



Biosorption of lead (II) on *Lactobacillus bulgaricus*

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Abstract

This study compared the ability of *Lactobacillus bulgaricus* to remove lead from single and mixed metal ion solution. The influence of pH was studied. The adsorption capacity of lead onto *Lactobacillus* obtained at pH 5.5 was 43.5 mg g⁻¹. The adsorption processes of lead by studied microorganism were evaluated and analyzed using the theories of Langmuir and Freundlich. The maximum metal uptake values were (q_{max} = 123 mg/g). Biosorption of lead metal ions by *Lactobacillus* is a promising technology that involves removal of toxic metals from industrial waste streams and natural waters.

1. Introduction

Increase in industrial activity during recent years and an agricultural activity has led to the contamination of environment, mainly in the aquatic systems. The wastewaters discharged from chemical industries contain heavy metal ions which have toxic effect on all living organisms [1, 2]. Heavy metals also accumulate throughout the food chain even in relatively low concentrations and have harmful effects on human being [3].

Extensive research has been carried out during the last years to find high efficiency and low-cost adsorbents for the removal of heavy metal from aqueous solution [4, 5]. The most widely used methods for removing heavy metals from waste waters include ion-exchange [6], coagulation, chemical precipitation, reverse osmosis, evaporation, membrane filtration, biological adsorption treatment. Recently, biosorption using microbial biomass [7] as the adsorbent, has emerged as a potential alternative technique to removal of toxic heavy metals to an environmentally friendly manner and environmentally safe level in a cost effective.

The employment of bacterial biomass for the metal removal treatment of effluents is a perspective suggested by many researches dealing with metal-bacteria interactions. Their cell walls contain a variety of surface organic functional groups, which exhibit a high affinity to bind metals and other chemical species [8, 9].

The aim of this study was to test bacterial cells (*Lactobacillus bulgaricus*) for their capacity to absorb Pb (II) at different parameters such as initial concentration of lead ions, contact time, pH, and biomass doses. Two adsorption models, the Langmuir model and the Freundlich equation have been applied to the experimental data.

2. Materials and methods:

2.1. Preparation of *Lactobacillus bulgaricus*

1. Preparation of bacterial strains:

A swab of dilutions of yogurt was transferred with a sealed pipette to agar MRS plate's media and streaked for isolation. The anaerobic bacteria isolation and biochemical testing were performed under anaerobic condition with suitable humidity using a modified technique.

The cultures were incubated at 35 °C in the anaerobic chamber. All media were prepared aerobically and passed into the chamber at least 24 hours before use.

Lactobacillus bulgaricus were prepared at microbiology laboratory- faculty of science-Suez Canal University *in vitro* as the following: 5.0 ml of the tested bacteria was added to 500 ml of MRS broth. After that it was incubated at the optimum temperature (37°C anaerobic conditions) to 24 hr., then it was centrifuged at (3000 r.p.m at 4°C for 20 min) to harvest the cells. Dehydration was obtained by addition 50 g of defatted soy protein (soy protein without fat) to cells in big Petri dishes and the cells were incubated under vacuum incubator at 40°C overnight until it seemed like as thin slice or skins. The viability of the cells was tested on MRS agar plates then, the strain was chopped and made as a powder containing 109 of bacteria/g.

2.2. Reagents and solution

The chemicals used for this study were analytical grades of lead acetate Pb (C₂H₃O₂)₂, nitric acid and sodium hydroxide. All chemicals and reagents used for experiments and analyses were of analytical grade. Stock solutions of 1000 mg L⁻¹ Pb (II) were prepared from Pb (C₂H₃O₂)₂ in distilled, deionized water that contained a few drops of 0.1 mol L⁻¹ HNO₃ to prevent the precipitation of Pb (II) by hydrolysis. The solutions were diluted as required to obtain working solutions. The initial pH of the working solutions was adjusted to 6.0 by addition of HNO₃ or NaOH solution, except the experiment examining the effect of pH. Fresh dilutions were used for each sorption study.

2.3. Lead ion analysis

The concentrations of the lead ions present in water will be measured by a Perkin Elmer Analyst 700 atomic absorption spectrometer with deuterium background corrector was used. All measurements were carried out in an air/acetylene flame. A 10 cm long slot-burner head, a lamp and an air-acetylene flame were used. The operating parameters for working elements were set as recommended by the manufacturer.

