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Biosorption of Cd, Cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, *Geobacillus toebii* sub.sp. *decanicus* and *Geobacillus thermoleovorans* sub.sp. *stromboliensis*: Equilibrium, kinetic and thermodynamic studies

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ABSTRACT

Biosorption of each of the ions Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} on *Geobacillus toebii* sub.sp. *decanicus* (G1) and *Geobacillus thermoleovorans* sub.sp. *stromboliensis* (G2) in a batch stirred system was investigated. The equilibrium adsorptive quantity was determined to be a function of the solution pH, contact time, biomass concentration, initial metal concentrations and temperature. The results obtained from biosorption experiments are used to understand the driving forces that govern the interaction between metal ions and a biosorbent. The experimental results were fitted well to the Scatchard plot, Langmuir, Freundlich, Dubinin–Radushkevich (D–R) isotherms. According to the parameters of the Langmuir isotherms, the maximum biosorption capacities of Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} for G2 were 38.8, 41.5, 42, 29 and 23.2 mg/g, respectively, while 29.2, 48.5, 21, 21.1 and 13.9 mg/g for G1, respectively. The mean free energy values evaluated from the D–R model indicated that the biosorptions of studied heavy metal ions onto bacteria were taken place by physical interaction. The biosorption mechanisms of studied metal ions on to the biosorbents were analysed by FT-IR spectra of G1 and G2 with and without metal ions. The first order and second order coefficients were obtained at 298, 308, 318 and 343 K. The experimental results were used to calculate the thermodynamic parameters.

1. Introduction

The increase of industrial activities has intensified environmental pollution problems [1] and the deterioration of several ecosystems with the accumulation of many pollutants, such as toxic metals [2–4]. Heavy metals are discharged from various industries such as electroplating, metallurgical processes, textile, storage batteries, pigment, fertilizers, plastic manufacturing, mining, ceramic and glass [5,6]. Heavy metals are persistent environmental contaminants since they cannot be degraded or destroyed [7]. Heavy metal pollution represents an important problem due to its toxic effect and accumulation throughout the food chain which leads to serious ecological and health problems [5,8,9].

Removal and recovery of heavy metals are very important with respect to environmental and economical considerations [10]. Conventional physicochemical methods such as electrochemical treatment, ion-exchange, precipitation, reverse osmosis, evaporation and oxidation/reduction for heavy metal removal from waste streams are expensive, not eco-friendly [11] and inefficient for metal removal from diluted solutions containing from 1 to 100 mg/l of dissolve metal [12–14].

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 [15]. The use of biological material, including living and non-living microorganisms, in the removal and possibly recovery of toxic or precious metals from industrial wastes, has gained important credibility during recent years, because of the good performance, minimization of chemical/biological sludge and low cost of these materials [16,17]. The main advantages of biological substrates are (a) the diversity of biological active binding sites, (b) small and uniform size and (c) less subject to interference from alkali and alkali-earth metals than ion-exchange resins [18]. Microorganisms including bacteria, algae, fungi and yeast uptake metal either actively (bioaccumulation) and/or passively (biosorption) [13,19,20]. More recently, attention has been focused on the use of microbial biomass particularly bacteria for removal of heavy metals from aqueous solutions [10,21].

The biosorption term is a metabolism-independent binding of heavy metals by dead/inactive biological materials [11]. The biosorption mechanism, which is complex and still understood,

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depends on whether the organism is living or dead, the type of microorganism and the element species [18]. The use of non-living microbial cells as a biosorbents has been shown to be an effective means for removal or recovery of heavy metals from aqueous systems [22]. Biomass exhibits this property, acting just as chemical substance, as an ion exchanger of biological origin. It is particularly the cell wall structure of certain algae, fungi and bacteria, which was found responsible for this phenomenon [21]. The metal ions in solution are adsorbed on the surface through interactions with chemical functional groups such as carboxylate, amine, amide, imidazole, phosphate, thioether, hydroxyl and other functional groups found in the cell wall biopolymers [11,18,23,24]. Biosorption by these microbes is attributed mainly to the ligands present in the biomolecules of their wall polymers. Biosorption includes a combination of several mechanisms such as electrostatic attraction, complexation, ion-exchange, covalent binding, Van der Waal's forces, adsorption and microprecipitation [25,26].

Most of the studies were carried out on the biosorption and bioaccumulation mechanisms in mesophilic bacteria, mainly in *Bacillus* and related genus [9,10,14–16,27–33]. However, a few investigations have been published about the metal adsorption on thermophilic microorganisms. Thermophilic microorganisms are able to grow at a wide range of temperatures (45–80 °C). Several adaptations are required for biological membranes for optimal functioning at high temperatures. In general, the phospholipid composition of bacteria changes with the growth temperature [34]. Thus, they may possess different metal adsorption mechanisms compared to the mesophilic species [35,36].

The present study was carried out to characterize the metal (Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+} and Zn^{2+}) biosorption behaviour of the thermophilic bacteria namely, *Geobacillus toebii* sub.sp. *decanicus* (G1) and *Geobacillus thermoleovorans* sub.sp. *stromboliensis* (G2). For this purpose, various factors affecting the biosorption, such as initial metal concentrations, contact time, the amount of biomass, the pH and temperature of the solution, were investigated using the batch method in thermophilic bacteria.

2. Materials and methods

2.1. Growth and preparation of the powdered dried dead cells

G1 and G2 were obtained from Istituto di Chimica Biomolecolare, CNR, Napoli/Italy. G1 was grown in 50 ml of medium containing 0.4% yeast extract, pepton 0.8% and 0.2% NaCl at pH 7.0, which is placed in 250 ml Erlenmeyer flasks on a shaker (Julabo SW72) at 67 °C for 24 h as described by [37]. G2 was similarly cultivated in 50 ml of medium containing 0.6% yeast extract and 0.4% NaCl at pH 6.0, on the shaker at 70 °C for 24 h [38].

Following bacterial growth, the samples were centrifuged (Sigma Christ 2K15) at 10,000 rpm for 10 min, then the pellets were washed twice with 0.9% NaCl and dried in an oven at 80 °C for 24 h. To obtain a fine powder, dried cells were ground in a porcelain mortar, then were autoclaved at 121 °C for 15 min to assess complete death of the dried cells. The cells were inoculated to liquid medium and the absence of any growth indicated positive results (complete death of the bacteria).

2.2. Preparation of metal solution

The heavy metal solutions were prepared from their chloride and sulfate salts as $CdCl_2$, $CuCl_2{\cdot}2H_2O$, $NiCl_2{\cdot}6H_2O$, $ZnSO_4$ and $MnCl_2{\cdot}4H_2O$. Stock solutions were prepared in distilled water, slightly acidified with HNO_3 (2–3 drops of concentrated HNO_3), and were sterilized at 121 °C for 15 min. These solutions, in various concentrations according to the metal tested, were kept at 25 °C.

Table 1
Operating conditions of the ICP-OES.

Parameter	
RF power (W)	1450
Plasma gas flow rate (l/min)	15
Auxiliary gas flow rate (l/min)	0.2
Nebulizer gas flow rate (l/min)	0.8
Sample flow rate (l/min)	1.5
View mode	Axial-radial
Read	Peak area
Source equilibration time (s)	15
Read delay (s)	60
Replicates	3
Background correction	2-point (manual point correction)
Spray chamber	Scott type spray chamber
Nebulizer	Cross-Flow GemTip Nebulizer (HF resistant)
Detector	CCD
Purge gas	Nitrogen
Shear gas	Air
Gas	Argon
Analytical wavelengths (nm)	Cd 228.802 Cu 327.393 Ni 231.604 Zn 206.200 Mn 257.610

2.3. Heavy metal biosorption studies

A set of 100 ml Erlenmeyer flasks containing 20 ml of the tested metal solutions were used in the batch experiments. The different concentrations of dried powdered dead cells (0.25–10 g/l) were exposed to various initial metal concentration (10–300 mg/l) for 15–120 min at 30–80 °C on a shaker (Julabo SW72) at 120 rpm. After the incubation, the dried powdered dead cells were centrifugated at 10,000 rpm for 10 min. Supernatant and pellet (after acid digestion by concentrated HNO_3) were separately used to estimate for residual metal concentration by using ICP-OES (PerkinElmer, Optima 2100 DV). The operating conditions of the ICP-OES are given in Table 1. All experiments were repeated three times. The Fourier transform infrared spectroscopy (FT-IR, Mattson 1000 model) was used to obtain the FT-IR spectra of the biosorbents with and without the metal in KBr pellet.

