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Original Article

Biosorption of Cd^{+2} and Pb^{+2} by Exopolysaccharide Extracted from *Lactobacillus fermentum 6b*; Adsorption Isotherm and Kinetic Studies

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Abstract

Background: In recent years, the biosorption of heavy metals by *Lactobacillus* strains has received attention from researchers. We aimed to remove of heavy metals lead and cadmium from *L. fermentum* 6b exopolysaccharide in 2021.

Methods: Extracellular exopolysaccharide was first extracted from selected probiotic strain, and then the effect of variables such as pH, the extracted exopolysaccharide adsorbent dose, contact time, heavy metal concentration, and temperature on the adsorption rate was investigated. The adsorption isotherms of Langmuir and Freundlich were also examined. Pseudo-first and pseudo-second-order kinetics equations were also investigated for the desired surface adsorption.

Results: The adsorption process at pH=6.5, contact time=80 min, pollutant concentration=100 $mg.L^{-1}$, adsorbent dose (extracted exopolysaccharide) =1500 $mg.L^{-1}$, temperature=35°C for cadmium; pH= 6, contact time=60 min, contaminant concentration of 100 $mg.L^{-1}$, adsorbent dose (extracted exopolysaccharide) =1500 $mg.L^{-1}$ temperature=of 35 °C for lead had optimum condition. The adsorption process corresponded to Freundlich isotherm with R^2=0.958 and R^2=0.988, and pseudo-second-order kinetic with R^2=0.99 and R^2=0.85 for cadmium and lead, respectively.

Conclusion: The exopolysaccharide extracted from *L. fermentum 6b* isolate can have an acceptable removal potential for lead and cadmium heavy metals.

Keywords: Cadmium; Lead; *L. fermentum 6b*; Adsorption process; Extracted exopolysaccharide

Introduction

Metals that have an atomic weight in the range of 63.5-200.6 Da and density of >5 $g.cm^{-3}$ are called heavy metals (1). Accumulation of heavy metals in water, air, and soil is an important environmental problem. On the other hand, long-term consumption of cadmium causes liver and kidney damage, lung cancer, osteomalacia, lack of essential nutrients, protein deficiency, and hypertension (2-5). Over 0.6% of the global burden of diseases in low- and middle-income countries is

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due to exposure to lead, which involves 98% of adults and 99% of children (6, 7). Therefore, the presence of heavy metals in the environment bears irreversible effects necessitating their removal.

In recent years, several technologies have been developed to reduce or remove heavy metals from contaminated environments. Bioremediation or removal of heavy metals with the help of microorganisms is an innovative and cost-effective technology (8). Today, the biological removal of heavy metals by *Lactobacillus* strains has received much attention from researchers (9). EPS produced by lactic acid bacteria (LAB) have been shown to possess the acceptable ability to remove heavy metals (10-12). Exopolysaccharides (EPS) produced by LABs are Generally Recognized as Safe (GRAS) and are divided into two groups: bound and released into the environment (5, 13-16).

In this study, cadmium (Cd) and lead (Pb) which are two toxic, abundant, unnecessary heavy metals that have a non-degradable structure and cause adverse effects on the health of humans and other living organisms were selected. In our previous study conducted in 2020, *L. acidophilus ATCC 4356* and *L. fermentum ATCC 9338* had good removal activities for lead (53.9± 0.01%) (17).

Therefore, this study was conducted in Al-zahra University of Iran, Iran to investigate the removal of lead and cadmium metal ions by EPS produced by *L. fermentum 6b* and finally to examine the isotherm and kinetics reactions under optimal conditions.

Materials and Methods

Heavy metals: Stock solutions (2 $mg.mL^{-1}$) of lead and cadmium were prepared from $Cd(NO_3)_2$ (CAS number 10325-94-7, Sigma-Aldrich) and $Pb(NO_3)_2$ (CAS number 10099-74-8, Merck), respectively (12).

