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Original Research Article

Biosorption of Ni (II) by *Bacillus* sp. Isolated from Desert-Maranjab Soil

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ABSTRACT

The objective of this research was to isolate microorganisms which produce uptake of nickel ions, from soils in Desert-Maranjab. In this study various soil samples were collected in Desert Maranjab and were cultured on nutrient agar and saboroud dextrose agar and the patent isolates were purified. Twenty soil samples were collected from various areas of Desert Maranjab, Iran .Initial screening of a total of 40 bacterial isolates at pH 5, resulted in the selection of one isolate with maximum uptake capacity of nickel ions 0.71 mmol.g^{-1} dry weights. A contact time of 10 min was sufficient to reach equilibrium. It was tentatively identified as *Bacillus* sp according to morphological and biochemical properties and named strain AEJ-89.This *Bacillus* sp gram positive bacteria were used to investigate the biosorption of Ni ions. In the next step the effects of some ecological parameters (temperature, pH, kinetics and isotherm were studied on the biosorption. The results obtained showed that the optimized conditions for the uptake nickel ions were as follow: Temperature 25ºC, pH= 7.8 - 7.2, and carbon sources (glucose and lactose 10 g / l). The equilibrium time was about 5 min and the adsorption equilibrium data were well described by the Langmuir`s equation.

Key Words: Desert-Maranjab, Nickel, Uptake, *Bacillus* sp.

INTRODUCTION

Heavy metal ions existing in wastewaters of various industries such as metal plating, mining operations, battery manufacturing and tannery fabrication are posing serious risks to human health and the environment (Nilanjana 2010). The term "biosorption" is used to describe metabolism-independent binding of heavy metals and/or radionuclides to nonliving biomass. The discovery and development of biosorption phenomena provide a basis for a whole new technology aimed at removal of heavy metallic species from dilute solutions in the range of 1 to 100 mg/L. Recovery of some of these metals is a possibility. Biomass from various natural or industrial origins can be used as complexing materials to recover toxic or strategic elements from industrial wastewaters (Jinsong and Paul Chen 2014). The conventional methods for removal heavy metal include chemical and physical methods are ineffective or expensive, and are not eco-friendly (Gadd and de Rome 1988). The active and passive removal of heavy metals by biomass of bacteria, actinomycetes, fungi, and algae has been described (Ashutosh *et al.* 2014, Treen-Sears 1984). The major mechanisms responsible for it include ionic interactions and complex formation between metal cations and ligands contained in the structure of the biomaterials (Fourest and Roux 1992, Klimmer *et al.* 2001). However, very little is known about the actual tissue structure and composition of different organisms, which also vary widely depending on the growth conditions for industrial or laboratory grown biomass, and the location or the season for natural harvested biomass (Crist *et al.* 1994). The unknown features of most biosorbents reduce their chance to be used as competitive products compared to well-known synthetic ion exchangers (Jinsong and Paul Chen 2014), even if their costs are expected to be significantly lower. The knowledge of the chemical structure of biosorbents is essential for modeling and predicting their metal binding performance in water purification systems. The overall effectiveness of biosorbent metal removal would also depend on the concentration range, the solution pH, the reaction kinetics, sorption equipment design, and the composition of the actual effluent handled. The identification of the binding sites in efficient biosorbents would be also helpful in the selection process for new biomass types as well as in attempts to improve their complexing properties by biological, chemical, or engineering processes.

Divalent nickel $Ni^{2+}$is a toxic heavy metal present in raw wastewater streams from industries such as electroplating, non-ferrous metal, mineral processing, paint formulation, porcelain enameling, copper sulfate and battery manufacture, as well as from steam-electric power plants (Percival and McDowell 1967). Among heavy

