

# Equilibrium and Kinetics Studies on Biosorption of Thallium (I) by Dead Biomass of *Pseudomonas fluorescens*

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## Abstract

We investigated the biosorption characteristics of thallium (I) ions using dead biomass of *Pseudomonas fluorescens* strains as biosorbents. The biosorbents were characterized by Fourier transform infrared spectroscopy (FT-IR) and a scanning electron microscope (SEM). The effects of different environmental factors such as initial Tl concentration, initial solution pH, biomass dosage, and contact time were evaluated. The maximum adsorption capacity was found to be 93.76 mg/g at an optimum initial pH of 5.0, a contact time of 60 min, a biomass of 0.5 g/L, and an initial Tl concentration of 50 µg/mL. The biosorption process can be well defined by the Langmuir isotherm ( $R^2 = 0.9967$ ). The biosorption kinetics were better described by the pseudo second-order model ( $R^2 = 0.9950$ ) than the pseudo first-order one. The analysis of (FT-IR) indicates that the main functional groups responsible for adsorption of Tl (I) were hydroxyl, carboxyl, and amino groups. SEM analysis verifies an obvious surface morphology change of adsorbed biomass. The results presented in this study show that the *Pseudomonas fluorescens* could be an effective, low-cost, and environmentally friendly biosorbent for removing Tl (I) from aqueous solution.

**Keywords:** Thallium (I), biosorption characteristics, biomass, *Pseudomonas fluorescens*

## Introduction

Heavy metals pollution has become a major concern among the most important world environmental issues that threaten the ecological environment and human

health due to its toxicity and accumulation throughout the food chain [1]. Thallium is regarded as a lethally toxic metal that usually arises from mining wastewater, and it is produced as a by-product in the refining of iron, lead, zinc, and pyrite [2]. Even at low concentrations, after exposure, Tl can easily enter the human body and cause great chemical toxicity to the whole organism, such as lung insufficiency, bone degradation, liver and kidney

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damage, and inducing sarcoma and leukemia [3-4]. The removal of Tl-containing wastewater has generally been implemented using traditional methods [5-6] such as chemical ion exchange, filtration, reduction, oxidation, and precipitation [8]. However, these techniques have been proven to be cost-ineffective and time-consuming in their practical application. Hence, it is essential to explore other effective methods for removing Tl from wastewater.

Biosorption is a potential technology for utilizing the cost-effective biomass to remove heavy metals from wastewater due to their economical operation, eco-friendliness, good performance, and availability, and it has been verified for its adsorptive characteristic in many published reports [9]. Numerous microbial materials such as bacteria, fungi, algae, and yeast, both the living biomass and dead biomass can bind the heavy metals from wastewater [10]. Among the microorganisms, owing to functional groups on cell wall such as carboxyl, hydroxyl, amino, or sulfhydryl [11-12] for heavy metal ions binding, bacteria have exploited various resistance mechanisms to resist heavy metal stresses [13]. The use of dead biomass could prove superior to living biomass regarding less sensitivity to toxic metal ions and relatively simple executing conditions, plus no requirements for nutrients and culture media, and easy recovery and desorption after the biosorption process [14-15].

Different living and dead biomasses of bacteria have been used as adsorbents to remove heavy metals from the wastewater. [16] used a Cd-resistant *Pseudomonas* strain to remove Cd, while [17] used *starfish* and *Pseudomonas putida* strain for the adsorption of heavy metals and uranium from wastewater. However, there are no reports on the adsorption of Tl by *Pseudomonas* sp. from the wastewater. In this study, one resistant strain identified as *Pseudomonas fluorescens*, which showed a tolerance to thallium, was isolated and screened from the wastewater of Yunfu pyrite mine in southern China.

The aim of this paper was to investigate the potential application of *Pseudomonas fluorescens* as a substitutable adsorbent for the removal of thallium ions from aqueous solutions. The biosorption characteristics of different environmental parameters, including the effect of initial Tl concentration, initial solution pH, biomass dosage, and contact time were evaluated. The isotherm and kinetic models of Tl adsorption were also constructed. In addition, the mechanism of biosorption was explored by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscope (SEM).

## Materials and Methods

### Preparation of Dead Biomass

*Pseudomonas fluorescens* was cultured in LB medium (150 mL) at 25°C in a 500 mL flask with shaking speed at 180 r/min for 24 h. After fermentation, cultures were centrifuged at 10,000 r/min for 8 min and washed thrice with deionized water to collect the biomass. The dead

biomass was acquired by heating at 121°C for 30 min.

### Structural Characterization by FT-IR Spectral and SEM

The biomass powder was mixed with dry KBr at a ratio of 1 mg/100 mg. The mixture was thoroughly incorporated and compressed into a small pellet [18]. The sample was analyzed using a Nicolet 380 spectrometer (USA) in the wavelength from 400-4,000cm<sup>-1</sup>. The morphological properties of the strain *Pseudomonas fluorescens* before and after biosorption was investigated using a JEM-1230 analyzer (Japan).