Bacterial strains: The native strain of *L. fermentum 6b* isolated from Iranian dairy products was selected due to its acceptable ability to produce EPS (18).

Extraction of exopolysaccharides (EPS): After incubation at 37 °C for 24 h in 10ml of MRS broth under microaerophilic conditions, the culture media were then centrifuged for 15 min at 4 °C (15000 g). The cell pellet was studied for attached EPS by adding ethanol and the supernatant was studied for released EPS by adding trichloroacetic acid (TCA) (19, 20). The amount of attached/released EPS produced by EPS-producing strain was determined using a spectrophotometric phenol-sulfuric acid method by the standard glucose curve at 490 nm (Amersham spectrophotometer (RS23)). The total amount of carbohydrates was expressed by the standard glucose curve using Microsoft Excel (2013) software (21).

Application of Extracted EPS in Adsorption of Heavy metal: Because of that environmental parameters as variables, affecting the adsorption process, the adsorption capacity of the extracted EPS for heavy metals was evaluated by examination of following parameters: Contact time (0, 20, 40, 60, 80, and 100 min), pH (4.5, 5, 5.5, 6, 6.5 and 7), the amount of extracted EPS (100, 500, 1000, 1500 and 2000 mg/l), initial concentration of heavy metals (200, 400, 600, 800, 1000 and 1200 ppm) and temperature (25, 35, 45, and 55 **°C**). After the adsorption process, the solutions were centrifuged and the residual cadmium and lead in the supernatant were determined by Inductively Coupled Plasma-optical emission spectrometry (ICP-MS) in triplicate (22, 23). Amount of removed heavy metals were determined by the following equation:

Removal efficiency (%) = $[(C_o - C_1)/ C_o] \times 100$, where C_o and C_1 are the initial and final concentration (ppm) of metal ions in solution, respectively

Analysis of Adsorption Isotherms and Kinetics: Adsorption modelling (isotherms and kinetics studies) provides helpful information for predicting in operating conditions of chemical processes. Freundlich and Langmuir isotherm models were used to study the form and manner of adsorption (24), and pseudo-first and pseudo-second-order kinetics models were employed to determine the adsorption rate (25-27).

Results

Extracted EPS production rate: The EPS production rate of *L. fermentum 6b* was calculated from a standard glucose calibration curve by the phenol-sulfuric acid method (Fig. 1). *L. fermentum 6b* had an EPS production capacity of 17.87±111.72 mg.l^{-1} in the released state (EPS-r) and 54.46±5.62 mg.l^{-1} in the bound state (EPS-b) over 24 hours.

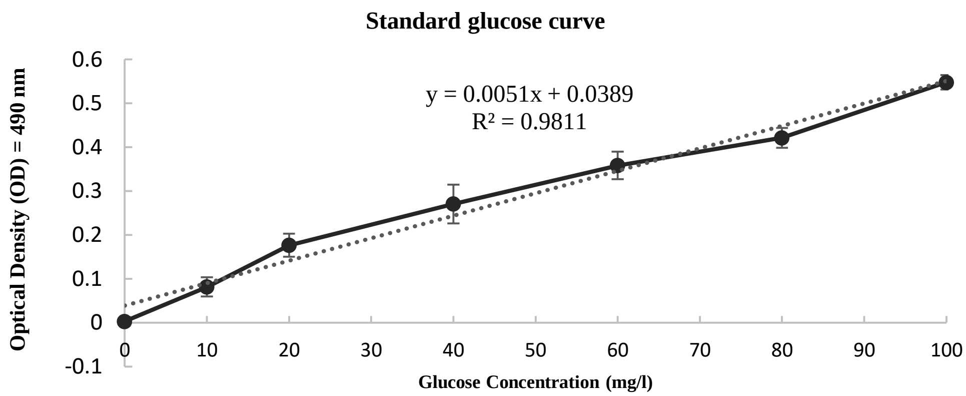

Fig. 1: Standard glucose curve for the phenol-sulfuric acid test

Effect of different parameters on the adsorption process

Effect of pH: The adsorption rate of cadmium at pH=6.5 and lead at pH=6 (Fig. 2) was significantly higher than other pHs (*P*<0.05). The adsorption efficiency of cadmium and lead was 18.8% and 35% at lower pH value and 52.7% and 46.5% at higher pH value, respectively.

