

BIOSORPTION OF Pb (II) AND Cd (II) BY *HALOMONAS SP.*

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(Received 2 February, 2021; Accepted 2 March, 2021)

Key words: Lead (II), Cadmium (II), Biosorption, Moderate halophile, *Halomonas sp.*

Abstract – In this study, the biosorption ability of the halophilic bacterial isolate H1S2 biomass was investigated for Pb (II) and Cd (II) removal from aqueous solution. The bacterial isolate H1S2 was identified using the 16S rRNA gene sequence analysis as *Halomonas meridiana* H2. The parameters influencing the biosorption process were optimized such as initial metal concentration, biomass dose and contact time. The optimum dried biomass dose of *H. meridiana* H2 for Pb (II) and Cd (II) metal ions removal was 50 mg. The optimal contact time for the removal of Pb (II) and Cd (II) metal ions by *H. meridiana* H2 was 10 and 15 minutes respectively. The Langmuir, Freundlich and Dubnin–Redushkevich isotherm models were applied. The equilibrium data obtained from the experiments fitted well with the Langmuir and Freundlich isotherms. The kinetic data was also studied and found to be following pseudo-second order model.

INTRODUCTION

Environmental contamination with heavy metals is increasing rapidly due to the development of industries that discharge these pollutants into the environment (Arivalagan *et al.*, 2014). Once heavy metals are released into the environment, they persist in the environment and tend to bioaccumulate in living tissues and bio magnify throughout the food chain causing harm to human health and the ecosystem (Ali *et al.*, 2019). Therefore the removal and detoxification of these metals is a crucial target that should be reached.

Among these metals, lead and cadmium are extremely poisonous even at low amounts and are considered among the non-essential heavy metals which are not needed by the living organisms (plants, animals and humans) (Ali *et al.*, 2019). Lead has many detrimental effects on the human health through harming the brain, kidney, reproductive system, nervous system, bones, immune system and heme production (Chen *et al.*, 2015; Cai *et al.*, 2018). The USEPA and WHO specified the permissible limit of lead in drinking water to 0.015 mg/l and 0.01 mg/l respectively (WHO, 2004; El-Naggar *et al.*, 2018). Cadmium also affects humans by causing

cancer, elevated blood pressure and affecting the kidneys, lungs and bones (Hou *et al.*, 2015). USEPA and WHO reported that the acceptable limit of Cd²⁺ in drinking water is 0.005 mg/l (Kumar and Puri, 2012), and it has categorized Cd²⁺ as group B1 carcinogen (Arivalagan *et al.*, 2014).

There are several conventional methods, such as ion exchange, precipitation, reverse osmosis, filtration and evaporation, applied to remove these heavy metals. However, these methods turned out to have many limitations at the level of cost and efficiency in addition to disadvantages and adverse effects on the environment. Biological processes including biosorption and bioaccumulation are considered a better alternative. Biosorption and bioaccumulation are defined as the use of biological materials to sequester metal ions from aqueous solutions either by passive removal or active uptake respectively. The use of biological material, including living and dead biomass, has gained a great attention since it is environment friendly and efficient with low cost (Mohapatra *et al.*, 2017).

The present study deals with the characterization of the Biosorption of lead and cadmium metal ions by halophilic bacteria. The parameters influencing the biosorption process of these metal ions, such as

the amount of biomass, initial metal concentrations and contact time were investigated using the batch method.

MATERIALS AND METHODS

Isolation and preparation of bacterial biomass

Water samples were collected from Solar Saltern located in Anfeh, North Lebanon. Halophilic bacterial isolate, H1S2 was isolated and cultured using marine broth medium, consisting of (g/L⁻¹) NaCl, 81; MgCl₂, 7; MgSO₄·7H₂O, 9.6; CaCl₂, 0.36; KCl, 2; NaHCO₃, 0.06; NaBr, 0.026; yeast extract, 10; protease peptone, 5; glucose, 1 and agar, 20 (Atlas, 2005). One milliliter of water sample was inoculated in 50 ml of marine broth medium in a 250 ml capacity Erlenmeyer flask and incubated overnight in a shaker incubator (ZHWY-2102C) with 150 rpm at 30 ± 2°C. Following the period of incubation, the bacterial culture suspension was autoclaved. Then the bacterial cells were harvested by centrifugation at 6000 rpm for 15 minutes and washed three times with sterile deionized distilled water and dried until constant weight was obtained.

