Biosorption of Pb(II) by the resistant *Enterobacter* sp.: Investigated by kinetics, equilibrium and thermodynamics

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Abstract: The Pb(II)-resistant bacterium was isolated from heavy metal-contained soils and used as a biosorbent to remove Pb(II). The strain was identified as *Enterobacter* sp. based on the 16S rRNA sequence analysis. The effect of biosorption properties (pH value, Pb(II) concentration, bacterial concentration and temperature) on Pb(II) was investigated by batch experiments. Results of FTIR and XPS showed that the biosorption process mainly involved some oxygen-containing groups (-OH and -COOH groups). The experimental results and equilibrium data were fitted by pseudo-second-order kinetic model and Langmuir model, respectively. The experimental biosorption isotherms fitted the Langmuir model, and the maximum biosorption capacity was 40.75 mg/g at 298 K. The calculated ΔG^o and ΔH^o were –4.06 and 14.91(kJ/mol), respectively, which indicated that biosorption process was spontaneous and endothermic. Results show that *Enterobacter* sp. will be an efficient biosorbent for Pb(II) removal.

Introduction

Pb(II) is one of heavy metals that are known to be highly toxic (Colak et al. 2011). The toxicity of Pb(II) and its compounds depends on its solubility in the organism. If it accumulates in the human body for a long time, it will seriously harm the nerves, hematopoietic system and digestive system (Tabaraki et al. 2014, Nseem et al. 2011, Wang et al. 2017). Pb(II) combines with active groups in enzymes and amino acids in the body, hinders the body's physiological and biochemical reactions, and produces poisoning (Nseem et al. 2011). Due to its long-term persistence in the environment and its strong potential toxicity to many living tissues, Pb(II) has been classified as a strong pollutant. Treatment of excessive Pb(II) ions in wastewater can effectively reduce the content of Pb(II) in animals and plants, and reduce harm to humans. At present, the main treatment methods for Pb(II) in wastewater are: chemical precipitation, ion exchange, electrolytic, activated carbon adsorption, solvent extraction, membrane separation technology, dilution water exchange method, biosorption, etc (Chen et al. 2016, Selatnia et al. 2004, Song et al. 2016). In contrast, Pb(II) biosorption has many advantages. The operating pH value and temperature range are wide, and researchers can choose the appropriate biosorbent according to different adsorption materials. Low-

-concentration ions can also be selectively adsorbed (Li et al. 2017). Metals recovered by biosorption can be recycled, but traditional methods cannot generally recover heavy metals in wastewater, while biosorbents can use their specificity to effectively recover some precious metals (Gupta et al. 2001). The biosorbent can be recycled, and the material source of the biosorbent is extensive. Separated microorganisms can be used as biosorbents, which can not only remove heavy metals in wastewater, but also recycle heavy metals after biosorption (Sag et al. 2000).

Biosorption method uses organisms such as fungi, bacteria, algae, moss, lignocellulose or cereal bark to absorb heavy metal ions dissolved in water (Holan et al. 1993, Lee et al. 2011, Kratochvil et al. 1998, Uzun et al. 2017). Then solid biosorbents are used in liquid-phase separation to remove heavy metal ions in an aqueous solution. Extracellular polymers are used to separate metal ions. Some bacteria release proteins during the growth process, which can convert soluble heavy metal ions in solution into precipitates and remove them (Song et al. 2018). The advantage of the biosorption method is that when the concentration of heavy metal ions is very low, it can also be removed. Other advantages include wide source, low price, strong adsorption capacity, easy separation and recovery of heavy metals, etc (Gupta et al. 2001). Baruah

et al. have found that *Enterobacter* sp. RC4 was capable of reducing crude oil content by 80%, which has been widely used (Baruah et al. 2016). Pb(II) biosorption by *Enterobacter* sp. is theoretically feasible, but to the best of our knowledge, there is no report on Pb(II) biosorption by *Enterobacter* sp., therefore, the use of *Enterobacter* sp. to adsorb Pb(II) has certain significance.

The objectives of this study are as follows:

(1) The strong Pb(II) resistant *Enterobacter* sp. was isolated from the Pb(II)-contaminated soils.

(2) The influence of solution pH value, uptake time and biosorption doses on biosorption were investigated systematically.

