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Original Article

Biosorption of nickel by *Halobacillus* sp. KN57 isolated from the Miankaleh Wetland, Iran

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Abstract: Heavy metals are generally discharged from untreated wastewater in the aquatic ecosystem. This study was aimed to investigating the effective parameters in the sorption of nickel metal by a native halophile bacterium isolated from sediments of the Miankaleh Wetland in the north of Iran. *Halobacillus* sp. KN57 as the most resistant Gram-positive strain was selected and identified by phenotypic and phylogenetic properties (16S rRNA). The optimum contact time, pH, temperature, salinity, and initial biosorbent concentration of nickel for this strain were 100 min, 7, 30°C, 10%, 1 $g.L^{-1}$, and 150 $mg.L^{-1}$, respectively. The nickel biosorption was 111.11 mg/g by the selected strain under the laboratory conditions, followed Langmuir isotherm with a correlation coefficient of more than 0.98 and the maximum single layer sorption. In addition, the kinetics of nickel biosorption of this strain correspond to a pseudo second order kinetic model with a correlation coefficient of more than 0.99. Scanning electron microscopy was applied to confirm the biosorption of Ni^{2+} by *Halobacillus* sp. KN57. Finally, the FT-IR spectrum identified that the amide, carbonyl, and amine functional groups were participated in binding to nickel ions. The results showed that the native bacterial strain (*Halobacillus* sp. KN57) isolated from Miankaleh Wetland, is potentially a promising biosorbent for sorption of nickel metal.

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Introduction

Heavy metals are one of the most important threats to the wetlands (Bai et al., 2011). Nowadays, various human activities have been led to entrance of a worrying amount of the heavy metals into aquatic ecosystems, including wetlands. These pollutants can impact the ecosystems, and ultimately, human beings due to their persistence and accumulation in the food chain (Pascoe et al., 1996). Nickel is found in industrial wastewater such as stainless steel, non-ferrous alloys and super alloys, mineral processes, color formulation, electrotyping, glazing, pumice, copper sulfate production, electronics products, welding products, textile, nickel-cadmium batteries, and fertilizers (Coman et al., 2013). Since the wastewaters enter aquatic ecosystems, if they are not properly cleaned, hence, removal of the heavy metals from industrial effluent and aquatic ecosystems need to be done using physicochemical and biological methods (Regine et al., 2000). In this regard, biological methods can preferred than physicochemical methods due to their cost-effectiveness, eco-friendly and less sludge production (Regine et al., 2000; Masoumi et al., 2017). Bacteria, fungi, algae, yeasts, agricultural waste and other polysaccharide materials are of the most potent to extract and remove heavy metals (Volesky, 1986; Padmavathy et al., 2003; Ozturk, 2007; Özer et al., 2008; Abdel-Ghani and El-Chaghaby, 2014).

Among the biological adsorbents, bacteria are good adsorbent because of their high surface-to-volume ratio, and having surfaces for the absorption of chemicals like teichoic acid (Ozturk, 2007; Masoumi et al., 2017). In addition, bacteria, due to their small size, growth ability under controlled conditions, and resistance to a wide range of environmental conditions can be considered as a good bioadsorbent (Ansari et al., 2011; Kulkarini et al., 2014). Halophile bacteria

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are used in this research due to their importance in biotechnology, and their industrial application to produce enzymes, polymers, proteins and so on (Kushner and Kamekura, 1998). In such a way that their growth in high salt concentrations can minimize the risk of contamination of the culture medium, and their simple nutritional needs help to use different combinations of carbon and energy sources (Ventosa et al., 1998). According to our knowledge, there is no information on nickel-resistant native halophile bacteria species from the Miankaleh wetland and biosorption capability of nickel metal by *Halobacillus* sp. are available. Hence, this study aimed to evaluate kinetics, isotherms and effective parameters in biosorption of nickel by a native halophile bacterium (*Halobacillus* sp.) from the Miankaleh Wetland.

Materials and Methods

Study area and sampling: Sediment samples were taken from the Miankaleh Wetland at latitudes of 36°49'24" and 36°56'45", and longitude of 53°24'50" and 541'20" in the Golestan Province, Iran during fall 2015. Sediments were sampled from the top 5 cm, and stored in a plastic bag and transferred to the laboratory.

Isolation and identification of bacteria: The isolation of Gram-positive strains was carried out by culturing of the samples on moderate Halophilic media (MH) (NaCl 1.1 $g.L^{-1}$, $MgCl_2$. $6H_2O$ 7 $g.L^{-1}$, $MgSO_4$. $7H_2O$ 9.6 $g.L^{-1}$, $CaCl_2$. $2H_2O$ 0.36 $g.L^{-1}$, KCl 2 $g.L^{-1}$, $NaHCO_3$ 0.06 $g.L^{-1}$, NaBr 0.026 $g.L^{-1}$, Yeast extract 10 $g.L^{-1}$, Peptone 5 $g.L^{-1}$, Glucose 1 $g.L^{-1}$, and Agar 15 $g.L^{-1}$) (Gaballa et al., 2003), and Gram staining for 48 h at 30°C. The adjusted pH of the growth media was 7 which was made using 0.1 N H_2So_4 and/or NaOH. Further steps for preparation of samples were the same.

A strain with the maximum growth at the highest concentration of nickel was selected as the most resistant Gram-positive strain to nickel metal. Morphological characteristics (color and form of colony), Gram staining method, and biochemical tests (Simon Citrate, triple sugar iron, motion, indole, sulfide production, Methyl Red and Vogues-Proskauer) were done using Bergey's manual by sequencing of 16S rRNA gene to identify *Halobacillus* sp. KN57 (Bergey et al., 1930; Krieg et al., 1984).