Characterization of the bacterial isolate

The isolate was examined for some of its characteristics such as Gram reaction, catalase, oxidase, citrate utilization, Voges-Proskauer, Hydrogen sulfide and indole production, gelatin liquefaction in addition to starch, lipids, cellulose and pectin hydrolysis (Barrow and Felthman, 1993).

Molecular identification of the bacterial isolate

The 16S rRNA sequence analysis was carried out for the molecular identification of the bacterial isolate H1S2 at Sigma Scientific Services Company, Egypt. The PCR product was sequenced by the GATC Company using ABI 3730xl DNA sequencer with universal primers (16S 27F and 16S 1492R). The forward primer was: 27F (AGA GTT TGA TCC TGG CTC AG) and the reverse primer was: U1492R (GGT TAC CTT GTT ACG ACT T) (Abd-Elnaby *et al.*, 2016). The purified product was sequenced and analyzed at the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST>) using the basic local alignment search tool (BLAST). Based on the percent homology scores, the bacterial isolate was identified. Phylogenetic tree was constructed using MEGA version 3 software (Kumar *et al.*, 2004). The partial sequence of 16S rRNA gene of the isolate was submitted to NCBI

GenBank, and an accession number was assigned.

Preparation of lead (II) and cadmium (II) solutions

Stock solutions were made for each heavy metal with a concentration of 1000 mg/l by dissolving lead (II) nitrate Pb(NO₃)₂ and cadmium (II) nitrate Cd(NO₃)₂ salts in deionized distilled water. Then each required concentration was prepared by dilution using deionized distilled water.

Biosorption of Pb(II) and Cd(II) by dead biomass

The parameters influencing the biosorption process including initial metal concentration, biomass dose and contact time were investigated. Biosorption experiments were conducted at 30 ± 2 °C in batch experiments in polypropylene tubes containing 20 ml of tested metal solution.

Effect of initial metal concentration

To study the effect of initial metal concentration of Pb (II) and Cd (II) metal ions on the biosorption process, different concentrations of each heavy metal were prepared (20, 100, 200 and 500 mg/L of Pb (II) and 10, 100, 200 and 500 mg/l of Cd (II)). Fifty mg of bacterial biomass were transferred to each metal solution and incubated for 1 hour at 30 ± 2 °C. Then the bacterial biomass was separated from the metal solution by filtration through 0.45 µm filters. The amount of residual metal in the filtered samples was measured using Atomic Absorption Spectrophotometer (Thermo Scientific iCE 3000 series). The percentage of Pb(II) and Cd(II) removal (% R) was calculated using the following equation:

$$\% (R) = (C_i - C_f) / C_i \times 100 \quad \dots \text{Eq. (1)}$$

C_i is the initial and C_f is the final concentration of heavy metal (mg/L) in water.

The uptake capacity, q_{eq} (mg.g⁻¹) amount of metal ion taken up per unit of mass of bacterial pellet, was calculated using the following equation:

$$q_{eq} = \frac{(C_i - C_{eq}) \times V}{m} \quad \dots \text{Eq. (2)}$$

In Eq. (2), V denotes the sample volume (ml), c_i and c_{eq} are the initial and equilibrium concentrations (mg/l) respectively, and m is the amount of the bacterial pellet used.

Effect of biomass dose

The effect of biomass dose on the removal of heavy metals (Pb(II) and Cd(II) metal ions) was studied at different dosages of the bacterial biomass (10, 15, 25,

50 and 100 ml dry weight). Each heavy metal solution (20 ml) (100 mg/l of Pb(II) and Cd(II)) was added separately to the bacterial biomass and incubated for 1 hour at 30 ± 2 °C. Then the samples were filtered and analyzed for the residual Pb(II) and Cd(II) concentrations as mentioned in the procedure of the previous section. The uptake capacity was calculated using Eq. (2).