(3) XPS and FTIR were used to analyze the change elements and different functional groups on the surface of bacteria, respectively, and the morphological changes were analyzed by TEM.

(4) The biosorption kinetics, thermodynamics and isotherm of *Enterobacter* sp. for Pb(II) were studied.

Materials and methods

Cultivation of biosorbent and identification

The biosorbent was isolated from the Pb(II)-contaminated soils, and the samples (Pb(II)-contaminated soils) were stored in the School of Environment and Chemical Engineering, Anhui Vocational and Technical College. The strain was isolated by a specific method and was similar to the previous studies (Liu et al. 2018, 2019). Firstly, 10 g of soil sample was dissolved in 100 mL of sterile water, then the supernatant was gradually diluted to 10^{-4} times. Secondly, 1 mL of the dilution was spread on a nutrient agar plate (5 g/L of peptone, 1 g/L of glucose, 2.5 g/L of yeast extract) containing 100 g/L of Pb(II) ion, which was incubated for 24 hours at 37°C. Finally, the single colony was obtained for molecular identification and biosorption experiments. The methods used for the cultivation of *Enterobacter* sp. were the same as the methods for screening and separating. DNA was extracted from the isolated bacteria for PCR amplification. The amplified sequences were searched for homology in NCBI database.

Reagents and Characterization methods

The Pb(II) stock solution (500 mg/L) was prepared by dissolving $Pb_2(NO_3)_2$ in deionized water. Other reagents were analytical grade. The morphology of Pb(II)-loaded *Enterobacter* sp. was investigated using carbon dioxide critical point dryer (Emitech K850) and Transmission Electron Microscope (TEM) (Hitachi, HT-7700). The specific method was as follows: firstly, the cultured cells were centrifuged at 3000 rpm for 5 minutes, resuspended in physiological saline, repeated 3 times, and then dehydrated with 25%, 50%, 70%, 80%, and 90% ethanol solutions. Secondly, the dehydrated cells were fixed in 25% glutaraldehyde at 4°C for 12 hours., then rinsed with phosphate buffer solution 3 times, and then the sample was fixed with 1% osmium acid. Finally, the sample was mixed with embedding agent and acetone, and then dried overnight at 70°C. The chemical groups of *Enterobacter* sp. were performed by Fourier Transform Infrared Spectrometer (FTIR) (Thermo Scientific IS10, USA) in the wave number range 4000–500 cm^{-1}. The Elemental analysis was measured using X-ray photoelectron spectroscopy (XPS) (Thermo, ESCALAB250Xi, USA).

Biosorption experiments

The biosorption experiments were conducted at pH 6.0 and 0.01 mol/L KCl. Briefly, 1.0 mL of 0.5 mol/L KCl, 44.0 mL sterile water, 5.0 mL of 500 mg/L Pb(II) stock solution and 0.1 g *Enterobacter* sp. suspension (wet cell) were mixed. After equilibrium of 2 h, the liquid phase was separated from solid phase by centrifugation at 8000 rpm for 5 min. The concentration of Pb(II) in supernatant solutions was measured by UV spectrophotometry. The *Enterobacter* sp. was collected by centrifugation, and then the cells were weighed according to the amount of biosorbent on the effectiveness of Pb(II) biosorption. In the 50 mL biosorption system, 0.1 mol/L of sodium hydroxide and hydrochloric acid were used to adjust the pH of the biosorption system. The removal percentage (R, %), biosorption capacity (Q, mg/g) and distribution coefficient (K, mL/g) were determined by the following Eqns. (1–3) (Bobik et al. 2017, Wang et al. 2017, Wang et al. 2009):

$$R = \frac{C_0 - C_e}{C_0} \times 100\% \tag{1}$$

$$Q = \frac{(C_0 - C_e) \times V}{m} \tag{2}$$

$$K = \frac{(C_0 - C_e) \times V}{C_e \times m} \tag{3}$$

where C_0 and C_e (mg/L) are original and equilibrated concentrations, respectively. V (mL) and m (g) are the suspension volume and the mass of biosorbent, respectively.