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metals in wastewaters, Ni^{2+} and Cu^{2+} has been a major focus in wastewater treatment because it is associated with many health hazards. The effluent emanating from these industries often contains high concentrations of Ni^{2+} ions, which are toxic to both higher and lower organisms (Percival and McDowell 1967, Ozturk 2007). In humans, Ni^{2+}can cause various types of acute and chronic health disorders, such as severe damage of lungs and kidney, skin dermatitis, nausea, vomiting, diarrhea, pulmonary fibrosis, renal edema, chest pain, rapid respiration, cyanosis and extreme weakness (Padmavathy *et al.* 2003, Pandey *et al.* 2007). Besides, it is well known that nickel is carcinogenic and this effect is probably related to its lipid-peroxidation properties, which induce DNA-strand gaps and breaks, and DNA–protein crosslinks (Subbaiah *et al.* 2009). Moreover, nickel has been implicated as a nephrotoxin (Savolainen 1996), teratogen and embryotoxin (Padmavathy *et al.* 2003). The United States Environmental Protection Agency (EPA) and the World Health Organization (WHO) have established drinking water guidelines for nickel of 0.1 mg l-1 (Malkoc 2006) and 0.07 mg l-1 , respectively. In order to meet the water quality standards for most countries, it is essential to reduce the concentration of Ni^{2+}ions and of other heavy metals in industrial wastewaters to their permissible limits before discharge to the environment.

We report here the biosorption of nickel ions by a bacterium isolated from Desert Maranjab on the province Isphahan. Furthermore, we present the effects of various environmental parameters in the removal of nickel ions.

MATERIALS AND METHODS

Biomass production

Twenty soil water samples were collected from the Desert Maranjab in Isphahan province (Fig 1). It is situated between 34°16' 54" North latitude and 51°48'16" East longitude in the Isphahan region of Iran. The bacterial strain *Bacillus sp* was isolated from Desert Maranjab soil. The bacterial strain was isolated on nutrient agar medium comprising (g/l): bacteriological peptone, 5.0; sodium chloride, 8.0; beef extract, 3.0, agar, 2% and pH 7.0. Pure bacterial culture was obtained by repeat streaking on basal agar medium using standard isolation techniques. For isolation, inculcated plates were incubated at 37°C for 72 h. The pure colony was obtained and identified from microbial type culture collection, University of Ilam. Morphological, physiology and biochemical characteristics of the bacterial Sp. Bacillus were shown in table 1.

Figure 1. Maranjab desert is located by Aran & Bidgol, a city in Isfahan province, Iran.

Preparation of bacterial biosorbents

For biosorption study, bacterial strain bacillus sp was cultivated aerobically in 250 ml Erlenmeyer flask containing sterile nutrient broth on a rotary shaker 130 rpm at 37°C. Cells were harvested at the end of experimental phase. After cultivation, the cells were centrifuged at 10.000×g for 20 min and then washed three times with deionized water and wet weight of the cells equivalent to 0.8 g dry weight/L was used in the experiments.

Table 1. Morphological, physiology and biochemical characteristics of *Bacillus* sp. strain AEJ-89.

Morphological characteristics	Colony	Cream, round, with rough surface
	Gram strain	+
	Motility	+
	Cell shape	Rod
	Endospore formation	+ (central)
Physiology characteristics	Growth temperature	25-45°C
	pH	6.5-9.5
	Growth on NaCl (%)	2-5
Biochemical characteristics	Glucose	+
	Sucrose	+
	Mannose	-
	Sorbitol	+
	Xylose	-
	Lactose	-
	Mannitol	-
	Arabinose	-
	Citrate utilization	-
	Casein hydrolysis	-
	Gelatin	+
	Urea	-
	Nitrate reduction	+
	Oxidase	-
	Starch hydrolysis	+
	Nitrate reduction	-
	Indol test	-
	Methyl Red test	+

Biosorption studies

In terms of initial pH of solution impacts on biosorption, the Ni^{2+} concentration of working solution was selected at 10 mg/L, and 1 mol/L NaOH and 1 mol/L HCl were used to regulate the initial pH to the investigating range, from 1.1 to 7.8 standard values. The determination of the equilibrium time of Ni^{2+} removal was carried out with three working solutions with the same pH at 4.8 and different Ni^{2+} concentration, 50, 200, and 400 mg/L. Samples via reaction for 1, 2, 3, 4, 5, 10, 20, 30, 60, 90, and 120 min. would be analyzed to determine the equilibrium time. In addition, metal solutions with various concentrations ranging from 50 to 500 mg/L were used to assess the effect of initial metal ion concentration on biosorption.