Effect of contact time

To evaluate the effect of kinetics on the biosorption of Pb (II) and Cd (II) metal ions by the dried bacterial biomass, the optimum metal concentration with the optimum biomass weight were applied but at different periods of time (5, 10, 15, 30, 45, 60 and 120 min). Then the samples were filtered and analyzed for the residual Pb(II) and Cd(II) concentrations as mentioned in the procedure of the previous section. The removal percentage and the uptake capacity were calculated using Eq. (1) and Eq. (2) respectively.

Biosorption kinetics

To describe the kinetics of biosorption, the pseudo-first-order and pseudo-second-order kinetic models were utilized. The pseudo-first order model points that the rate of adsorption sites occupation is proportional to the number of unoccupied sites (Cruz *et al.*, 2004).

The linear equation for this model is:

$$\text{Log}(q_e - q_t) = \text{Log} q_e - (K_1 / 2.303) t \quad \text{Eq. (3)}$$

where q_e and q_t are the amounts of metal ions adsorbed at equilibrium and at given time (t), respectively (mg/g) onto the biosorbent surface, and K_1 is the rate constant of the first-order biosorption. Linear plots of $\text{Log}(q_e - q_t)$ versus t indicate if this kinetic model is applicable.

In the pseudo-second order model, it is assumed that the rate of the occupation of adsorption sites is proportional to the square of the number of unoccupied sites (Cruz *et al.*, 2004).

Linear equation for this model is:

$$t/q_t = (1/K_2 \cdot q_e) + (1/q_e) t \quad \text{Eq. (4)}$$

where q_e and q_t are the amounts of metal ions adsorbed on the biosorbent at equilibrium and at any time (t), respectively (mg/g), and K_2 is the rate constant of second-order biosorption (mg/mg min). The plot of t/q_t versus t shows a straight line if second order kinetics is applicable.

The well fitted data to the model was evaluated by the coefficient of determination R^2 value.

Biosorption Isotherms

The evaluation of the equilibrium data for the biosorption of Pb (II) and Cd (II) metal ions onto H1S2 bacterial biomass was carried out by the Langmuir, Freundlich and Dubinin Radushkevich isotherm models. The Langmuir and Freundlich isotherm models represent the equilibrium amount of metal removed (q_e) as a function of the equilibrium concentration (C_e) of metal ions in the solution (Dada *et al.*, 2012).

The linear Langmuir equation is written as follows:

$$C_e/q_e = (C_e/q_{\max}) + (1/b \cdot q_{\max}) \quad \text{.. Eq. (5)}$$

where q_e is the equilibrium biosorption capacity of biomass in mg heavy metal/mg of biomass, C_e is the equilibrium concentration of metal ion in mg/l, q_{\max} is the maximum amount of metal sorbed in mg heavy metal/mg of biomass, and b is the constant that is referred to the bonding energy of sorption in mg/l. Langmuir isotherm assumes that the solid surface presents a finite number of identical sites where a monolayer is formed when the solid surface reaches saturation.

The linear Freundlich equation is written as follows:

$$\text{Ln} q_e = \text{Ln} K + (1/n) \text{Ln} C_e \quad \text{Eq. (6)}$$

where q_e is the equilibrium biosorption capacity of the biomass in mg heavy metal/mg biomass, C_e is the equilibrium concentration of metal ion in mg/l, and K (in mg/l) and 1/n are constants related to the sorption capacity and intensity, respectively. Freundlich isotherm model can be applied to multilayer adsorption, with non-uniform distribution of adsorption heat and affinities over the heterogeneous surface. Moreover, the correlation coefficient R^2 values were used to obtain the best-fit linear equation.

The Dubinin–Radushkevich isotherm model (Dubinin and Radushkevich, 1947) was utilized to estimate the biosorption mechanism whether physical or chemical with a Gaussian energy distribution onto a heterogeneous surface.