Biosorption time and kinetic study: A solution containing 150 mg nickel nitrate and 1000 mL deionized water was prepared in an Erlenmeyer flask. A sample of zero time in kinetics (control) was isolated from this solution. Then, time was recorded by adding 1 $g.L^{-1}$ of bacterial biomass to the solution and placing flask in an incubator shaker at 30°C and 150 rpm. Samples were removed from the solution and filtered at different time periods (5, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180 and 240 min). Concentration of the residual nickel ion in the solutions (Ce) were measured by a flame atomic absorption spectroscopy (Agilent Varian AA240 FS) with three replications. Then, the biosorption was calculated at different times. The sorption rate was calculated using following equation:

$$q_e = (C_i - C_e)\frac{v}{m} \quad \text{eq. (1)}$$

Where qe is the metal uptake or biosorption ($mg.g^{-1}$), Ci=initial concentration of metal ($mg.L^{-1}$), Ce=metal concentration in solution ($mg.L^{-1}$), V=solution volume (L), and M=starting sorbent weight (g). The data of this stage were compared with the pseudo-first and second kinetic models (Ho and McKay, 1999; Ho, 2006). Pseudo-first kinetic model (equation 2) and its linear form (equation 3) are as follow (Lagergren, 1898; Ertugay and Bayhan, 2008):

$$\frac{dq}{dt} = k_1(q_e - q_t) \quad \text{eq. (2)}$$

$$\ln\frac{(q_e - q)}{q_e} = -k_1 t \quad \text{eq. (3)}$$

Pseudo-second kinetic model (equation 4) and its linear form (equation 5) also were (Chojnacka, 2010):

$$\frac{dq}{dt} = k_2(q_e - q)^2 \quad \text{eq. (4)}$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{1}{q_e}t \quad \text{eq. (5)}$$

Where qe and qt are the amount of metal biosorbed per unit weight ($mg.g^{-1}$ dry weight) of biosorbent at equilibrium at any time, t time (min),and k_1 and k_2 the rate constant of pseudo-first-order sorption (min^{-1}) and rate constant of pseudo-second-order sorption ($mg.g^{-1}$ min^{-1}), respectively.

Initial metal concentration and biosorption isotherms: To evaluate the isotherm of equilibrium sorption of nickel, amount of the nickel biosorption at various initial concentrations of metal ions (25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275 and 300 $mg.L^{-1}$) were investigated under constant conditions (pH=7, temperature=30°C, adsorbent concentration=1 $g.L^{-1}$, and salinity=10%). At last, the compatibility of the findings was investigated which resulted in the effect of the initial concentration of metal on biosorption with Langmuir and Freundlich isotherm models. Based on Langmuir isotherm model, biosorbent surface assumes homogeneous, no interaction between adsorbed molecules, and once a site is occupied by a species, no more sorption take place at that site, while the Freundlich isotherm model assumes adsorption on a heterogeneous surface, and there is interaction between adsorbed molecules in this model. Langmuir equations (Equation 6) and Freundlich (Equation 7) are as follows (Langmuir, 1918; Freundlich, 1906):

$$q_e = \frac{bq_m C_e}{1+bC_e} \quad \text{eq. (6)}$$

$$q_e = k_F C_e^{1/n} \quad \text{eq. (7)}$$

Where q_e is the amount of metal absorbed per unit weight of biosorbent ($mg.g^{-1}$), C_e = equilibrium metal concentration ($mg.L^{-1}$), q_m = maximum metal uptake of biosorbent ($mg.g^{-1}$), b = Langmuir constant ($L.mg^{-1}$), K_F = distribution coefficient, and n = correction factor (n>1).

Optimum pH, temperature, salinity, and adsorbent concentration: After obtaining the best initial concentration for optimal sorption (150 $mg.L^{-1}$), the experiments were performed to find the effect of pH (4-9), temperature (5-40°C), salinity (4-16% sodium chloride), and adsorbent concentration (0.5-2 $g.L^{-1}$) on the nickel biosorption in three replication. Unless otherwise stated, for the experiments of nickel sorption, pH, temperature, adsorbent concentration, and salinity were 7, 30°C, 1 $g.L^{-1}$, and 10%, respectively.

Biosorption by live and dead bacteria: In an Erlenmeyer flask, the nickel solution was prepared by solving 150 g of the nickel nitrate in 1000 mL deionized water (150 $g.L^{-1}$). Next, a reference sample was taken from the prepared solution, then it was shared between two 250 mL Erlenmeyer flask. The dead biomass, due to autoclaving of the living bacteria biomass, and living bacteria were added to solutions with the same concentration (1 $g.L^{-1}$) at temperature of 30°C for 10 min. At the end of experiment, the samples were taken by syringe and filtered, then concentration of the nickel in a solution was measured using AAS.

Surface morphology of the selected strain using scanning electron microscope: The liquid culture medium containing bacteria was centrifuged at 10000 rpm for 10 min, after filtering a wet biomass of bacteria was obtained. This biomass was divided into two parts, one part was exposed to the nickel metal and another one not. Then, both portions were dried in an oven for 48 h at 40°C. Scanning Electron microscopy (VEGA-LMU model manufactured by TESCAN USA) was applied to observe the bacteria surface without and with present of the nickel (KulKarini et al., 2014).

Fourier transforms infrared (FT-IR) spectroscopy: To identify the functional groups for sorption of Ni by bacteria, a FT-IR spectrophotometer was used. In order to prepare solid samples, the bacterial biomass without and with present of the nickel were placed in an oven at 40°C for 48 h and dried. The dried samples, which were completely powdered, mixed with Potassium Bromide (KBr) and converted to pill-shaped under a high pressure, and finally pills were examined by the FT-IR spectroscopy (Verma et al., 2017).

