrates (dextrose, mannitol and sucrose) utilisation test, starch hydrolysis, and gelatine liquefaction test were determined by the standard methods given in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994).

2.4. 16S rRNA based identification

The partial sequencing of 16S rRNA gene sequence of the strain OSM29 was carried out commercially by DNA Sequencing Service, Macrogen Inc., Seoul, South Korea using universal primers, 518F (5′-CCAGCAGCCGCGGTAATACG-3′) and 800R (5'-TACCAGGGTATCTAATCC-3′). Later, nucleotide sequence data were deposited in the Gen-Bank sequence database. The online program BLASTn was used to find out the related sequences with known taxonomic information in the databank at NCBI website (http://www.ncbi.nlm.nih.gov/BLAST) to accurately identify the strain OSM29.

2.5. Bacterial biomass production

Selected bacterial strain was maintained on nutrient agar medium. Biomass of *B. thuringiensis* OSM29 was produced by growing bacterial culture in nutrient broth (pH 7) at 30 ± 2 °C for 24 h. Cells were harvested by centrifugation at 5724 g for 20 min. Cell pellets were washed three times with distilled water and dry biomass was prepared by vacuum oven drying at 90 °C. Dry biomass of *B. thuringiensis* OSM29 was then used for biosorption studies.

2.6. Metal solutions

A separate stock solution of $CdSO_4$, $K_2Cr_2O_7$, $CuSO_4.5H_2O$, $PbCl_2$ and $NiCl_2.6H_2O$ was prepared in 100 ml capacity flask

by dissolving appropriate quantities of pure metal powders in 1% nitric acid double distilled water.

2.7. Metal sorption

A batch equilibrium method (Khodaverdiloo and Samadi, 2011) was used to determine the sorption of cadmium, chromium, copper, lead and nickel by *B. thuringiensis* OSM29. All set of experiments was done in fixed volume (100 ml) of single metal ion solution in a 250 ml Erlenmeyer flask. Bacterial biomass was exposed to metal solutions for 72 h on an orbital shaking incubator (Remi, India) at 160 r/min. Biomass was separated by centrifugation at 5724*g* for 15 min. and the supernatant was analysed for residual metal concentration by flame atomic absorption spectrophotometer.

Measurement of metal uptake

The amount of metal bound by the biosorbent was calculated as:

$$Q = \frac{V(C_i - C_f)}{M}$$

where,Q = Metal ion uptake capacity (mg g^{-1})C_i = initial concentration of metal in solution before the sorption analysis (mg g^{-1})C_f = final concentration of metal in solution after the sorption analysis (mg g^{-1})M = Dry weight of biosorbent (g)V = solution volume (l)

The difference between the initial metal ion concentration and final metal ion concentration was considered as metal bound to the biosorbent.

Freundlich and Langmuir model

Langmuir and Freundlich model was used to describe biosorption isotherms.

$$Q = Q_{\max} bC_f / 1 + bC_f$$

It is linearised to the form

$$\frac{1}{Q} = \frac{1}{Q_{max}} + \frac{1}{b \cdot Q_{max}}(C_f)$$

where, $Q_{\max}$ and b are the Langmuir constants. The Freundlich equation of adsorption isotherm is

$$Q = K(C_f)^{1/n}$$

Its linearised form is represented by the equation

$$logQ = logK + (1/n)logC_f$$

where, Q is the amount adsorbed per unit mass of adsorbent and is equilibrium concentration. The plot of $\log Q$ vs. $\log C_f$ is line and constants K and n are evaluated from slopes and intercepts.Separation factor (S_f) and Surface coverage (Ø)

The shape of the isotherm can be used to predict whether adsorption system is favourable or unfavourable in a batch adsorption system. Accordingly, the essential feature of Langmuir isotherm was expressed in terms of dimensionless constant called the separation factor as

$$S_f = 1/(1 + bC_i)$$

Surface coverage (Ø) is number of adsorption sites occupied divided by number of adsorption sites available. The adsorption behaviour of the metal ions on the biomass was determined by the formula:-

$$bC_i = Ø/(1 - Ø)$$

From above equation, surface coverage was calculated as:-

$$Ø = bC_i/1 + bC_i$$

2.8. Effect of initial metal concentration

To examine the effect of the initial metal concentration, the experiments were performed at different initial metal concentrations such as 25, 50, 75, 100,125 and 150 mg l^{-1} at optimum temperature and pH for each metal, by using 100 mg dried biomass of bacterial cells of *B. thuringiensis* incubated for 60 min. on orbital shaking incubator at 160 r/min.