Isotherm and kinetics analysis

Langmuir and Freundlich models are showed by Eqns. (4) and (5), respectively (Abdi et al. 2015, Ozdemir et al. 2009, Saha et al. 2017):

$$Q_e = \frac{Q_m b C_e}{1 + b C_e} \tag{4}$$

$$Q_e = K_F C_e^{1/n} \tag{5}$$

$$Q_e = \frac{RT}{b_T} \ln A_T + \frac{RT}{b_T} \ln C_e \tag{6}$$

Where:

Q_e (mg/g) and C_e (mg/L) are the equilibrium concentration of Pb(II) at solid and solution respectively;

Q_m is the maximum sorption capacity (mg/g), and n is the advantage of biosorption;

b (L/mg) and K_F ((mg/g)/(mg·L)$^{-n}$) are Langmuir Freundlich constant, respectively;

b_T is a dimensionless Tempkin isotherm constant (J/mol);

A_T is the Temkin isotherm equilibrium binding constant (L/mg).

The pseudo-first-order and pseudo-second-order are expressed as Eqns. (7) and (8), respectively (Bulut et al. 2007, Chen et al. 2016):

$$Q_t = Q_e(1 - e^{-k_1 t}) \tag{7}$$

$$Q_t = \frac{k_2 Q_e^2 t}{1 + k_2 Q_e t} \tag{8}$$

Where:

Q_t and Q_e (mg/g) are the biosorption capacity at time t and equilibrium, respectively;

k_1 (min^{-1}) and k_2 (g·mg^{-1}·min^{-1}) are the equilibrium constants of pseudo-first-order and pseudo-second-order models, respectively.

Thermodynamics analysis

The thermodynamic parameters (standard Gibbs free energy change -ΔG^o, standard enthalpy change -ΔH^o and standard entropy change -ΔS^o) can be calculated by the following Eqns. (8) and (9) (Naik et al. 2013, Ren et al. 2015):

$$\ln K = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \tag{9}$$

$$\Delta G^o = \Delta H^o - T\Delta S^o \tag{10}$$

Where:

R and T are gas constants (8.314 J/(mol·K)) and K – Kelvin temperature, respectively.

The values of ΔH^o and ΔS^o can be calculated by the intercept and slope of the linear eqn. (8), respectively.

Result and discussion

TEM analysis

Enterobacter sp. before and after Pb(II) biosorption was sliced and observed under Transmission Electron Microscope (TEM). The principle is to project the accelerated and concentrated electronbeams onto very thin samples to form different light and dark images (Li et al. 2017). Fig.1 shows the morphology of cell wall of *Enterobacter* sp. with and without Pb(II). Compared with the control group, the cell wall of *Enterobacter* sp. after Pb(II) biosorption became rough and fuzzy, and the contents of the cell aggregated and more heavy metal particles appeared. Baysal et al. found that electron denseness was present on the cell walls of *Candida albicans carrying* Pb(II), whereas it was absent on the cell walls not carrying Pb(II) (Baysal et al. 2009).
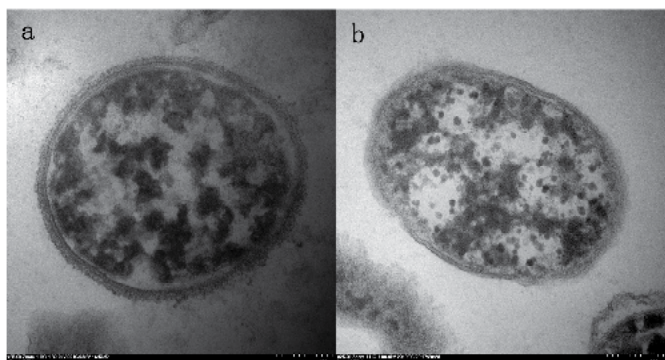
FTIR spectral analysis

As shown in Fig. 2, the spectra of unloaded and Pb(II)-loaded *Enterobacter* sp. in the range of 500–4000 cm^{-1} were analyzed. It was found that chemical groups of *Enterobacter* sp. were responsible for biosorption. Compared with the spectra of the control group in the range of 1000–2000 cm^{-1} and 2700–3200 cm^{-1}, the spectra after the Pb(II) biosorption disappeared. According to the analysis, the peak at 1697 cm^{-1} was caused by the vibration of the C-O group, in addition, the peak at 1540 cm^{-1} was caused by the O-H and C-C vibration (Boyanov et al. 2003). The spectra of *Enterobacter* sp. loaded-Pb(II) were slightly lower than those of the unload--metal biomass. After the addition of Pb(II), the metal-ligand interaction was more obvious due to the interaction between Pb-N and O-S-O with Pb(II). These results confirmed that the biosorption of Pb(II) was due to some groups on the surface of *Enterobacter* sp. (Chojnacka et al 2004, Chojnacka et al 2005). The cell walls are made up of large peptides that can be attached to teichoic acids and polysaccharides. These molecules and intracellular substances have functional groups of Pb(II) biosorption. The groups (-NH, carboxyl (-COOH), hydroxyl (-OH)) present the ability to bind to metallic ions (Tang et al. 2017).