3. Results and discussion

The ratio of adsorbed metal ion concentration at equilibrium to the initial concentration of metal ion, which is defined as the adsorption yield, is calculated from the equations:

$$\mathrm{Ad}\% = \frac{c_o - c_{eq}}{c_o} \times 100 \quad (1)$$

where c_o is the initial metal ion concentration (mg/l) and c_{eq} is the residual metal ion concentration in solution at equilibrium (mg/l). Adsorbed quantities at equilibrium and adsorption yields of metal ions obtained at different initial metal ion concentrations are given in Table 2.

3.1. Effect of pH

The pH of the solution is considered one of the most important environmental factors affecting the biosorption process. The pH affects not only the solution chemistry of the metals but also the ionization state of the functional groups like carboxylate, phosphate, imidazole, and amino groups of the cell wall [39–41]. At lower pH, the overall surface charge on the cells become positive, which inhibits the approach of positively charged metal cations. It is likely that protons compete with metal ions for binding sites, thereby decreasing the interaction of metal ions with the microbial

Table 2
Equilibrium adsorbed quantities and adsorption yields of metal ions obtained at different initial metal ion concentrations.

Cd[a]			Cu[a]			Ni[a]			Zn[a]			Mn[a]		
c_o (mg/l)	q_{eq} (mg/g)	Ad %	c_o (mg/l)	q_{eq} (mg/g)	Ad %	c_o (mg/l)	q_{eq} (mg/g)	Ad %	c_o (mg/l)	q_{eq} (mg/g)	Ad %	c_o (mg/l)	q_{eq} (mg/g)	Ad %
G1														
10.1	3.8	94.3	9.9	3.1	78.7	10	3	75.6	9.8	3.6	91.9	9.9	3.2	79.8
25.3	9.5	93.6	23.5	7.4	79	23	5.9	64.6	23.8	8.7	91	24.7	7.2	72.7
49.5	18	90.9	46.6	15.2	81.5	48.9	12.2	62.5	47.8	15.2	79.5	49.6	12.8	64.4
96	31.6	82.2	96.5	27	69.9	98.9	21.1	53.2	98.1	22.9	58.5	99.1	17.5	44.2
191	35.3	46.2	180.5	31.2	43.2	193.4	29	37.5	194.1	27.8	35.9	198.1	22.9	28.8
280	38.2	34.1	291	38.4	33	290.6	35.6	30.6	295.2	28	23.7	296.8	21.5	18.1
G2														
9.2	3.5	96.2	9.9	2	49.2	10	2.5	61.2	9.5	3.34	88.1	9.8	2.7	68.8
24.4	9.1	93.3	23.5	4.7	50.1	23	4.5	49	23.3	7.9	85.4	24.1	5.2	54.6
47	16.2	85.9	46.6	7.9	42.5	48.9	7.6	38.9	47.3	11.6	61.5	48.6	8	41.2
93.8	24.4	65.1	96.5	15.5	40.1	98.9	10.4	26.2	97.4	19.3	49.4	98.3	11.1	28.6
191.3	27	35.3	180.5	20.6	28.4	193.4	13.4	17.3	193.3	20.7	26.8	196.6	11.7	14.8
279.3	29	26	291	33	28.3	290.6	19.5	16.7	292.9	20.3	17.4	295	13.4	11.4

[a] Conditions: 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn in case of G1 and 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2, 60 min of contact time, 50 mg of powdered dried cell, pH: 6.0 for the Cd, pH: 4.0 for Cu and Ni, pH: 5.0 for Zn and pH: 6.0 in the case of G1, pH: 4.0 for Cd, Cu, Ni, Zn and pH: 5.0 for Mn in the case of G2.

cells [39]. At higher pH, the solubility of metal complexes decreases to a great extent allowing metal hydroxide precipitation, which may complicate the sorption process [42]. In general, pH 3.0–6.0 has been found favorable for the biosorption of metal ions by microbial biomass [11].

To evaluate the effect of pH on the biosorption, 50 mg dried powdered cells of G1 and G2 were suspended in metal solutions with different pH values, ranging from 2.0 to 10.0, for 60 min at 70 °C on a shaker at 120 rpm. The pH of each test solution was adjusted to the required value by adding 0.1 M HCl and 0.1 M NaOH before mixing the microorganisms. After the centrifugation at 10,000 rpm for 10 min, the supernatant fractions were separated and analysed by ICP-OES for the remaining metal ions. Fig. 1a and b shows the effect of the pH on the biosorption capacity of heavy metals. The results revealed that the optimum biosorption pH values for Cd, Cu, Ni, Zn and Mn in G1 were 6.0, 4.0, 4.0, 5.0 and 6.0, respectively, while they were 4.0, 4.0, 4.0, 4.0 and 5.0 for G2, respectively.

Several researchers have also investigated the effect of pH on biosorption of heavy metals by using different biomass. The optimum pH for the biosorption of Cu^{2+}, Ni^{2+}, Zn^{2+}, Pb^{2+} and Cr^{3+} ions by Gram positive (*Micrococcus*) and Gram negative (*Pseudomanas*) bacteria was found to be 5.0 [42]. Furthermore, the optimum pH for Pb^{2+} uptake in Gram−ve capsulated and non-capsulated bacteria was determined as 4.0 [43]. Ianis et al. also found the optimum pH to be 4.5 for Cu^{2+} ion adsorption by *Penicillum cyclopium* [44].

3.2. Effect of temperature and the activation energy

Fig. 2a and b shows that the temperature of the adsorption medium (30–80 °C) was less significant when compared with the effect of pH of the adsorption medium. Temperature affects the interaction between the biomass and the metal ions, usually by influencing the stability of the metal–sorbent complex, and the ionization of the cell wall moieties [39]. The temperature of the biosorption solution could be important for energy dependent mechanisms in metal binding process [14]. Energy-independent mechanisms are less likely to be affected by temperature, since the processes responsible for removal are largely physicochemical in nature [31]. The biosorption results obtained in the present study showed that no significant changes were observed between the lowest and highest temperatures studied. The q_{eq} values were changed from 14.7 to 15.7 mg/g for Cu and from 5.9 to 6.1 mg/g for Ni.

The optimum temperature values were obtained as 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn in case of G1 and 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2.

3.3. Effect of initial metal concentration

To examine the effect of the initial metal concentration, the biosorption experiments were carried out at different initial metal concentrations (10, 25, 50, 100, 200 and 300 mg/l) at the optimum temperature and pH for each metal, using 50 mg dried powdered cells of G1 and G2 incubated for 60 min on a shaker at 120 rpm.

As shown in Fig. 3a and b, G1 and G2 show similar behaviour in the biosorption process. It can be clearly seen that the biosorption

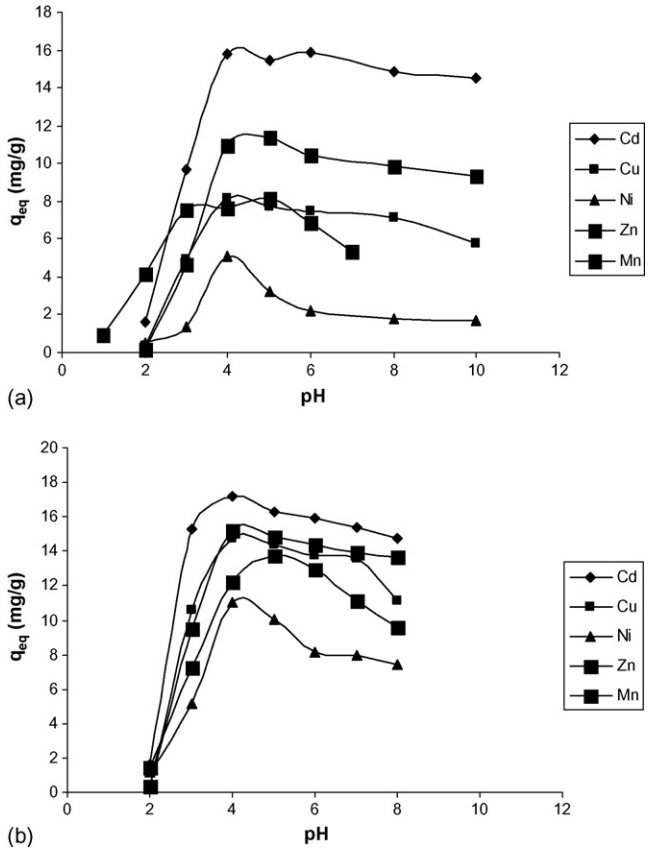

Fig. 1. Effect of initial pH on equilibrium biosorption capacity of Cd, Cu, Ni, Zn and Mn ions (conditions: 50 mg/l of metal concentration, 70 °C of temperature, 60 min of contact time, 50 mg of powdered dried cell) by the dried cells of G1 (a) and G2 (b).