Recovery of nickel ions from the metal-laden biomass was examined using 30 ml of various desorbent agents including EDTA, NaCl, HNO_3, KOH and CH_3COOH at 1 M concentration in a batch stirred system on a rotary shaker for 30 min at 37°C. The biomass was separated from each eluents solution by centrifugation 10.000 g for 20 min at 4°C, and concentration of the nickel ions released into the eluents solution was determined. The treated biomass was extensively washed with distilled water and then used in another sorption cycle. The experiments were repeated for five cycles. The desorption rate of *Bacillus* sp. Strain AEJ-89 was calculated by the ratio between the amount of metal ions desorbed and the amount of metal ions adsorbed.

Metal analysis was carried out by an inductively an, atomic absorption spectrometer (Chem., Tech, Analytical CTA 2000). The metal adsorption uptake (q, mg/g) was calculated with Eq. (1):

$$q = \frac{V(C_0 - C_e)}{W}$$

Where, V (L) is the volume of solution in flask, C_0 (mg/L) is the initial metal concentration, C_e (mg/L) is the residual metal concentration, and W (g) is the mass weight of adsorbent.

Similarly, biosorption efficiency R (%) was calculated as Eq. (2):

$$R = 100 * (C_i - C_f) / C_i$$

The sorption results have been subjected to Langmuir isotherms.
The Langmuir adsorption isotherm was tested in the following linearized form:

$$\frac{1}{q_e} = \frac{1}{b_L . q_m} \frac{1}{C_e} + \frac{1}{q_m}$$

where q_{max} (mmol/g of dry weight) is the maximum uptake capacity corresponding to complete monolayer coverage, C_e (mmol/L) the equilibrium solute concentration, and b_L the equilibrium constant related to the energy of adsorption (L/mmol).

Q_m, corresponding to the saturation of the adsorption layer, and b_L, the equilibrium constant of the biosorption reaction:

$$\overline{Bsp} \quad + \quad Metal \quad \Leftrightarrow \quad \overline{Bsp. - Metal}$$

RESULTS AND DISCUSSION

Adsorption equilibrium

Because pH is one of the main controlling parameters affecting the adsorption process ,the biosorption of Ni^{2+} on bacillus sp were measured at initial Ni^{2+} concentration of 100 mg/L, with pH ranging from 1.0 to 7.8 (Fig.2). The increase of pH has a positive effect on metal uptake. The maximum adsorption of Ni^{2+} ions on *Bacillus* sp. was observed at pH 5.2. These results revealed that the Ni^{2+} adsorption on bacillus was mainly attributed to the effort of ionic attraction. At low ph, a high concentration of protons resulted in the protonation of functional groups (such as carboxylate group), thus, the negative charged intensity on the binding was decreased (Yan and Viraraghavan 2003) .That was the reason why the attraction between Ni^{2+} and biosorbent decreased. In contrast, at high pH, biosorbent was more negatively charged because of the deprotonation of the metal binding sites, which was in favor of the Ni^{2+}biosorption. These results were similar to other adsorbent systems related to the biosorption on Ni^{2+} in the laboratory (Yan and Viraraghavan 2003).