2.4. Methods of adsorption studies:

Batch adsorption experiments were carried out by shaking the flasks at 120 rpm for a period of contact time using a water bath mechanical shaker. Following a systematic process, the absorption uptake capacity of Pb (II) in batch system was studied in the present work. The data obtained in batch mode studies was used to calculate the equilibrium metal adsorptive quantity. It was calculated for each sample of Pb (II) by using the following expression:

$$q_e = \frac{V(C_0 - C_e)}{m}$$

Where q_e is the amount of heavy metal ions adsorbed onto per unit weight of beer yeast in mmol g^{-1} , v the volume of solution treated in L, c_0 the initial metal ion concentration in m mol L^{-1} , C_e the equilibrium metal ion concentration in m mol L^{-1} , and m is the dry weight of the biomass in g.

2.4.1. Effect of contact time on biosorption:

Batch biosorption tests were done at different contact time at the initial concentration of 60 mg L^{-1} for metal cations, and *Lactobacillus* dose concentration is 0.012 g L^{-1} in 10 mL solution. The temperature was controlled with a water bath at the temperature of 25°C . The samples were then collected at different intervals and were centrifuged and filtrated. The concentration of metal ions in the supernatant solution was analyzed using flame atomic absorption spectrometry. Each determination was repeated three times and the results given were the average values. The deviation is less than 6%.

2.4.2. Effect of biomass concentration on biosorption:

Batch sorption tests were done at different *Lactobacillus* concentrations 0.003 to 0.024 g L^{-1} at pH 6.0, for a contact time (60 min), and 25°C . The initial concentrations of Pb (II) were 60 mg L^{-1} . Agitation was made for 60 min. The samples were then centrifuged and the left out concentration in the supernatant solution was analyzed as said before.

2.4.3. Effect of solution pH on biosorption:

The effect of pH on the adsorption capacity of *Lactobacillus bulgaricus* was investigated using solution of 60 mg L^{-1} metal cations for a pH range of 2–9.0 at 25°C . An aliquot of biomass (0.012 g L^{-1}) was added to 10 mL of Pb (II) solution. Experiments could not be performed at higher pH values due to hydrolysis and precipitation of the lead ions. Flasks were agitated on a shaker for 60 min to ensure that equilibrium was reached. Then the mixture was centrifuged and the concentration of metals in the supernatant solution was filtrated and measured.

2.4.4. Equilibrium modeling

The Langmuir and Freundlich equations are in common use for describing adsorption isotherms at a constant temperature for water and wastewater treatment applications. The well known expression of the Langmuir model is given

by Eq. (2):
$$\frac{C_e}{q_e} = \frac{1}{K_L q_{\max}} + \frac{1}{q_{\max}} C_e$$

Where q_e and q_{\max} are the observed and maximum uptake capacities (mg / g), C_e is the equilibrium concentration (mg L^{-1}), and K_L is the equilibrium constant (Lmg^{-1}).

The empirical Freundlich equation based on sorption onto a heterogeneous surface is given below by Eq. (2):

$$q_e = K_F C_e^{1/n} \quad 2$$

Where K_F and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the sorbent, respectively (Ho and McKay, 1998;; Aksu, 2002).

2.2.5. Kinetic modeling

One of the most important characteristics in evaluating adsorption efficiency is kinetics. Several models can be used to express the kinetics of the sorption processes such as pseudo-first and pseudo-second-order reaction model. The pseudo-first order rate expression of Lagergren is generally described by equation 3.

$$\frac{dq_t}{dt} = K_1 (q_e - q_t) \quad 3$$

Where q_e and q_t are the amounts of metal ion (mg/g) adsorbed on the sorbent at equilibrium, and at time t , respectively and K_1 is the rate constant, (min^{-1}). Integrating and applying the boundary conditions, $t=0$ and $q_t=0$ to $t=t$ and $q_t=q_e$

The pseudo-second order chemisorption kinetic rate equation is expressed as shown in equations 4 and 5.

$$\frac{d q_t}{d t} = K_2 (q_e - q_t)^2 \quad 4$$

Where K_2 is pseudo-second order rate constant ($\text{g mg}^{-1} \text{min}^{-1}$). After integrating and applying boundary conditions for $t=0, q=0$, Eq. (5) becomes

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \quad 5$$

3. Results and discussion

3.1. Effect of contact time:

The effect of contact time on metals was found that the adsorptive quantity of lead metal on *Lactobacillus* was relatively stable as the contact time increased. The biosorption of Pb(II) onto studied *Lactobacillus bulgaricus* microorganisms was rapid for first 10 min and equilibrium was nearly reached after 15 min followed by a constant adsorption upon further increasing the contact time as shown in (Fig.1). So, in the present experiment, 60 min was chosen as the equilibrium time.