2.9. Effect of contact time on biosorption

Experiment to determine the equilibrium time required for biosorption was performed using 100 mg cell biomass from the initial metal concentration of each metal ion in 100 ml of metal solution at pH 6 for Cd, Cu, Pb and pH 7 for Ni and Cr and at 32 ± 2 °C. Metal solutions were taken at the desired intervals (from 0 to 60 min.) and subsequently centrifuged at 5724*g* for 10 min. The heavy metal concentration in the supernatant was analysed by flame atomic absorption spectroscopy (GBC, Australia).

2.10. Effect of pH on biosorption

In order to evaluate the impact of pH on biosorption, the biomass of *B. thuringiensis* OSM29 strain was subjected to different pH regimes (varying between 2 and 10). Suspensions of pH-conditioned biomass (10 ml) were then allowed to contact with metal solutions of the corresponding pH 2, 4, 6, 8 and 10. For contact time analysis, a 10 ml of cell suspension (10 mg of dry cell biomass) was mixed with 100 ml aliquots of metal solutions in a 250 ml Erlenmeyer flask. Each experiment was repeated two times with appropriate controls. Flasks were incubated on an orbital shaking incubator (120 r/min) at 32 ± 2 °C. Samples of metal solutions were removed from

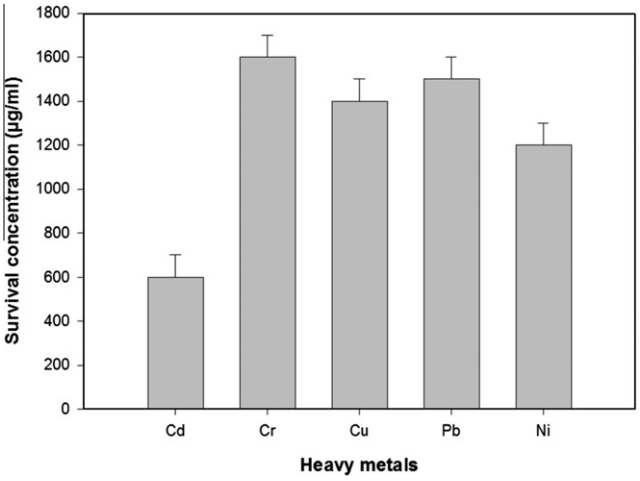

Figure 1 Heavy metal tolerant pattern of *B. thuringiensis* OSM29.

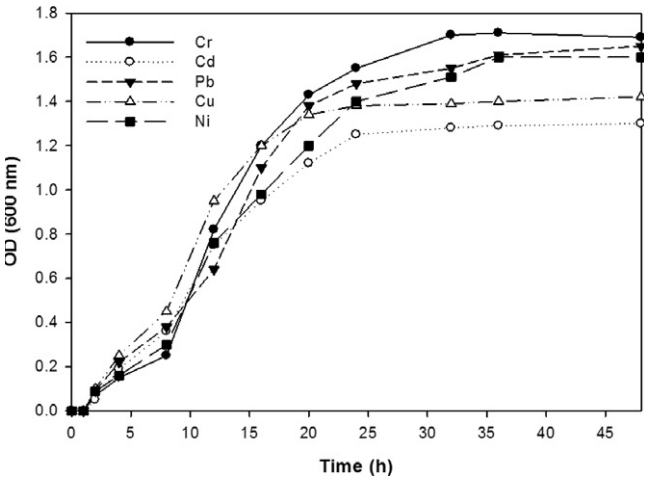

Figure 2 Growth pattern of *B. thuringiensis* OSM29 grown in nutrient broth amended with 100 mg l^{-1} each of Cd, Cu, Cr, Ni, and Pb.

each flask at different time intervals (0–72 h) and were analysed for residual metal content.