The linear equation for this model is:

$$\text{Ln} q_e = \text{Ln} q_D - 2B_D RT \text{Ln} \left(1 + \frac{1}{C_e} \right) \quad \text{Eq. (7)}$$

Where, q_e is the amount of heavy metal adsorbed (mg/g) at equilibrium per unit weight of bacteria, q_D is the maximum biosorption capacity (mg/g), B_D constant of Dubinin-Radushkevich, R the gas

constant (8.314 kJ /mol K), T the absolute temperature (K), and C_e is the equilibrium concentration of heavy metal in solution (mg/L). The mean free energy expresses the energy for taking out a molecule from its location in the sorption space to the infinity, the model was usually useful to distinguish whether the biosorption was physical or chemical (Dubinin, 1960). E per molecule of biosorbent can be calculated by:

$$E = \frac{1}{\sqrt{2BD}} \quad \text{Eq. (8)}$$

RESULTS AND DISCUSSION

Characterization of the halophilic bacterial isolate

The isolate H1S2 was characterized and identified at the morphological, biochemical and molecular levels. The isolate was gram negative, motile and rod shaped. It was positive for catalase, oxidase, Voges-Proskauer, lipase and pectinase. It revealed a negative result for the production of amylase, gelatinase, cellulase, urease, hydrogen sulfide and indole. And it was unable to utilize citrate. The 16S rRNA gene sequence of the isolate H1S2 search was performed by using the BLAST program that showed a close genetic relation of H1S2 with the rRNA sequence of *Halomonas meridiana* strain NBRC 15608 (NR 113779.1) with 99% similarity. The isolate

was depicted as *Halomonas meridiana* H2 and was submitted to Gen-Bank (GenBank accession number MK 357744). A phylogenetic tree constructed by MEGA version 3 software based on 16S rRNA partial sequence is presented in Figure 1.

Biosorption of Pb(II) and Cd(II) by dead biomass

Determination of the optimum parameters affecting the biosorption of metal ions was done by changing the values of the following factors, such as the initial metal ion concentration, the amount of dry cell and contact time.

Effect of initial metal concentration

The results (Figures 2 and 3) showed that Biosorption of both heavy metals increased as the initial concentrations of both heavy metals become higher, and this was due to an increase in electrostatic interaction between surface sites and metal ions. When initial metal concentration of Pb (II) metal ion increased from 20 to 500 mg l⁻¹, the biosorption increased from 3.5 to 76 mg g⁻¹ dry weight. When the initial metal concentration of Cd (II) increased from 10 to 500 mg l⁻¹, the biosorption increased from 0.6 to 25.9 mg g⁻¹ dry weight. But the removal percentage of both heavy metals by the bacterial isolate decreased with the increase in initial metal concentration. These results were in agreement with the findings of Özdemir *et al.*,

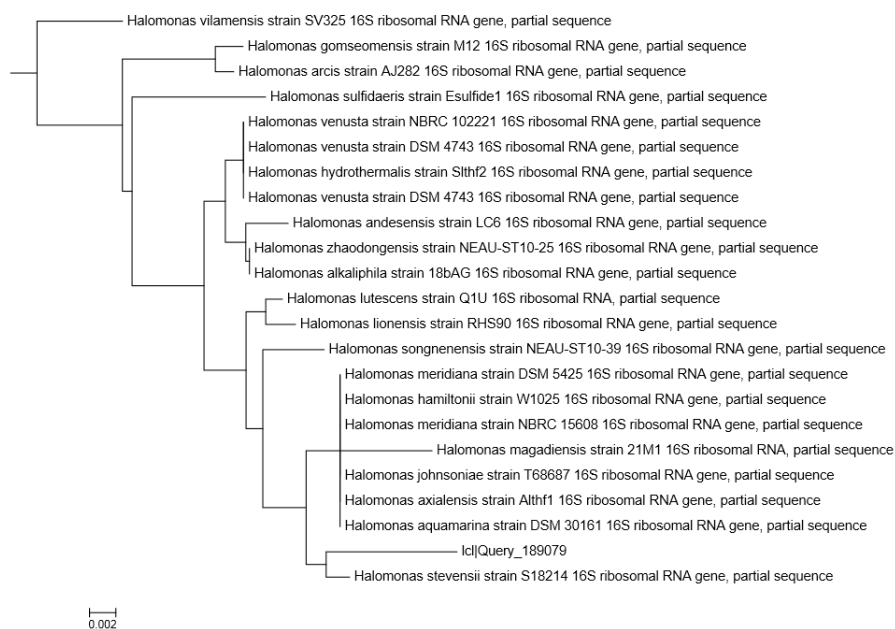


Fig. 1. Phylogenetic tree showing the position of the isolate H1S2 with the other members of *Halomonas* genus based on 16S rRNA gene sequence. The selected isolate was identified as *Halomonas meridiana* H2 and submitted under accession number (MK 357744)