Results and Discussions

Isolation and purification: The bacterial strains (n=14) were isolated and purified from the sediment of the Miankaleh Wetland. After Gram staining, culturing of 9 isolated gram-positive halophile bacteria strains on salt-free nutrient agar culture medium resulted in the separation of halophile strains from salt tolerant, and 7 Gram-positive strains were selected to continue the experiment. Then, KN57 strain was selected as the most resistant strain to nickel by testing the minimum

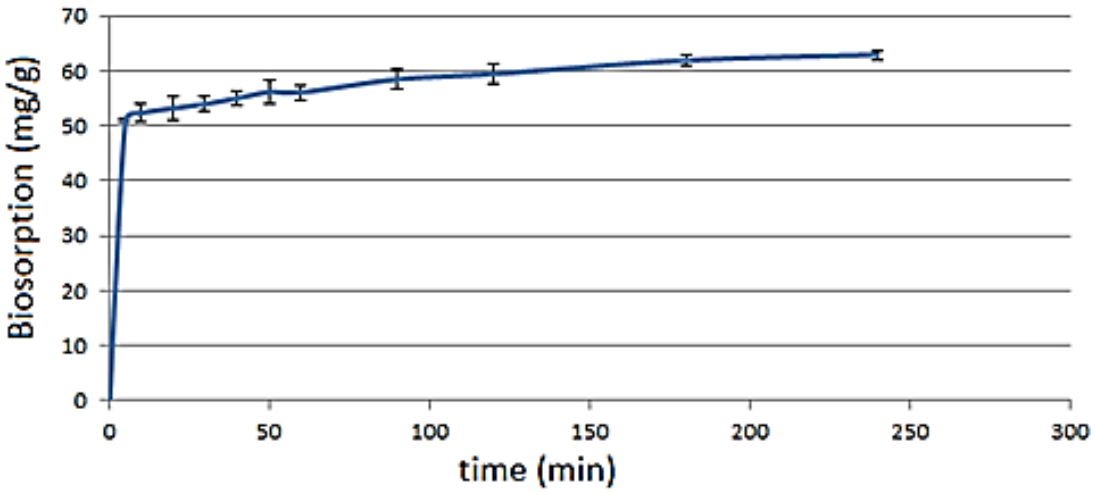

Figure 1. Biosorption of nickel metal by *Halobacillus* sp. (KN57) at different contact time.

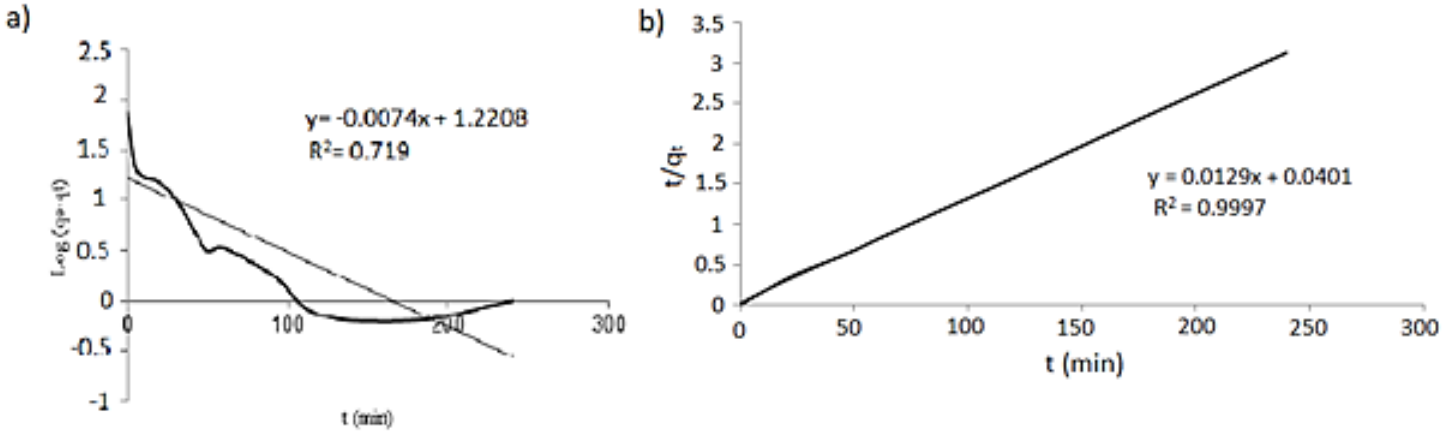

Figure 2. Pseudo first (a) and pseudo second order model (b) for the biosorption kinetics of Ni (II) by *Halobacillus* sp. (KN57).

concentration inhibiting bacterial growth.

Identification: According to the morphological characteristics of bacteria, the strain of *Halobacillus* sp. was identified by Gram staining and biochemical tests. The 16S rRNA gene sequence of the KN57 strain with access number of KY490583 has shown the highest match (98%) with *Halobacillus* sp. strains (Supplementaries 1-2).

Optimum biosorption time and kinetics: Biological sorption of bacteria occurs in most cases with two different mechanisms of biosorption (which is a fast, inactive, and independent of metabolism) and bioaccumulation (which is a slow, active, and dependent on cellular metabolism) (Goyal et al., 2003). Study of sorption time showed that biosorption of the nickel by KN57 strain had two stages. At the first, the absorption rate reached 54.52 $mg.g^{-1}$ over 5 min. In fact, at an initial 5 min of the experiment, 36.14% of the nickel metal was absorbed by the selected strain indicating metal binding to the bacteria surface, due to a large available surface area at the initial times, which accrues very fast. Up to 240 min, the absorption rate reached 76.75 $mg.g^{-1}$ of nickel, i.e. an average of 50.87% of the nickel was absorbed (Fig. 1). The biosorption over time showed no significant increase in sorption of metal after 100 min, due to the competition between metal ions for binding to active sites on bacteria surface in solution. Therefore, 100 min is selected as an optimum biosorption time.

