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Biosorption of Pb^{2+} from aqueous solutions by *Bacillus licheniformis* isolated from Tigris river with a comparative study

M.Firat Baran*
Mardin Artuklu University, Medical Laboratory Techniques, Vocational Higher School of Healthcare Studies, 47200 Mardin, Turkey

M. Zahir Duz
Dicle University, Science Faculty, Chemistry Department, Diyarbakir, Turkey

[*]*Corresponding authors*

Abstract: Biosorption by bacteria is an effective method for the removal of toxic elements from drinking water and waste water. Biosorption of Pb^{2+} from aqueous solutions was studied in a batch method by using death bacteria Bacillus Subtilis obtained from ATCC 6051(B1) and Bacillus Licheniformis was isolated from soil in the area of Tigris River. The concentration of lead was measured by AAS, ICP-OES and ICP-MS. The isotherm data, kinetic models and thermodynamic parameters were calculated to describe the adsorption behaviour of bacteria and the data showed that the mechanism of reaction was found to be endothermic from values of $\Delta G < 0$, $\Delta H > 0$ and $\Delta S > 0$. Uptake kinetic model follows the pseudo-second-order kinetic equation and the equilibrium is well described by Langmuir model. The maximum monolayer adsorption capacity of Pb^{2+} was determined as 40.82, 43.48, 42.92 mg/g, respectively for Bacillus subtilis and Licheniformis according to the Langmuir model data in the optimum adsorption conditions. The characterization of Bacillus subtilis and Licheniformis were determined by using FTIR, TGA, DTA, SEM and EDAX. The results of analysis showed that the capacity adsorption of Bacillus subtilis was found to be better than Bacillus licheniformis.
Keywords: Bacillus licheniformis; Biosorption; Environmental pollution,; ICP-OES

Introduction

Recently toxic metals in drinking and wastewater have become a problem due to hazardous effects on human, environmental, water pollution, fauna and flora. It is impotent that researching methods for removal toxic metals from aqueous solution have a great importance [1] . Pb, As, Cd, Hg and Sb ions are among the most toxic elements affecting on the environment[2,3]. These elements come into water through the combustion of the smelting of sulphide, fossil fuels, into lakes and streams by acid mine drainage. Manufacturing industries, such as battery, metal plating and petroleum product are also prime source which lead pollution [4-6] . According to WHO drinking water standard for Pb is 0.05 mg/L and 10 g/L, respectively. Pb causes many serious disorders like anaemia, kidney diseases, nervous disorders and sickness even cancer [7,8]. Therefore, it is required to remove from drinking and wastewater before using which many different research techniques are developed to remove excessive amount of toxic metals from aqueous solutions include chemical processes such as precipitation, evaporation, ion exchange, membrane and electroplating processes. But these methods are not effective enough especially for removing metal ions having low concentration in waters and their high cost of application are economically rather challenging [8,9]. Therefore, it is necessary to find new technologies or biomaterials for removing of toxic metal ions from drinking and wastewater [10,11]. The biological technique for the removal of lead from the aqueous solution can be promising more effective as an alternative method physical and chemical processes because of its high metal binding capacity, low cost, high efficiency in dilute solution effluents, easily obtained in large quantities, water resistant and re-applicability[12,13]. Biomass has many benefits both by metabolically mediated, physico-chemical methods and with maximum adsorption capacity in wastewater [14]. Therefore; there are many studies and compilations on biosorbent / biomass obtained from various microbial sources such as water algae, aquatic plants, soil and leaf based adsorbents for the removal of Pb ion with different methods[15,16]. Among these main biomass types such as fungal and algea have proved to possess the maximum adsorption capacities due to the presence of proteins, polysaccharides or lipid on the surface of their cell walls containing some functional groups such as hydroxyl amino, carboxyl, sulphate and amino, which can be act as binding sites for elements [17,18]. In some studies; the algal biomass used for biosorption, *Spirogyra sp.* is a green filamentous, readily available source of biomass for trace element removal from drinking and wastewater. Investigations conducted by several researchers show that *Spirogyra sp.* is capable of accumulating elements such as chromium, copper and zinc [19-21]. Recently, the biosorption technique continues to attract interest to be developed into a potentially cost-effective process for the removing multi-element from industrial wastes [22]. The biosorbent was characterized by employing instrumental techniques, atomic absorption (AAS), inductively coupled plasma optical emission spectrometer (ICP-OES) [23]. Fourier transform infrared spectroscopy (FTIR), thermo gravimetric analysis (TGA, DTA), scanning electron microscope and energy-dispersive X-ray spectroscopy (SEM-EDX) [24,25]. This study involves the removal abilities of *Bacillus subtilis* and *Licheniformis* for Pb^{2+} ions were evaluated in aqueous solutions of these metals with using batch method. In this context, in order to determine optimum conditions, several parameters including initial concentration, temperature, pH, time, amount of biosorbent, kinetics studies, activation energy, Freundlich and Langmuir isotherm models, TGA-DTA analyses, thermodynamic functions such as free energy change (ΔG), enthalpy change (ΔH) and entropy change (ΔS) were investigated using experimantal data [26,27].

Materials and methods Reagents

Instrumentation and Standard Solutions

Chemical (HNO_3, HCL, NaOH, $Pb(NO_3)_2$) used in this study were analytical grade obtained either from Merck, Germany. Purified water was prepared using a Millipore Milli-Q (Direct-Q UV 3, USA) water purification system. Calibration solutions were prepared from certified Pb^{2+} solution (1004 ± 4 μg/mL, Plasma CAL, SCP science, USA). Standard base and acid solutions (0.1N NaOH and 0.1N HCl) were used for pH adjustments. pH measurements, agitation and precipitation were made using a pH meter (Hanna HI-2211700,USA), shaker (Nuve) controlled temperature and centrifuge (ALC-4235A) models. The concentration of Pb(II) were measured by atomic absorption spectrometry (AAS, Unicam 929, USA) and inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin-Elmer Optima 5300) and inductively coupled plasma mass spectrometry (ICP-MS) depending on its concentration. The scanning electron microscopy EVO 40 LEQ model was used for surface investigation. Shimadzu TGA-50 was used for TGA (thermogravimetric analysis) and DTA (differential thermal analysis) studies in the temperature range 20–750 ◦C. BET (Brunauer-Emmett-Teller) surface area measurement was performed by quantasorb surface analyser method. Infrared spectra was recorded by Mattson-1000 model FTIR (fourier transform infrared) spectrophotometer.