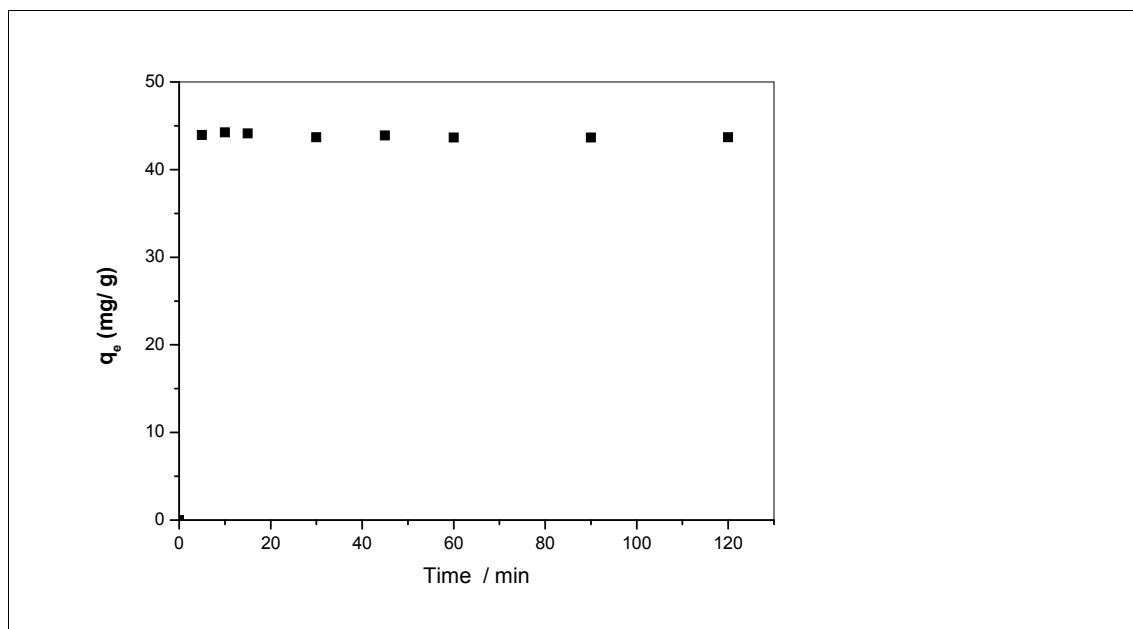


Fig. (1) The effect of contact time on biosorption of Pb(II) *Lactobacillus* biomass.

3.2. The effect of biomass dose on biosorption:

The influence of adsorbent dosage in equilibrium uptake was investigated as shown in (Fig. 2). The biosorptive capacity of the metal ions is inversely proportional to the initial concentration of the biomass when the initial concentration of metal ions is kept constant, i.e., with the dose of biosorbent increasing, the values of q_e were both decreased.

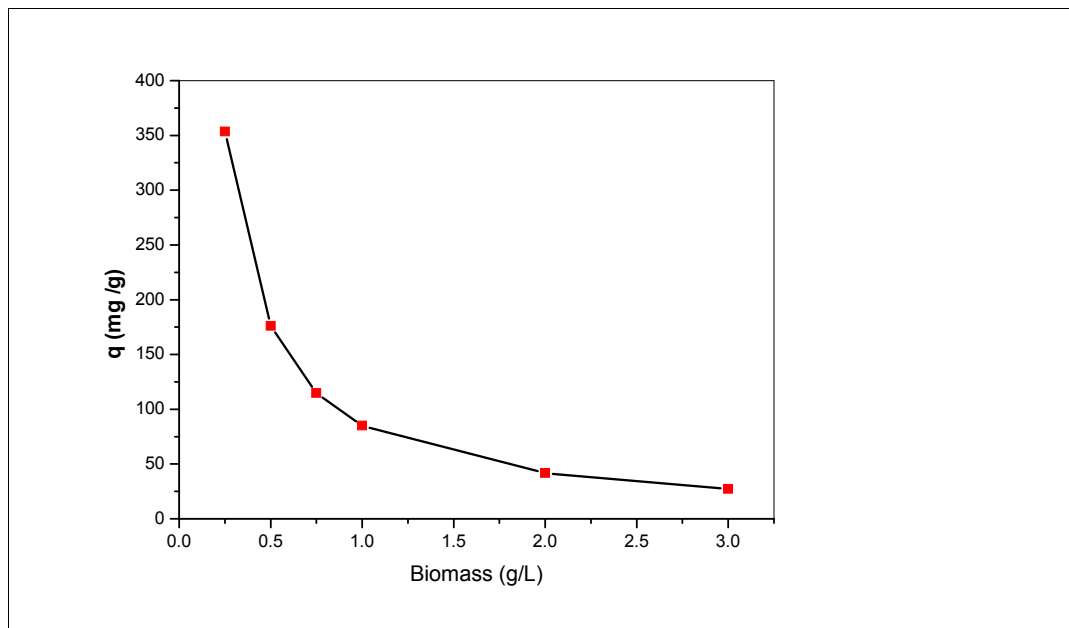


Fig (2): The effect of biomass dosage on the adsorption capacity for lead metal solutions.