2.11. FTIR analysis

Fourier Transform infrared Spectroscopy (FT-IR) was used to identify the main chemical functional groups of *B. thuringiensis* OSM29 biomass. FT-IR analysis of the *B. thuringiensis* OSM29 biomass Infrared spectra of the unloaded original and metal-loaded biomass was performed with a FT-IR spectrometer (Thermo Nicolet, Nexus 670). For this, a 2.5 mg of dried bacterial biomass was mixed and ground with 75 mg of KBr in an agate mortar. The translucent discs were prepared by pressing the ground material with the aid of 8 tonnes of pressure bench press. The tablet was immediately analysed with a spectrophotometer in the range of 1000–4000 cm^{-1} with a resolution of 5 cm^{-1}. The influence of atmospheric water and CO_2 was always subtracted.

3. Results and discussion

3.1. Screening and selection of heavy metal tolerant bacteria

In this study, a total of 22 bacterial strains able to grow in the presence of toxic metals such as Cd, Cr, Cu, Pb and Ni, added to the nutrient agar medium were isolated from the rhizosphere of cauliflower, grown in field with a history of long time application of polluted water. These bacterial strains when grown in nutrient broth amended with varying concentrations of different heavy metals, showed a variable tolerance level to each of the five tested metals (Cd, Cr, Cu, Pb and Ni). Among bacterial strains, strain OSM29 was selected due to- (i) high degree of metal tolerance and (ii) its ability to produce significantly higher amounts of biomass very rapidly (Fig. 1). Among various metals and different concentrations, strain OSM29 could survive at 1600, 1500, 1400, 1200 and 600 mg l^{-1} each of chromium, lead, copper, nickel and cadmium, respectively. Moreover, varied growth behaviour of strain OSM29 was observed when it was grown in nutrient broth treated with fixed concentration (100 mgl^{-1}) of each metal (Fig. 2).

3.2. Characterisation and molecular identification of the strain OSM29

The selected bacterial strain was characterised and identified by using standard morphological, physiological and biochemical tests (Table 1). On the basis of the characteristics observed

Table 1 Morphological and biochemical characteristics of strain *B. thuringiensis* OSM29.

Tests employed	Characteristics observed
Accession number	HM222647
Morphology	
Gram reaction	+
Shape	Rod
Pigments	–
Biochemical reactions	
Citrate utilisation	+
Indole	+
Methyl red	+
Nitrate reduction	–
Oxidase	–
Catalase	+
Voges Proskauer	+
Carbohydrate utilisation	
Glucose	+
Mannitol	–
Sucrose	+
Hydrolysis	
Starch	+
Gelatin	+