The effect of initial Ni^{2+} concentration on the biosorption capacity of biosorbent was examined at pH of 3.3, 4.4 and 5.5. The Ni^{2+} concentrations varied from 50 to 500 mg/L, the loading capacity increased from 4.54 to 42.12 mg/g biosorbent. The initial Ni^{2+} concentration also had significant influence on removal efficiency (fig .2 b). The lowest efficiency turned up at the maximum Ni^{2+} concentration of 500 mg/L. That is because the number of metal ions increased to an extent that redundantly exceeded the available binding site in the biosorbent for complexation. In other works, the metal ions competing for the available binding sites increased, which led to the lack of the binding sites for complexation of Ni^{2+} ions (Ekmekyapar *et al.* 2006). The Ni^{2+} biosorption on biosorbent was studied at *bacillus* concentration ranging from 500 to 1,500 mg/L. The results are graphically shown in Fig 4. The increase of biosorbent concentration caused a decrease in the metal specific uptake and an increase in the biosorption removal efficiency. Indeed, the specific uptakes dropped from 142 to 61 mg/g biomass and the biosorption removal efficiency increased from 71.2% to 91.2%, respectively, as the biomass dose increased from 500 to 1500 mg/L. such a trend is mainly attributed to an increase in the sorptive

surface area and the availability of more adsorption sites. Quite similar tendency was reported for Ni^{2+} adsorption onto *Bacillus subtilis* (Aksu and Balibek 2007).

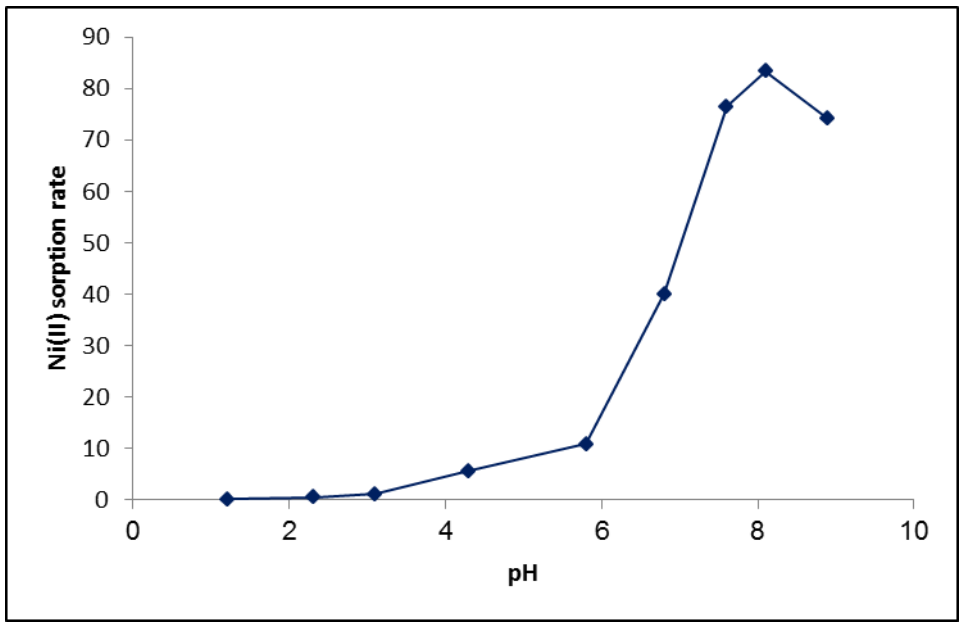

Figure 2. Effect of pH on Ni^{2+} biosorption by biosorbent (C_i: 100 mg/L; biosorbent concentration: 1 g/L; 25°C; 150 r/min).