3.3. The effect of solution pH:

Effect of pH on adsorption of lead ions was investigated at 30°C with agitation at 150 rpm as shown in (Fig.3). The maximum equilibrium uptakes for Pb (II) ions by *Lactobacillus bulgaricus* were observed at the pH between 5.5 and 6.0. The results indicate that the adsorption capacity of *Lactobacillus* for Pb (II) reached its highest value of 43.76 mg/g (83.5%) at pH 6.0. The adsorption showed the lowest value of 36.0 mg/g (73.0%) at pH 2.0. There was an increase in biosorption capacity of biomass with increasing pH from 2.0 to 6.0 for lead metal ions.

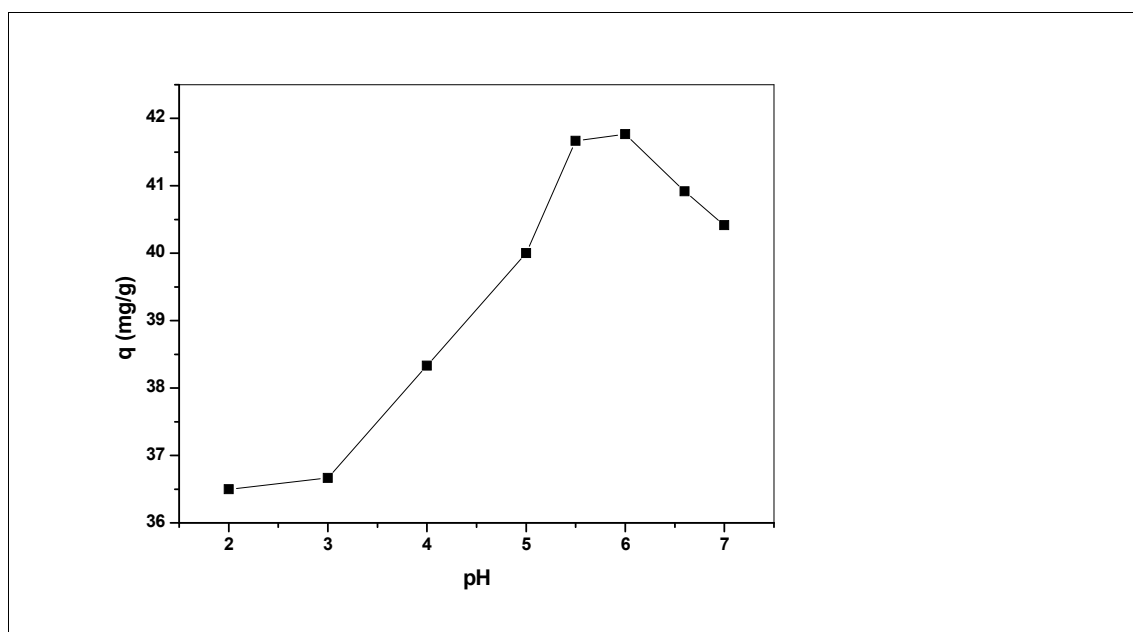


Fig (3): Adsorption capacity of Pb (II) as a function of pH

3.4. Initial concentration of metal ions and biomass:

The effect of the initial metal ion concentration on the adsorptive capacity of the biomasses was investigated as shown in (Fig.4) For the determination of adsorption characteristics of Pb (II) ions, the initial concentrations of the metal ions were varied between 10 and 100 mg L⁻¹ while the biomass weight of the adsorbent was kept constant at 1.2 g L⁻¹ at pH 5.5. From (Fig. 4), equilibrium uptake increased with the increasing of initial metal ions concentrations at the range of experimental concentration.