XPS analysis

FTIR analysis was performed, in order to further verify whether there is a physical and chemical reaction between bacteria and Pb(II). Fig. 3(a) shows the XPS results of cell walls before and after Pb(II) biosorption. The diagrams clearly show that the spectral line of O is very strong, that of N is relatively weak, and those of P and S are almost absent. The adsorption spectra of Pb(II) before and after the adsorption of Pb(II) were compared, and the binding energy range of the two obvious peaks (130–150 eV) proved that Pb(II) was adsorbed on the cells (Naik et al. 2017, Ku and Jung 2001, Chuah et al. 2005). As shown in Fig. 3 (b), compared with the control group, a lead peak appeared after Pb(II) biosorption. At the same time, the analysis of the spectrum also showed that the content of O on the cell wall was high. According to Fig. 3 (c-f), the peak area and shape of elements C, O, N and P have changed, which indicates that these elements participate in the reaction and the results of sample detection show that the content of these elements has changed (Chen et al. 2020, Ramrakhiani et al. 2016). From the above analysis, it can be seen that *Enterobacter* sp. has obvious treatment effect for heavy metal.
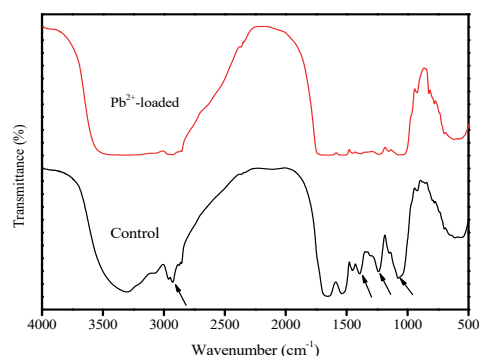

Fig. 1. TEM images of *Enterobacter* sp. (a) – control and (b) – loaded-Pb(II), respectively

Fig. 2. FTIR spectra of *Enterobacter* sp. before and after biosorption of Pb(II)

Effect of solution pH

Fig. 4(a) shows the effect of pH on Pb(II) biosorption by *Enterobacter* sp. Fig. 4(b) shows the distribution of Pb(II) speciation in aqueous solutions under different pH using visual MINTEQ 3.1 software. 1 mL of 50 mg/L lead ion solution and 7mL of sterile water were placed in labeled conical bottles 1, 2, 3, 4, 5 and 6, and pH was adjusted to 3 ~ 8 respectively. Then 2 mL of bacterial suspension was added, and the concentration of residual lead ion was determined by visible spectrophotometer with the supernatant centrifugation (Tunali et al. 2006) .