In order to understand the mechanism of biosorption process, the experimental data of biosorption at different times (q_t) with first and second order kinetic models, diagrams of log (q_e-q_t) and t/qt versus t (time) were plotted, respectively. The results

Table 1. Parameters for pseudo first and second order models for Ni (II) biosorption kinetic by *Halobacillus* sp. (KN57).

Biosorption	Pseudo first order kinetic model			Pseudo second order kinetic model		
	q_e(mg.g^{-1})	k_1	R^2	q_e (mg.g^{-1})	k_2	R^2
KN57 strain	549.540	0.101	0.719	83.330	0.003	0.999

Table 2. Parameters of Langmuir and Freundlich model for adsorption of Ni (II) on *Halobacillus* sp. (KN57).

Biosorption	Langmuir			Frendlich		
	q_{max} (mg.g^{-1})	b (L.mg^{-1})	R^2	K_F (L.g^{-1})	n	R^2
KN57 strain	111.110	0.009	0.987	4.139	1.865	0.872

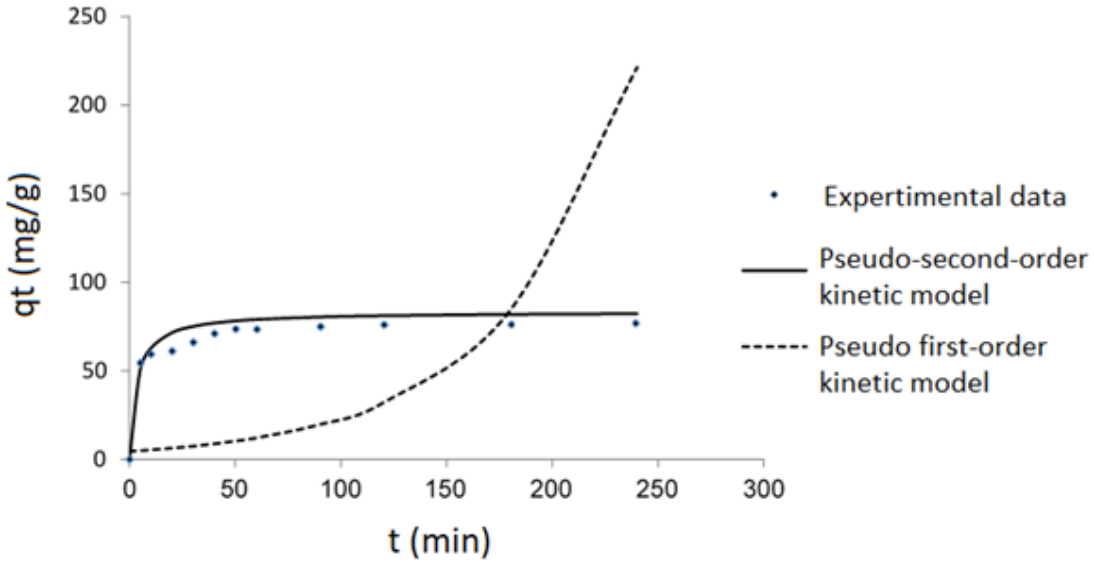

Figure 3. Comparing the experimental data with the non-linear form of pseudo-first and pseudo second order model for the biosorption of Ni (II) by *Halobacillus* sp. (KN57).

showed a higher value for correlation coefficient (R^2) of the pseudo second order model (R^2=0.999) than to the pseudo first order model (R^2=0.719) (Table 1). Moreover, the qt values derived from second order kinetic model were more fitted to experimental qt values at different times than first order kinetic model (Table 1, Fig. 2). Thus, our findings follow a second order kinetic model which this model explains chemisorption of Ni^{2+} by *Halobacillus* sp. (KN57) biomass as rate limiting step (Fig. 3). Some former studies on biosorption of metal by bacteria also reported that the kinetics of metal biosorption follow a pseudo-second order kinetics (Kulkarini et al., 2014; Masoumi et al., 2016; Tabaraki et al., 2013).

Optimum concentration of metal and biosorption isotherm: The initial concentration of metal is playing an important role for the metal absorption by a biosorbent in the solution (Abdel-Ghani and El-Chaghaby, 2014). In this study, biosorption of the nickel at various concentrations (25-300 mg.L^{-1}) showed that by increasing the nickel up to 150 mg.L^{-1}, biosorption increased under constant conditions (Fig. 4). At lower concentrations of nickel, an increase in concentration resulted in an increase of biosorption per unit weight of the sorbent which could be related to the greatest surface area of bacteria being available

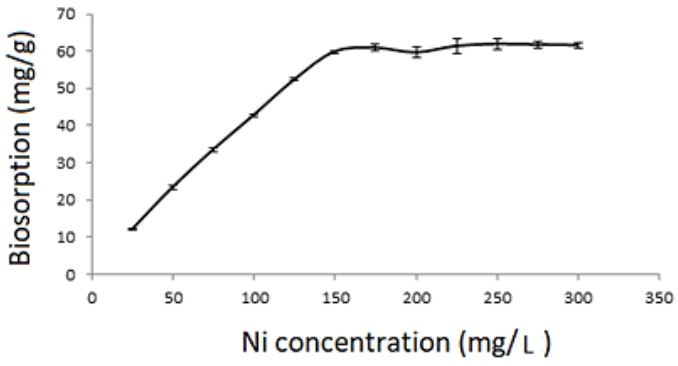

Figure 4. Effect of initial concentration of Ni (II) on biosorption of this metal by *Halobacillus* sp. KN57 (error bars represent ± standard deviations).