### Sorption Isotherms

The dynamic process of the solute adsorbed to the adsorbents depends on the biosorption equilibrium between solid and liquid phase, which could be described by the most typical adsorption isotherm models such as the Langmuir and Freundlich model. The Langmuir model simplifies the monolayer sorption, in which the solid surface is uniform and each adsorption can only be a molecular or atomic adsorption and with lateral interaction between the sorbed molecules. The Freundlich model describes the heterogenous adsorption and multilayer biosorption to the binding sites located on the surface of the adsorption equilibrium [19].

The Langmuir model [20] is described by the following equation:

$$q_{eq} = \frac{q_{max} b C_e}{1 + b C_e}$$

...where  $q_{eq}$  is the adsorbing capacity per gram of biomass at equilibrium (mg/g),  $q_{max}$  is the maximum adsorbent amount per unit weight of biomass, and  $b$  is the adsorbent constant that concerns the binding force (mg/g). The Freundlich isotherm model [21] equation can be expressed by the form:

$$q = K_f C_e^{1/n}$$

...where  $q$  represents adsorption capacity for mass per unit mass of biomass at equilibrium;  $C_e$  represents the concentration of metal remaining in solution at equilibrium; and  $k$  and  $n$  are the Freundlich constants that help to describe the adsorption process.

### Batch Adsorption Experiments

The batch adsorption of Tl experiments proceeded in an isothermal shaker. In order to determine the optimal initial concentration, pH range, biomass, and contact time we used Tl(I) solutions of 50 mg/L. The effect of pH was investigated at the range of 2.0-8.0, biomass at the range of 1.0-6.0 g/L, concussion agitation speed was 180 r/min and temperature was 25°C was set for

the biosorption experiments. In most of the experiments (except biomass variation study), 0.45 g of sorbent was added to 100 mL solution of Tl. The biosorption capacity of Tl was calculated according to the following formula [22]:

$$q = \frac{V(C_0 - C_{eq})}{M}$$

...where  $q$  means the biosorption capacity of heavy metal ions (mg/g),  $C_0$  and  $C_{eq}$  mean the initial and equilibrium metal concentrations in the solution (mg/L), respectively;  $V$  is the volume of solution (L); and  $M$  is the mass of adsorbent (g).

## Results and Discussion

### FT-IR and SEM Analysis

The FT-IR spectroscopy technique was applied to reveal which functional groups were responsible for the adsorption of Tl(I). The FT-IR spectra of before and after adsorption from the range of 400–4,000  $\text{cm}^{-1}$  is presented in Fig. 1. It is demonstrated that functional groups on the cell wall were consistent with the adsorption bands. The broad bands at about 3,359  $\text{cm}^{-1}$  represented the bonded –NH or –OH groups. The peaks near 2,928  $\text{cm}^{-1}$  could be attributed to the –CH stretching [23]. The bands appearing at about 1,398  $\text{cm}^{-1}$  were distributed carboxyl group (–C=O) stretching. The peaks observed at about 1,093  $\text{cm}^{-1}$  were caused by C–O stretching [24]. The peaks observed at about 727  $\text{cm}^{-1}$  were assigned to the –CH stretching of the aromatic series.

The peak at 3,359  $\text{cm}^{-1}$  was shifted to 3,376  $\text{cm}^{-1}$  for Tl(I)-loaded biomass. The peak of the –CH group was shifted from 2,928  $\text{cm}^{-1}$  to 2,911  $\text{cm}^{-1}$ , the carboxyl

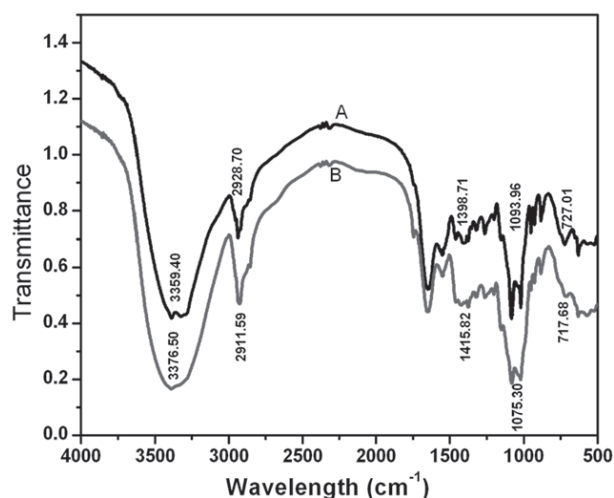


Fig. 1. FT-IR analysis for *Pseudomonas fluorescens* biomass (A) meaned strain before biosorption and (B) meaned strain after biosorption.