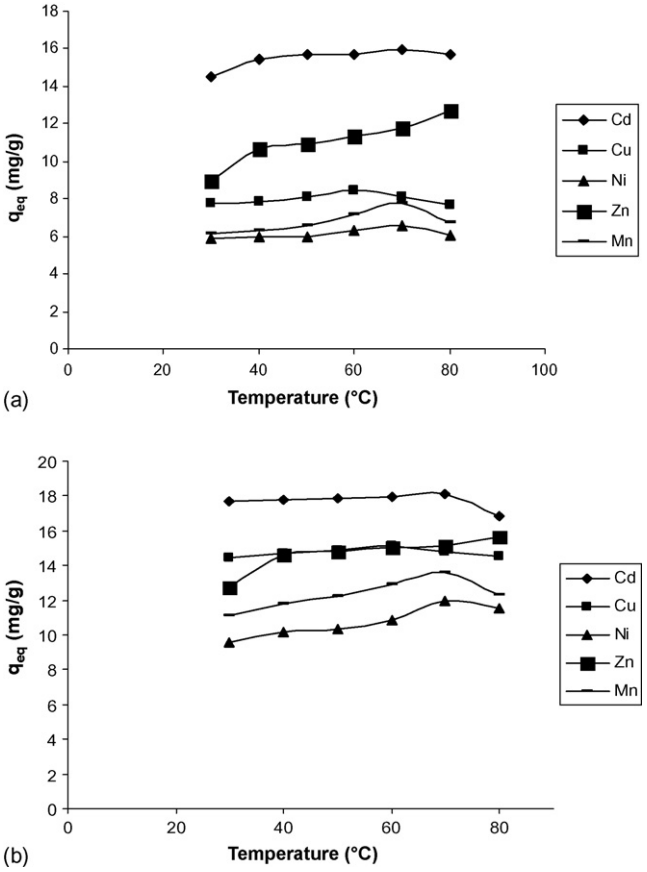

Fig. 2. Effect of temperature on equilibrium biosorption capacity of Cd, Cu, Ni, Zn and Mn ions (conditions: 50 mg/l of metal concentration, 60 min of contact time, 50 mg of powdered dried cell, pH: 6.0 for the Cd, pH: 4.0 for Cu and Ni, pH: 5.0 for Zn and pH: 6.0 in the case of *G. toebii* sub.sp. *decanicus*, pH: 4.0 for Cd, Cu, Ni, Zn and pH: 5.0 for Mn in the case of *G. thermoleovorans* sub.sp. *stromboliensis*) by the dried cells of G1 (a) and G2 (b).

capacities (q_{eq}) of both bacteria increased with increasing initial metal concentrations. The enhancement in metal sorption could be due to an increase in electrostatic interactions, involving sites of progressively lower affinity for metal ions [43]. When initial metal concentrations increased from 10 to 300 mg/l, Cu biosorption increased from 3.1 to 38.4 mg/g dry weight, respectively for G2 and 2.0–33.0 mg/g dry weight, respectively for G1. However, as it was expected, the percentages of biosorbed metals were decreased with increasing initial metal concentrations.

3.4. Effect of dried powdered cell concentration

Dried powdered cells of G1 and G2 (5, 10, 25, 50, 100 and 200 mg) were exposed to 20 ml of metal solution (50 mg/l) at the optimum temperature and pH of each metal for 60 min on a shaker at 120 rpm.

Fig. 4a and b shows that the metal adsorptions (mg metal/g dried biomass) obtained at various concentrations (0.25–10 g/l) of dried biomass of G1 and G2 were decreased with increasing dry mass concentrations, despite increases in metal removal (the biosorbed metal %). This could be attributed to interference between the binding sites at higher concentrations. Higher specific metal uptake at lower dry mass concentrations could be due to an increased metal-to-biosorbent ratio, which decreases upon an increase in dry mass concentration [43]. When the dried biomass concentrations increased from 0.25 to 10 g/l, the Cd biosorption decreased sharply from 38.1 to 4.7 mg/g dry weight, respectively for G2 and from 30.3 to 4.4 mg/g dry weight, respectively for G1, while there were increases in the percentage of the biosorbed metal (e.g. Cd removal in 0.25 and 10 g/l dry biomass for G1 were 16.1 and 94.7%, respectively). There is an agreement in the literature that the increased biomass concentration of the microbial cells results in an attainment with metal adsorption as g/l.

3.5. Effect of contact time

50 mg dried powdered cells of G1 and G2 were suspended in metal solutions (50 mg/l) with optimum temperature and pH of each metal at different contact times (15, 30, 45, 60, 90 and 120 min) on a shaker at 120 rpm.

The time course of metal adsorption by dried dead cells is of great importance in metal removal, as it depends on the nature of adsorbent used. It is known that metal ion adsorption by non-living cells, which is metabolism-independent passive binding to cell walls, reaches equilibrium within 5–15 min in mesophilic organisms [23]. It can be seen from Fig. 5a and b that the metal adsorption by the bacteria is also rapid process, which take place within few minutes. The Cu uptake by dried cells of G1 and G2 in 15 min were 6.3 mg/g dry weight and 14.5 mg/g dry weight, respectively, which did not increase significantly up to 120 min. It is also known that various steps are involved in the transfer of metal from metal solution to binding sites. The most important and rapid step is the first phase at which the bulk transport of metal ions onto biomass takes place in few minutes due to mixing and advective flow [5,30,31].

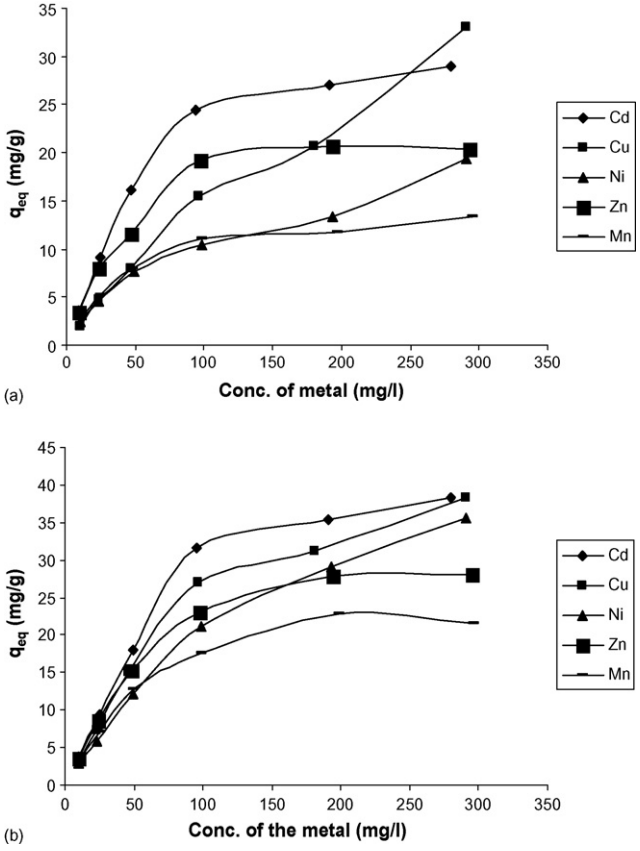

Fig. 3. Effect of initial metal concentration on equilibrium biosorption capacity of ions (conditions: 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn in case of G1 and 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2, 60 min of contact time, 50 mg of powdered dried cell, pH: 6.0 for the Cd, pH: 4.0 for Cu and Ni, pH: 5.0 for Zn and pH: 6.0 in the case of *G. toebii* sub.sp. *decanicus*, pH: 4.0 for Cd, Cu, Ni, Zn and pH: 5.0 for Mn in the case of *G. thermoleovorans* sub.sp. *stromboliensis*) by the dried cells of G1 (a) and G2 (b).

