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Removal of nickel from aqueous solution by the bacterium *Bacillus thuringiensis*

Ayten Öztürk *

Department of Biology, Faculty of Sciences and Arts, University of Nigde, 51200 Nigde, Turkey

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Abstract

The biosorption of the toxic metal (nickel) from aqueous solutions on dried vegetative cell and spore–crystal mixture of *Bacillus thuringiensis* var. *thuringiensis* was tested under laboratory conditions as a function of pH, initial metal ion concentration and temperature. The characteristics of the adsorption process were investigated using Scatchard analysis. Scatchard analysis of the equilibrium binding data for metal ions on vegetative cell and spore–crystal mixture of *B. thuringiensis* gave rise to a linear plot, indicating that the Langmuir model could be applied successfully. Adsorption behaviour of nickel(II) ion on *B. thuringiensis* is expressed by both Langmuir and Freundlich isotherms. The adsorption data with respect to the metal provided an excellent fit to both isotherms. Ni(II) ion uptake of *B. thuringiensis*'s spore–crystal mixture at 250 mg l^{-1} was 15.7%, whereas its vegetative cell metal uptake was 10%. The best temperature for ion uptake was found to be at 35 °C.

Keywords: *B. thuringiensis*; Biosorption; Nickel ions; Toxic metal removal

1. Introduction

It is known that the presence of heavy metals in the environment has resulted in a number of environmental problems. Hence the removal and recovery of these metals from wastewater streams is very important. Nickel(II) is such a heavy metal frequently existed in raw wastewater streams from industries such as non-ferrous metal, mineral processing, paint formulation, electroplating, porcelain enameling, copper sulphate manufacture and steam-electric power plants [1,2]. While the nickel(II) ion concentration in plating rinse can approach 2–900 mg $^{-1}$, wastewater from paint and ink formulation, porcelain enameling, copper sulphate manufacture industries record effluent nickel(II) ion concentrations that varies from 0 to 40, 0.25 to 67 and around 22 mg l^{-1} [3]. A number of conventional methods have been used to remove nickel(II) ion from wastewater streams, such as adsorption on activated carbon [4]; chemical precipitation [5]; crystallization in the form of nickel carbonate [6]. However, these methods are ineffective or expensive when initial metal concentrations in wastewaters are low, or when very low concentrations of heavy metals in treated water is required [7]. In the past few decades, biosorption using microbial biomass as the adsorbent has emerged as a potential alternative technique to the existing methods for metal removal.

Because of the good performance and low cost of these materials the use of living and non-living microorganisms, in the removal and possibly recovery of toxic or precious metals from industrial wastewaters, has gained important credibility during recent years. The natural affinity of biological compounds for metallic elements could contribute to the purification of metal-loaded wastewater, a fact which has been already proved in many cases and by many researchers [7–10]. The sorption capacity of nickel(II) ion by these materials ranges from as low as 0.798 mg/g by *Streptomyces noursei* [10], to as high as 264.69 mg/g by *Pseudomonas aeruginosa* [11].

Most of these studies focused on the uptake of nickel in batch mode and also by using native biomass itself as the biosorbent [12,13]. In other words, there are certain cations already present in the biomass such as Na^+, Ca^{2+} which may affect the kinetics and extent of biosorption [8]. Although many different mechanisms may take place simultaneously during bioaccumulation (the term generally applied, when living microorganisms are being used) or biosorption (considered as a collective term, when using non-living biomass), up to present, only a few of

* Fax: +90 388 2250114.
E-mail address: aozturk@nigde.edu.tr.

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Nomenclature

A_s (mg g^{-1}) saturation capacity
C_{eq} (mg l^{-1}) unadsorbed metal ion concentration in solution at equilibrium
C_0 (mg l^{-1}) initial metal ion concentration at equilibrium
K_b (l mg g^{-1}) adsorption binding constant of metal ion
K_F (mg g^{-1}) Freundlich adsorption constant of metal ion related to the adsorption capacity
n adsorption intensity of an adsorbent.
q (mg g^{-1}) uptake
q_{eq} (mg g^{-1}) adsorbed quantity of the metal ion per gram of dried biosorbent at equilibrium
q_m (mg g^{-1}) Langmuir maximum uptake
X (g l^{-1}) bacterium concentration

them have been clearly identified and very little information exists on correlating metal uptake with the speciation of the metal in dilute aqueous solutions, which in turn is depending on the physicochemical conditions of the solution [8–10].