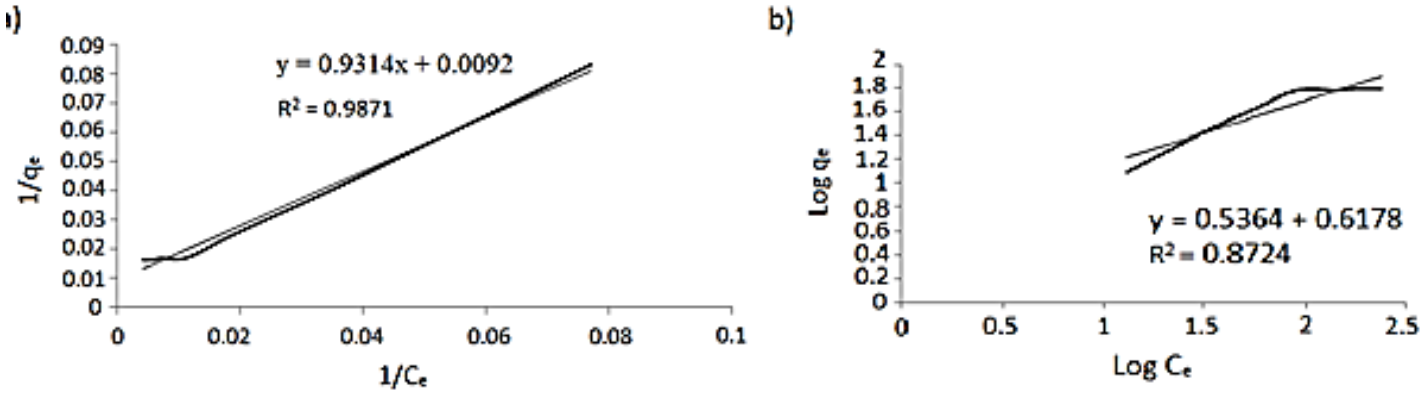

Figure 5. Plots for Langmuir (a) and Freundlich model (b) for adsorption of Ni (II) on *Halobacillus* sp. (KN57).

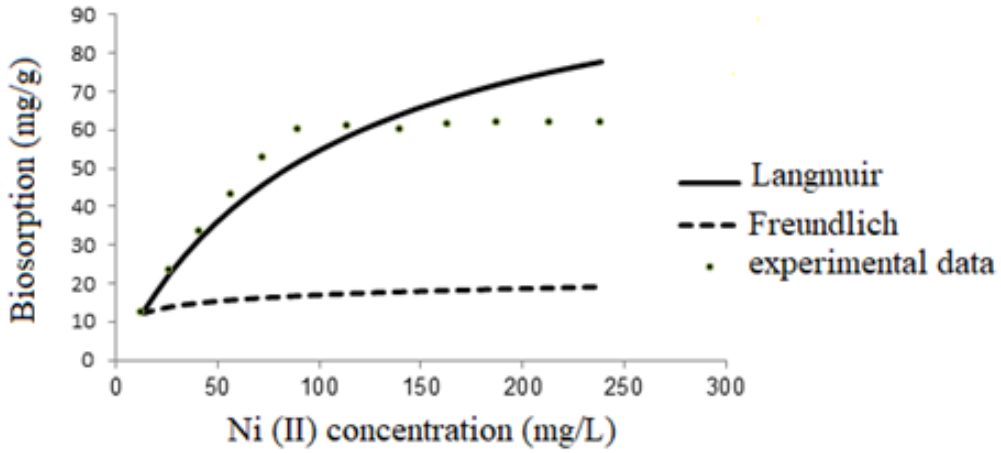

Figure 6. The non-linear form of Langmuir and Freundlich biosorption isotherms of Ni (II) by *Halobacillus* sp. (KN57).

(Kulkarini et al., 2014). At higher levels of initial concentration of the metal (> 150 $mg.L^{-1}$), this increase did not affect the biosorption rate, which could be due to the reduction of existing sites on the adsorbent surface comparing to the existing nickel ions in the solution. The similar results also reported in other studies (Kulkarini et al., 2014; Masoumi et al., 2016; Tabaraki et al., 2013).

Biosorption isotherms was investigated using Langmuir and Freundlich model at different concentration of the nickel metal to describe the relationship between the amount of metal adsorbed by bacterial biomass and concentration of the metal in the solution. The obtained experimental data was plotted the 1/qe versus 1/Ce based on Langmuir model, and also was plotted the log qe versus log C_e based on Freundlich model (Fig. 5a, b). The R^2 value obtained from Langmuir (R^2=0.987) and Freundlich model (R^2=0.872) (Table 2) showed that the experimental data were more consistent with the Langmuir model than the Freundlich model (Fig. 6). Thus based on the Langmuir model, *Halobacillus* sp. (KN57) surface is homogeneous and no more sorption take place at the saturated sites. This results can be applied for further design of adsorption species by *Halobacillus* sp. (KN57).

Effect of pH: pH is an important parameter for absorption of metal in aquatic solution by a bacterium (Cayllahua et al., 2009; Fan et al., 2014; Sarada et al., 2014; Masoumi et al., 2016). The effect of pH on the biosorption of nickel by KN57 strain is shown in Figure 7. The biosorption rate was small in low pH due to the presence of proton ions (H^+) and their competition with nickel ions. With increasing pH and decreasing H^+, the biosorption rate increased so that the maximum biosorption was observed at neutral pH (pH=7) (Huang and Liu, 2013). The absorption rate decreased with increasing pH from 7 to 9, due to low solubility of ions at high pH, and consequently nickel could be deposited in the form of hydroxide (Kulkarini