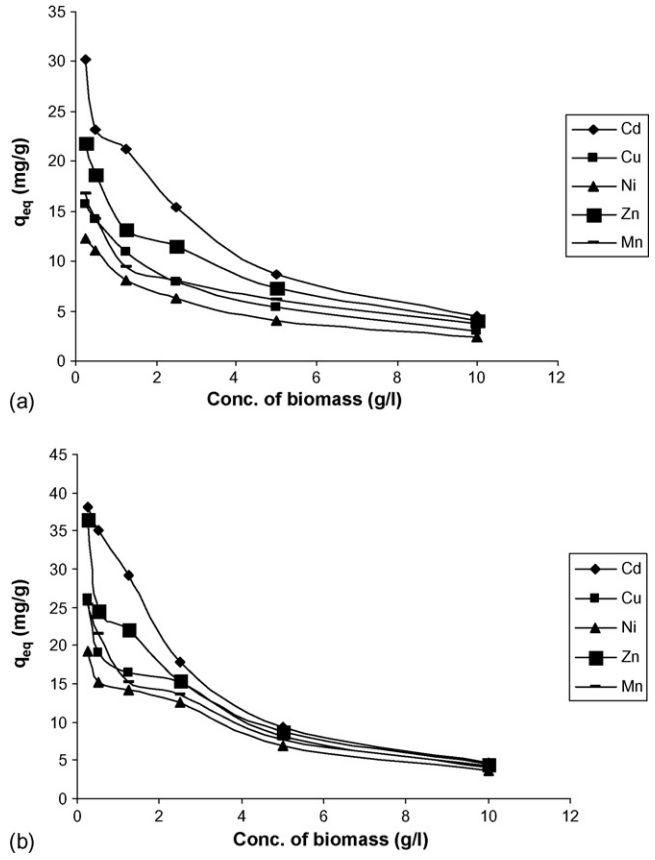

Fig. 4. Effect of dried powdered cell concentration on equilibrium biosorption capacity of Cd, Cu, Ni, Zn and Mn ions (conditions: 50 mg/l of metal concentration, 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn in case of G1 and 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2, 60 min of contact time, pH: 6.0 for the Cd, pH: 4.0 for Cu and Ni, pH: 5.0 for Zn and pH: 6.0 in the case of G1, pH: 4.0 for Cd, Cu, Ni, Zn and pH: 5.0 for Mn in the case of G2) by the dried cells of G1 (a) and G2 (b).

3.6. Biosorption isotherms

The biosorption capacity of a biosorbent which is obtained from the mass balance on the sorbate in a system with solution volume V, is often used to acquire the experimental adsorption isotherms. Under the optimum conditions, the biosorption capacities (q_{eq}) of both biosorbents for each concentration of studied metal ions at equilibrium were calculated from the following equation:

$$q_{eq} = \frac{(c_o - c_{eq})V}{X} \quad (2)$$

where c_o is the initial concentration of solution, c_{eq}, the concentration of solution at equilibrium, V the volume of solution, X the mass of biosorbent.

The nonlinearized adsorption isotherms (q_{eq} versus c_{eq}) of the metal ions on the biosorbents were shown in Fig. 6. These isotherms show that the amount of metal adsorbed increases as its equilibrium concentration increases in solution. As evident from these data, adsorption isotherms of metal ions were steep, indicating a greater affinity of metal ions on the powdered forms of both biosorbents. However, the optimization of a biosorption process using a batch technique requires an understanding of the driving forces that govern the interaction between metal ions and a biosorbent. Although the classical Langmuir and Freundlich isotherm models are applied to account for these interactions as a first approach. In this study, we have used the Scatchard plot in order to obtain more compact information about the interaction between metal ions and the biosorbent [16]. The Scatchard linearized form (Eq. (3)) of the Langmuir equation is used here not only to determine the adsorption parameters, but also to have a preliminary prediction about the types of interaction and biosorbent affinity for metal ions.

$$\frac{q_{eq}}{c_{eq}} = q_m K_b - q_{eq} K_b \quad (3)$$

where q_{eq} is the amount of metal ion adsorbed per unit weight, c_{eq} was the equilibrium concentration of metal ion, K_b and q_m are the adsorption binding constant and maximum biosorption capacity, respectively. The shape of the plot of q_{eq}/c_{eq} versus q_{eq} is related to the type of interaction of the sorbate with a sorbent. If the Scatchard plot is linear with a negative slope, it is related to independent interaction between the sorbate and the binding sites in the sorbent, which follows the Langmuir model [16]. In this work, the Scatchard plots were straight lines. This indicates that there was no change in the affinity of the binding sites for metal ions over the whole range of concentrations used (Fig. 7). Scatchard plot of the results of equilibrium adsorption indicated the presence of one type of binding site for metal ions on the biosorbents. Binding constants and correlation coefficients calculated from the Scatchard analysis are also given in Table 3. The results show that the adsorption of Cu on the biosorbents was very different from the other metal ions. This situation is probably due to a high affinity of Cu for the active site of the biosorbent. Namely, the interaction between the Cu and active sites of the biosorbent, which are likely functional groups such as carboxyl and amine groups, should be stronger compared to other

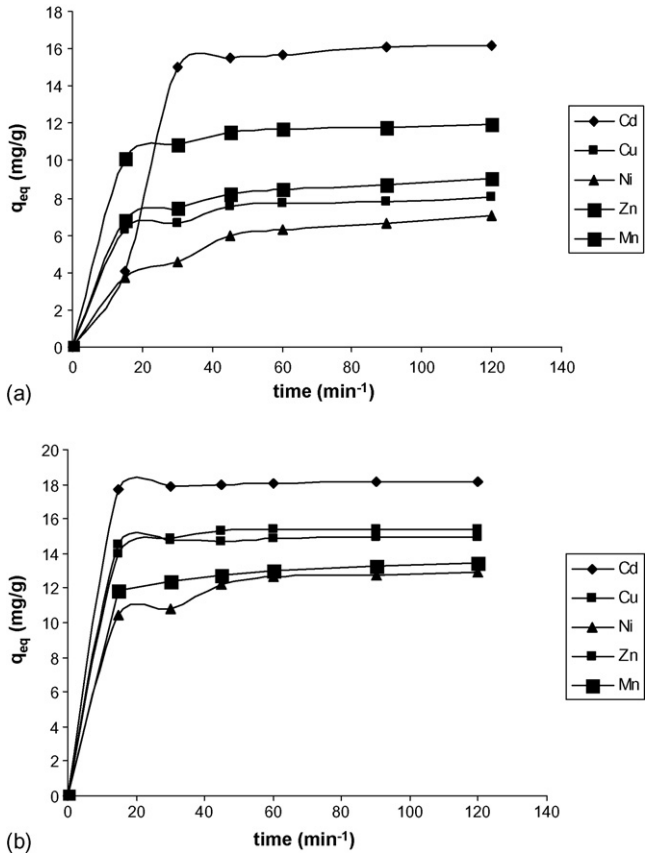

Fig. 5. Effect of contact time on equilibrium biosorption capacity of Cd, Cu, Ni, Zn and Mn ions (conditions: 50 mg/l of metal concentration, 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn in case of G1 and 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2, 50 mg of powdered dried cell, pH: 6.0 for the Cd, pH: 4.0 for Cu and Ni, pH: 5.0 for Zn and pH: 6.0 in the case of G1, pH: 4.0 for Cd, Cu, Ni, Zn and pH: 5.0 for Mn in the case of G2) by the dried cells of G1 (a) and G2 (b).

Table 3
Adsorption isotherm parameters for Cd, Cu, Ni, Zn and Mn by using G1 and G2 as biosorbents.

Metal	Biosorbent	Langmuir isotherm			Freundlich isotherm			Scatchard plot analysis			Dubinin–Radushkevich isotherm			
		A_s (mg/g)	K_b (l/mg)	r^2	K_F	n	r^2	K_b	q_m	r^2	β (mol^2/kJ^2)	q_m (mmol/g)	r^2	E (kJ/mol)
Cd	G2	38.8	0.180	0.9989	7.3	2.7	0.8574	0.19	39.2	0.9902	0.1294	4.73	0.8574	1.96
	G1	29.2	0.200	0.9986	6.7	3.2	0.9109	0.37	27.0	0.9194	0.1097	2.27	0.9109	2.13
Cu	G2	41.5	0.047	0.9906	3.3	2.0	0.8752	0.04	44.8	0.7930	0.1853	15.1	0.8754	1.66
	G1	48.5	0.008	0.8982	1.4	1.4	0.9847	0.01	47.0	0.8424	0.2620	33.5	0.9847	1.38
Ni	G2	42.0	0.023	0.9890	1.9	1.8	0.9793	0.03	38.9	0.8560	0.2001	17.6	0.9794	1.58
	G1	21.0	0.019	0.9233	1.4	2.1	0.9880	0.03	18.3	0.7551	0.1661	4.44	0.9880	1.73
Zn	G2	29.0	0.140	0.9993	5.5	2.9	0.9120	0.19	27.5	0.9449	0.1202	3.74	0.9121	2.04
	G1	21.1	0.136	0.9980	4.3	3.1	0.9061	0.16	20.7	0.8916	0.1110	2.27	0.9063	2.12
Mn	G2	23.2	0.074	0.9960	3.1	2.5	0.9227	0.07	23.2	0.9671	0.1404	4.42	0.9229	1.88
	G1	13.9	0.053	0.9940	2.1	2.8	0.9487	0.07	13.0	0.9183	0.1237	1.80	0.9489	2.01

metal ions. In the present study, the maximum biosorption capacity was obtained with Cu ions using G1 as biosorbent.