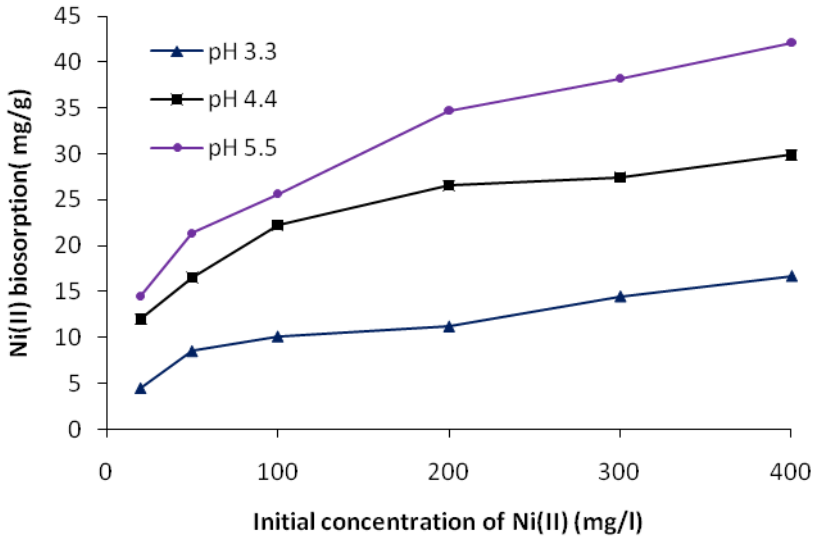

Figure 3. Effect of initial Ni (II) concentration on biosorption capacity by *Bacillus* sp (concentration biosorbent 1 g/l; 25°C, 150 rpm).

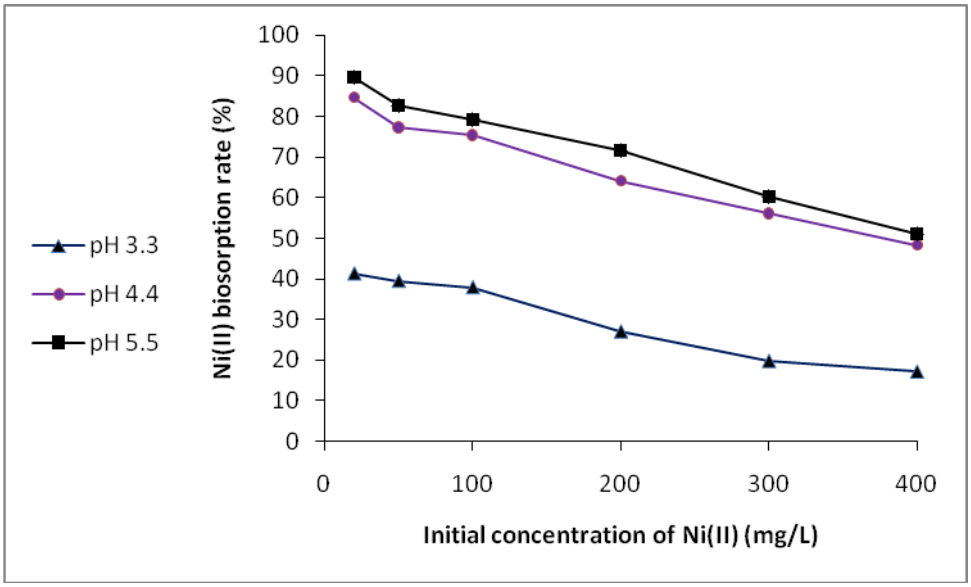

Figure 3. Effect of initial Ni (II) concentration on removal efficiency by *Bacillus* sp. (concentration biosorbent 1 g/l; 25°C, 150 rpm).

Fourier transforms infrared spectroscopy

In order to understand better the nature of the functional groups responsible for the biosorption, FT-IR analysis of the biomass *Bacillus sp.* was carried out. The spectrum of the biomass is shown in Fig.3 and the IR adsorption bands with corresponding possible groups are shown in Table 2. The spectrum (Fig.3) exhibits a broad absorption band between 3500 and 3200 cm−1 due to bonded –OH stretching vibration, and in this range, stretching vibration of –NH groups located at 3306.48 cm−1 can be observed. The peak at 2930.84 cm^{-1} is the indicator of alkyl chains –CH stretching vibration. The C==O of the carboxylic groups or esters groups stretching vibration, appears at 1728.68 cm−1. The typical amide I band, C==O stretching vibration, appears strongly at 1655.19 cm^{-1}. The peak at 1544.20 cm−1, known as amide II, is contributed to a motion combining both the –NH bending and the –CN stretching vibration of the group –C(==O)–NH– in its transform, and this peak appears as a shoulder with a moderate to strong intensity on C==O stretching vibration absorption. Sometimes, the C (==O)–O− anti-symmetric stretching vibration in carboxylate so-called νC==O (I) appears around 1544.20 cm−1 wavenumbers. The small absorbance peak at 1453.60 cm−1 are due to –CH2 scissoring or –CH3 anti symmetrical bending vibration. The other typical amide band (amide III) located in 1381.11 cm−1 is identified, and in general, the C (==O)–O− symmetric stretching vibration in carboxylate is overlapped in the wavenumbers. The C(==O)–O− stretching vibration coupled to the –OH in plane deformation, aromatic amines (–C–N–), P==O and S==O stretching vibration all exhibit the moderate band at about 1291.09 cm−1. The peaks at 1184.29 and 1055.95 cm−1 may be attributed to C–N stretching vibration of amine groups, P–O–C links of the organic phosphate groups and P–O vibration of (C–PO3 2−) moiety (Aksu and Balibek 2007, Dogru *et al.* 2007, Romero-Gozalen *et al.* 2001, Socrates 2001) . Therefore, infrared spectra of *B. cereus* biomass showed the presence of amine R–NH2 (Amino acids, proteins, glycoproteins, etc.), carboxylic acid (fatty acids, lipopolysaccharides, etc.), hydroxyls, and phosphate (Selatina et al, 2004, Naja *et al.* 2005).