"+" and "–" indicate positive and negative reactions, respectively

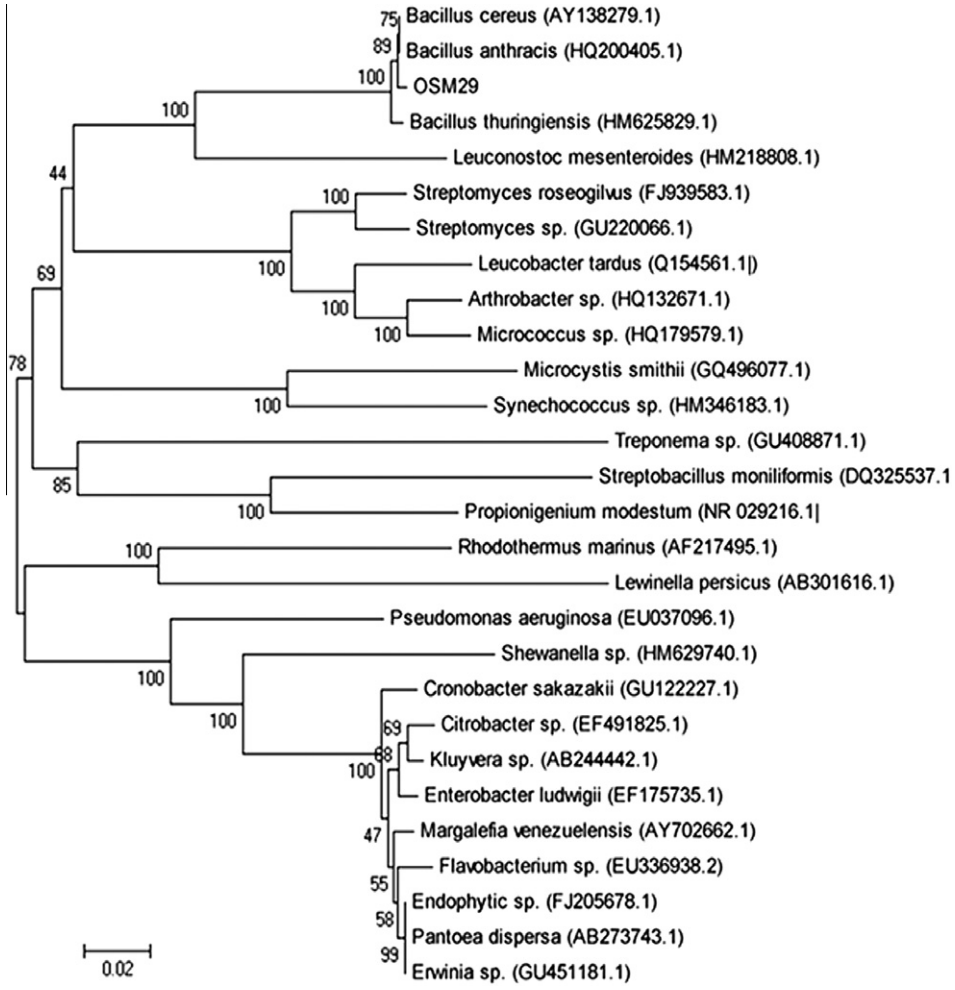

Figure 3 Phylogenetic tree constructed from the 16S rRNA gene sequence of *B. thuringiensis* OSM29 (GenBank accession no. HM222647) and related organisms using NCBI BLAST (*n*) analysis and neighbour-joining (NJ) algorithm from the alignment (Clustal W sequence alignment) of 1486 nucleotides.

Table 2 Parameters obtained in Langmuir and Freundlich isotherm models applied.

Metal	Langmuir isotherm parameters					Freundlich isotherm parameters		
	Q_{max}	b	r^2	S_f	Ø	k	$1/n$	r^2
Cd	59.17	0.031	0.999	0.244	0.75	2.576	0.736	0.991
Cr	71.94	0.034	0.989	0.238	0.76	3.265	0.704	0.983
Cu	39.84	0.080	0.985	0.110	0.88	3.459	0.685	0.996
Pb	30.76	0.094	0.994	0.096	0.90	3.672	0.559	0.998
Ni	43.13	0.153	0.997	0.061	0.94	5.482	0.538	0.986

for strain OSM29 and compared with those listed in Bergey's Manual of Determinative Bacteriology (Holt et al., 1994), strain OSM29 was presumptively identified as *Bacillus* sp. In order to further validate this strain OSM29, it was subjected to 16S rRNA gene sequence analysis. The sequence of 16S rRNA of strain OSM29 was submitted to Gen-Bank (Gen-Bank accession number HM222647). A similar search was performed by using the BLAST program that indicated a close genetic relatedness of strain OSM29 with the rRNA sequence of *B. thuringiensis* HM 625829.1 (16S: 99% similarity with the reference strain HM222647) in NCBI database. Such a higher identical value confirmed the strain OSM29 to be *B. thuringiensis*. A phylogenetic tree constructed by MEGA4 software based on 16S rRNA partial sequence is presented in Fig. 3.

3.3. Biosorption profile

The Langmuir and Freundlich isotherms of heavy metal biosorption by the biomass of strain *B. thuringiensis* OSM29 are presented in Table 2 while the best fit of curve generated in accordance with this model is depicted in Fig. 4a and b. Considering the fact that the rate of absorption is a function of