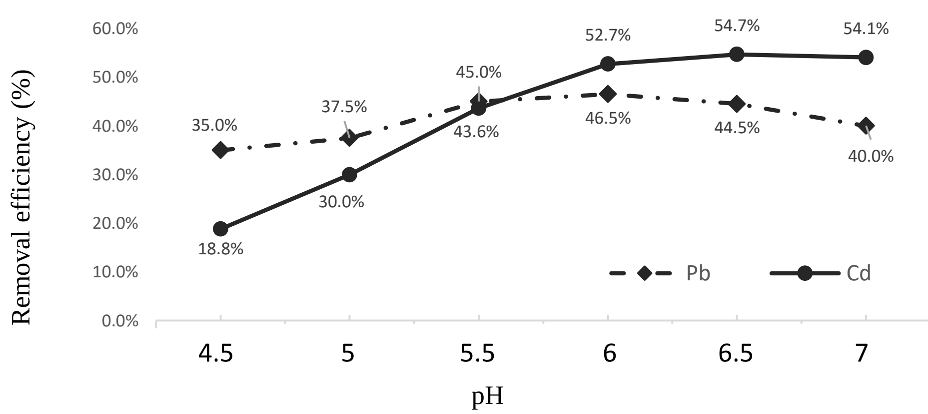

Fig. 2: Effect of pH of aqueous solution on cadmium and lead uptake by extracted EPS (heavy metal concentration 100 mg.l^{-1}, EPS concentration 2 g.l^{-1}, at 37°C for 60 min)

Effect of contact time: The adsorption efficiency reached 55.8% and 49% during 80 min of contact for cadmium and lead respectively. For cadmium, a slight change in the amount of adsorption at 100 min (adsorption efficiency of 56%) was observed. For lead minor increase in adsorption efficiency was equal to 49.8% and 50% at 80 and 100 min respectively was observed (Fig. 3). The adsorption rate of both heavy metals after 60 and 80 min was almost constant ($P<0.05$).

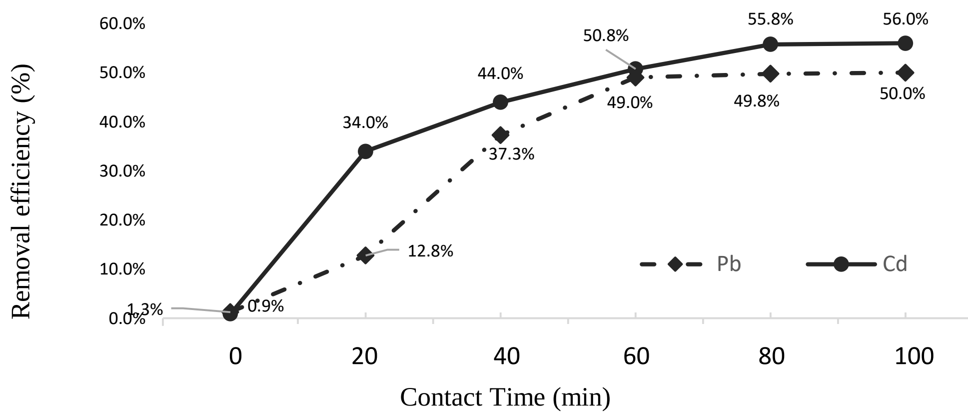

Fig. 3: Effect of contact time on the adsorption rate of cadmium and lead by the extracted EPS adsorbent (heavy metal concentration 100 $mg.l^{-1}$, EPS adsorbent concentration $2g.l^{-1}$, 37°C and pH=7)

Effect of initial concentration of heavy metals: In the study of initial concentrations of cadmium and lead in the concentration range of 100-1200 $mg.l^{-1}$, the highest adsorption was at 100 $mg.l^{-1}$ concentration with an efficiency of 81.8% for cadmium, and the lowest adsorption was observed at 1200 $mg.l^{-1}$ with a removal efficiency of 46.3% for lead ($P<0.05$) (Fig. 4).