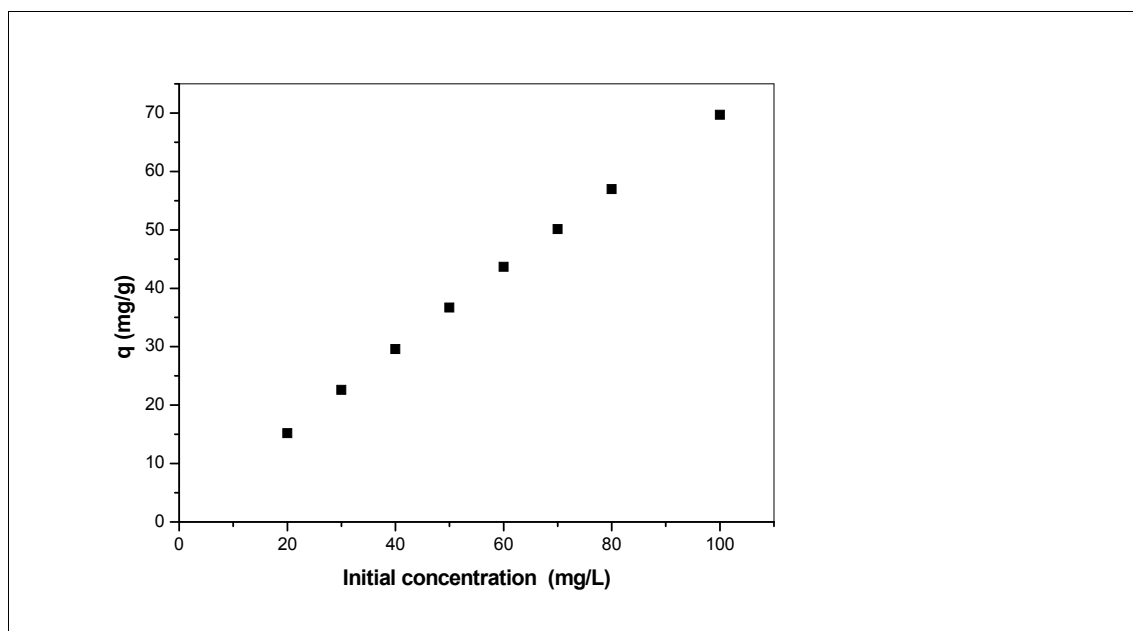


Fig (4): Effect of initial metal ion concentration on the different adsorbent

3.5. Equilibrium modeling

The Langmuir and Freundlich equations are in common use for describing adsorption isotherms at a constant temperature for water and wastewater treatment applications. The Langmuir model is valid for monolayer sorption on to a surface with a finite number of identical sites. Analysis of the equilibrium data is important to develop an equation which accurately represents the results and which could be used for design purposes. The linearized Langmuir isotherm of Pb (II) ions obtained at the temperature of 30 C is given in (Fig. 5). The experimental data were analyzed by linear regression analysis and then the Langmuir isotherm constants and correlation coefficients are listed in Table 1. The well

known expression of the Langmuir model is given by Eq. (2):

$$\frac{C_e}{q_e} = \frac{1}{K_L q_{\max}} + \frac{1}{q_{\max}} C_e$$

2

Where q_e and q_{\max} are the observed and maximum uptake capacities (mg g⁻¹), C_e is the equilibrium concentration (mg L⁻¹), and K_L is the equilibrium constant (Lmg⁻¹).

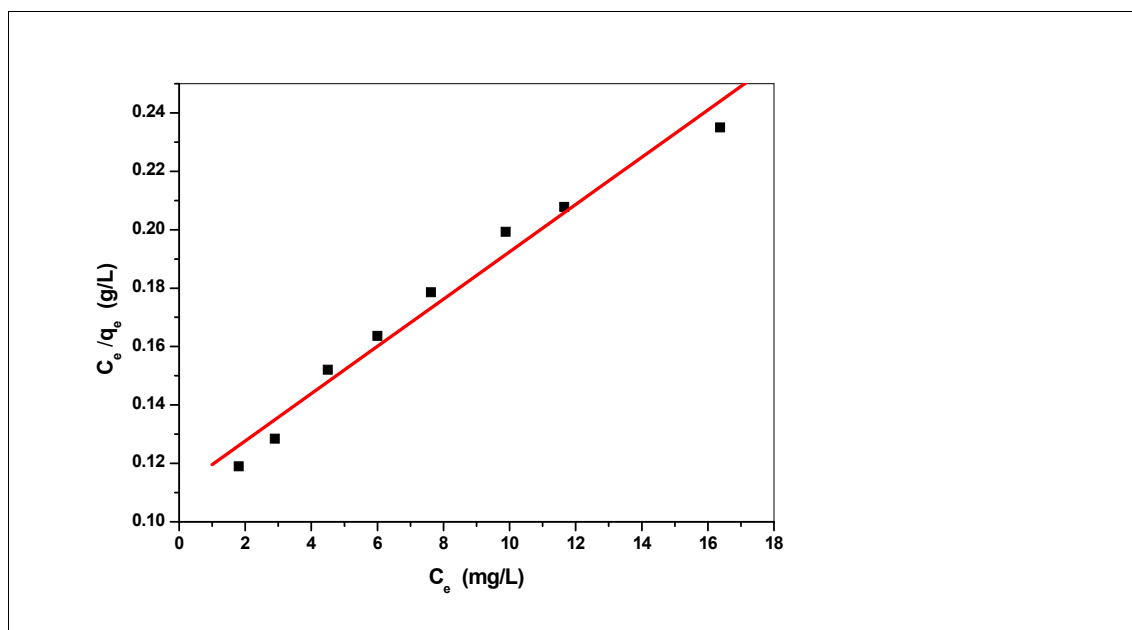


Fig (5): Linearized Langmuir adsorption isotherms (dry weight = 1.2 gL⁻¹, initial pH value = 5.5).