Preparation of Biosorbents

The *Bacillus subtilis* was obtained from ATCC 6051(B1). The single species of *Bacillus licheniformis* was isolated from soil in the different area of Tigris river by Dr.Fikret Uyar and Dr. Zübeyde Baysal from Dicle University. They were placed in sterile glass bottles and transferred to the laboratory within 2 h. The moist soil was shade-dried and stored at 4^oC. The morphological characterization of the organism was also done with the bacterial culture such as Gram and endospore staining. Each of microorganisms was inoculated to 1 L of liquid Nutrient Broth and it was left to incubation in a shaker at 37^oC for 24 h. The biomass was centrifuged at 7000 rpm in 15 min and was extracted by decantation, washed twice by sterile water. Then it was dried at the room temperature for 24h followed by drying in a oven at 65 ◦C for 24h and then it was sieved to select the particles 180 μm size and protected in sterile sample bottles [28,29] .

Analytical sensitive and accuracy of the method for AAS, ICP-OES and ICP-MS

The limit of detection (LOD) and limit of quantification (LOQ) for Pb element were determined by using analytical curves performed with 10 independent analysis of a blank solution spiked with the metal at a level of lower concentration for the analytical sensitive and accuracy of AAS, ICP-OES and ICP-MS. The standard curves were found to be linear with a correlation coefficient of 0.999. The LOD and LOQ were calculated from the standard deviation (S_d) (LOD = $X_{avr.} + 3\ S_d$ and LOQ = $X_{avr.} + 10\ S_d$) (Table 1) .

Table 1 The analytical sensitive and accuracy for measurements

Atomic spectroskopy	Wavlengts (nm)	LOD (µg/ml)	LOQ (µg/ml)	Certified values (µg/L)	Confidence measured (µg/L)	Profesion testing (µg/L)
AAS	217.0	0.0410(μg/ml)	0.1362(μg/ml)	631±0.046	ND	-
ICP-OES	220,351	0.0206(μg/ml)	0.0635(μg/ml)	631±0.046	700±0.043	536-726
ICP-MS	^{208} Pb	0.4020 (μg/L)	1.3390(μg/L)	631±0.046	705±0.062	536-726

Batch experiments

In this study, the adsorption of the biosorbent *Bacillus subtilis* and wild strain *licheniformis sp.* were studied as a function of biosorbent dose, pH, Pb^{2+} concentration, time and temperature. The optimum conditions were investigated by batch experiments, using 250 mL flasks containing 50 mL of Pb^{2+} solutions and the pH value was adjusted to the desired value by adding 0.1 M NaOH or 0.1 M HCl throughout the experiment. At the end of adsorption, samples were taken out at different time intervals, 3 mL sample transferred to centrifuge tubes and centrifuged at 4000 rpm for 5 min in a centrifuge to remove the suspended biomass. The concentration of Pb^{2+} in residual solution was measured [30,31].

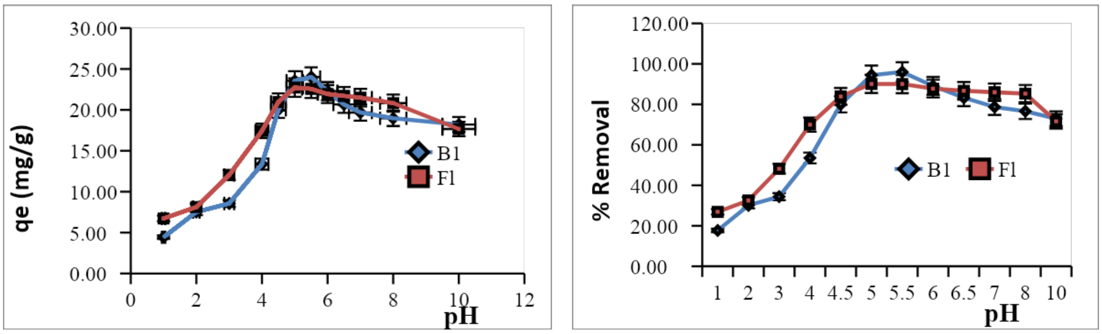
Results and discussion

Effect of pH

The uptake of Pb^{2+} is well known that the functional groups of pH could affect the protonation on the biomass as well as the metal chemistry. From this point of view, effect of pH was investigated for pH 1-10, The equilibrium sorption capacity (q_e) and removal of Pb^{2+} (%) were calculated from differences initial (C_0) and equilibrium (C_e) concentration (Akar et al. 2005). The equilibrium sorption capacity (q_e) is the amount of metal ion sorbet at equilibrium (mg/g), C_0 initial concentration of Pb^{2+}, C_e equilibrium concentration, V volume of solution Pb^{2+}, m biomass dose and it can be expressed as follows:

$$qe = \frac{(Co - Ce).V}{m} \qquad \%\,Removal = \frac{(Co - Ce).100}{C0} \qquad equation\ (1)$$

As seen in the Fig.1 the maximum adsorption capacity; 24,01 mg/g, removal of lead 96 % at pH 5,5 and 22,73 mg/g, removal of lead 91 % at pH 6.0 for *Bacillus subtilis and Licheniformis* were found respectively and it was observed that the capacity and removal of lead decreased at the upper these pH values. The maximum adsorption of *B. Licheniformis* near neutrality may be more suitable for metal removal from water. The decrease of adsorption capacity on pH 7 values suggests that the occurrence of Pb^{2+} precipitation and interfered with the accumulation may be due to the increase of negative groups such as OH^- ion or biomass deterioration. At the higher pH, more of ligands may be exposed and carried out negative charges, with subsequent attraction of cations with positive charge and biosorption onto the cell surface [32]. Therefore; the precipitation of insoluble some metal hydroxides takes place restricting the true biosorption capacity. At low pH, negative cell wall components are closely related to H_30^+ hydronium ions and restrict the approach of Pb^{2+} cation as a consequence of the thrust. The dependence to pH of Pb^{2+} mechanism can be explained by the nature of the cell surface metal ion binding sites or the solution chemistry of metal ions in water[21,33] .



a ***b***

Fig.1 The effect of pH on the adsorption capacity (a) and removal (b) of Pb^{2+}

The effect of adsorbent dose

To determine the effect of adsorbent dose, the different amounts of biosorbent were varied as 10–50 mg at pH; 5,5 - 6.0 for *Bacillus subtilis and Licheniformis* while other parameters were constant. The results are shown in Fig.2 that the amount of biosorbent significantly influenced the removal of Pb^{2+}. In case of 20 mg biomass, maximum equilibrium dose capacities were determined as 9.79 mg/g capacity and 98 % removal Pb^{2+} and 9.66 mg/g capacity and 96.6 % removal Pb^{2+} for *Bacillus subtilis and Licheniformis.* As shown in the Fig. 2, the adsorption capacity of each biosorbent is close to each other with a maximum dose of 20 mg. Therefore; there was only a slow change in the extent of Pb^{2+} adsorption when the adsorbent dose was increasing [22,34]. Furthermore, higher adsorbent dose was significantly influenced in lower adsorption.