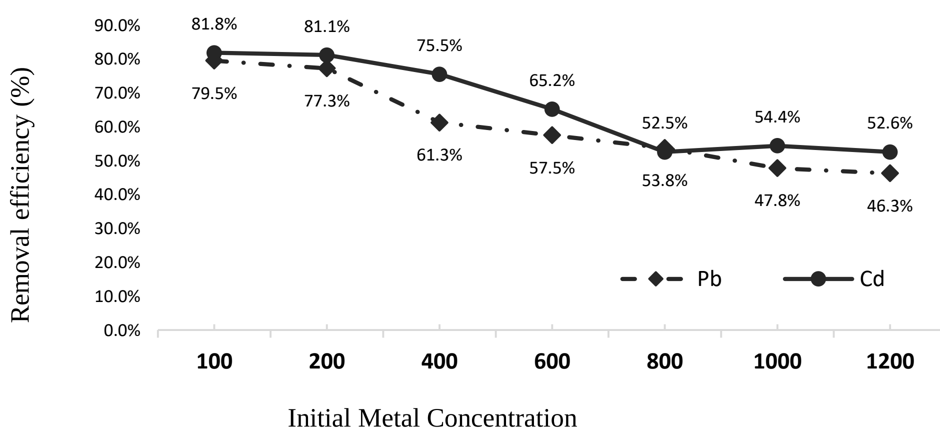

Fig. 4: Effect of initial metal concentration on the removal rate of cadmium and lead by EPS adsorbent (EPS adsorbent concentration 2 $g.l^{-1}$, at 37 °C, for 60 min and pH=7)

Effect of extracted EPS adsorbent concentration: As can be seen in Fig. 5, at extracted EPS concentration of 1500 $mg.l^{-1}$, the highest adsorption rate was 34% and 40% for cadmium and lead respectively. No significant changes in adsorption efficiency were observed at higher concentrations of extracted EPS ($P<0.05$).

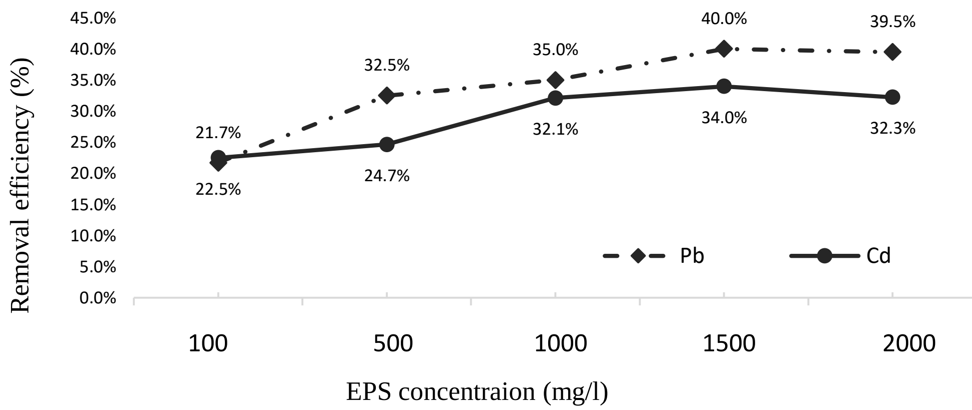

Fig. 5: Effect of EPS concentration (mg.l-1) on cadmium and lead removal rate (heavy metal concentration 100 mg.l-1, at 37 °C, for 60 min and pH=7)

The effect of temperature: The highest amount of adsorption was observed at 35 °C with efficiencies of 53.3% and 58.8% for cadmium and lead, respectively ($P<0.05$) (Fig. 6).