The equilibrium established between the adsorbed metal ions (q_e) and that remained free in the solution (c_e) was also represented by the Freundlich adsorption isotherm. The empirical Freundlich equation based on sorption onto a heterogeneous surface is given below by Eq. (3):

$$q_e = K_F C_e^{1/n} \quad 3$$

Where K_F and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the sorbent, respectively (Ho and McKay, 1998;; Aksu, 2002).

The above equation is conveniently used in the linear form by taking the logarithm of both sides as:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln C_e \quad 4$$

The equilibrium data of lead ions adsorption by lactobacillus obtained at 298 K was applied to Langmuir and Freundlich models (Figs. 5 & 6). The relative parameters and correlation coefficients (R) and standard deviations (S.D.) were listed in Table 1. The high values of K_F showed ready uptake of the heavy metals from wastewater with high adsorptive capacities of these biosorbents. In addition, n was greater than unity, indicating that the metal ions were favorably adsorbed by *Lactobucillus* biomasses. It is generally stated that the values of n in the range 2–10 represent good adsorption (Ho and McKay, 1998).

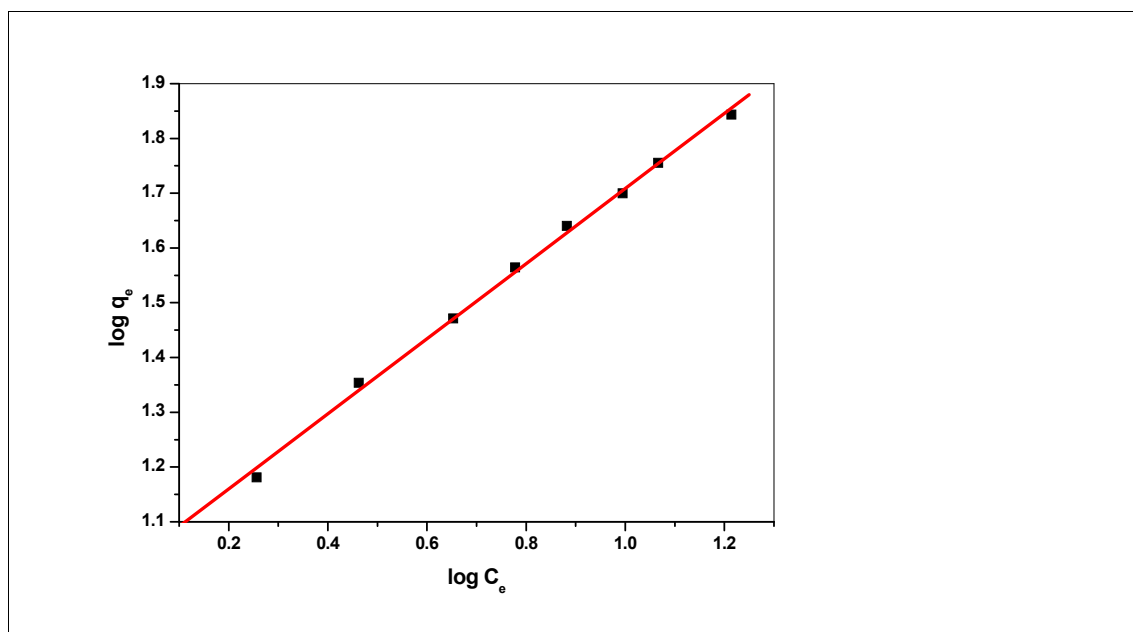


Fig (6): Linearized Freundlich adsorption isotherms for (A) Pb(II) ions (dry weight = 1.2 gL⁻¹, initial pH value = 5.5).

Table11: Parameters for the application of Langmuir and Freundlich model to biosorption of Pb(II).