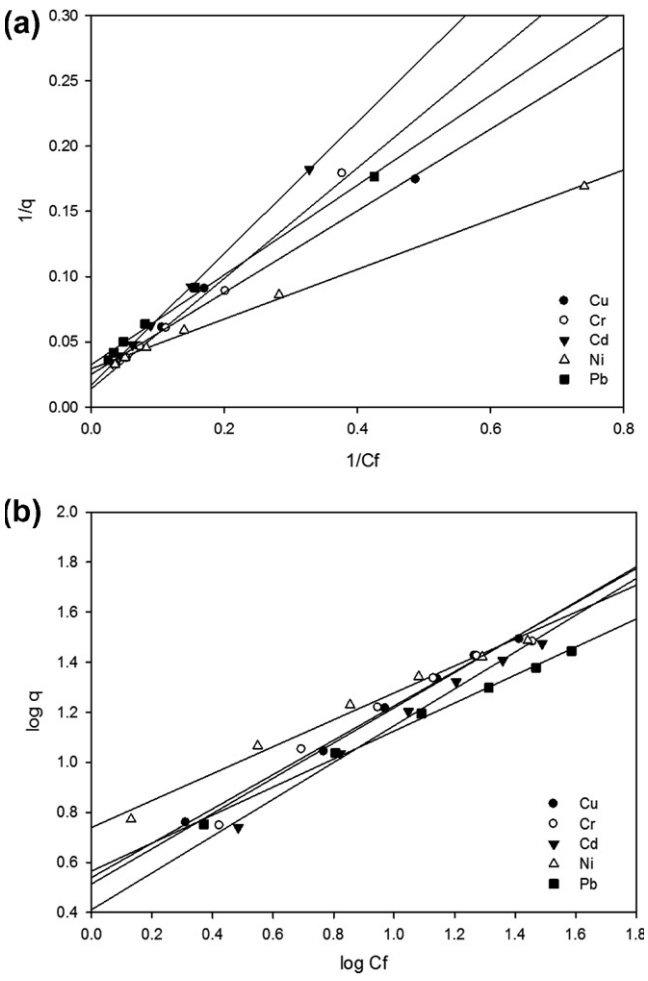

Figure 4 Linearised Langmuir (a) and Freundlich (b) adsorption isotherm for heavy metal ions on biosorbent biomass of *B. thuringiensis* OSM29.

initial concentration of metal ions; an important factor to be measured for more effective biosorption, we determined the effect of various factors on biosorbing ability of the test bacterial strain. In this context, our data revealed that the bio-sorbent potential was higher at lower initial concentration of each metal ions added to aqueous solution. These characteristics indicated that the surface saturation of adsorbent depends on the initial concentration of metal ions. The greater uptake of metal by the biosorbent materials at the lowest concentration could probably be due to a rapid metal absorbing ability of the bacterial biomass. In contrast, at higher metal concentrations metal ion diffuses onto the biomass surface by intraparticle diffusion and therefore, the hydrolysed ions are likely to diffuse very slowly (Horsfall and Spiff, 2005). In other study, it has also been observed that with a decrease in metal ion concentration the biosorption rate increases rapidly while with higher metal ion concentrations a substantial decline in metal removal rate is reported which could probably be due to the saturation of a number of adsorption sites (Wang and Chen, 2009). Furthermore, the Langmuir and Freundlich adsorption constants were evaluated from the isotherms with correlation coefficients (>0.98). Both models described a better absorption process as indicated by correlation coefficient (R^2). In the Langmuir isotherm b is a Langmuir constant related to energy of sorption. If value of b is higher then the affinity of biosorbent is enhanced for metal ions. In this study, the Langmuir constant b value was highest for Ni (0.153) and lowest for Cd (0.031). According to the b value observed here, the bacterial biomass could adsorb the metal ions in the order: Ni > Cu > Pb > Cr > Cd.

Adsorption partition constant of metals was further determined by Freundlich isotherm where K and n are constants. The constants K and $1/n$ were determined by linear regression from the plot of $\log q$ against $\log C_f$. K is the degree of adsorption. Conceptually, when K value is low it indicates minimal adsorption of heavy metals whereas the higher K value suggests greater sorption ability. In our study, K value was highest for Ni (5.482) and it was lowest for cadmium (2.576) indicating a favourable adsorption according to Freundlich isotherm. The value of $1/n$ was lowest for Ni (0.538) but was highest for Cd (0.7358) suggesting a maximum biosorption of Ni and least of Cd. All values however, favoured Freundlich isotherm and correspondingly the order of adsorption were similar to Langmuir isotherm.