Microorganisms based technologies must compete with both operational and economical terms in the existing metal-removal treatment systems. Non-living biomass appears to present specific advantages in comparison to the use of living microorganisms. For instance, the former may be obtained with much lower (if any) cost, it is not subjected to metal toxicity, the nutrient supply is not necessary in addition to their greater binding capacities for toxic metals. Metal sorption performance depends on some external factors such as pH, temperature, other metals, organic materials, cell metabolic products in solution [7,8,14,15,16] and intrinsic factors such as cell walls which contain polysaccharides as basic building blocks. The polysaccharides of cell wall provide amino, carboxyl, phosphate and sulphate groups for metal binding [17].

Bacillus thuringiensis is an aerobic, Gram-positive, endospore-forming soil bacterium in which during sporulation, produces a parasporal protein toxin called insecticidal crystal protein. These crystals consist of different major protein units with different molecular weights and are generally present in all of the of *B. thuringiensis* strains. Since these proteins are toxic to the larvae of dipterans, lepidopteran and coleopteran insects, the bacteria have been used as bioinsecticide worldwide for many years [18].

Our objective of the study is to contribute to the understanding and modeling of the equilibrium of adsorption process. For this purpose, various factors affecting the adsorption, such as treatment time, initial pH of solution, metal ion concentration, were investigated by the batch equilibration technique. *B. thuringiensis*'s unique ability of producing spore and crystal is thought to affect their sorption abilities compared with those that are vegetative cell forms only. Adsorption ability of each of these vegetative cells and spore–crystal mixture was therefore studied and adsorption data obtained were applied to both Langmuir and Freundlich isotherms which were found to describe adsorption equilibrium adequately.

2. Materials and methods

2.1. Microorganism and growth conditions

B. thuringiensis var. *thuringiensis* was obtained from University of Ankara, Department of Food Science and Technology (Turkey). The strain was grown and maintained on both nutrient broth and nutrient agar. Cells were inoculated on Petri dishes by scratching and were left over night at 35 °C in the incubator. Vegetative cell cultures were collected from Petri dishes by the aid of serum physiologic and were harvested by means of centrifugation at 13,000 rpm for 5 min, then were washed twice with serum physiologic. The pellets were put in Petri dishes and dried at 60 °C for 24 h. However, spore–crystal mixture was kept for 15 days at room temperature after incubation and the same procedure was followed as that for vegetative cell. The pellets of spore–crystal mixtures were dried at 35 °C for 24 h.

2.2. Preparation of metal solution

Test solutions in 500 ml Erlenmeyer flask containing 200 ml of nickel(II) ion were prepared from analytical grade (nickel nitrate). The concentrations of nickel(II) ion prepared from stock solutions ranged from 25 to 250 mg l^{-1}. Before mixing the microorganisms, the pH of each test solution was adjusted to the required value by using 1 M NaOH and HNO_3 or H_2SO_4.

2.3. Biosorption experiments

The biosorption experiments were carried out using the batch equilibrium technique at different pH (4–8) values and temperatures (25, 35, 45 °C). Equilibrium biosorption was determined by using 1 g l^{-1} of the dried and ground bacterium suspension sample to which different initial metal concentrations was added. Solution concentrations were varied from 25 to 250 mg l^{-1} and were agitated (100 rpm) on a shaker for 8 h which is more than ample time for adsorption equilibrium. Samples were taken at definite intervals for their residual metal ion concentrations in the solution. Solid–liquid separation was performed by centrifugation (13,000 rpm for 5 min) and analyzed for the remaining metal ions spectrophotometrically at 460 nm using sodium diethyldithiocarbamate as the complexing agent [19]. For each sample a blank test without microorganisms was also performed in parallel to avoid confusion between biosorption and possible metal precipitation. However, these experiments were carried out in duplicates and mathematical average values were used in calculations. The difference in results between duplicates was typically less than 5%.