The Scatchard plots have indicated that adsorption of metal ions followed the Langmuir model. However, we have tested the adsorption data against the Standard isotherm models, the Langmuir and Freundlich in order to investigate in detail the adsorption characteristics and to compare adsorption performance of the adsorbents for the biosorption of metal ions. The Langmuir (Eq. (4)) and Freundlich (Eq. (5)) isotherms are represented by the equations below:

$$\frac{c_{eq}}{q_{eq}} = \frac{1}{K_b A_s} + \frac{c_{eq}}{A_s} \quad (4)$$

$$\ln\ q_{eq} = \ln K_F + \frac{1}{n} \ln c_{eq} \quad (5)$$

where q_{eq} and c_{eq} are the equilibrium metal adsorption capacity of the biosorbent and the equilibrium metal concentration in the aqueous solution, respectively; A_s, K_b, K_F and n are the adsorption isotherm parameters. A_s is the maximum amount of the metal ions per unit weight of biosorbents to form a complete monolayer on the surface bound at high c_{eq}, and K_b is a constant related to the affinity of the binding sites. A_s represents a practical limiting adsorption capacity when the surface is fully covered with metal ions and assists in the comparison of adsorption performance, particularly in cases where the biosorbent did not reach its full saturation in experiments. The Langmuir equation is valid for monolayer sorption onto a homogeneous surface with a finite number of identical sites. The empirical Freundlich equation given above is based on a monolayer adsorption by the adsorbent with a heterogeneous energy distribution of active sites. K_F and n (it is desired to be $0.1 < n < 1$) are indicators of adsorption capacity and adsorption intensity, respec-

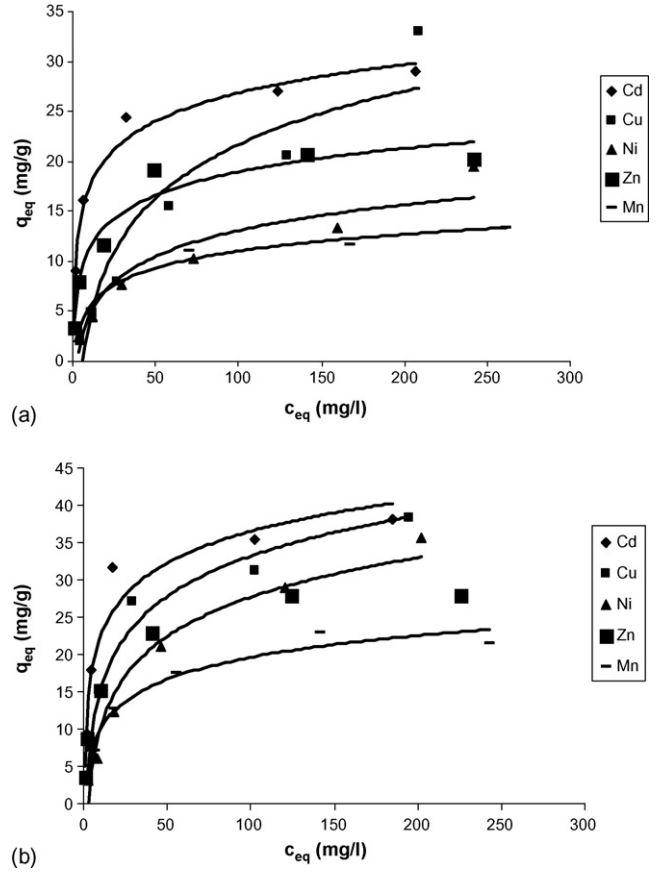

Fig. 6. Nonlinear isotherms for the equilibrium binding of metal ions on G1 (a) and G2 (b).

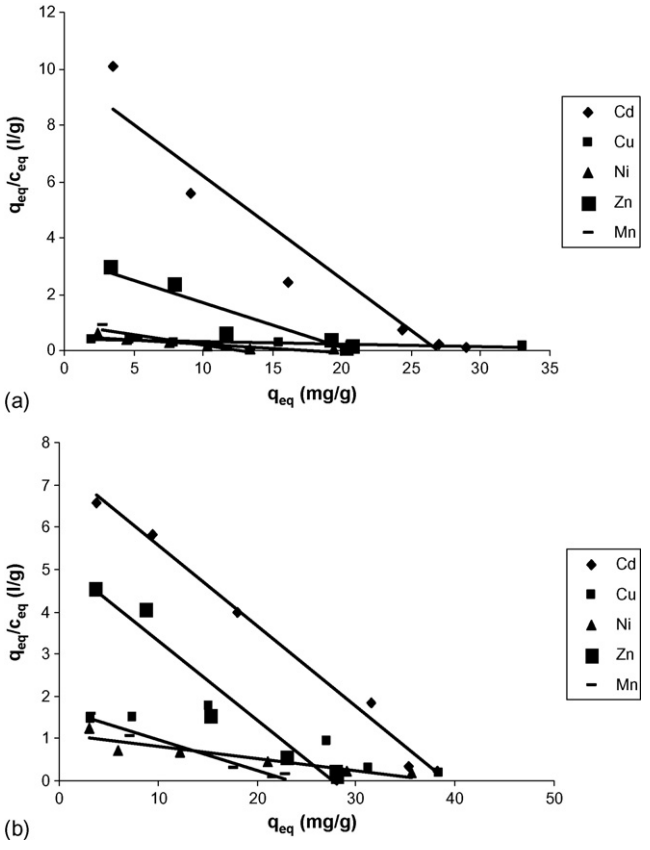

Fig. 7. Scatchard plots for the metal ions adsorption on G1 (a) and G2 (b).

tively [45]. The Freundlich isotherm is also more widely used but provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model [46].

Figs. 8 and 9 show the Langmuir and Freundlich adsorption isotherms of metal ions obtained at 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn using G1, whereas 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2. Table 3 also shows the adsorption constants and correlation coefficients obtained from the Langmuir and Freundlich isotherms at 60 °C for Cu, 70 °C for Cd, Ni and Mn, 80 °C for Zn using G1, while 60 °C for Cu and 70 °C for Cd, Ni, Zn and Mn in the case of G2. According to the A_s values, G1 and G2 highly adsorbed Cu ions. The comparison of the maximum adsorption capacities for both bacteria showed that G2 has a higher sorption capacity. As can be seen in the table, high regression correlation coefficients (>0.99) were obtained from Langmuir isotherms and the results showed that the adsorption equilibrium data has fitted Freundlich adsorption models in the concentration range studied. The values of K_F showed easy uptake of metal ions with high adsorptive capacities of the studied bacteria. There was an agreement between the values of the A_s and K_F. The comparison of K_F values for both bacteria showed that the affinity of the metal ions bound to the active sites of G2 were higher than that of G1. The n values obtained from calculations were in the range between 1.4 and 3.2. These values indicated that the metal ions were favorably adsorbed by both bacteria in the present study. The applicability of both Langmuir and Freundlich isotherms to the biosorption of metal ions shows that both monolayer adsorption and heterogenous energetic distribution of active sites on the surface of adsorbent conditions exist under the experimental conditions employed.