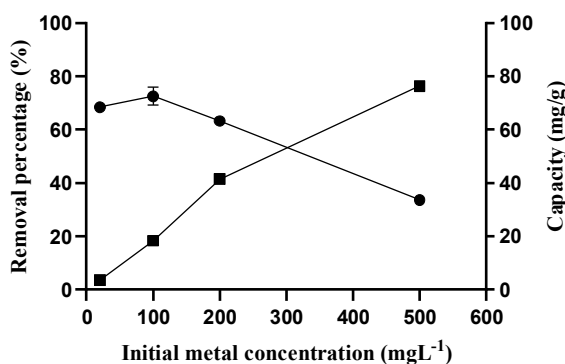


Fig. 2. Removal of Pb(II) by *Halomonas meridiana* H2 (50 mg dead biomass) incubated at 30 ± 2 °C for 1 hour.

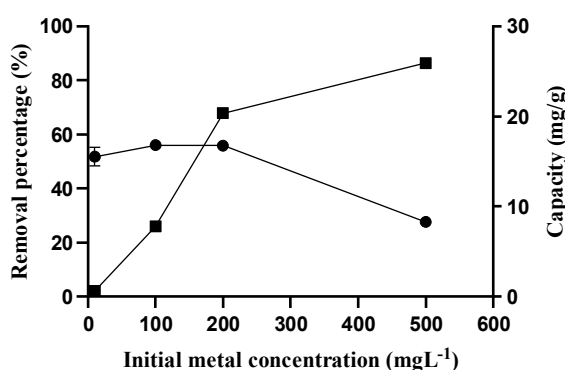


Fig. 3. Removal of Cd(II) by *Halomonas meridiana* H2 (50 mg dead biomass) incubated at 30 ± 2 °C for 1 hour.

(2009); Özdemir *et al.*, (2013) and Wang and Chen, (2009).

Effect of biomass dose

Figures 4 and 5 showed that when the dried biomass weight increased from 10 to 50 mg, the Pb

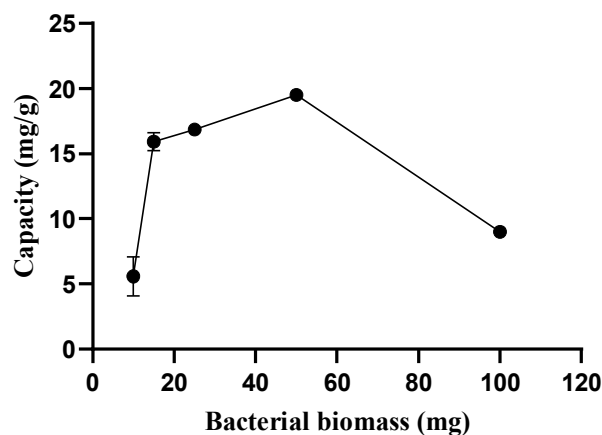


Fig. 4. Effect of bacterial biomass of *Halomonas meridiana* H2 on the removal of Pb(II) and Cd(II) (100 ppm) incubated at 30 ± 2 °C.

(II) biosorption increased from 5.4 to 19.5 mg g⁻¹ and the Cd (II) biosorption also increased from 1.6 to 8 mg g⁻¹. Beyond this mass, the biosorption capacity decreased to 9 and 3.9 mg g⁻¹ for Pb (II) and Cd (II) metal ions, respectively. This has been found to be due to interference between binding sites at higher biomass concentrations (Al-Garni, 2005; Özdemir *et al.*, 2013).

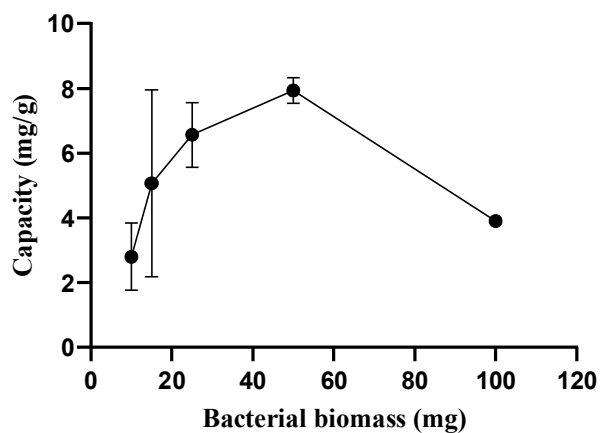


Fig. 5. Effect of bacterial biomass of *Halomonas meridiana* H2 on the removal of Cd(II) (100 ppm) incubated at 30 ± 2 °C.