3.4. Separation factor

Langmuir isotherm can also be used to predict the conditions of biosorption system. The separation factor (S_f) indicates the shape and nature of biosorption process and principally the separation factor value between 0 and 1 represents favourable isotherm. Our results in this respect were favourable according

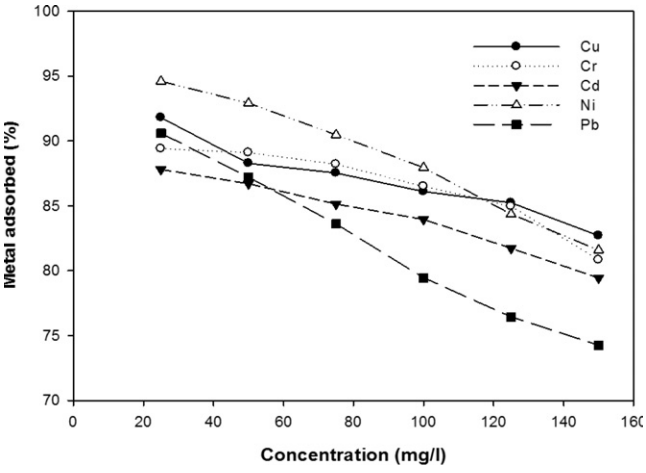

Figure 5 Effect of initial metal ion concentration on biosorption.

Table 3 Effect of initial metal concentration on biosorption.

Initial metal concentration (mg l^{-1})	Removal of metals (%)				
	Cd	Cr	Cu	Pb	Ni
25	87.8	89.4	91.8	90.6	94.6
50	86.7	89.1	88.3	87.2	92.9
75	85.1	88.2	87.5	83.6	90.4
100	83.9	86.5	86.1	79.4	87.9
125	81.7	84.9	85.2	76.4	84.3
150	79.4	80.8	82.7	74.2	81.5

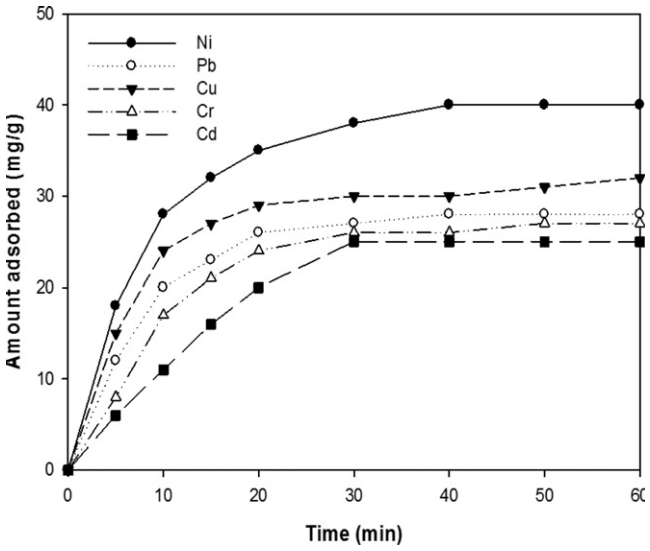

Figure 6 Biosorption of heavy metals by strain *B. thuringiensis* OSM29 over the reaction time at initial concentration of 100 mg l^{-1}, pH 7 and 30 ± 2 °C temperature.

to adsorption equation because all values of S_f were >0 and less than 1. The S_f values of metal ions were 0.061, 0.096, 0.110, 0.238 and 0.244 for Ni, Pb, Cu, Cr and Cd, respectively (Table 2). Moreover, the surface coverage factor (Ø) of sorption was determined according to Langmuir isotherm. Surface coverage value for metal ions adsorbed onto the biomass of bacterial strain *B. thuringiensis* OSM29 was in the order: Ni > Pb > Cu > Cr > Cd (Table 2). From the surface coverage value, it was inferred that strain *B. thuringiensis* OSM29 could effectively and maximally biosorbed Ni metal ions in aqueous solution than other test metals.

3.5. Effect of initial metal concentration, contact time and pH on biosorption

The effect of initial metal concentration on metal biosorption by dry biomass of *B. thuringiensis* OSM29 was evaluated under reaction condition, set at pH 6 and 30 ± 2 °C for equilibrium time half hour as shown in Fig. 5. Here, it was observed that the rate of biosorption decreased with an increase in metal ion concentration. The maximum biosorption of metal was recovered at a low initial metal ion concentration for example it was 94.6% for Ni at 25 mg l^{-1} while it was 81.56% at 150 mg l^{-1} Ni (Table 3). A trend similar to Ni was also observed for other metals. The decrease in the percentage of biosorption may be attributed to the lack of sufficient free sites for metal biosorption. At lower concentrations, all metal ions present in the solution however, could interact with the binding sites and thus the biosorption percentage is likely to become higher than that at higher ion concentrations as found in this study. At higher concentrations, a lower adsorption yield is due to the saturation of adsorption sites. Similar results have been reported by others (Kadukova and Vircikova, 2005; Lu et al., 2006; Pandiyan and Mahendradas, 2011).