	Langmuir				Freundlich			
	k_L (L/mg)	q_{max} (mg/g)	R	S.D	K_F (mg/g)(L/mg) ^{1/n}	1/n	R	S.D
Pb(II)								
<i>Lactobacillus bulgaricus</i>	0.0725	123.61	0.9866	0.0071	10.54144	0.6857	0.9987	0.0122

3.6 Kinetic modeling

One of the most important characteristics in evaluating adsorption efficiency is kinetics. The kinetic parameters, which are helpful for the prediction of adsorption rate, give important information for designing and modeling the adsorption processes. Adsorption kinetics are studied to estimate the time required for obtaining equilibrium concentration of adsorbate for the adsorption of heavy metals on the adsorbent. According to literature, several models can be used to express the kinetics of the sorption processes such as pseudo-first and pseudo-second-order reaction model. The pseudo-first order rate expression of Lagergren is generally described by the following equation.

$$\frac{dq_t}{dt} = K_1 (q_e - q_t) \tag{5}$$

where q_e and q_t are the amounts of metal ion (mg/g) adsorbed on the sorbent at equilibrium, and at time t , respectively and K_1 is the rate constant, (min⁻¹). Integrating and applying the boundary conditions, $t=0$ and $q_t=0$ to $t=t$ and $q_t=q_e$ Eq. (4) takes the form:

$$\log (q_e - q_t) = \log q_e - \frac{K_1}{2.303} t \tag{6}$$

The rate constant K_1 can be obtained from the slope of the linear plots of $\log (q_e - q_t)$ against t . The experimental data of adsorption lead metal ions by *lactobacillus* microorganisms based on $\log (q_e - q_t)$ versus t show that the resulting fit

curves deviate from the experimental data (Fig. 7). Therefore, this biosorption system is not the pseudo first-order reaction.

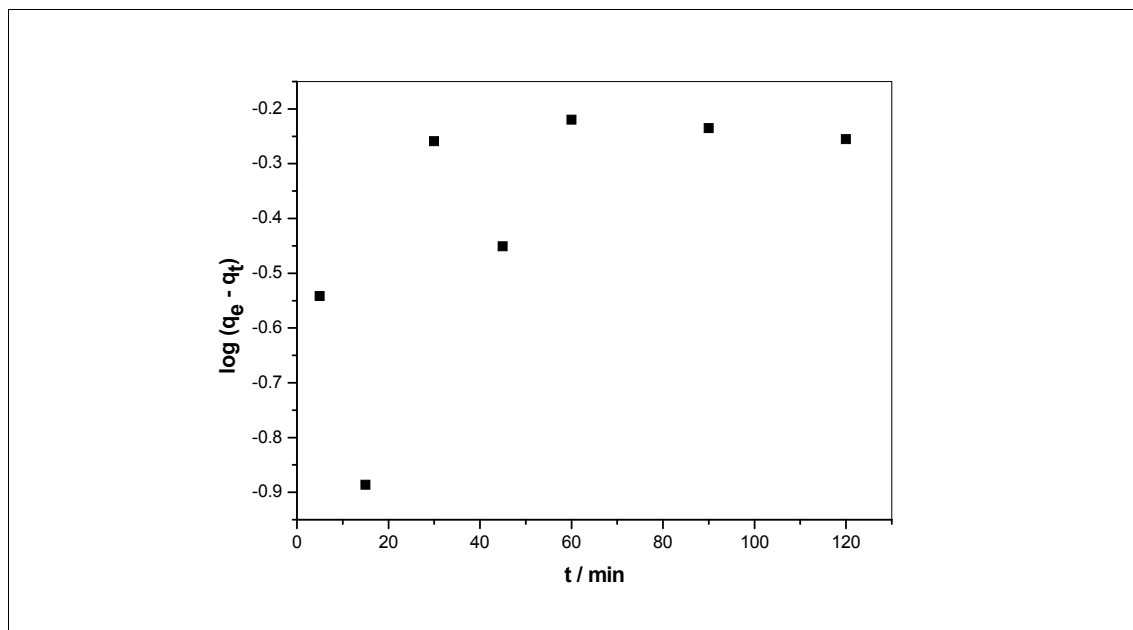


Fig (7): Experimental data obtained using pseudo-first order kinetic model (log(q_e-q) versus t).