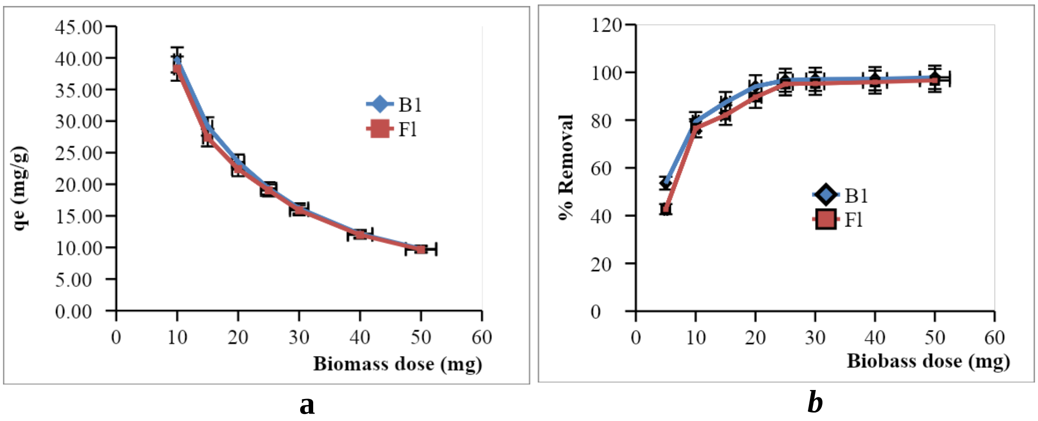


Fig.2 The effect of adsorbent dose on adsorption capacity(a) and removal(b) of Pb^{2+}

Adsorption isotherm studies

The equilibrium adsorption isotherm is one of the most important methods to understand the adsorption process. In this study, Freundlich and Langmuir adsorption isotherms were applied to investigate the interaction between the biosorbent and Pb^{2+}. To determine the effect of temperature on Pb^{2+}, the different concentration and temperature were studied using between 20 mg/L of Pb^{2+}, at 25°C, 35°C and 45°C temperatures. As shown in the Fig.3, the adsorption capacity of Pb^{2+}, for each biomass was not significantly influenced with increasing of temperature but increased the adsorption rate. Freundlich and Langmuir adsorption isotherm models are generally based on the isotherm equations given in literatures [35]. The best fit of each isotherm model for biosorbents was evaluated in terms of correlation coefficient (R^2). The Langmuir equation is given as follows:

$$q_e = Q_{max} \cdot \frac{b.Ce}{1+Ce} \quad \rightarrow \quad \frac{Ce}{Qe} = \frac{1}{Qmax\ .b} + \frac{1}{Qmax}.Ce \qquad eq.(2)$$

where q_e is the equilibrium metal concentration on the biosorbent (mg/g), Ce is the equilibrium metal concentration in solution (mg/L), Q_{max} is the monolayer capacity of the biosorbent (mg/g) and b is the Langmuir constant. The determination of Langmuir constants for biosorbents were plotted the graph C_e and C_e/q_e and calculated Q_{max} and b constants from eq.2, R^2 from Fig.3 [15,32] . The Freundlich isotherm model equationis expressed as follows;

$$= logk + \frac{1}{n} logCe \qquad eq.(3)$$

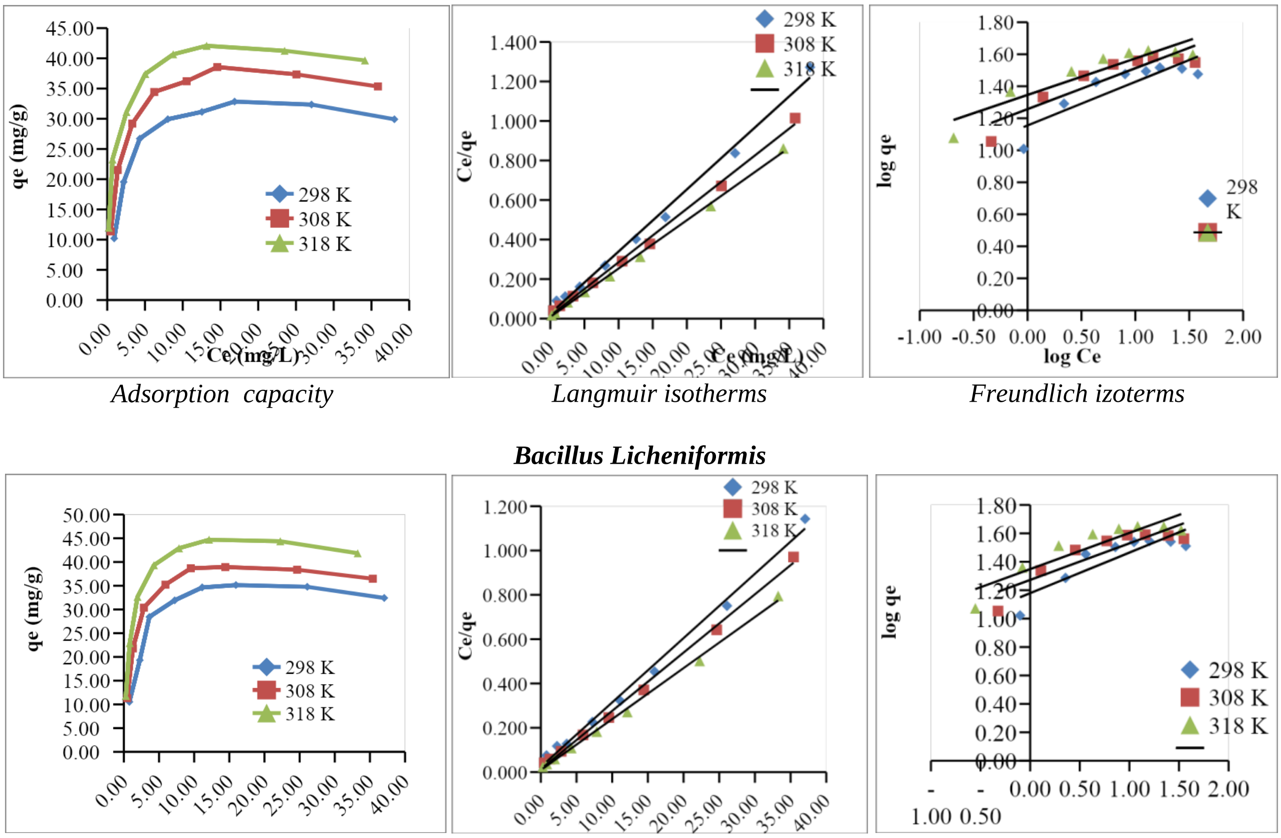
where K_F is the Freundlich adsorbtion capacity, Ce is the equilibrium metal concentration in solution (mg/L), n is the Freundlich constant. The determination the Freundlich constants for biosorbents were plotted the graph $Logq_e$ / $LogC_e$, and calculated K_F and n constants from eq.3 (Table 2), correlation coefficient (R^2) from Fig.3