3. Results and discussion

The effect of initial pH, temperature and initial metal ion concentration on the adsorption of nickel(II) ion on *B. thuringiensis* was investigated. Adsorption yields (Ad%) is defined as the ratio of adsorbed quantity of metal ion per gram of bacterium at equilibrium to the initial amount of metal ions and is calculated from

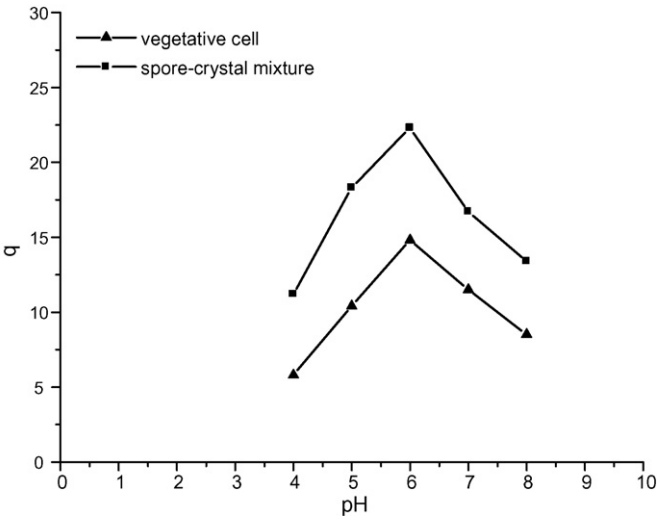

Fig. 1. The effect of initial pH on equilibrium adsorption of Ni(II) ion (C_0: 100 mg l^{-1}; X: 1.0 g l^{-1}; temperature: 25 °C; agitation rate: 100 rpm).

Eqs. (1) and (2):

$$\text{Ad}\% = \frac{q_{\text{eq}} X}{C_0} \quad (1)$$

$$q = \frac{C_0 - C_{\text{eq}}}{X} \quad (2)$$

3.1. Effect of pH on nickel(II) biosorption

Earlier studies on heavy metal biosorption have shown that pH was the single most important parameter affecting the biosorption process [10,14]. The variation of equilibrium uptake for Ni(II) ion at initial pH values for each of the vegetative cells and spore–crystal mixture are given in Fig. 1 which shows that nickel(II) was adsorbed by the spore–crystal mixture more strongly than the vegetative cells.

The optimum pH value for biosorption of nickel(II) ion was determined as 6.0 for both of the spore–crystal mixture and vegetative cells. However, it is believed that, different pH binding profiles for Ni(II) ion are due to the nature of the chemical interactions of metal with the bacterial cells [9–11,14]. Since solution pH influences cell surface metal binding sites and as Ni(II) ions are ionic in nature, the interaction of these ions with bacteria may be primarily electrostatic in nature.

The metal-binding properties of Gram-positive bacteria are largely due to the existence of specific anionic polymers in the cell wall structure, consisting mainly of peptidoglycan, teichoic or teichuronic acids [16,17,20]. Due to this high fixed anionic content of the cell forms which are obviously present in *B. thuringiensis* var. *thuringiensis* too, they may exhibit high sorption capacities that would be very important aspect in future because of its industrial application as biosorbent for the metal cations, since they belong to the transition metal ions that have high affinity not only to surface ligands, such as phosphoryl, SO_3^{2-} RNH_2 and R_2NH, but also to carbonyl (COO^-) groups too.

Table 1
Equilibrium adsorbed quantities and adsorption yields of nickel(II) ion obtained at different initial metal ion concentrations

Vegetative cell			Spore–crystal mixture		
C_0 (mg l^{-1})	q_{eq} (mg g^{-1})	Ad%	C_0 (mg l^{-1})	q_{eq} (mg g^{-1})	Ad%
59.8	15.6	26.0	58.4	26.6	45.5
119.2	21.5	18.0	122.5	34.3	28.0
159.1	23.7	14.9	152.1	36.3	23.9
230.1	26.8	11.6	221.2	39.1	17.7
291.8	29.2	10	265.6	41.8	15.7

X: 1.0 g l^{-1}; temperature: 35 °C; agitation rate: 100 rpm; pH: 6.0.