The Dubinin–Radushkevich (D–R) isotherm is more general than the Langmuir isotherm, because it does not assume a homogenous surface or constant sorption potential [47–49]. The D–R equation

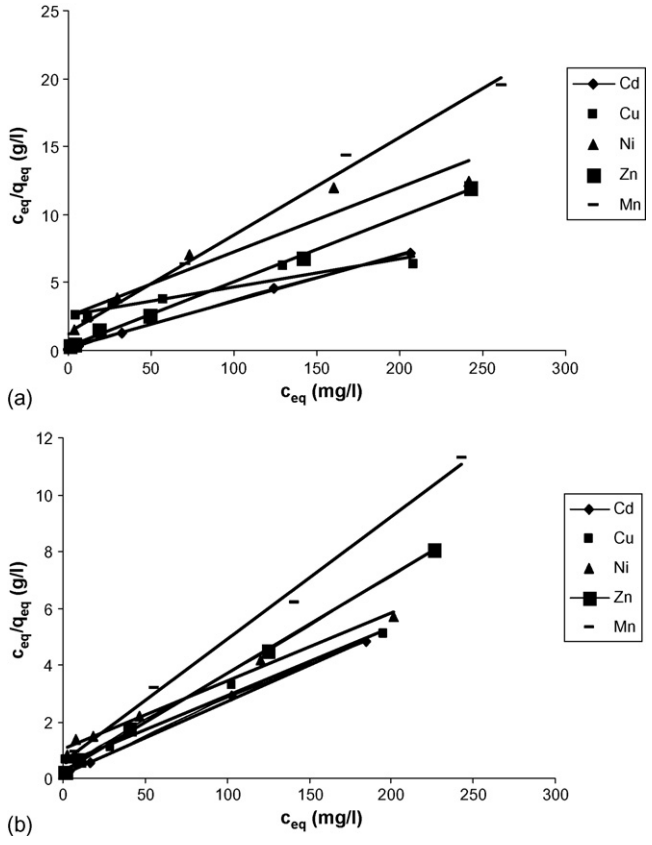

Fig. 8. Langmuir adsorption isotherms of the metal ions adsorption on G1 (a) and G2 (b).

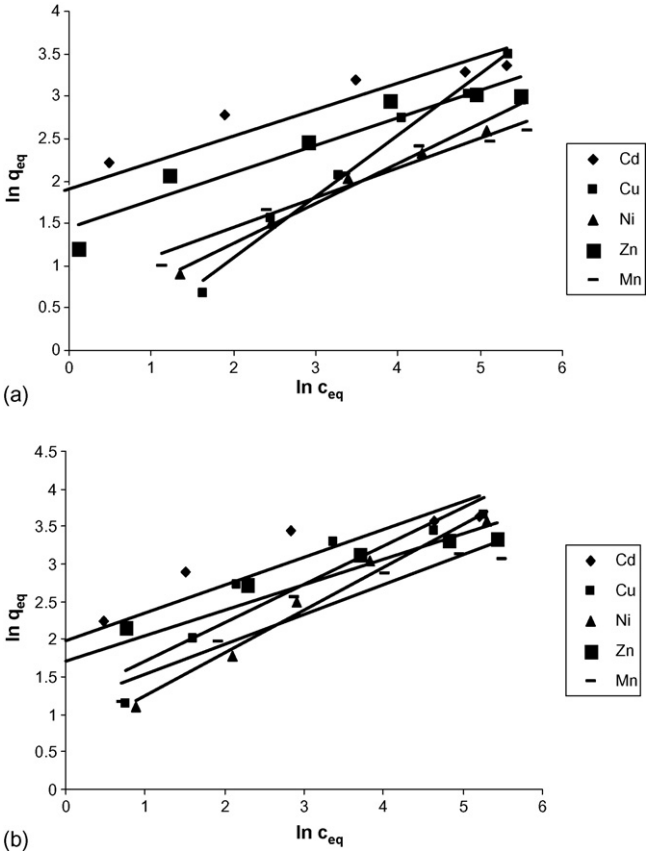

Fig. 9. Freundlich adsorption isotherms of the metal ions adsorption on G1 (a) and G2 (b).

is,

$$q_e = q_m \exp(-\beta\varepsilon^2) \quad (6)$$

where q_e is the amount of metal ions adsorbed at equilibrium, β is a constant related to the adsorption energy, q_m is the theoretical saturation capacity, ε is the Polanyi potential, that is equal to $RT\ln(1+1/c_e)$. The linear form of Eq. (6) is,

$$\ln\ q_e = \ln q_m - \beta\varepsilon^2 \quad (7)$$

The D–R isotherm model well fitted the equilibrium data since the r^2 values were found in the range of 0.8574 and 0.9880 for biosorption of metal ions (Fig. 10). The q_m and β values were calculated from the slop and intercept of the plots and presented in Table 3. The biosorption mean free energy (E; kJ mol^{-1}) is as follows:

$$E = \frac{1}{\sqrt{-2\beta}} \quad (8)$$

The biosorption mean free energy gives information about biosorption mechanism. If E value is between 8 and 16 kJ/mol, the biosorption process follows by chemical ion-exchange and if E < 8 kJ/mol, the biosorption process is of a physical nature [47–49]. The mean biosorption energy was calculated and given in Table 3. These results suggest that the biosorption processes of heavy metal ions onto the studied bacteria is likely to take place by physical mechanism because the sorption energies are below the 8 kJ/mol.

The results presented in Table 3 showed that the highest theoretical saturation capacity was obtained with G2. Although all biosorption models applied to the biosorption results in the present study clearly showed that Cu ions were highly adsorbed on both G1 and G2, theoretical saturation capacity of G2 calculated from Dubinin–Radushkevich isotherms was higher than that of G1.

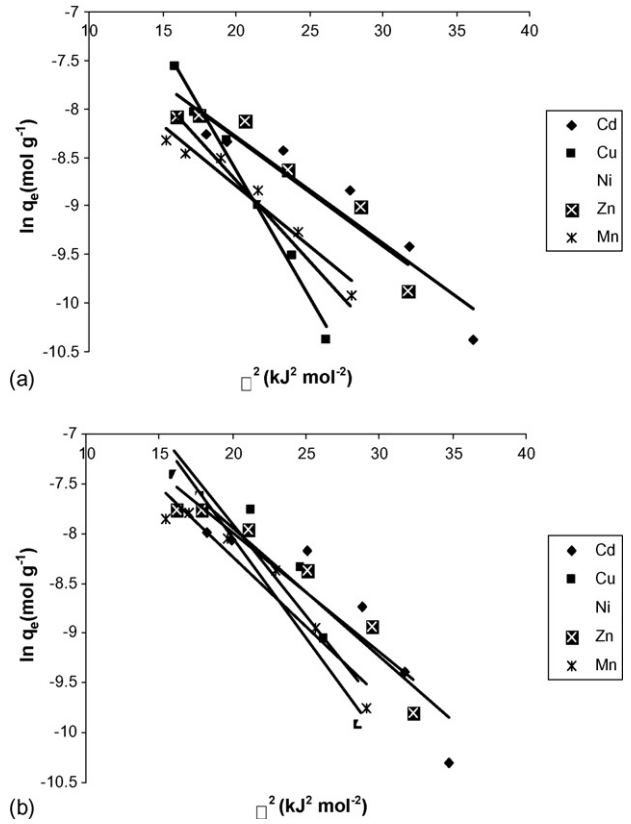

Fig. 10. (D–R) isotherms of the metal ions adsorption on G1 (a) and G2 (b).

3.7. Biosorption kinetics

In order to investigate the mechanism of biosorption, characteristic constants of adsorption rate were determined at individual temperatures for each thermophilic bacteria and at different time by using a pseudo-first-order equation (Eq. (9)) of Lagergren based on solid capacity, and pseudo-second-order equation (Eq. (10)) based on solid phase adsorption

$$\ln(q_e - q_t) = \ln\ q_e - k_1 t \tag{9}$$

$$\left(\frac{t}{q_t}\right) = \frac{1}{k_2 q_e{}^2} + \left(\frac{1}{q_e}\right) t \tag{10}$$

where q_t and q_e (mg/g) are the amounts of the metal ions biosorbed at equilibrium (mg/g) and t (min), respectively and k_1 is the rate constant of the equation (min^{-1}), k_2 (g/mg min) is the rate constant of the second order equation, q_t (mg/g) is the amount of biosorption time t (min) and q_e is the amount of biosorption at equilibrium (mg/g). The biosorption rate constants (k_1) can be determined experimentally by plotting of $\ln(q_e - q_t)$ versus t.