The pH value of the solution directly affects the effect of Pb(II) biosorption, and the influence degree can be divided into two stages (Fig. 4). When pH < 6, the adsorption amount decreased significantly with the decrease of pH. In the range of 6 < pH < 8,and the adsorption amount also decreased significantly with the increase of pH, and the adsorption amount was the largest when pH was 6.0 (Sahin et al. 2005). Since proteins on cell walls are partially decomposed into amino acids during chemical pretreatment, both amino and carboxyl groups on amino acids have a lone pair of electrons
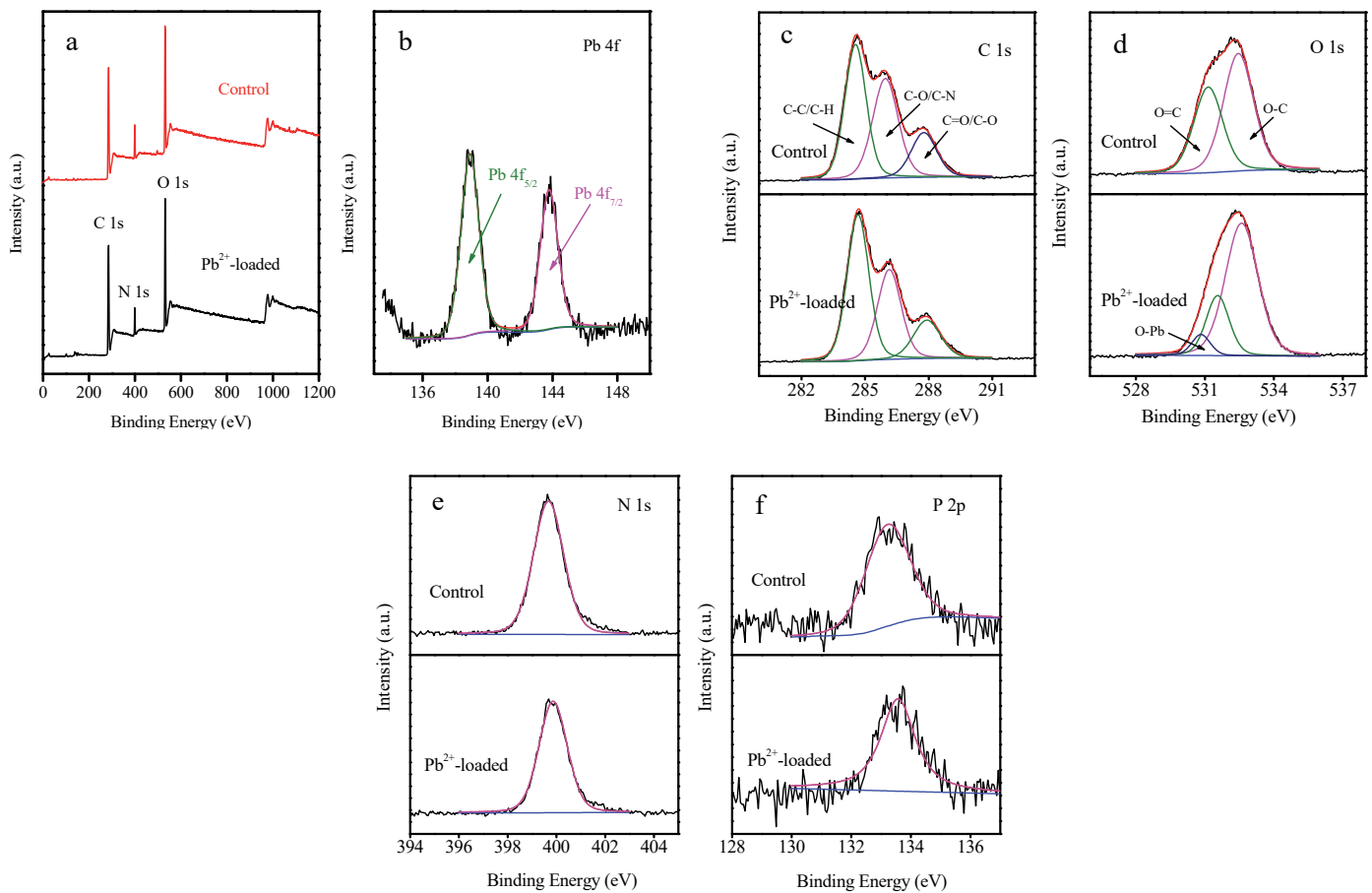

Fig. 3. XPS Intensity curves of Pb(II) biosorption on *Enterobacter* sp., (a) – total survey scans, (b) – Pb4f as a function of the binding energy, (c–f) – C 1s,O 1s, N 1s, P 2p
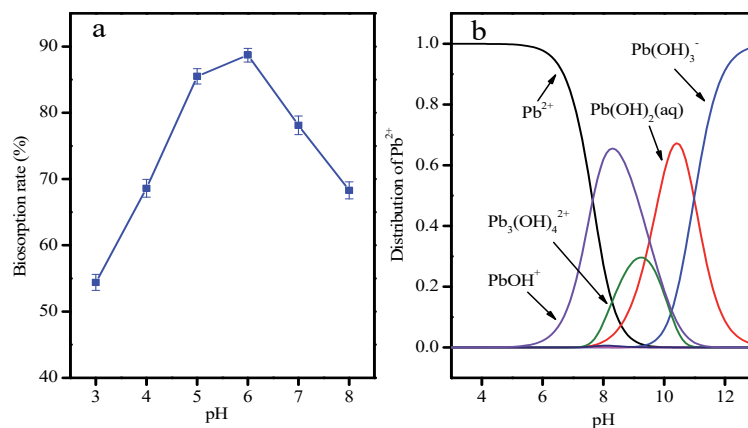