Contact time is one of the important factors of biosorption process. The biosorption of Cd, Cr, Cu, Ni and Pb by bacterial biomass is shown in Fig. 6. The rate of metal ion biosorption was highest in the beginning due to high affinity of free metal ion binding sites on bio sorbent but after few minutes the rate of biosorption slowed and reached to equilibrium. Here, in our experiment, the initial sorption rate was highest and moved to equilibrium within half hour and hence, the order of biosorption rate was Ni > Cu > Pb > Cr > Cd. These indicate the equilibrium time at which an equilibrium metal ion concentration is presumed to have been attained. In this context, Zoubolis et al. (2004)) and Volesky (1990) here also observed that the initial shortest time period of sorption process is important for a high rate of metal sorption. Similar results have also been determined by Gabr et al. (2008) for Ni and Pb biosorption.

In order to see whether pH plays any role in the biosorption process or not we set an experiment with varying pH to evaluate their effect on biosorption capacity of microbial biomass using a fixed (100 mg l^{-1}) amounts of Cd, Cr, Cu, Ni and Pb (Fig. 7). The biosorption capacities for each metal ion increased with an increase in pH. The optimum biosorption

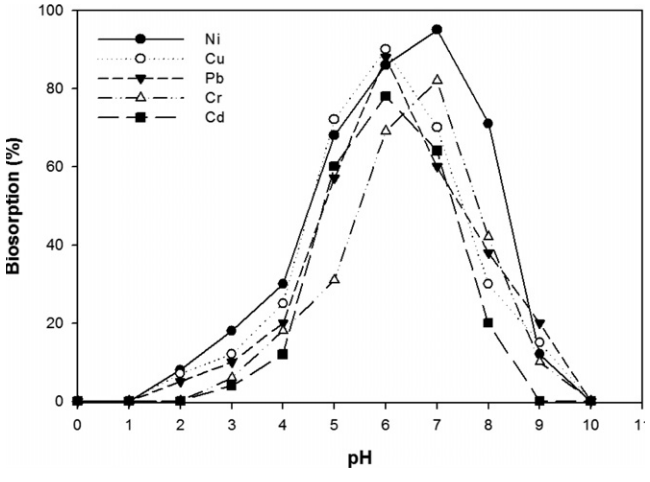

Figure 7 Effect of pH on biosorption by dry biomass of *B. thuringiensis* OSM29 at initial concentration of 100 mg l^{-1} for each metal incubated at 30 ± 2 °C for one hour.

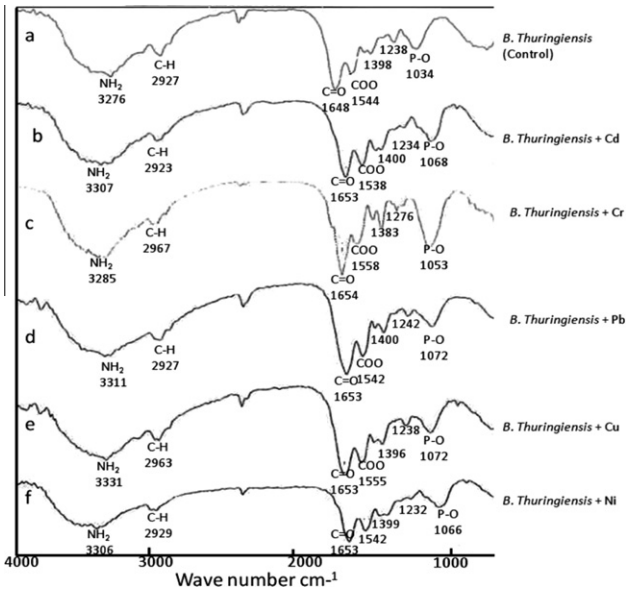

Figure 8 The IR spectra of bacterial biomass treated with heavy metals.

occurred at 6 for Cd, Pb and Cu and while it was maximum for Ni and Cr at pH 7. The variation in biosorption of heavy metals by microbial biomass at different pH could be due to the differences in the sensitivity of cell wall molecules of the bacterial cells to pH. For instance, at a low pH, cell wall ligands tightly bind with the hydronium ions H_3O^- and hence restrict the approach of metal cations due to repulsive force. On the contrary, at higher pH values, more ligands like carboxyl, phosphate, imidazole and amino group would be exposed and carry negative charges with a subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface (Pardo et al., 2003). Most of the living organisms have been shown to biosorb heavy metals such as Cd and Cu at a low pH, due to their physiological properties (Sar et al., 1999; Ok et al., 2007).