3.2. Time course of nickel uptake and effect of initial metal ion concentration

The initial metal ion concentration remarkably influenced the equilibrium metal uptake and adsorption yield as shown in Table 1. The higher the initial concentration of the metal ion, the larger amount of metal ion adsorbed. When the initial Ni(II) concentration varied from 50.0 to 250.0 mg l^{-1}, the loading capacity of *B. thuringiensis*'s vegetative cell has increased form 15.6 to 29.2 mg g^{-1} while its spore–crystal mixture's uptake has increased from 26.6 to 41.8 mg g^{-1}. However, at 250 mg l^{-1} initial Ni(II) ion concentration, the maximum nickel(II) equilibrium uptake of the vegetative cell was determined as 29.2 while that of the spore–crystal mixture was found to be as high as 41.8. The increase of loading capacities of biosorbents with the increase of metal ion concentration is probably due to higher interaction between metal ions and biosorbents. Table 2 shows a comparison of adsorption yields determined at different initial metal ion concentrations for the cell forms (vegetative and spore–crystal mixture) with those reported in the literature. From this data it is evident that higher adsorption yields were observed at lower concentrations of metal ion.

According to literature [8–10,15] metal-ion adsorption reaches equilibrium within 15 min. Time of contact of adsorbate and adsorbent is of great importance in adsorption, because it depends on the nature of the system used. Microbial metal uptake by non-living cells, which is metabolism-independent passive binding to cell walls (adsorption), and other external surfaces, is generally considered as a rapid process, taking place within a few minutes. This supports very well our observation of nickel–bacterium system equilibrium where adsorption was achieved within 15 min as shown in Fig. 2.

3.3. Effect of temperature

The effect of initial Ni(II) concentration on biosorption was investigated at 25, 35 and at 45 °C. As is shown in Fig. 3, there is a significant increase in sorption capacity as the temperature has increased to 35 °C. This increase was much more pronounced (highest) when the temperature was 35 °C and was less at 45 °C which is yet still higher than the sorption at 25 °C. However, when our results (Table 2) are compared with those reported in the literatures, the values of Ni(II) specific uptake of

Table 2
Comparison of our Ni(II) ion adsorption results with those found in the literature

Biosorbent[a]	Operating conditions				q (mg g^{-1})	Ref.
	pH	T (°C)	C (mg/l)[b]	Biomass (g/l)		
Ascopphyllum nodosum (1)	6	25	200 (e)	n.a.[c]	70.0	[22]
Fucus vesiculosus (1)	3.5	25	200 (e)	n.a.[c]	17.0	[22]
Chlorella vulgaris (1)	5	25	100 (i)	1.0	42.3	[14]
Scenedesmus obliquus (1)	5	25	100 (i)	1.0	18.7	[14]
Synechocystis sp. (1)	5	25	100 (i)	1.0	15.8	[14]
Pseudomonas syringae (2)	n.a.[c]	22	0–12 (i)	0.28	6.0	[23]
Streptomyces coelicolor (2)	8	25	150 (i)	1	11.1	[15]
Streptomyces noursei (2)	5.9	30	0.6–60 (i)	3.5	0.8	[10]
Artrobacter sp. (2)	5–5.5	30	150 (e)	1.4	13.0	[24]
Rhizopus arrhizus (3)	6–7	n.a.[c]	10–600 (i)	3.0	18.7	[25]
Bacillus thuringiensis (vegetative cell) (2)	6	35	100 (i)	1.0	21.5	t.w.
Bacillus thuringiensis (spore–crystal mixture) (2)	6	35	100 (i)	1.0	34.3	t.w.

t.w.: this work.
[a] (1) Algae; (2) bacterium; (3) fungus.
[b] i: initial metal concentration; e: metal equilibrium concentration.
[c] Not available.