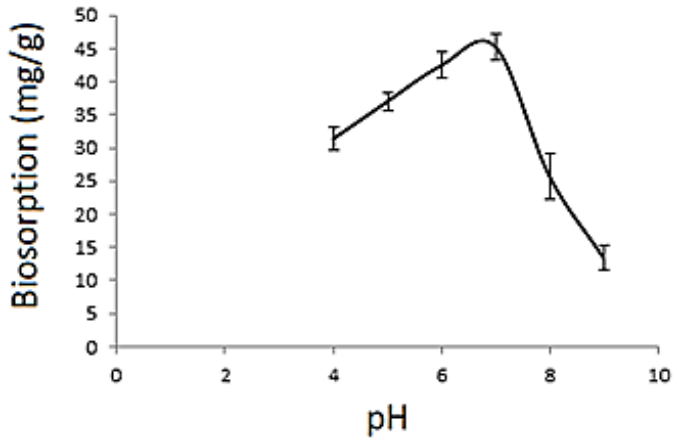

Figure 7. Effect of pH on biosorption of Ni^{2+} by *Halobacillus* sp. KN57 (error bars represent ± standard deviations).

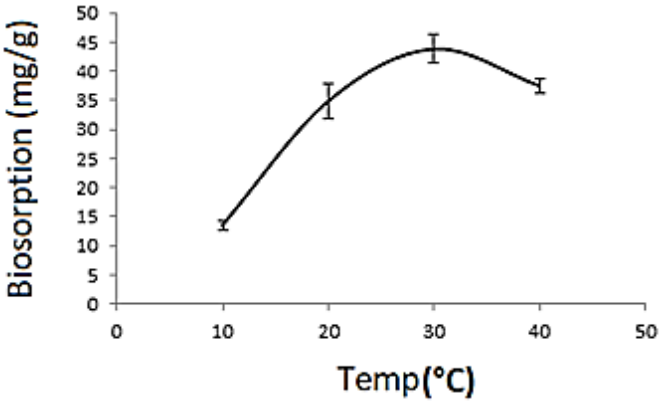

Figure 8. Effect of temperature on biosorption of Ni^{2+} by *Halobacillus* sp. KN57 (error bars represent ± standard deviations).

et al., 2014; Masoumi et al., 2016). Our finding was in line with those of Kulkarini et al. (2014).

Effect of temperature: Effect of temperature on biosorption of metal by microorganisms is species specific (Fan et al., 2014; Kulkarini et al., 2014; Masoumi et al., 2016). It is well-known that temperature is an important parameter which affects microbial growth and metabolism (Fan et al., 2014). In this study, nickel biosorption at different temperature under constant conditions showed that biosorption increased by increasing temperature up to 30°C and after that, the biosorption was decreased (Fig. 8). Increasing biosorption by temperature rise can be due to increasing the size of pores on the adsorbent surface, creating new active sites, increase the speed of some slow sorption steps or increase mobility of metal ions. However, excessive increase in this factor can lead to the destruction of the physical

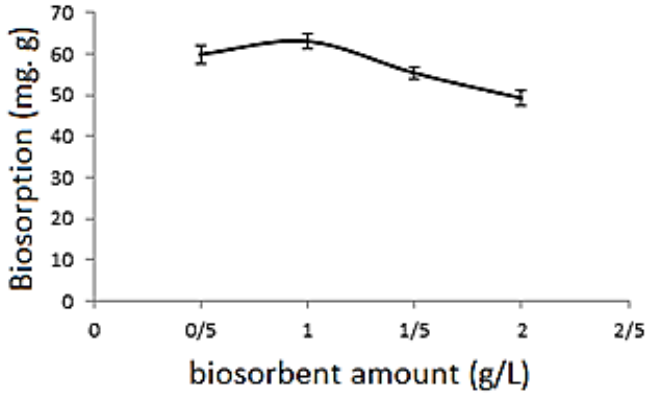

Figure 9. Effect of Ni concentration on biosorption of metal by *Halobacillus* sp. KN57 (error bars represent ± standard deviations).

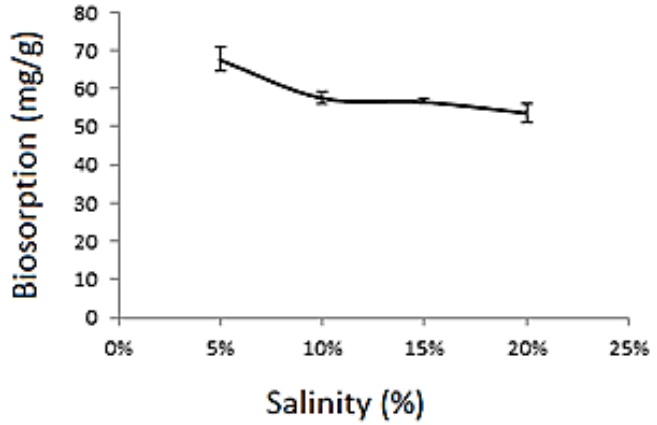

Figure 10. Effect of salinity on biosorption of Ni^{2+} by *Halobacillus* sp. KN57 (error bars represent ± standard deviations).

Figure 11. Biosorption of Ni^{2+} by live and dead biomass of *Halobacillus* sp. KN57 (error bars represent ± standard deviations).

structure (Tabaraki et al., 2013).