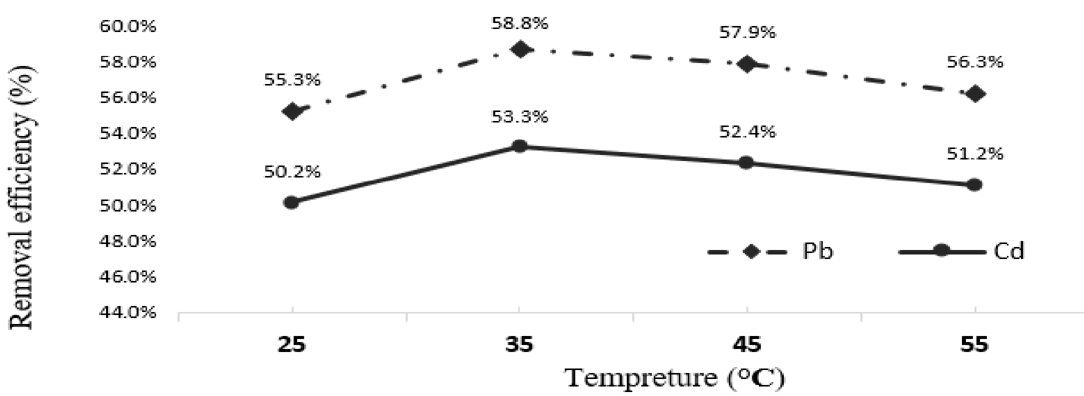

Fig. 6: Effect of temperature on cadmium and lead removal efficiency (heavy metal concentration 100mg.l1, EPS adsorbent concentration 2 g.l-1, for 60 min and pH=7)

Isotherm and kinetics results of adsorption process: Isothermal and kinetic models of metals adsorption process to describe the distribution of metal ions between the liquid phase and extracted EPS have been shown in Figs. 7 and 8 for cadmium and lead respectively. The constants of Langmuir and Freundlich isotherms and pseudo-first and pseudo-second-order absorption kinetics of cadmium and lead onto extracted EPS were evaluated from their corresponding linear plots and presented in Table 1. The absorption kinetics of cadmium and lead onto extracted EPS fit well with the Freundlich isotherm with R^2=0.958 and R^2=0.988, and pseudo-second-order kinetic with R^2=0.99 and R^2=0.85 for cadmium and lead, respectively.