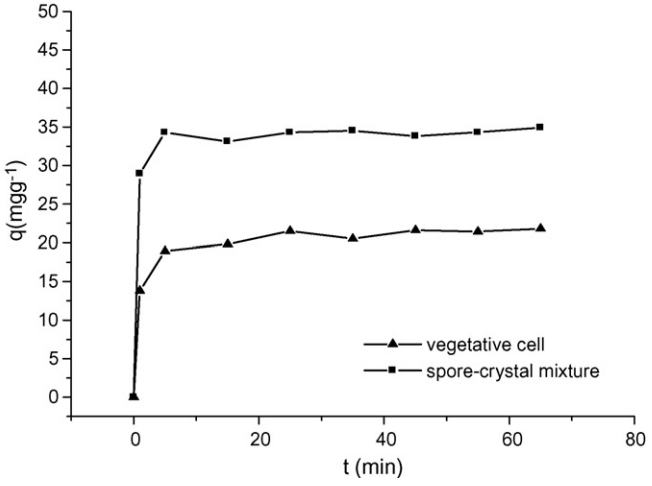

Fig. 2. Time course of nickel uptake (C_0: 100 mg l^{-1}; X: 1.0 g l^{-1}; temperature: 35 °C; pH: 6.0; agitation rate: 100 rpm).

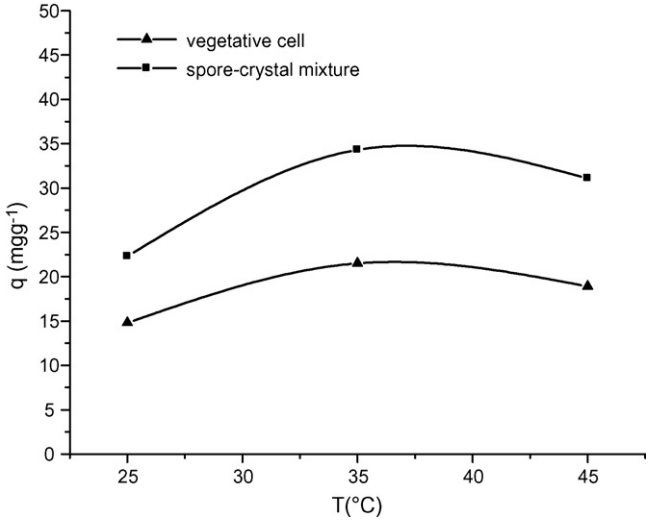

Fig. 3. The effect of temperature (C_0: 100 mg l^{-1}; X: 1.0 g l^{-1}; pH: 6.0; agitation rate: 100 rpm).

spore–crystal mixture are found to be significantly higher than those given elsewhere.

3.4. Adsorption isotherms

An adsorption isotherm is characterized by certain constants, the values of which express the surface properties and affinity of the biosorbent and can also be used to compare biosorptive capacity of the biomass for different metal ions. Out of the several isotherm equations two of them have been applied for this study, the Freundlich and Langmuir isotherms.

Isotherm plots, showing the nickel concentration dependence of the adsorptive capacity for each dilution, are presented in Fig. 4. From this figure, it readily appears that, all isotherms are somewhat curved and the equilibrium is established between nickel ions and the biomass. The adsorption isotherms (Figs. 5–7) show that the amount of metal adsorbed increases as

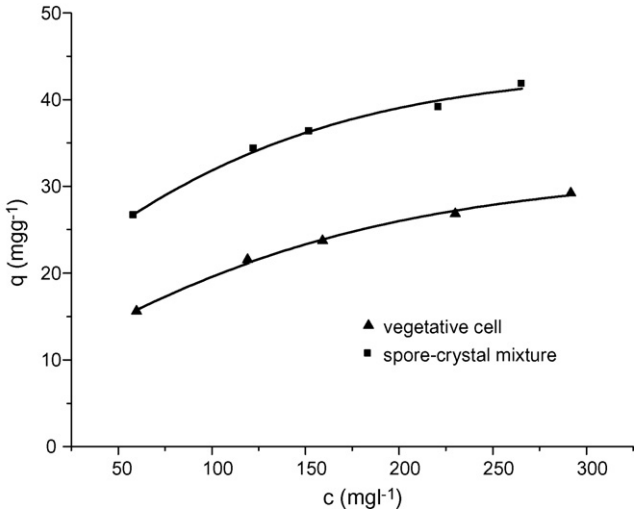

Fig. 4. Isotherms for the equilibrium binding of Ni(II) ion on *Bacillus thuringiensis*.