Fig. 4. (a) – Effect of pH on Pb(II) biosorption by *Enterobacter* sp. (C_{Pb2+} = 50 mg/ L, V = 50 mL, m/V = 2.0 g/L, T = 298 K), (b) – the relative species distribution of 50 mg/L Pb^{2+}

that can be provided to Pb(II), and some other functional groups containing N, O and S can also be combined with Pb(II) (Fourest et al. 1992). It can be seen that the biosorption amount has a great relationship with the functional groups on the cell wall.

Effects of biosorption doses and isotherm
Effects of biosorption doses
The amount of biological adsorbent is an important parameter to determine the adsorption amount of heavy metal ions. The influence of biosorption dose on Pb(II) is shown in Fig. 5 (a). As the *Enterobacter* sp. concentration increases, the removal rate of Pb(II) also increases, however, the amount of Pb(II) biosorption decreases. In the late stage of biosorption, the *Enterobacter* sp. cells aggregate with each other (Ma et al. 2015), thereby reducing the effective binding sites on the surface of the cell walls.

Isotherm analysis
Fig. 5 (b & c) shows the adsorption isotherm of Pb(II) by *Enterobacter* sp. at pH 6.0. The three isothermal models, i.e., Langmuir, Freundlich and Temkin isotherms, are the most common isothermal models. Langmuir model represents monolayer biosorption, whereas Freundlich and Temkin models represent multi-molecular layer biosorption. The experimental data were regressively analyzed according to the Langmuir, Freundlich and Temkin isothermal equations, and the biosorption parameters and regression equations were obtained (see Table 2). According to the analysis (R^2), in the low concentration, the Pb(II) biosorption by *Enterobacter* sp. relatively conforms to the Langmuir monolayer biosorption (Han et al. 2006, Shroff et al. 2011, Liu et al. 2021). Nonetheless, the Pb(II) biosorption was more consistent with the Temkin model. The Temkin isotherm is shown in Fig. 5 (c). According to this model, the decrease in the heat of biosorption due to the interaction between the biosorbents had a linear relationship with the surface coverage of the biosorbent. The Temkin isotherm can be used to explain the results. When the Pb(II) concentration is low, single-layer biosorption is the main cause, while at higher concentration, the multi-layer biosorption is dominant. With the increase of the equilibrium concentration of Pb(II) in the solution, the biosorption amount of bacteria increases, and when the equilibrium concentration is greater than a certain value, the biosorption reaches the equilibrium and tends to saturation. The Pb(II) biosorption is closely related to the Temkin model.
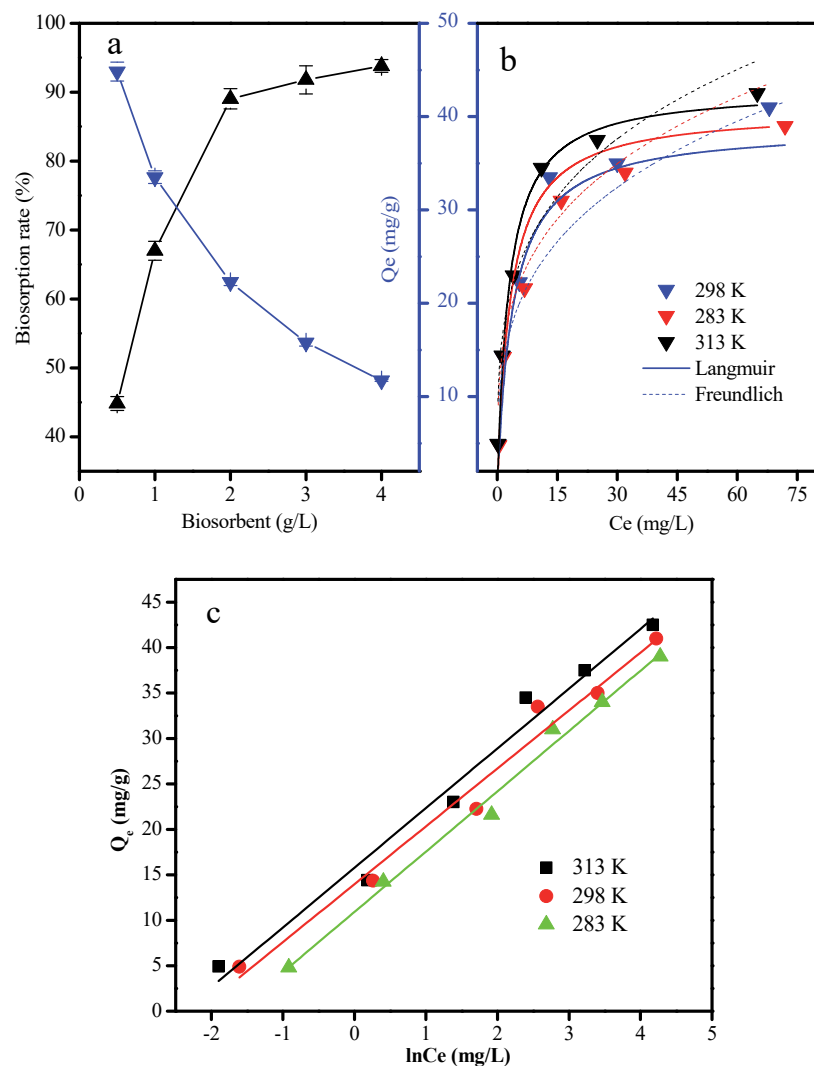