Effect of contact time

Figures 6 and 7 showed that metal Biosorption by *Halomonas meridiana* H2 was a rapid process that took place within few minutes. The highest Pb (II) uptake by dried cells of *Halomonas meridiana* H2 was 23.4 mg g⁻¹ dry weight at 10 minutes, and that of Cd (II) was 11.6 mg g⁻¹ dry weight at 15 minutes. This capacity did not show any significant increase when contact time increases up to 120 minutes. This finding was in agreement with Boyanov *et al.*,

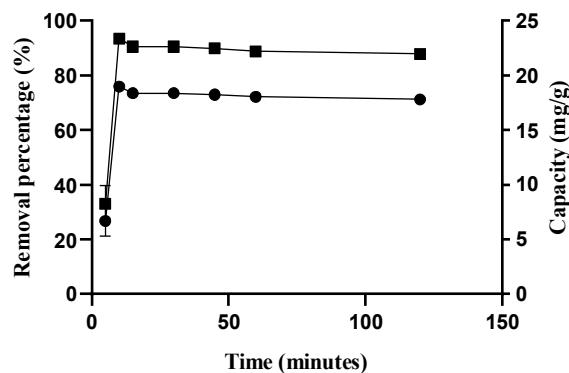


Fig. 6. Kinetics of the removal of Pb(II) (100 ppm) by *Halomonas meridiana* H2 (50mg) incubated at 30 ± 2 °C.

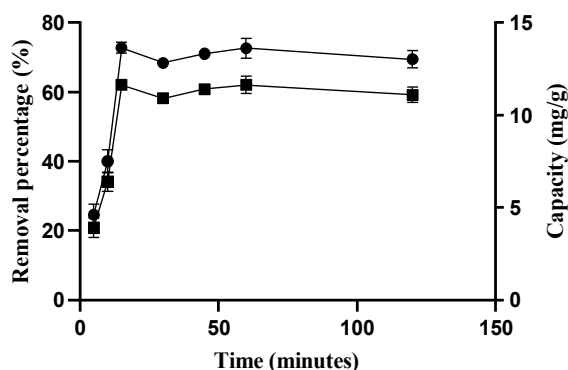


Fig. 7. Kinetics of the removal of Cd(II) (100 ppm) by *Halomonas meridiana* H2 (50 mg) incubated at 30 ± 2 °C.

(2003); Zouboulis *et al.*, (2004) and Sahin and Öztürk, 2005.

Biosorption kinetics

The pseudo-first-order kinetic model is the plot of $\text{Log}(q_e - q_t)$ versus time. The linear regression of the pseudo-first-order kinetic model did not fit the experimental data. Therefore, the pseudo-second-

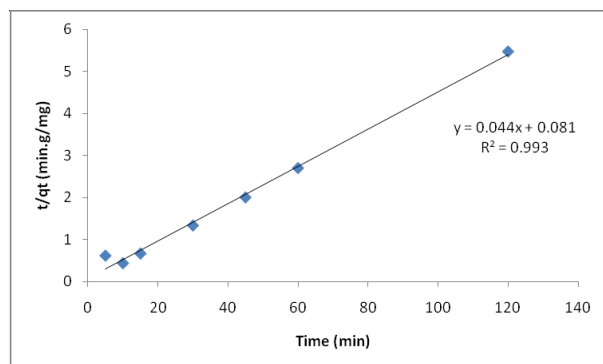


Fig. 8. Pseudo second-order biosorption kinetics of Pb(II) by *Halomonas meridiana* H2 biomass