Table 2

The isotherm constants of Langmuir and Freundlich for biosorbents in different temperature

Biomass	Freundlich constants				Langmuir constants		
	T (K)	**K_F (min^{-1})**	**n**	**R^2**	**Q_m (mg/g)**	**b (L/mg)**	**R^2**
Bacillus subtilis	298	14,29	3,67	0,7567	31,85	1,246	0,9934
	308	18,08	3,92	0,8182	36,90	1,844	0,9966
	318	22,17	4,39	0,8517	40,82	2,917	0,9984
Bacillus licheniformis	298	15,10	3,51	0,7659	34,48	1,213	0,9936
	308	18,71	3,89	0,7866	38,02	1,977	0,9970
	318	22,29	3,88	0,8117	43,48	2,556	0,9975

Bacillus Subtilis



Adsorption capacity *Langmuir isotherms* *Freundlich isotherms*

Fig.3 The adsorption capacity and Langmuir - Freundlich isotherms plots for biosorption of Pb^{2+} on biosorbents in different temperature.

The experimental data on biosorbents of Pb^{2+} shows that the isotherms graph curves are agreement with the Langmuir type, as can be seen from the magnitude of the R^2 values in the table 2. As seen in the Table 2, monolayer biosorption capacities (Q_m) were found to vary between 32 and 44 mg/g depending to the temperature but there were no significant difference between of biosorbents [36] .

Effect of contact time on adsorption capacity of Pb^{2+}
Kinetics studies

The effect on adsorption of kinetic studies is one of the important characteristics on defining of reaction rate. The adsorption of Pb with 20 mg of biomass was carried out at pH 5,5-6.0 for *Bacillus subtilis and Licheniformis* in concentration 10 mg/l in 50 volume solution, at 150 rpm, by the following in different time intervals 5 min. in 75 min. at the different temperature 25°C, 35°C and 45°C. The equilibrium sorption capacity (q_t) were calculated from equation [37]. As shown in the fig.4 (a), the adsorption rate of Pb^{2+} for each biomass increases rapidly in the first part within 10 min. of contact time with increasing of temperature. After that the rate reaction continues in till reach a constant value of Pb^{2+} concentration within 75 min. These changes in Pb^{2+} uptake may be due to the fact that initially all adsorbent fields are empty and the solute concentration is high. After this time, there was a very small increase in metal involvement, as the bacteria had little active surface area on the cell wall. There are several kinetic models to understand the control mechanism of the adsorption process and to test the experimental data [38]. The reaction mechanisms of biosorption process were used as the pseudo- second-order and pseudo -first-order to interpret the experimental data. The rate constant of adsorption was determined from the following first-order rate expression given by Lagergren [26,39]. The pseudo-first and second-order equations can be expressed as follows:

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The pseudo-first-order equation;

$$\frac{dqt}{dt} = k_1(qt - qe) \;\rightarrow\; log\, log\, \frac{qe}{qe - qt} = \frac{k_1}{2{,}303}t \;\rightarrow\; log\, log\,(qe - qt) = logqe - \frac{k_1}{2{,}303}t \qquad eq.(4)$$

The pseudo second-order equation ;

$$\frac{dqt}{dt} = k_2(qe - qt) \;\rightarrow\; \frac{1}{qe - qt} = \frac{1}{qe} + k_2 \;\rightarrow\; \frac{1}{qt} = \frac{1}{qe^2} + \frac{1}{qe}t \qquad eq.(5)$$

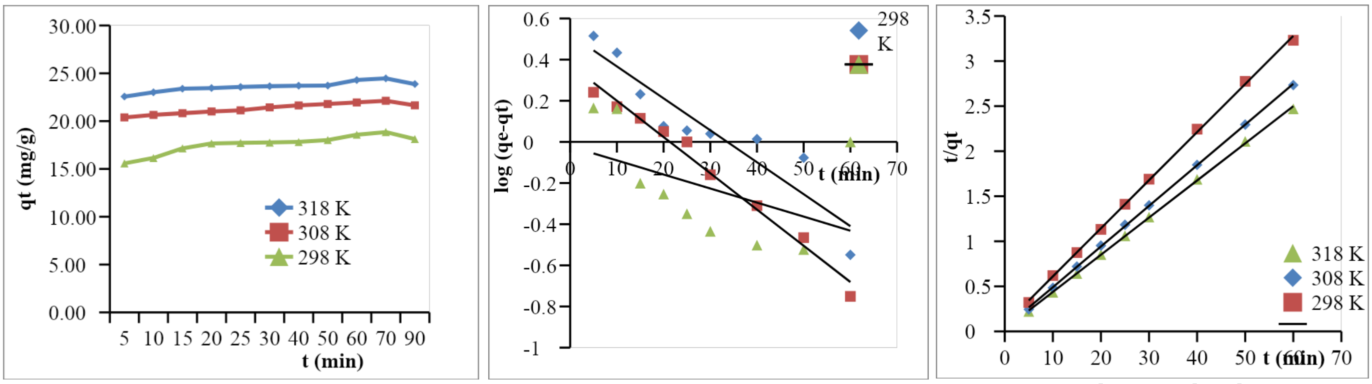
where q_e and q_t are the amount of Pb^{2+} biosorbed (mg/g) in equilibrium at any time (t), k_1 and k_2 is the rate constant for the pseudo-first and second-order kinetics (min^{-1}). The best fit of each kinetic model for biosorbents was evaluated in terms of correlation coefficient (R^2). The determination of the pseudo-first and second equation constant for biosorbent in different time(min.) and temperature (^oC) were plotted the graph Log(q_e-q_t) / t (min.) for the first- order, t (min)/q_t for the second-order, and calculated k_1 (pf) and k_2(ps) constant from eq.4,5 (Table 3), correlation coefficient (R^2) from fig.4. [37-40].