Biosorbent concentration: For, absorption of the nickel at different concentrations of biosorbent (0.5, 1, 1.5 and 2 $g.L^{-1}$) under constant conditions, it was observed that absorption increased by raising the concentration of biosorbent up to 1 $g.L^{-1}$, but when the

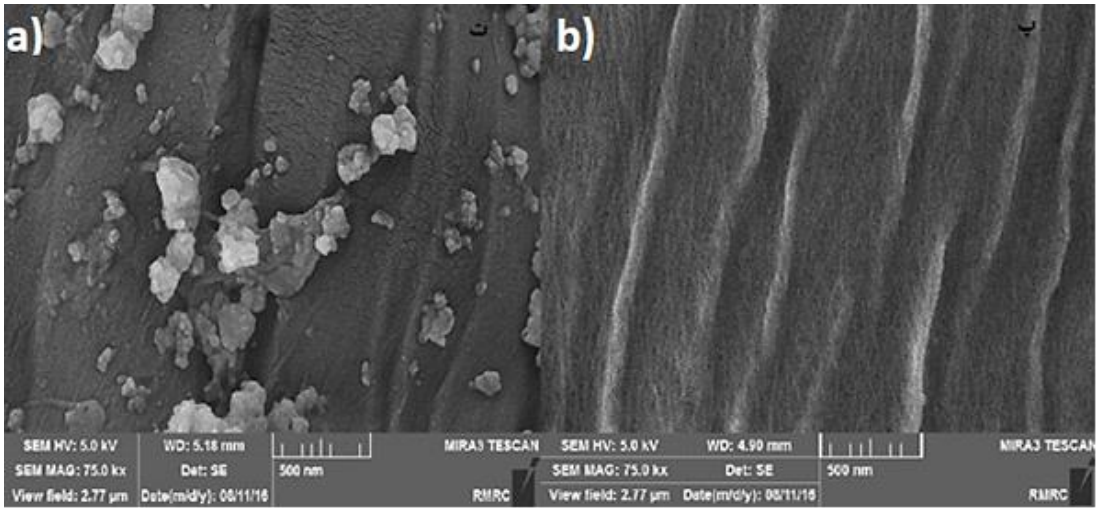

Figure 12. Morphology of the surface of *Halobacillus* sp. KN57 before (a) and after (b) adsorption of nickel with scanning electron microscopy (SEM).

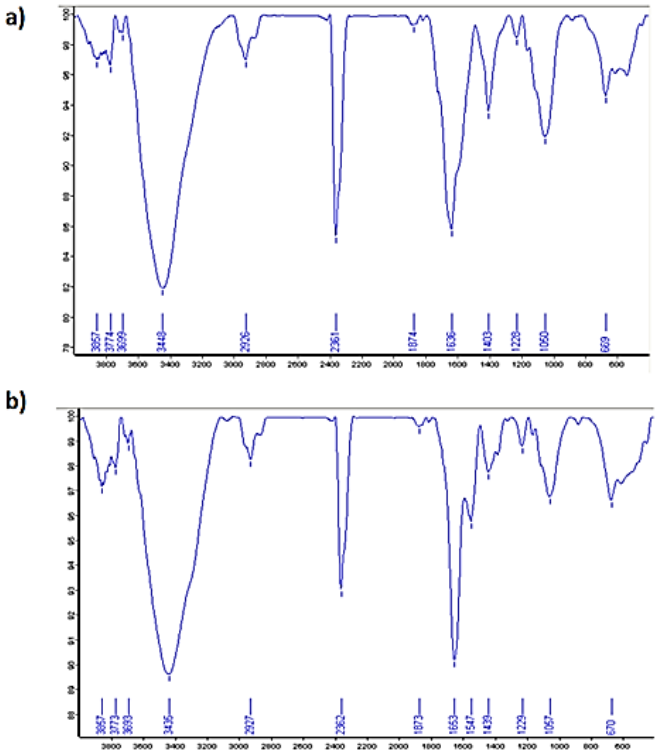

Figure 13. FT-IR spectrum for *Halobacillus* sp. KN57 before (a) and after (b) adsorption of Ni^{2+}.

biosorbent was more than 1 $g.L^{-1}$, the adsorption was reduced (Fig. 9). The increase of biosorbent concentration led to the increase of metal absorption attributed to the availability of the more surface area, and the biosorption reduction at a high concentration of biosorbent could be related to the complex interaction of different factors in the solution (Masoumi et al., 2016).

Effect of salinity: The effect of salinity on adsorption under constant conditions showed that the absorption rate decrease with increasing salt content (Fig. 10). The increase in Na^+ ion resulted in declining absorption due to the competition between Na^+ ions and heavy metal cations in absorption to the bacterial binding sites (functional groups with negative charge) (Deschatre et al., 2013).

Biosorption potential of live and dead bacteria: The nickel sorption by live and dead mass under constant conditions revealed that the absorption rate was 69.76 $mg.g^{-1}$ in living and 44.08 $mg.g^{-1}$ in the dead (autoclaved) bacteria (Fig. 11). Therefore, the highest rate of biosorption was inactive and independent of metabolism that occurred quickly. The cell metabolism depended active biosorption was carried out at a lower rate with a lesser extent which obtained by the difference between the biosorption of live and dead mass (i.e. 25.68 $mg.g^{-1}$). Therefore, the live mass of the selected bacteria as native bacterium has a high capacity for removal of metal from the Miankaleh Wetland, and dead mass has a high potential to be produced on large scale in industries due to its metabolic independent sorption process.

Scanning electron microscopy (SEM): SEM examination revealed the adsorption of Ni^{2+} by the surface of *Halobacillus* sp. KN57 (Fig. 12).

Fourier transform infrared (FTIR) analysis: The results of the Fourier transform infrared spectroscopy

in the absence and presence of nickel showed a shifts of the bands and change in the intensity of biosorption peaks (Fig. 13). The change in the biosorption peak intensity at wavelengths of 3773 cm^{-1} and 3857 cm^{-1} indicates interaction of the nickel metal with amide groups. At a wavelength of 3435 cm^{-1}, the stretching band of hydroxyl (O-H) was observed, which after exposure to nickel does not differ significantly in terms of the biosorption and displacement. The stretching band at the wavelength of 12362 cm^{-1} is related to the cyanide functional group (≡ NC), which does not have an effect on biosorption as this functional group does not form a complex with heavy metals. Biosorption peaks in the regions of 1653 cm^{-1} and 1547 cm^{-1} are related to the carbonyl (C-O) and amine (C-N) groups that considering the change in sorption peak intensity and peak displacement of 100 nm. Then it is concluded that the functional groups of carbonyl and amine are highly effective in biosorption. In the wavelength of 1057 cm^{-1}, the C-H bending band can be observed, which has no effect on biosorption.