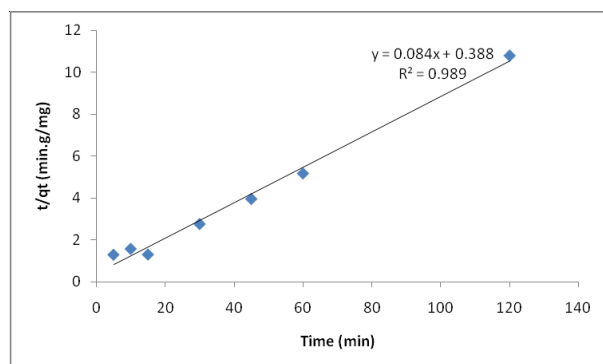


Fig. 9. Pseudo second-order biosorption kinetics of Cd(II) by *Halomonas meridiana* H2 biomass

order kinetic model was used to analyze the biosorption kinetics of Pb (II) and Cd (II) metal ions. The pseudo-second-order kinetic model is the plot of t/q_t versus time. The pseudo-second-order model fitted the experimental data with $R^2 = 0.99$ (Figures 8 and 9) (Masoudzadeh *et al.*, 2011; Chakravarty and Banerjee, 2012; Huang and Liu, 2013; Tafakori *et al.*, 2017).

Biosorption isotherms

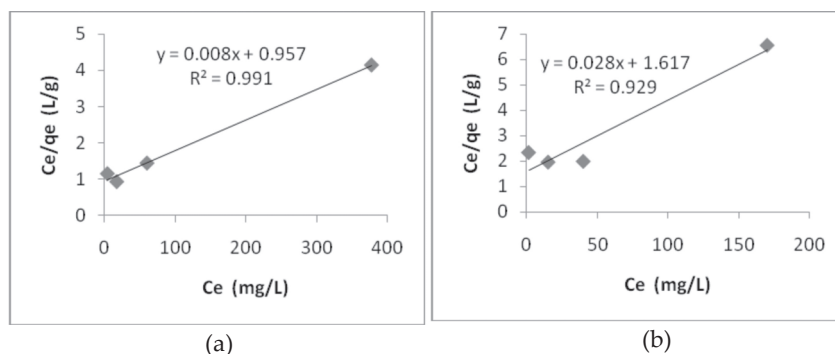
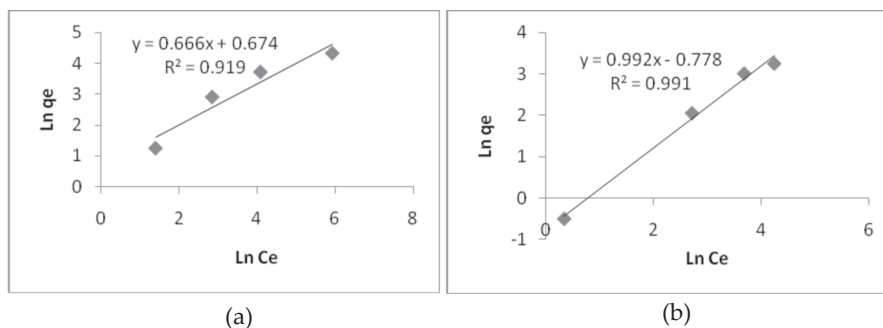
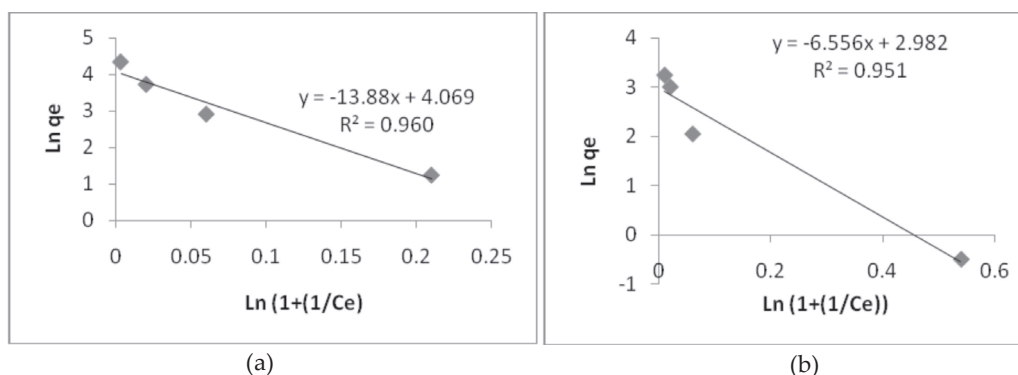
The Langmuir, Freundlich and Dubinin-Radushkevich isotherms data of heavy metal biosorption onto the biomass of *Halomonas meridiana* H2 are provided in Table 1 and illustrated in Figures 10, 11 and 12 respectively.