Table 3

Rate constants of kinetic models for biosorbents in different temperature

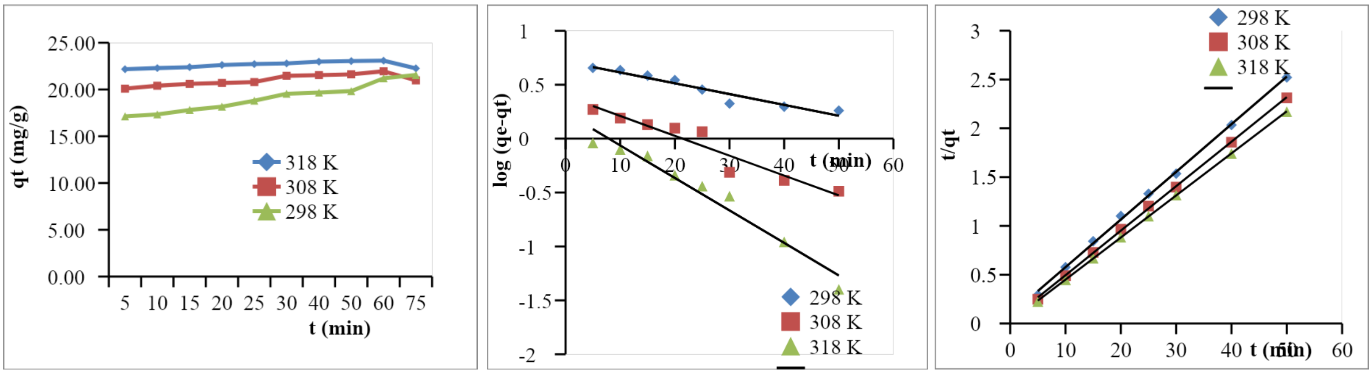
Biomass	Temp. (K)	Psedo- first order constants: K_1 (1/dak)	q_e (mg/g)	R^2	Psedo- second order constants: K_2 (g/mg.dak)	q_e (mg/g)	R^2
Bacillus subtilis	298	0,0357	3,3274	0,8677	0,0374	18,7265	0,9960
	308	0,0405	2,3768	0,9831	0,0516	22,1729	0,9998
	318	0,0157	0,9510	0,2217	0,0655	24,2718	0,9993
Bacillus licheniformis	298	0,0253	5,3003	0,9666	0,0278	20,4499	0,9989
	308	0,0424	2,4814	0,9159	0,0534	21,9298	0,9996
	318	0,6932	1,7302	0,9602	0,0938	23,2018	0,9959

Bacillus Subtilis



Adsorption capacity   Pseudo-first-order   Pseudo second-order

Bacillus Licheniformis



Adsorption capacity | Pseudo-first-order | Pseudo second-order

Fig.4 The adsorption capacity and Pseudo-first- second -order plots for biosorption of Pb^{2+} in different temperature

The results of two kinetic equations given in Table 3 show that the pseudo-first-order plot does not adequately describe the adsorption results with a low correlation coefficient. Generally, the first-order model is valid for the initial stage of biosorption processes but is not compatible well throughout the entire contact time because the correlation coefficients are low (Fig.4). As you can see Table 3 and fig.4 , the second order plots are all linear and the correlation coefficients are higher than 0.999, which shows that the pseudo-second-order kinetic model compatible well with the experimental data. The second-order kinetic rate constants (k_2) decrease with the increase of the initial Pb^{2+} concentration and increases with increasing temperature. This may be due to Pb^{2+} ions present at high concentrations in the solution. They compete with each other and cause a delay in reaching the equilibrium of low k_2 values. The high applicability of the second-order equation of Pb^{2+} ions to the various adsorbents was also determined.

The thermodynamic functions and activation energy on biosorption of Pb^{2+}

The activation energy of the biosorption Pb^{2+} in the optimum conditions were calculated from the kinetic model compatible with k_2 (second order) rate constant values at different temperatures and determined correlation coefficient R^2. Calculation was made using the Arrhenius equation given below

$$lnk_2 = lnA_0 - \frac{E_a}{RT} \qquad eq.6$$

Where k_2 is the rate constant for the pseudo - second-order kinetics, A_0 Arrhenius constant, E_a activation energy and T Kelvin temperature. The thermodynamic functions of the biosorption Pb^{2+} were calculated from K_e equilibrium constant values at different temperatures and determined correlation coffition R^2. The calculation was made using the Clasius-Clapeyron equation given below. The results are given Table 4

$$K_e = \frac{Ca}{Cs} \rightarrow \Delta G^o = -RT\, lnK_e \rightarrow \Delta G^o = \Delta H^o - T\Delta S^o \rightarrow lnK_e = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \qquad eq.7$$

Where K, is the equilibrium constant, C_a amount of Pb^{2+} ions (mg/L) adsorbed by biomass in solution, C_s the amount of Pb^{2+} ions remaining in the equilibrium solution. The ΔG° is free energy, ΔH° (standard entropy) temperature exchange and ΔS° (entropy) irregularity in reaction [26,35].