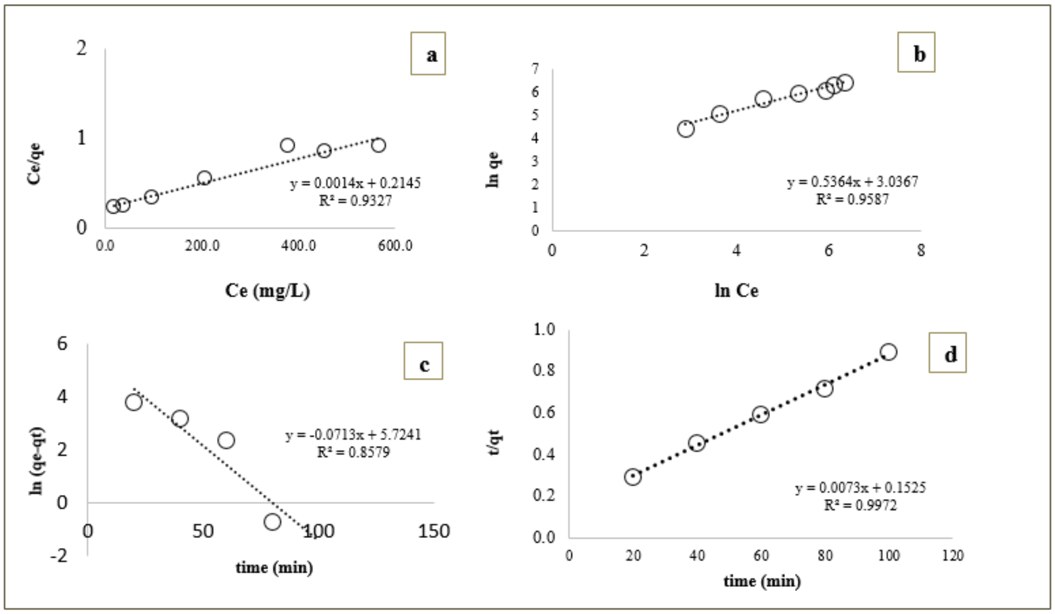

Fig. 7: Isothermal and kinetic models of cadmium adsorption process by extracted EPS; a) Langmuir isotherm model b) Freundlich isotherm model c) A pseudo-first-order kinetic model d) A pseudo-second-order kinetic model for cadmium uptake by extracted EPS. Explanation of Abbreviations: C_e: Cadmium concentration at equilibrium ($mg.L^{-1}$), q_e: Cadmium uptake at equilibrium time ($mg.g^{-1}$), Time: Equilibrium time

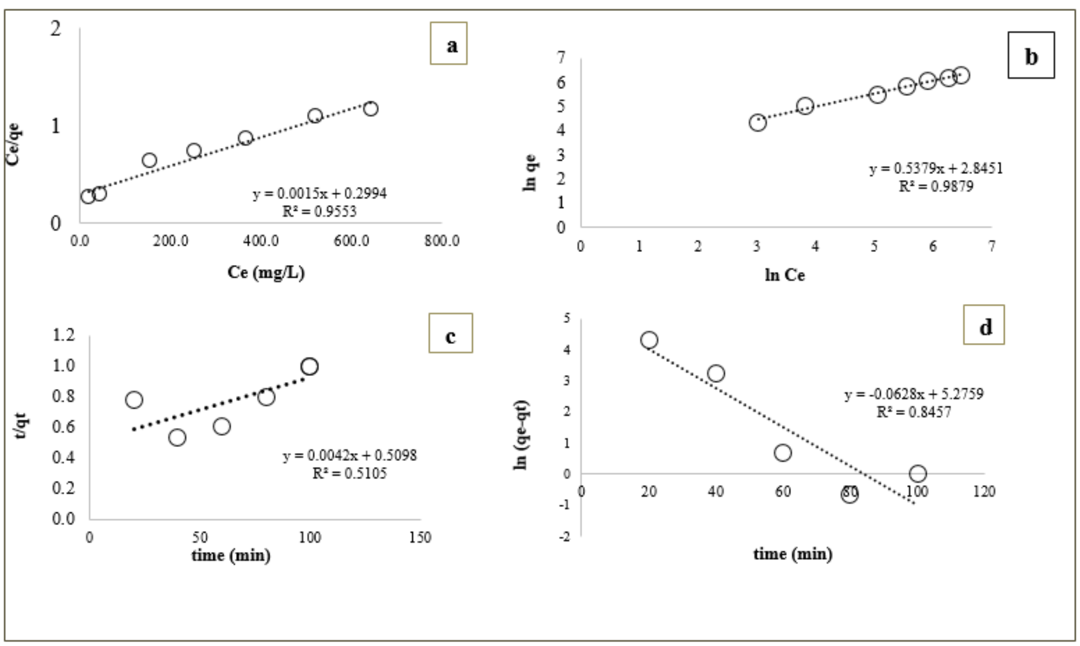

Fig. 8: Isothermal and kinetic models of lead adsorption process by extracted EPS; a) Langmuir isotherm model b) Freundlich isotherm model c) A pseudo-first-order kinetic model d) A pseudo-second-order kinetic model for lead uptake by extracted EPS. Explanation of Abbreviations: C_e: Lead concentration at equilibrium ($mg.L^{-1}$), q_e: Lead uptake at equilibrium time ($mg.g^{-1}$), Time: Equilibrium time

Table 1: Constants of isotherm models and parameters of kinetic models for the biosorption of cadmium and lead by extracted EPS