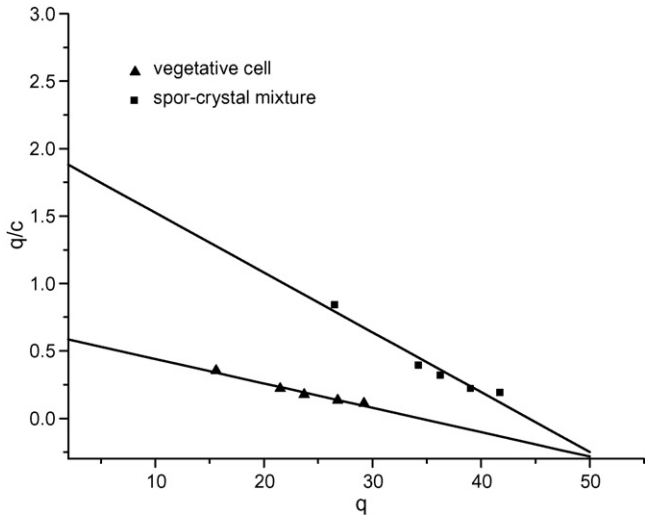

Fig. 5. Scatchard plots for nickel adsorption by *Bacillus thuringiensis*.

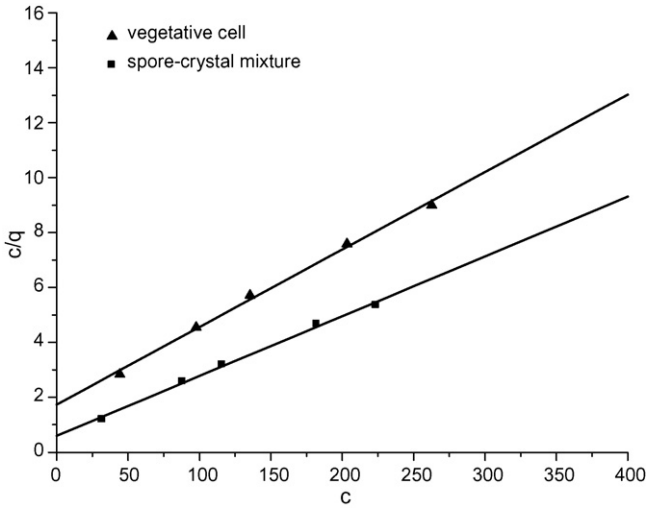

Fig. 6. Langmuir adsorption isotherms of Ni(II) ion on *Bacillus thuringiensis*.

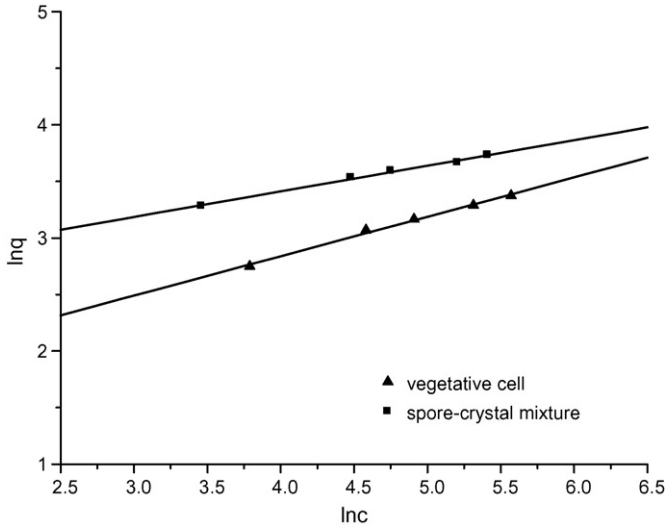

Fig. 7. Freundlich adsorption isotherms of Ni(II) ion on *Bacillus thuringiensis*.

their equilibrium concentration in solution increases. As evident from these data, both of the adsorption isotherms of Ni(II) ion were steep, indicating a greater affinity of nickel(II) ion for the cell forms of *B. thuringiensis* (Figs. 6 and 7).