Table 4
The activation energy and thermodynamic functions on biosorbtion of Pb^{2+} ion

	Activation Energy					Thermodynamic Functions			
Biomass	**T (K)**	**1/T**	**K_2**	**LnA_o**	**E_a (kj/mol)**	**LnK_e**	**□ G°**	**□ H°**	**□ S°**
	298	$3,36.10^{-3}$	0,0374	-3,2868		0,220	0,552		
Bacillus subtilis	308	$3,25.10^{-3}$	0,0516	-2,9638	22,15	0,612	1,693	33,45	114,08
	318	$3,14.10^{-3}$	0,0655	-2,7251		1,070	2,834		
	298	$3,36.10^{-3}$	0,0278	-3,5826		0,193	-0,678		
Bacillus licheniformis	308	$3,25.10^{-3}$	0,0534	-2,9295	47,93	0,682	-1,688	29,42	100,77
	318	$3,14.10^{-3}$	0,0938	-2,3664		0,938	-2,698		

The table 4 indicate that the adsorption mechanism is voluntary due to the negative values of ΔG^o, and it is observed that the biosorption occurs more negatively at higher temperatures. The positive ΔS^o value confirms an increase in irregularity at the solid-liquid interface during biosorption. In other hand, the positive affinity of ΔS^o can be explained by the release of metal biosorption in the solution and it is forming a regular structure surrounding the surfactant of the biosorbent with solution. The positive values of ΔH^o indicates that the biosorption process is endothermic [36]. The table 4 also provides information on the physical or chemical adsorption of the study as seen in activation energy values in some studies expressed that if the values of the activation energy (E_a) are between 5 - 50 kJ /mol, is considered the physical adsorption, the values between 60 - 800 kJ / mol. is chemical adsorption. In this study, the activation energy of *Bacillus subtilis and Licheniformis* (E_a) was found to be 22.15 and 47.93 kJ /mol, respectively as seen in table 4. Therefore; this study was determined to be physical adsorption

Characterization of the *Bacillus subtilis and Licheniformis*
FT-IR spectral studies

The FT-IR spectrum of treated and untreated biomass were determined using the KBr disc technique to analyse current functional groups in the biomass. The transmission FT-IR spectra were then recorded using a Mattson 1000 model Spectrum FTIR-ATR model between 4000 - 400 cm^{-1}. The results of FT-IR spectra analysis showed that the current of functional groups such as carboxyl, amino, amide, phosphodiester and hydroxyl could interact with protons or metal ions for biomass [3]. The results of FT-IR spectrum in the Fig.5 and Table 5 obtained give an idea about the presence of functional groups on the biomass cell surfaces.

Table 5
The FT-IR functional groups of biomass untreated and treated with Pb^{2+} ions

Biomass	**OH, –NH**	**–CH_2-**	**Amide** I	**Amide** II	**–OH** bending	**C–H, COO–**	**P=O** phosphodiester	**C–O-** stretching
Untreated *B.Sub.*	3263,69	2926,35	1630,60	1530,31	1445,80	1395,82	1219,00	1040,41
Treated - Pb^{2+}	3272,59	2928,57	1634,15	1525,39	1443,10	1382,74	1211,20	1029,00
Untreated *B.Lich.*	3265,84	2928,57	1629,7	1526,25	1443,1	1384,36	1229,53	1045,85
Treated - Pb^{2+}	3275,47	2927,97	1637,1	1512,97	1445,8	1385,90	1230,68	1043,92

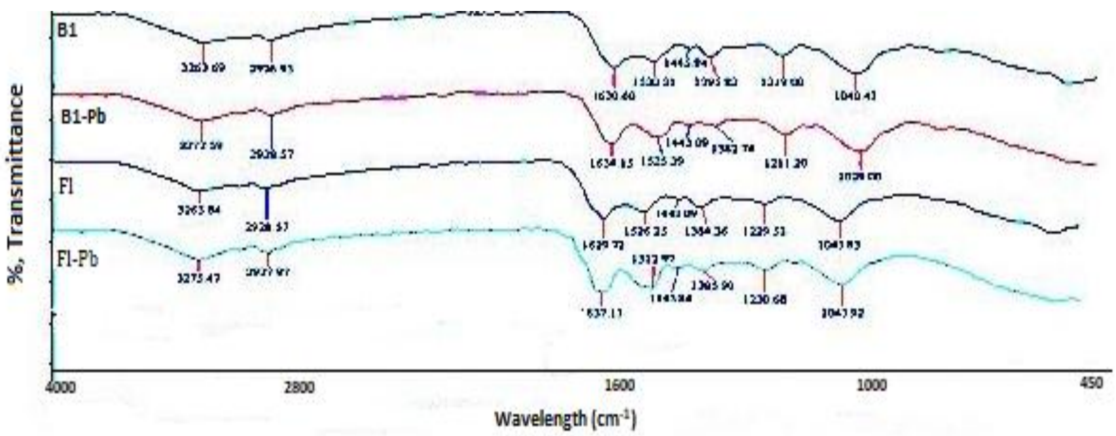


Fig. 5 FT-IR spectra of untreated biosorbents and treated with Pb(II)

As seen in the table 5 and FT-IR spectrum, strong asymmetric stretching bands belonging to functional groups such as OH^-, -NH_2, amid (I,II), P = O, COO^-, C-O- on surface biosorbent were observed. After then the biosorbents was activated with Pb^{2+} and it was found that there were some shifts in functional group bands (Fig.5). The results FT-IR spectra indicated that the Pb^{2+} ions was adsorbed by these functional groups on the biomass surface. The spectra of *B.licheniformis sp.* obtained from Tigris river area are similar to nearly FTIR spectra of *B.subtilis ATCC 6051 (B1)* used by other workers[41].