Fig. 5. (a) – The effect of biosorbent dosage on Pb(II) biosorption by *Enterobacter* sp., (b) – Langmuir & Freundlich isotherms (c) Temkin isotherm (C_{Pb2+} = 50 mg/L, pH = 6.0, *V* = 50 mL, *T* = 298 K)

Biosorption kinetics analysis

Biosorption is a dynamic process. In order to investigate the adsorption mechanism and rate of *Enterobacter* sp., pseudo--first-order and pseudo-second-order adsorption rate equations have been adopted in this study (Lu et al. 2013) and their expressions are shown in formulas (6) and (7). The process of Pb(II) biosorption by *Enterobacter* sp. can be divided into two stages. In the first stage, the biosorption rate is very fast; in the second stage, the biosorption rate is very slow and the biosorption amount increases very slowly (Zheng et al. 2018, Lu et al. 2020). The values of Qe, k1, k2 and correlation coefficient (R^2) values were obtained from the graph and listed in Table 1. The present kinetic results showed best fitting with the pseudo-second-order model (Fig. 6a, b and c) as it has higher correlation coefficient values (R2 = 0. 99) than that of pseudo-first order model (R^2 = 0.89), indicating the chemisorptions type sorption of Pb(II) on to *Enterobacter* sp.

Thermodynamic parameter analysis

In the experiment, entropy and gibbs free energy factors need to be considered to determine what happens spontaneously. Thermodynamic parameters such as enthalpy change, gibbs free energy change and entropy change can be obtained by using the change of equilibrium constant with temperature (Raize et al. 2004). Thermodynamic parameters of *Enterobacter* sp. for Pb(II) biosorption are listed in Table 3, and the calculation formulas of the parameters are shown in (8) and (9). Table 3 shows that the negative value of gibbs free energy proves the feasibility of heavy metal ions' spontaneous adsorption of this biosorbent (Siripongvutikorn et al. 2016). In addition, the positive value of enthalpy change also indicates the endothermic property of the biosorption process, i.e., the amount of biosorption increases with the increase of temperature (Ahalya et al. 2003), which is also confirmed in Fig. 7.

Conclusion

The Pb(II)-resistant bacterium designated as *Enterobacter* sp. was isolated from heavy metal contaminated soil; it exhibited highly efficient removal rate and uptake capacity for Pb(II). The results show that the biosorption process was affected by pH, biosorbent doses, temperature and initial concentration of Pb(II) ions. The maximum uptake capacity was 40.75 mg/g by the Langmuir isotherm model. According to the thermodynamic results, the temperature rise is conducive to the increase of the biosorption capacity. Based on XPS and FTIR analyses,