Effect of temperature on nickel ions biosorption:

The effect of reactor medium temperature on biosorption of nickel (II) in with *Bacillus sp* is shown in Fig2. The optimum temperature is 41°C at which the maximum nickel ion biosorption occurs. The maximum nickel biosorption in 89.89% (w/w) at optimum temperature of 41°C for 700 ppm of initial nickel ion loading was observed. The biosorption of nickel ion are observed as 79.13, 83.25 and 76.5 (w/w %) for temperature of 27, 34

and 46 °C, respectively with 700 ppm of initial nickel ion loading. With increase in temperature beyond 41°C, the biosorption of nickel ion decrease with bacteria bacillus sp. strain AEJ-89.

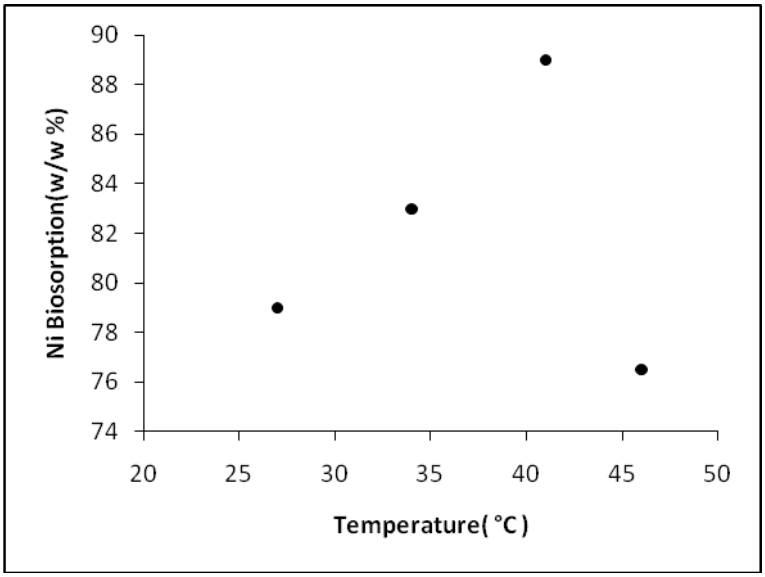

Figure 3. Effect of temperature on Nickel biosorption.

The results of desorption of nickel ions using various desorbing agents at 1 M concentration demonstrated that EDTA was a highly efficient desorbent and enabled the complete recovery of nickel ions from biosorbent. These results showed that nickel ions adsorption without considerable loss in its initial adsorption capacity. Nickel ions was removed from metal-laden biomass after desorption treatments by addition of different desorbing solutions with the results EDTA (98.24%)> NaCl (88.56%)> CH_3COOH (76.98%)> HNO3 (54.87%)> KOH (49.37%).Desorption of nickel ions from bacillus sp. strain AEJ-89 was completely achieved applying 1M EDTA and no significant decrease in activity took place after 5 sorption/desorption cycles.

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