The thermal analysis (TGA and DTA) studies

The main purpose of the analyses with TGA is to examine the disruption processes of the biosorbents depending on the temperature [42]. In this study, the biosorbents were analysed at 25-1000 °C with TGA and

DTA data at a flow rate of 20 mL / min., in a N_2 (g) atmosphere, at a heating rate of 10 °C / min. using Shimadzu TGA-50 series model. The results are shown in the Fig.6

Bacillus Subtilis		***Bacillus Licheniformis***	
Starting and ending temperature (°C)	Weight loss %	Starting and ending temperature(oC)	Weight loss %
25-109	5,17	25-100	4,72
109-211	7,21	100-235	9,41
211-343	35,08	235-344	31,83
343-900	40,92	344-900	47,33

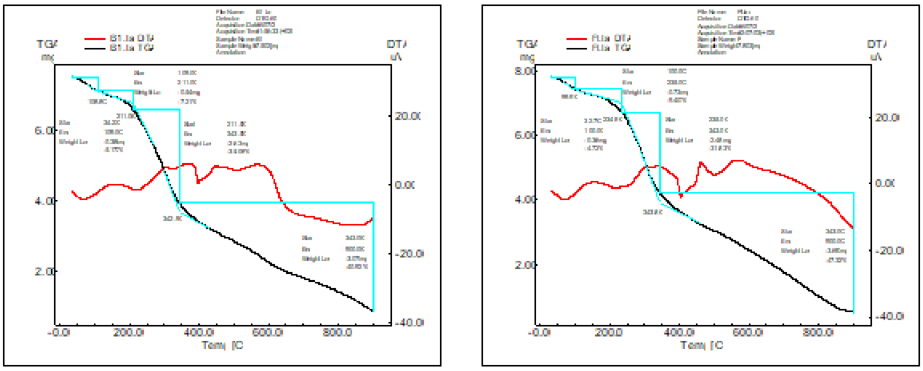


Fig.6 The TGA and DTA data of biosorbents

According to the result of TGA and DTA data, the first mass loss of the adsorbents appears to be between 25 and 109 °C due to physically adsorbed water on the surface of the adsorbents and at temperatures between 109-211°C is due to water trapped in the bacteria. The carbonation appears to occur between 211-343°C and they are burning in temperatures above 343°C. Therefore; TGA and DTA data show that the biosobents can be used for adsorption studies up to 211°C and resistant to heat [43].

SEM And EDX studies on biosorbents

The images of scanning electron microscope (SEM) were obtained with high vacuum EVO-440 model device under 20 kV. The SEM clearly revealed the surface texture and morphology of the biomass (Fig.7) at different magnifications. The surface areas of biosorbents untreated and treated with Pb^{2+} ion were imaged by SEM and it was clearly observed a lot of tiny interspace structure distributing on the surface of untreated and treated with Pb^{2+} shown that the surface of biosorbents observed rougher and more protrusions as seen in the Fig.7. This could be attribute to reactions occurring on the surface of biosorbents which treated with Pb^{2+} changed the structure of biosorbents. Also, there was a great number of crystal and white granular substances adhered to the surface of biosorbents in the SEM images, which could be the adsorbed Pb^{2+} ions particles[44,45].