Because the coefficients determined for this model at studied temperatures is low, the plots of $\ln(q_e - q_t)$ versus t for the pseudo-first-order model were not shown in a figure. The r^2 values in Tables 4 and 5 indicate that the biosorption mechanisms of heavy metal ions onto G1 and G2 biomass do not follow the pseudo-first-order kinetic model. When $q_{e,exp}$ values obtained for the biosorption experiments were considered, the second order kinetics were fitted well for the metal ions and both bacteria studied. Moreover, it could be seen in Tables 4 and 5 that the experimental values of $q_{e,exp}$ found for biosorption experiments after 24 h were not in good agreement with the theoretical values calculated ($q_{e1,cal}$) from Eq. (9). Therefore, the pseudo-first-order model is not suitable for modeling the biosorption of heavy metal ions onto G1 and G2.

This model is more likely to predict kinetic behaviour of biosorption with chemical sorption being the rate-controlling step. The linear plots of t/q_t versus t for the pseudo-second-order model for the biosorption of heavy metal ions onto G1 and G2 were not shown in the manuscript since there were totally 10 figures for the second order graphics. The rate constants (k_2), the r^2 and q_e values are given in Tables 4 and 5, showing that the r^2 values from the second order kinetics are very high in the range of 0.9913–1.0000 for the heavy metals studied. In the view of these results, it is clear that the pseudo-second-order kinetic model provided a good correlation for the biosorption of heavy metals onto both bacteria in comparison to the pseudo-first-order model.

Table 4
Kinetic parameters obtained from pseudo-first-order ($q_{e,exp}$: mg g^{-1}, k_1: min^{-1}) and pseudo-second-order (k_2: g/mg min, q_e: mg/g) for Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} biosorption onto G2.

Metal	Temperature (°C)	$q_{e,exp}$	Pseudo-first-order kinetics			Pseudo-second-order kinetics		
			k_1	$q_{e1,cal}$	r^2	k_2	$q_{e2,cal}$	r^2
Cd	25	20.0	0.0019	2.6	0.9469	0.0745	18.0	1
	35	18.9	0.0027	2.6	0.9084	0.0590	18.2	1
	45	17.3	0.0028	2.5	0.8823	0.0638	18.3	1
	70	16.1	0.0019	2.2	0.8497	0.1040	18.3	1
Cu	25	17.5	0.0015	2.9	0.9894	0.0758	14.6	0.9999
	35	16.8	0.0026	2.8	0.9123	0.0514	15.0	0.9999
	45	16.6	0.0023	2.6	0.9489	0.0653	15.1	1
	70	15.8	0.0019	2.4	0.7088	0.1020	15.1	1
Ni	25	15.3	0.0031	5.3	0.9586	0.0221	11.5	0.9994
	35	13.2	0.0030	5.1	0.9788	0.0228	11.7	0.9994
	45	12.1	0.0052	4.9	0.9538	0.0139	12.8	0.9979
	70	11.8	0.0072	4.5	0.8495	0.0139	13.5	0.9986
Zn	25	15.4	0.0031	6.5	0.9420	0.0185	13.1	0.9995
	35	14.5	0.0035	5.3	0.9395	0.0210	14.1	0.9996
	45	12.8	0.0051	4.5	0.8831	0.0199	15.1	0.9998
	70	10.9	0.0039	2.9	0.7149	0.0449	16.7	1
Mn	25	15.5	0.0047	4.9	0.8299	0.0191	12.9	0.9999
	35	13.7	0.0044	4.5	0.8566	0.0223	13.0	0.9999
	45	12.2	0.0062	4.3	0.8887	0.0176	13.7	0.9999
	70	10.6	0.0066	3.7	0.9065	0.0203	14.1	0.9999

Table 5
Kinetic parameters obtained from pseudo-first-order ($q_{e,exp}$: mg g^{-1}, k_1: min^{-1}) and pseudo-second-order (k_2: g/mg min, q_e: mg/g) for Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} biosorption onto G1.

Metal	Temperature (°C)	$q_{e,exp}$	Pseudo-first-order kinetics			Pseudo-second-order kinetics		
			k_1	$q_{e1,cal}$	r^2	k_2	$q_{e2,cal}$	r^2
Cd	25	18.1	0.0016	3.94	0.9912	0.0858	14.74	0.9997
	35	18.0	0.0015	3.41	0.7656	0.0725	15.19	1
	45	17.6	0.0058	3.21	0.9404	0.0311	16.1	0.9999
	70	16.9	0.0041	3.32	0.9502	0.0331	16.44	0.9991
Cu	25	10.0	0.0051	3.3	0.8802	0.0260	8.4	0.9998
	35	9.8	0.0051	3.1	0.9110	0.0257	8.5	0.9994
	45	9.5	0.0056	2.9	0.9688	0.0240	8.8	0.9989
	70	9.3	0.0058	2.8	0.9881	0.0225	8.8	0.9983
Ni	25	9.0	0.0085	5.3	0.9675	0.0068	7.9	0.9923
	35	8.8	0.0073	4.9	0.8742	0.0096	7.6	0.9938
	45	8.8	0.0102	5.2	0.9852	0.0065	8.4	0.9932
	70	8.7	0.0113	5.0	0.9628	0.0068	8.6	0.9922
Zn	25	14.0	0.0038	6.1	0.9039	0.0156	10.4	0.9992
	35	13.4	0.0036	5.5	0.9071	0.0190	10.7	0.9996
	45	12.8	0.0050	4.3	0.9238	0.0194	11.9	0.9996
	70	12.6	0.0070	4.2	0.9355	0.0149	12.6	0.9977
Mn	25	11.0	0.0052	4.3	0.9816	0.0157	9.1	0.9974
	35	10.4	0.0057	4.1	0.9767	0.0150	9.3	0.9966
	45	9.9	0.0068	4.1	0.9803	0.0150	9.6	0.9963
	70	9.6	0.0109	4.6	0.9547	0.0089	10.5	0.9913

3.8. Thermodynamic parameters

In order to describe thermodynamic behaviour of the biosorption of Cd(II) and Cr(III) ions onto *H. splendens* biomass, thermodynamic parameters including the change in free energy, enthalpy and entropy were calculated from the general equations [47,48,50].

The general equations are as follows [48], which are accepted as standard thermodynamic parameters when the experiments are carried out at standard conditions:

$$\Delta G^\circ = -RT \ln K_D \quad (11)$$

where R is the universal gas constant (8.314 J/mol K), T the temperature (K) and K_D (q_e/c_e) is the distribution coefficient [47–49].

The enthalpy (ΔH°) and entropy (ΔS°) parameters were estimated from the following equation,

$$\ln K_D = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad (12)$$

According to Eq. (11), the ΔH^a and ΔS^a parameters can be calculated from the slope and intercept of the plot of $\ln K_D$ versus $1/T$ yields, respectively (Table 6). The graphics of the $\ln K_D$ versus $1/T$ were not given because of there were a large number of figures. In a solution, these parameters were evaluated as integral functions and showed ΔG^a for Gibbs energy, ΔH^a for enthalpy, ΔS^a for entropy in Table 6. The results show that Gibbs energies, enthalpies and entropies change from negative and positive, the biosorption process could be endothermic or exothermic. Negative values indicate thermodynamically feasible and spontaneous nature of the biosorption. It could be seen that the biosorption of Cd on to bacteria is more possible because of the higher negative values. On the contrary, other metal ions were required energy. The positive enthalpy values indicate the endothermic nature of the process at the studied temperatures. Moreover, the positive entropy values suggest an increase in the randomness at the solid/solution interface during the biosorption process.

3.9. FT-IR spectral analysis

The metal ions are divided into four categories, while functional groups as ligands are divided into three [51–55]. Cd, Cu, Mn, Ni and Zn were classified as borderline ions. These metal ions could bind to the ligands with different preferences. Biosorption of the metal ions on the biomass highly depends on the functional groups on the active sites of biomass. The interactions on the biomass are highly depends on the physicochemical conditions of the solution. To understand the biosorption mechanisms of studied metal ions on to G1 and G2, FT-IR spectra of G1 and G2 with and without metal ions were evaluated (Figs. 11 and 12).