Langmuir isotherm					*Freundlich isotherm*		
Parameter	R_L	K_L	q_{max} (mg.g^{-1})	R^2	n	K_f	R^2
Cadmium	0.16	0.700	714.286	0.933	1.866	20.836	0.958
Lead	0.23	0.005	666.667	0.955	1.859	17.203	0.988
Pseudo -first order				*Pseudo -second order*			
Parameter	K_1 (min^{-1})	q_e (mg.g^{-1})		R^2	K_2 ((g.mg^{-1}.min)	q_e (mg.g^{-1})	R^2
Cadmium	0.0713	306.16		0.86	0.00035	136.99	0.99
Lead	0.00003	238.10		0.51	0.0628	195.57	0.85

Explanation of Abbreviations: R^2: Correlation coefficient, q_{max}: Maximum ion adsorption by adsorbent (mg.g^{-1}), K_L: Langmuir isotherm coefficient, R_L: isolation factor or equilibrium parameter, K_f: Freundlich isotherm constant, n: Freundlich isotherm power, q_e: Metal absorption rate at equilibrium (mg.g^{-1})), K_1: Adsorption rate constant for pseudo-first-order kinetic model (min^{-1}) and K_2: Adsorption rate constant for pseudo-second-order kinetic model ((g.mg^{-1}.min)

Discussion

Gram-positive bacteria, especially *Lactobacillus* spices, have a high adsorption capacity due to their peptidoglycan, high teichoic acid composition, and EPS in their cell walls (28). In this regard, the ability of extracted EPS from *L. fermentum 6b* to remove heavy metals such as lead and cadmium was examined. In this study, the production range of EPS in the released state from this bacterium is 17.87-111.72 mg.L^{-1} according to the related equations, which was consistent with the findings of Amatayakul et al. (29).

The role of *Lactobacillus* surface structures as an effective parameters in the removal of heavy metals was reported in many studies that was supported by the results of this study. *Lactobacillus* and *Bifidobacterium* species can bind lead and cadmium in a soluble state rapidly within 5 min to 1 hour and remain strongly bound to the cell up to 48 h after initial binding. Rapid adsorption of metals from the solution indicates that superficial links are involved in this phenomenon (30). The surface structures of *L.acidophilus* and *L. crispatus DSM20584* was examined and carboxylic and phosphate groups were the most abundant ionic groups on the surface of *Lactobacillus*. Therefore, these strains can efficiently remove positively charged metals such as cadmium and lead due to the production of negatively charged surface structures (31). Similar results were reported on *L. rhamnosus GG (ATCC 53103)* (32) and on *L. plantarum ATCC 8014* (33) which does support the findings of this study.

In this study, environmental parameters as variables affecting the adsorption process were examined (Fig. 2-6). Finally, the percentage of lead/cadmium removal and adsorption isotherms for all phases were calculated using the relevant equations, respectively (Figs. 7 and 8).

Uptake and bioaccumulation of cadmium and lead ions are affected by pH changes (Fig. 2). The pH of the solution has a significant effect on adsorption processes because it affects not only the solubility of metal ions but also the ionization status of bacterial surface groups (34). The pH values higher or lower than the optimum values led to a decrease of lead and cadmium uptake by the extracted EPS adsorbent. At low pH values, because the concentration of H^+ ions is high and also metals are present as cations, the protons compete with metal ions for adsorption sites (35). When the pH reaches about 6, the carboxyl, hydroxyl, phosphate, and amine radical groups are gradually exposed and combined with metal cations, which increases the removal of lead and cadmium ions. At high pH values, OH^- ions compete with metal ions for adsorption sites, and the efficiency of the adsorbent is eventually decreased. Besides in alkaline environments, metals converted to salts form and separate from the adsorbent surface. Similar results have been observed in other studies (23, 36-39) which is compatible with our findings.