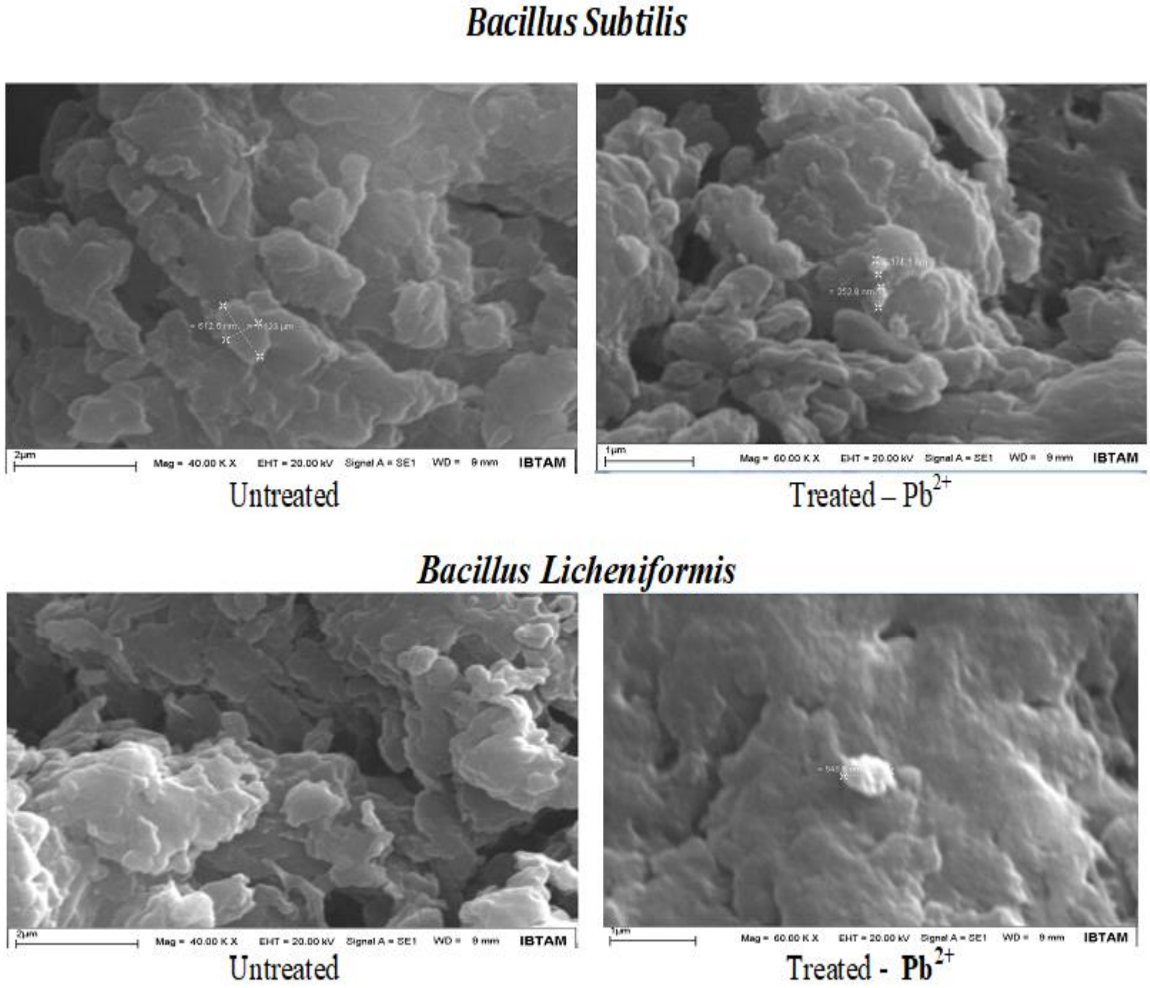


Fig.7 The images SEM of untreated and treated with Pb^{2+}ion of biosorbents

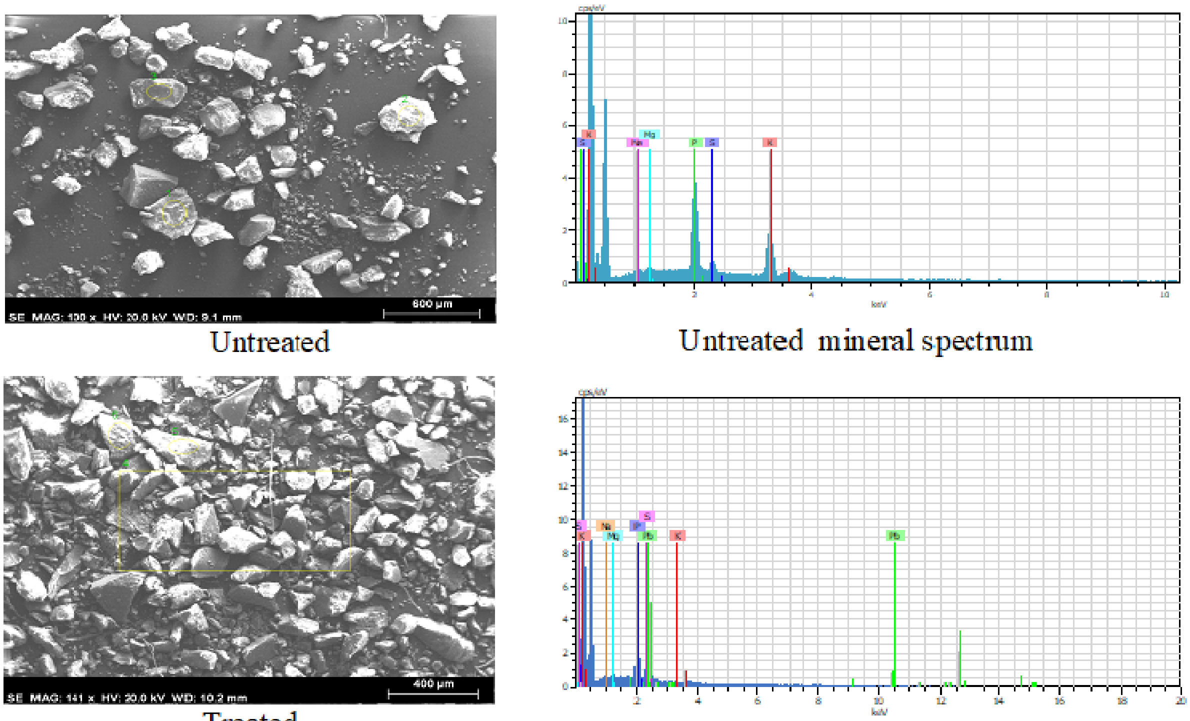
EDAX studies

In this research, the technique of SEM coupled with X-ray energy dispersion analysis was investigated to study the interaction of Pb^{2+} on *B.subtilis ATCC 6051 (B1)* treated and untreated. As seen in the Table 6 and Fig.8 the results of images and spectrum of EDAX indicate that the *B.subtilis* ATCC 6051 (B1) structure of was observed trace elements Na, Mg, P, S and K metal. The sample of *B.subtilis* was interacted with Pb^{2+} and the spectrum was observed on the EDAX where some spectrum rates of Na, Mg, P, S and K metal decreased. This indicated that Pb^{2+} ion could be exchanged with some metal ions on the cell wall of *B.subtilis*, so it would be better to suggest ion exchange mechanism[25,42].

Table 6
The spectra rates of mineral for Bacillus subtilis

Bacillus subtilis	Element /A. no	Measured amount (wt. %)	Mass amount (wt. %)	Atomic ratio (%)	Error rate (%)
Untreated Series	Na:11	0,503	4,4	6,33	0,1
	Mg:12	0,471	4,15	5,89	0,1
	P:15	4,713	41,003	43,86	0,2
	S:16	0,794	6,916	7,16	0,1
	K:19	5,063	43,526	37,02	0,2
	Na:11	1,05	1,56	7,44	0,133

Treated-Pb^{2+} Series	Mg:12	0,343	0,506	2,876	0,66
	P:15	3,873	5,633	25,9	0,2
	S:16	0,73	1,08	3,81	0,1
	K:19	1,21	2,19	5,23	0,1
	Pb:82	67,38	91,246	62,856	3,66



Untreated   Untreated mineral spectrum

Treated   Treated mineral spectrum and Pb^{2+}

Fig.8 The images of EDAX and mineral sepectrum for Bacillus subtilis

Recovery studies

In this study, the *B.subtilis* and *B.licheniformis sp.* saturated with Pb^{2+} were treated at different concentrations (0,01,0,05 and 0,1mol/L) with HCl and HNO_3 acid. The results showed that with 0.1M HNO_3 was removed in between 97-99 % from the biosorbents. However, HCL was little effect on the recovery because of the formation precipitate $PbCl_2$ in solution. The recovered bacteria was re-examined on the adsorption and observed to retain the adsorption capacities, which these features proved that bacteria can be reused in the adsorption of the metals[21].

Similar studies

As seen in the Table 7, some literature studies have used some types of bacteria on Pb^{2+} adsorption and the results are compatible with our studies[1,40,19,10].

Table 7

Comparison of our study and some studies

Referances	Mattuschka, at all.	Sulaymon, at all.	Pardo, at all.	Çolak, at all.	**Our studies**	
Biosorbent typ	*S.noursei*	*Chlamydomonas reinhardtii*	*Pseudomonas putida*	*B.cereus B.pumilus*	*Bacillus subtilis*	*Bacillus licheniformis*
Ads. capacity of Pb^{2+}(q_e) (mg/g)	36,50	24,90	56,20	22,10 28,20	40,82	40,82

Conclusions

The *Bacillus subtilis* obtained from ATCC 6051(B1) and *Bacillus licheniformis sp.* isolated from soil in area of Tigris River determined similar characteristics and adsorption capacities. There are no significant differences between *B.subtilis and B.licheniformis sp.* As a result of the studies carried out, it has been determined that it can be easily applied to water and wastewater treatments because of have a good morphological characteristics, recovery, temperature and water resistance. The *Bacillus licheniformis* for the removal of Pb^{2+} from the aqueous solution can be promising more effective as an alternative method physical and chemical processes because of its high metal binding capacity, low cost, high efficiency in dilute solution effluents, easily obtained in large quantities, water resistant and re-applicability

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