In FT-IR spectra of the unloaded G1 and G2 (Figs. 11a and 12a), the peaks at 3315 cm^{-1} are caused by the O–H and N–H stretching. The peaks at 1661 and 1545 cm^{-1} are caused by the vibration of –C=O and –C=C–, respectively. The bands observed about 1403 cm^{-1} are

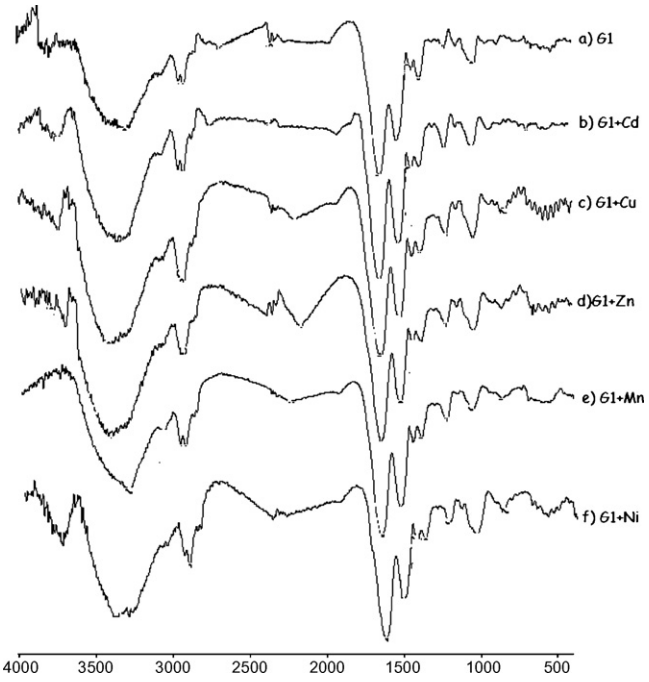

Fig. 11. IR spectra of G1 (a), G1 loaded with Cd (b), G1 loaded with Cu (c), G1 loaded with Zn (d), G1 loaded with Mn (e) and G1 loaded with Ni (f).

Table 6
Thermodynamic parameters for the biosorption process by general approach.

Metal	Temperature (°C)	G1			G2		
		ΔG^a (kJ/mol)	ΔH^a (kJ/mol)	ΔS^a (J/mol)	ΔG^a (kJ/mol)	ΔH^a (kJ/mol)	ΔS^a (J/mol)
Cd	25	−3.14	3.02	2.08	−0.69	7.61	28.40
	35	−3.38			−1.20		
	45	−3.67			−1.63		
	70	−4.07			−1.99		
Cu	25	−0.69	3.85	15.41	3.07	0.90	−7.23
	35	−0.92			3.12		
	45	−1.11			3.19		
	70	−1.39			3.39		
Ni	25	1.78	8.33	21.66	4.28	2.32	−6.44
	35	1.76			4.28		
	45	1.48			4.33		
	70	0.85			4.56		
Zn	25	0.89	15.25	48.72	2.22	7.95	19.32
	35	0.20			2.12		
	45	−0.50			1.53		
	70	−1.31			1.42		
Mn	25	1.18	5.17	13.39	3.07	0.56	−8.41
	35	1.08			3.14		
	45	0.87			3.22		
	70	0.59			3.45		

C–H bending of the aromatic ring. The double peaks at about 2930 and 2990 cm^{-1} are the aliphatic stretching. The C–H bending in benzene ring was observed at about 1460 cm^{-1}. Evaluation of the spectra after adsorption of the metal ions on G1 and G2 indicated that the bands at 3315, 1661 and 1545 cm^{-1} were shifted as about 10 cm^{-1}. However, there was a different peak at about 1240 cm^{-1} in the loaded G1, which is not seen in the unloaded G1. In addition, there was an unknown peak at 3870 cm^{-1} in the G2 loaded with Ni, but not with other metals. These changes of the spectra clearly show the complexation/coordination of the metal ions during the adsorption process. However, it is difficult to explain the exact mechanism of the biosorption of metal ions on G1 and G2 due to the unidentified peaks. Further studies are needed in order to determine the nature of the exact interaction mechanisms in the thermophilic bacteria used as biosorbent.

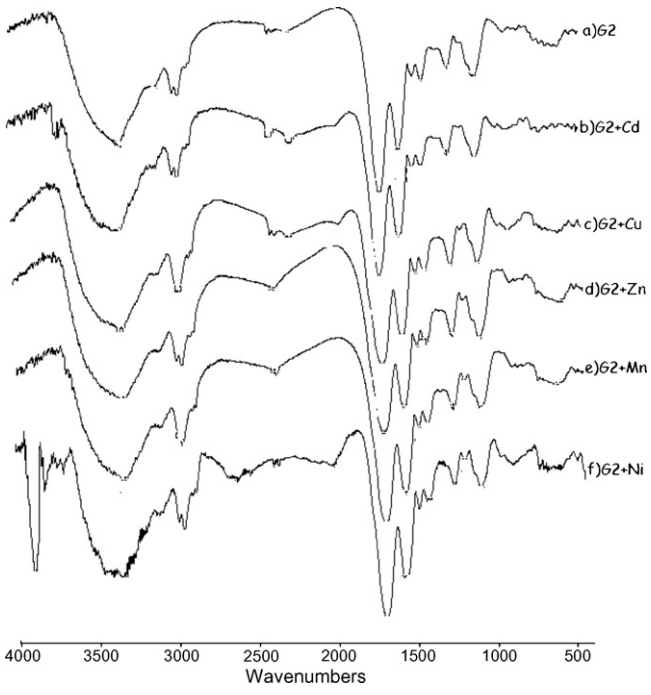

Fig. 12. IR spectra of G2 (a), G2 loaded with Cd (b), G2 loaded with Cu (c), G2 loaded with Zn (d), G2 loaded with Mn (e) and G2 loaded with Ni (f).

4. Conclusion

G. toebii sub.sp. *decanicus* (G1) and *G. thermoleovorans* sub.sp. *stromboliensis* (G2) were used as biosorbents for the Cd^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+} and Mn^{2+} ions in a batch stirred system. The parameters such as initial metal concentration, contact time, the amount of biomass, the pH and temperature of the solution which influence the biosorption were examined for each bacteria. The biosorption results obtained from experiments were applied to the Scatchard plot analysis, Langmuir and Freundlich isotherms. The excellent applicability of Langmuir isotherm to the metal ions biosorption showed the occurrence of monolayer adsorption of active sites on the surface of biosorbent. According to the correlation equations of the Langmuir equations of the each bacteria, the maximum adsorption capacities were obtained for Cu. The free energy changes of metal ions sorption onto the studied bacteria were also determined at individual conditions for each metal. The negative values of the Gibbs free energy change indicate spontaneous nature of the process. The higher negative values for the free energy changes were obtained for the Cd. These results show that the different chemical behaviour of the metals cause different biosorption characteristics. The energy changes of the biosorption process confirmed to the differentiation of the metal ions. According to thermodynamic parameter, the thermodynamic behaviours of the heavy metal ions specifically change from one metal ion to the other.

The results obtained from FT-IR spectra showed that the active surface of the biomass mainly included amin, carboxyl and aromatic groups. The shifts were attributed to the complexation/coordination of the metal ions on the biomass.

In view of the eco-friendly feature and generally easy procedures, the biosorption has being an alternative to the separation and preconcentration methods. This technology provides the using of the various types of biomass as a source to remove heavy metals from different matrices. Our biosorption results utilising the thermophilic bacteria provided the above-mentioned features. Hence, this method can be used for the removal of the Cd, Cu, Ni, Zn and Mn from the water.

The following results can be highlighted from the results obtained from the this study:

(a) According to the Langmuir isotherms, G1 and G2 highly biosorbed the Cu ion with high A_S values.
(b) From the Freundlich isotherms, adsorption capacity of the G2 was higher than that of G1. The adsorption intensities were not so different from each other.
(c) Dubinin–Radushkevich isotherms showed that theoretical saturation capacity of G2 was higher than that of G1. Because the sorption energies were lower than 8 kJ/mol, biosorption of the metal ions on G1 and G2 occurs by physical interaction.
(d) By analysing differential adsorption enthalpies it is seen that G1 and G2 were a good biosorbent for the metal ions.
(e) Biosorption kinetics results showed that the process contained rate-controlling steps and it agreed with pseudo-second-order kinetic model provided a good correlation for the biosorption of heavy metals onto both bacteria in comparison to the pseudo-first-order model.
(f) The results show that Gibbs energies were changed from positive to negative. This explains that biosorption of the Ni and Mn were required extra energy whereas Cd, Cu and Zn were not required energy in the case of G1. When G2 was used as biosorbent thermodynamically feasible and spontaneous nature of the biosorption was valid for Cd. The positive enthalpy values indicated the endothermic nature of the biosorption processes at studied temperatures. The positive entropy values suggested a increasing in the randomness at the solid/solution interface during the biosorption process.

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