The Langmuir parameters indicated a maximum biosorption capacity of 119.05 and 35.71 mg/g, with energy parameter of 0.009 and 0.017 for Pb (II) and Cd (II), respectively. Moreover, these results revealed that the biosorption of Pb (II) and Cd (II) by *Halomonas meridiana* H2 biomass fit the Langmuir biosorption isotherm, since the correlation coefficient was 0.99 and 0.93, respectively. As for the Freundlich model, the correlation coefficient (R^2) for biosorption of Pb (II) and Cd (II) by *H. meridiana* H2 biomass showed that this model also fits the experimental data with values of 0.92 and 0.99, respectively. According to Freundlich treatment the parameter K in Pb (II) was higher than that of Cd (II) this indicates that lead ion had higher bond strength to *Halomonas meridiana* H2 biomass than cadmium ion. In addition to the parameter $1/n$ which is the biosorption intensity. The $1/n$ values specify whether isotherm is irreversible ($1/n = 0$), favorable ($0 < 1/n < 1$) or unfavorable ($1/n > 1$). From the data in Table 1, the values of $1/n$ were 0.67 and 0.99 indicating that the biosorption of Pb (II) and Cd (II) metal ions onto *Halomonas meridiana* H2 biomass is favourable (Lu *et al.*, 2006; Gabr *et al.*, 2008; Oves *et al.*, 2013).

In order to determine if the biosorption of metal ions was physical or chemical, the Dubinin-Radushkevich isotherm model was used with the aid of the calculation of mean free energy value (E), if the value of (E) is less than 8 KJ/mol, then the biosorption is physisorption and if its value is higher than 8 KJ/mol, then biosorption is chemisorption. The E values calculated for the interaction of Pb (II) and Cd (II) with *Halomonas meridiana* H2 biomass were 13.51 and 19.61 (kJ/mol), respectively. Therefore, the biosorption mechanism of Pb (II) and Cd (II) metal ions with *Halomonas*

Table 1. Parameters obtained in Langmuir, Freundlich and Dubinin-Radushkevich isotherm models.

Metal	Langmuir isotherm			Freundlich isotherm			Dubinin-Radushkevich			
	q_{\max} (mg/g)	b (L/mg)	R^2	k (mg/g)	1/n	R^2	q_D (mg/g)	BD (mol ² /kJ ²)	E (kJ/mol)	R^2
Pb	119.05	0.009	0.99	1.96	0.67	0.92	4.07	2.75 10 ⁻³	13.51	0.96
Cd	35.71	0.017	0.93	0.46	0.99	0.99	2.98	1.30 10 ⁻³	19.61	0.95

**Fig. 10.** Langmuir Isotherm for (a) Lead and (b) Cadmium by *Halomonas meridiana* H2 biomass**Fig. 11.** Freundlich Isotherm for (c) Lead and (d) Cadmium by *Halomonas meridiana* H2 biomass**Fig. 12.** Dubinin-Radushkevich Isotherm for (e) Lead and (f) Cadmium by *Halomonas meridiana* H2 biomass

meridiana H2 demonstrated a chemisorption process (Samuel *et al.*, 2015).

CONCLUSION

The biomass of halophilic bacterial isolate

Halomonas meridiana H2 was able to remove Pb (II) and Cd (II) metal ions from aqueous solution. The potential of this isolate in biosorption process was investigated by studying the influence of initial metal concentration, amount of biomass and contact

time. It was found that the biosorption process was rapid within few minutes and following the pseudo-second-order kinetics model. The Biosorption data obtained from experiments fitted well with the Langmuir and Freundlich isotherms. The mechanism of Pb (II) and Cd (II) biosorption onto *Halomonas meridiana* H2 biomass was found to be a chemisorption process. Based on the results; the biomass of this halophilic bacterial isolate could be used as a promising, inexpensive and environment friendly biosorbent for removal of Pb (II) and Cd (II) metal ions from contaminated wastewaters.

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