Conclusion

This study demonstrated that biosorption of the nickel by *Halobacillus* sp. KN57 is highly dependent on pH, temperature, biosorbent concentration, salinity, and contact time. The live biomass of the selected bacteria had a higher biosorption of metal compared to dead biomass which suggests both dead and live bacteria from the Miankaleh Wetland can play an important role for sorption of metals in wastewater treatment and aqueous ecosystems. The SEM examination confirmed the biosorption of metal on the surface area of *Halobacillus* sp. KN57. The FT-IR analysis identified functional groups involved for metal absorption by *Halobacillus* sp. KN57. The biosorption of nickel ions by *Halobacillus* sp. KN57 follows pseudo-second order kinetic model indicating chemisorption as rate limiting step. Based on Langmuir model, the maximum biosorption capacity of nickel ions by *Halobacillus* sp. KN57 was 111.11 $mg.g^{-1}$. In conclusion, the kinetic and isotherm models allowed to gain insight into the biosorption mechanism of nickel ions by *Halobacillus* sp. KN57. This study based on kinetics study, Langmuir and Frendlich model, effective factors, biosorption capacity of dead and live bacteria, SEM examination, and FT-IR analysis suggests that *Halobacillus* sp. KN57 is a promising biosorbent for removal of metal specially nickel from contaminated sites.

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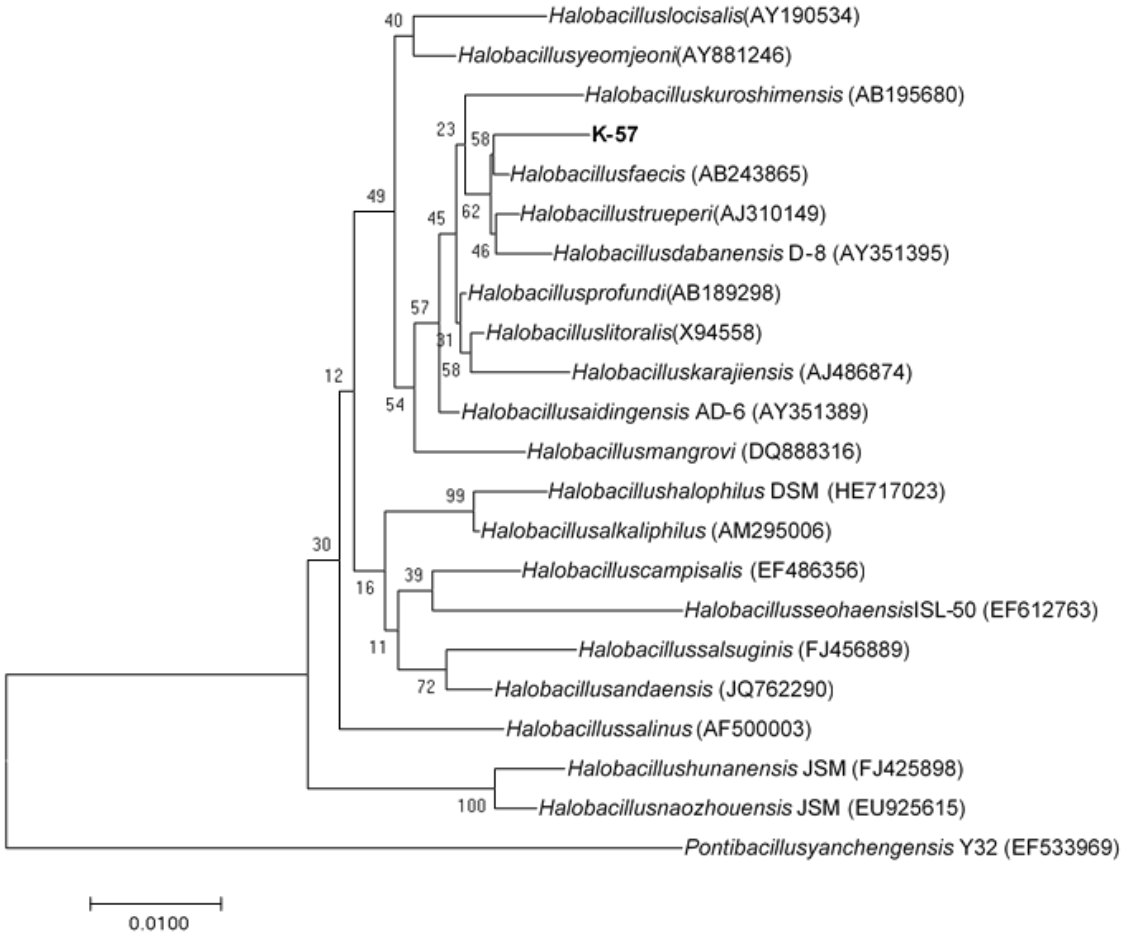
Supplementary 1: The results of morphological investigation and biochemical test for KN57 strain.

Colonies color	Dark cream
Colonies shape	Spherical
Gram staining	Positive
Bacterial cell shape	Bacillus
Motility	Negative
Sulfide	Negative
Indole	Negative
Triple sugar iron	Negative
Simmons citrate	Negative
Methyl-red	Negative
Vogesproskaure	Pink ring negative
Gelatinase	Negative
Identification	*Halobacillus* sp.



Supplementary 2: Phylogenetic tree of KN57 using Neighborhood-joining and the Bootstrap based on thousand replications.