To evaluate and compare the saturation capacities of *B. thuringiensis* toward Ni(II) ion, the adsorption isotherms were applied and analyzed by Scatchard equation (Fig. 5). Scatchard analysis used here not only to determine the adjustable parameters, but also to estimate the number of site type and their relative affinity for metal ion. The presence of more than one inflection point on a plot based on Scatchard analysis usually indicates the presence of more than one type of binding site. However, when the Scatchard plot showed deviation from linearity, emphasis was focused on the analysis of the adsorption data in terms of the Freundlich model, in order to construct the adsorption isotherms of the ligands at particular concentrations in solutions. Equilibrium binding data for metals gave rise to a linear plot, indicating that the Langmuir model could be applied for adsorption process [21,22]. Fig. 4 shows the adsorption isotherms of metal on *B. thuringiensis*, while Fig. 5 presents the adsorption characteristics assessed from the Scatchard plot.

To examine the fit of data, the Freundlich and Langmuir isotherm models were applied to this study (Figs. 6 and 7). These mathematical models provide information on the biosorption mechanisms and surface behaviour of biosorbent. However, in order to understand and explain the pattern of metal biosorption of vegetative cell and spore–crystal mixture of *B. thuringiensis* the mathematical models developed by Langmuir and Freundlich have been used.

Freundlich isotherm ensures heterogeneous energetic distribution of active sites on the surface of adsorbent which is a reversible binding interaction type. This isotherm can be explained by the following linear form of Freundlich equation:

$$\ln q = \ln k + \frac{1}{n} \ln C \tag{3}$$

The values of k and $1/n$ were evaluated from the intercept and the slope, of the linear plot of $\ln q$ versus $\ln C$ based on experimental data, respectively.

Due to physio-chemical binding without transmigration of electrons, this isotherm shows monolayer irreversible binding. The linearized Langmuir isotherm model is given as:

$$\frac{C}{q} = \frac{1}{K_b A_s} + \frac{C}{A_s} \tag{4}$$

Adsorption constants, metal binding constant, and correlation coefficients for the metals which were calculated from Langmuir, Freundlich isotherms and Scatchard analysis are given in Table 3. The adsorption data with respect to both cell forms provided an excellent fit to the Freundlich isotherm. However, when the Langmuir isotherm model and Scatchard analysis were applied to these data, a good fit for the nickel(II) ion adsorption was also obtained.

Table 3
Adsorption isotherm parameters for Ni(II) ions on *B. thuringiensis*

Cell form	Langmuir isotherm			Scatchard analysis			Freundlich isotherm		
	A_s (mg g^{-1})	K_b (l mg^{-1})	r^2	K_b	q_m	r^2	K_F	n	r^2
Vegetative	35.46	0.016	0.99	0.018	34.3	0.97	5.70	2.87	0.99
Mixture[a]	45.87	0.036	0.99	0.044	44.36	0.95	12.26	4.42	0.99

[a] Spore–crystal mixture.

4. Conclusions

From the present study, the following conclusions could be drawn:

- *B. thuringiensis* has relatively higher affinity for Ni(II) ion when compared with other microbial species.
- *B. thuringiensis*'s unique ability in producing spore and crystal is thought to provide better sorption abilities when compared to those cell forms that are vegetative type only. The experimental results obtained at different parameters showed that vegetative cells and spore–crystal mixture of *B. thuringiensis* var. *thuringiensis* strain are excellent adsorbents for Ni(II) ion.
- Ni(II) biosorption capacity of *B. thuringiensis* varies with temperature. The best temperature for ion uptake was found to be at 35 °C. However, at 35 °C the maximum adsorption capacity of spore–crystal mixture was 45.87 mg Ni(II)/g dry biomass, while the maximum capacity of the vegetative cell was 35.46 mg Ni(II)/g dry biomass.
- When adsorption ability of each of the vegetative cell and spore–crystal mixture was examined and adsorption data obtained were applied to both Langmuir and Freundlich isotherms they were found to describe adsorption equilibrium adequately. The adsorption data with respect to both cell forms provided an excellent fit to the Freundlich and Langmuir isotherms and showed good agreement to Scatchard analysis.

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