As the contact time increases, the adsorption rate decreases because the binding sites are gradually occupied by metal ions, which reduces the concentration of lead and cadmium ions in the solution (40). In this study, the uptake of lead and cadmium by the extracted EPS was so rapid, that it reached equilibrium within 60 min. At the beginning of the adsorption process, many adsorption sites are available, and lead and cadmium ions are easily adsorbed in these places. Similar results were obtained in other studies (36, 41, 42) that support our theories.

By increasing the concentration of metals, the percentage of their removal from extracted EPS decreases. This phenomenon can be described as follows; at low metal concentrations, the available sites on the surface of extracted EPS adsorbent is more than the amount of metals ions. However, with increasing metals concentration in solution, the available places on the surface of adsorbent are greatly reduced and thus the adsorption percentage is decreased. Other researchers have obtained similar results in their studies (35, 43-45)

As expected, increasing the adsorbent efficiency with increasing the extracted EPS adsorbent concentration is more related to a larger number of adsorption sites on the surface of EPS structures (23). At a very low level of adsorbent concentration, the surface area of the adsorbent is rapidly saturated with lead and cadmium metal ions (46, 47). In this study, at higher concentrations of extracted EPS, no significant changes in adsorption efficiency are observed. At concentrations more than in 1500 $mg.l^{-1}$ of the extracted EPS adsorbent, many of the bonds on the adsorbent overlap or integrate. Similar results obtained in other studies (48,49) show the validity of this claim.

The temperature of the adsorption medium may have a positive or negative effect on the adsorption process of metal ions by bacteria. In the present study, the adsorption capacity increased with increasing temperature and the highest adsorption occurred at 35 °C. Similar results were observed (41).

Isotherm and kinetics studies are useful to determining the amount of adsorption capacity and also the amount of adsorbent (extracted EPS) concentration required for the feasibility study of industrial application and design of adsorption systems. Langmuir isotherm shows that adsorption is homogeneous, uniform, and monolayer, in which no reaction occurs between the adsorbent molecules (extracted EPS) (50), while the Freundlich isotherm represents heterogeneous adsorption where the adsorption action is a multilayer (24). The first-order kinetic model, widely used in aqueous solutions, reflects the linearity of adsorption rate and the proportionality of adsorption process over time with the saturation concentration (51). After comparing the determination coefficients of linear isotherms in adsorption, the Freundlich model was selected due to the higher R^2 coefficient for cadmium (0.95) and lead (0.98). Therefore, the adsorption process is appropriate for the Freundlich model and the K_f coefficient showed a high adsorption capacity for cadmium and lead. The n constant which is an index of adsorption shows the degree of heterogeneity of the adsorbent surface. The $^1/_n$ coefficient of the Freundlich model that is a criterion for adsorption intensity (52), indicates appropriate adsorption under experimental conditions and showed an acceptable adsorption process. Various other studies have reported that Freundlich isotherm models are better to explain the adsorption behavior of microorganisms (53-55). In this study, based on the R^2 constant of pseudo-first and pseudo-second-order kinetics equations, the adsorption of lead and cadmium ions by extracted EPS has a heterogeneous diffusion manner. Similar results indicating that the adsorption process occurs by an ion-exchange mechanism (electron transfer) that affects the overall adsorption rate (41, 55-58), which is compatible with the results of this study.

Conclusion

The extracted EPS from *L. fermentum 6b* isolate had acceptable removal potential to bind cationic ions such as lead and cadmium due to the different types of charged groups such as carboxyl, hydroxyl, and phosphate groups on their surfaces. Therefore, these bacteria can be a good option for use in biosorption of lead and cadmium. The extracted EPS can be tested for biosorption of other heavy metals from waste water. Although the benefits of extracted properties from LAB bacteria in bioremediation of heavy metals from food and environmental contamination are well known, their involved mechanisms are still controversial. In this regard, studying the mechanism of accumulation-dependent metabolism for cadmium and lead and also the relationship between the surface properties of the studied bacteria with the removal of the heavy metals cadmium and lead are suggested.

Journalism Ethics considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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Conflict of interest

The authors declare that there is no conflict of interest.

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