The pseudo-second order equation assumes that the sorption process involves chemisorption mechanism and the rate of site occupation is proportional to the square of the number of unoccupied sites. The pseudo-second order chemisorption kinetic rate equation is expressed as:

$$\frac{d q_t}{d t} = K_2 (q_e - q_t)^2 \quad 7$$

Where K_2 is pseudo-second order rate constant ($\text{g mg}^{-1} \text{min}^{-1}$). After integrating and applying boundary conditions for $t=0, q=0$, Eq. (6) becomes

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \quad 8$$

The rate constant K_2 can be obtained from the intercept of the linearized pseudo-second order rate equation. If the pseudo-second order equation can fit the sorption data, there should be good linearity between t/q and t . The initial sorption rate K_i can be defined as

$$K_i = K_2 q_e^2 \quad 9$$

The relationship between $\log (q_e - q_t)$ versus time t in this work was not linear over the entire time range (Fig. 7). However, Fig. 8 showed that the pseudo-second order equation was ideally applicable to the sorption data. The best fit of the second-order expression suggests that the chemisorptions mechanisms involved in the adsorption. The initial sorption rates K_i ($\text{mg g}^{-1} \text{min}^{-1}$), the correlation coefficients for the second-order kinetic model were listed in Table 1. The calculated values (q_e) matched well with the experimental ones. These results implied that the biosorption of metal ions could be best described by the pseudo-second order model.

The pseudo-second order equation assumes that the sorption process involves chemisorption mechanism and the rate of site occupation is proportional to the square of the number of unoccupied sites.

The correlation coefficients for the second-order kinetic model were 1.00 for all the concentrations studied. The theoretical q_e values also agreed very well with the experimental q_e values in the case of pseudo-second-order kinetics. The studies on the kinetics of the various sorption processes have revealed that over long period of time pseudo-second order model provides the best correlation.

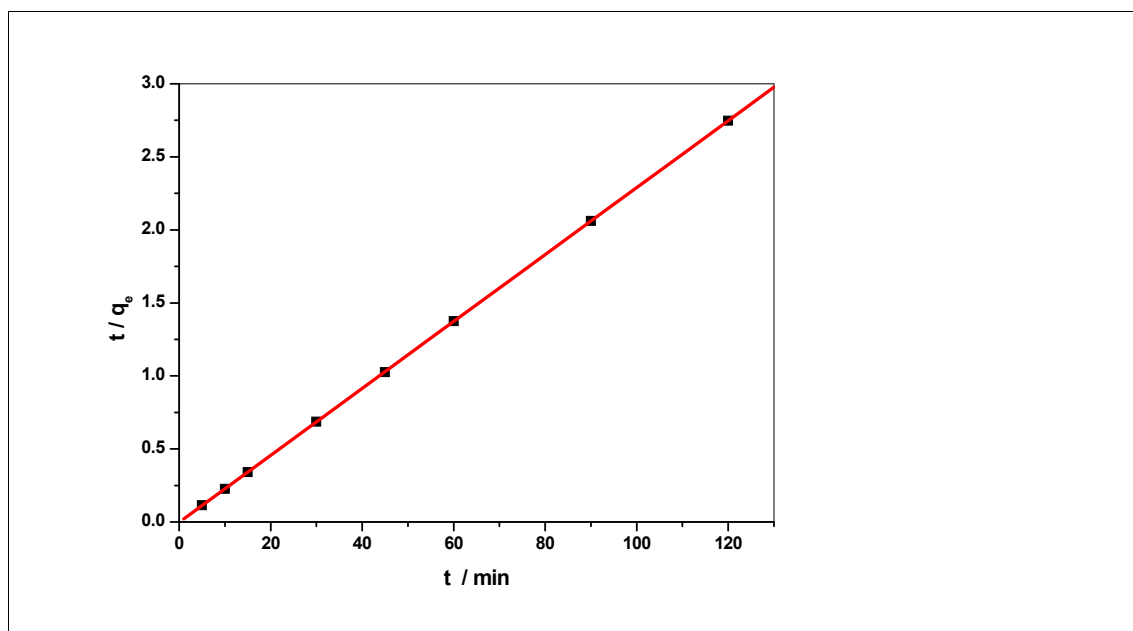


Fig (8): Linear fit of experimental data obtained using pseudo second order kinetic model (t/q versus t).

Table 2: pseudo-second-order models for different initial concentrations of Pb (II) ions at 30 °C.

Micro-organism	Intercept $1/K_2 q_e^2$	Slope $1/q_e$	q_e (Exp)	q_e (Calc) mg/g	K_2 g/mg min	K_i mg/g min	R
<i>lactobacillus</i>	0.0024	0.023	43.6	43.5	0.2202	416	0.999

4. Conclusion

The biosorption properties lead ions removal on *Lactobacillus bulgaricus* was studied. Lead ion concentration and pH of solution were found to effect the biosorption capacity of the biomass. The Langmuir adsorption model and Freundlich equation were used for mathematical description of the biosorption of lead ions onto bacterial biomass and it was found that the adsorption equilibrium data fitted well to the Langmuir model. In competitive biosorption experiments, the biosorption capacities of the bacteria biomass for lead ions were found to be lower than non-competitive conditions. The use of readily available *Lactobacillus* biomass may offer an alternative in the removal of metals from industrial wastewaters.

5. References

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