Table 1. Kinetic parameters of Pb(II) biosorption on *Enterobacter sp.*

Kinetic model	T (K)	k_1 (min^{-1})/ k_2 (g/mg min)	Q_e (mg/g)	R^2
Pseudo-first order	298	0.00486	11.97	0.893
Pseudo-second order	298	0.00498	22.76	0,999

Table 2. Parameters for the Langmuir and Freundlich isotherm models

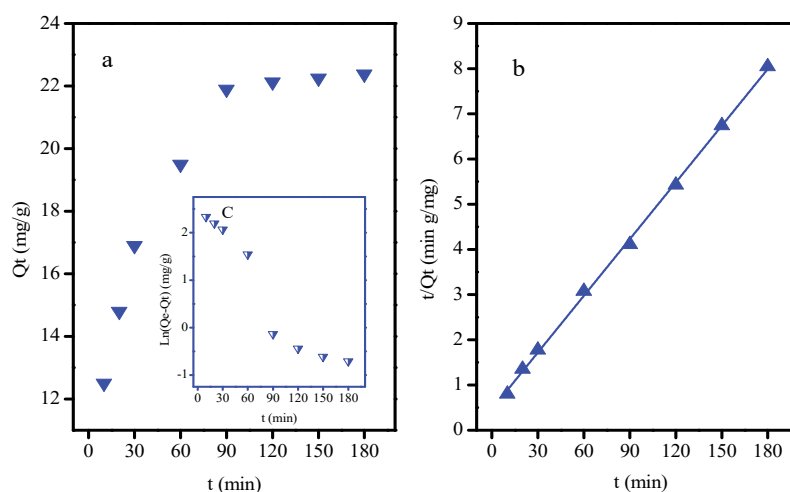| T (°K)	Longmuir			Freundlich			Temkin		
	Q_m (mg/g)	b (L/mg)	R^2	K_F (mg/g)/ (mg·L)$^{-n}$	n	R^2	A_T (L/mg)	b_T (J/mol)	R^2
283	38.92	0.259	0.959	12.28	0.285	0.933	5.19	354.56	0.989
298	40.75	0.307	0.956	14.02	0.269	0.927	8.97	388.96	0.973
313	43.02	0.349	0.979	15.53	0.260	0.918	11.06	396.33	0.974

Fig. 6. (a) – Biosorption kinetics of *Enterobacter* sp. on Pb(II); (**b**) –The pseudo-second-order model; (**c**) – The pseudo-first-order model. (*m/V* = 2.0 g/L, C_{Pb2+}= 50 mg/L, *T* = 298 K, pH = 6.0)

Table 3. Thermodynamic parameters for Pb(II) biosorption on *Enterobacter sp*

T(K)	ΔG° (kJ/mol)	ΔH° (kJ/mol)	ΔS° (J/(mol·K))	R^2
283	-2.52			
298	-3.44	1491	6159	0.942
313	-4.37			
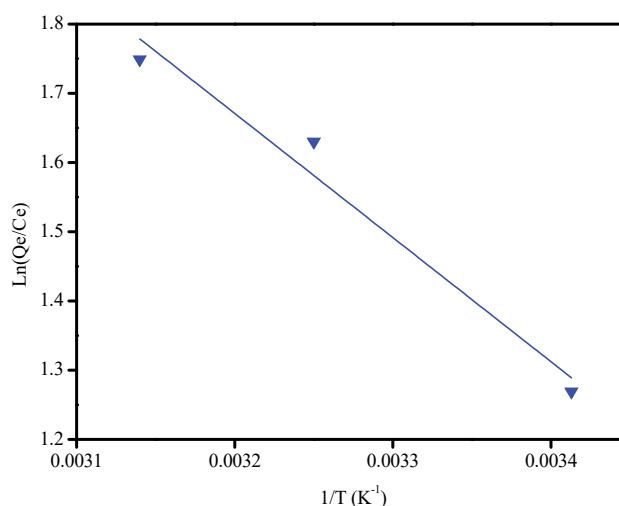
Fig. 7. Van, T Hoff plot for biosorption of Pb by *Enterobacter* sp. (m/V = 2.0 g/L, C_{Pb2+} = 50 mg/L, pH = 6.0)

the main groups of *Enterobacter* sp. that combine with Pb(II) during biosorption are some oxygen-containing groups. The resistant *Enterobacter* sp. will have potential application value in removing heavy metal.

Conflicts of interest

All authors declare that they have no actual or potential conflicts.

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