3.6. FTIR analysis

Biosorption of metal ions by the microbial biomass depends largely on the functional groups present on the active sites of bacterial cells and physiochemical conditions of the solution. And hence, in order to understand better the kinds of the functional groups involved in the biosorption process, FT-IR analysis of the biomass *B. thuringiensis* was carried out. The FTIR spectra of unloaded and metal loaded *Bacillus* sp. biomass in the range of 1000–4000 cm^{-1} were taken just to ascertain the presence of functional groups that could possibly be involved in the biosorption process (Fig. 8a). The absorption band characterisation including hydroxyl and amine group peaks were assigned at 3400–3200 cm^{-1}, alkyl and CHO had a broad band ranging between 2921 and 2851 cm^{-1}, C═O of amide groups at 1648 cm, COO^- of the carboxylate groups appeared at 1544 cm^{-1}, the band located at 1238, 1398 and 1740 cm^{-1} represented COO^- anions whereas those located at 726 cm^{-1} was assigned SO_3^- groups. Furthermore, the peaks located at 1034 and 1075 cm^{-1} were indicative of organic phosphate groups and P–O of the ($C–PO_2^{-3}$) moiety, respectively. The IR spectra of the loaded biomass varied with the metal species such as Cd (Fig. 8b), Cr (Fig. 8c), Pb (Fig. 8d), Cu (Fig. 8e) and Ni (Fig. 8f). The IR spectra revealed a stretching of band appearing at 1068, 1053, 1072, 1072, and 1066 cm^{-1} which was attributed to the interaction of sorbed metals such as Cd, Cr, Pb, Cu, and Ni with phosphate groups, respectively. Additionally, shifting of bands observed at 1648–1653 cm^{-1} (Cd, Pb, Cu, Ni), and 1654 cm^{-1} (Cr) to after biosorption could be due to the involvement of carboxyl groups. Likewise, stretching of bands from 1400 to 1383 cm^{-1} was due to involvement of H-bonds as also reported by Sar et al. (1999). The bands located between 3284 and 3423 cm^{-1} however, verified the interaction of hydroxyl and amine groups. The transmittance of the peaks in the loaded biomass was substantially lower than the unloaded bacteria biomass. These changes suggest that bond stretching occurs to a lesser degree due to the presence of metals and therefore, peak transmittance is consequently reduced. In agreement to our findings, numerous workers have also reported similar results (Norton et al., 2004; Lodeiro et al., 2006; Tunali et al., 2006; Gabr et al., 2008; Giotta et al., 2011). Conclusively, the formation of varying spectra following adsorption of the metal ions on bacterial biomass validated the contribution of functional groups in metal binding. However, it is difficult to pinpoint the exact mechanism as to how metals are adsorbed onto the microbial biomass due to some unidentified peaks appearing in this experiment.

4. Conclusion

The biomass of metal tolerant *B. thuringiensis* OSM29 successfully removed the metals such as Cd, Cr, Cu, Pb and Ni from aqueous solution. The biosorption of heavy metals by the bacterial strain OSM29 was affected by initial metal concentrations, pH, and contact time. The maximum biosorption of heavy metals by *B. thuringiensis* OSM29 occurred at pH 6 within 30 min at 32 ± 2 °C. The functional groups identified on bacterial surface by FTIR technique included amino, carboxyl, hydroxyl and carbonyl groups, which could possibly be involved in the biosorption of Cd, Cr, Cu, Pb and Ni. Biosorption data fitted well with the Langmuir and Freundlich adsorption isotherm equations and indicated sufficient biosorption by the test bacterial strain at varying range of metal ion concentration. This study validates that the biomass of *B. thuringiensis* OSM19 could be used as an inexpensive and highly efficient reliable biosorbing bio-agent for effectively removing heavy metals from aqueous environment.

Conflict of interest

The authors declare that there are no conflicts of interest.

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