peak was shifted from 1,398  $\text{cm}^{-1}$  to 1,415  $\text{cm}^{-1}$ , the peak of –CO group was shifted from 1,093  $\text{cm}^{-1}$  to 1,075  $\text{cm}^{-1}$ , and the stretching vibration at 727  $\text{cm}^{-1}$  was shifted to 717  $\text{cm}^{-1}$  after Tl(I) biosorption. These phenomena indicate that several functional groups have important roles in Tl(I) biosorption because of the components on the microbial cell wall and membrane [25–26]. These functional groups like carboxyl, hydroxyl, and amine actively participate in binding of metal ions by chemical interactions. The results favored the chemical process as ion-exchange between carboxyl, hydroxyl, and amine groups of the biomass, and the metal ions mainly participated in the adsorption of Tl(I) onto *Pseudomonas fluorescens*. Similar FT-IR results [27] for Cd and Ni adsorption onto bacterial biomass were reported, which are consistent with our findings.

The surface morphology of Tl explored by SEM images is shown in Fig. 2. Before biosorption (Fig. 2a), the adsorbent appeared as a smallish and porous surface structure. Nevertheless, after biosorption (Fig. 2b) the surface became rough. Such roughness of the surface might be because of the adsorption on thallium over the surface that makes the surface coarser than its original form. This finding is similar to that reported by Cui Pang et al. [28], in which biosorption of rubidium was conducted using rubidium-bearing bacteria.

### Effect of pH on Biosorption

pH is a significant factor influencing the biosorption of heavy metal ions, which could affect the ionization of functional groups on cell walls and the precipitation of heavy metal in solution. The influence of pH on the biosorption of Tl(I) into *Pseudomonas fluorescens* was explored over the pH range 2.0 to 8.0 using 50  $\mu\text{g/mL}$  initial Tl(I) concentration at normal temperature. The experimental data are exhibited in Fig. 3. Solutions of 0.5 M HCl and NaOH were used to adjust the pH of the Tl(I) solution. As can be seen from Fig. 3, the biosorption capacity of Tl(I) increased as the pH increased from 2.0 to 5.0 and reached a maximum at a pH of 5.0, which was applied to all the subsequent experiments. The low adsorption capacity at lower pH values could be explained by that  $\text{H}^+$  rather than Tl(I) in cells combined with negatively charged functional groups on the surface of binding sites. The gradual increase in solution pH will bring about the increase in the degree of ionization

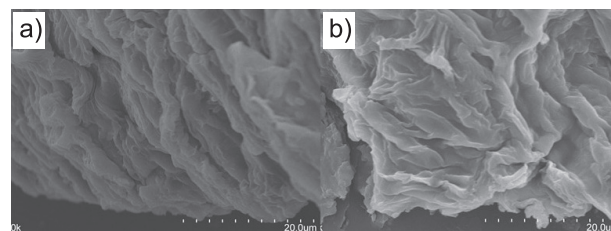


Fig. 2. Scanning electron microscope (SEM) analysis for *Pseudomonas fluorescens* biomass a) means before biosorption and b) means after biosorption.

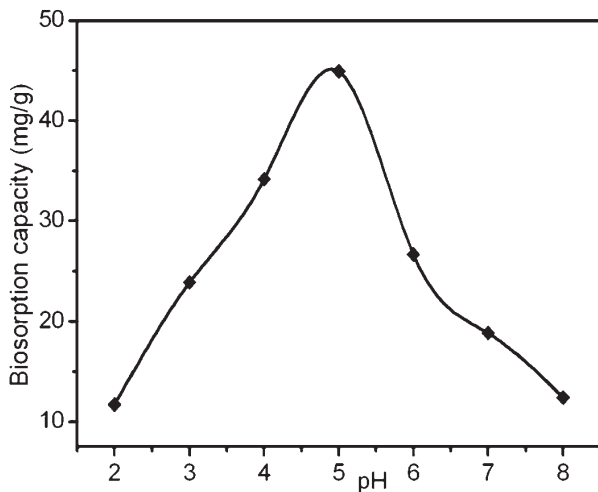


Fig. 3. Effect of pH on thallium(I) biosorption by *Pseudomonas fluorescens* (m: 0.45 g, V: 100 mL, Tl: 50  $\mu$ g/mL, 25°C).

functional groups from the biomass surface, and therefore the degree of electrostatic interactions will also be strengthened. However, the adsorption capacity decreased as the solution pH continued to increase from the range 6.0 to 8.0, which is probably due to the change in the form of heavy metals, as at higher pH, the hydroxyl, bicarbonate ions in solution increased and competed binding sites with Tl(I), which ultimately lead to a reduction in adsorption capacity [29-30].

#### Effect of Biomass on Biosorption

The biomass of adsorbent could play an important role on biosorption. The influence of biomass quantity on biosorption is listed in Fig. 4. In this study, with the biomass from the range 0.1 to 0.6 g/L, the biosorption

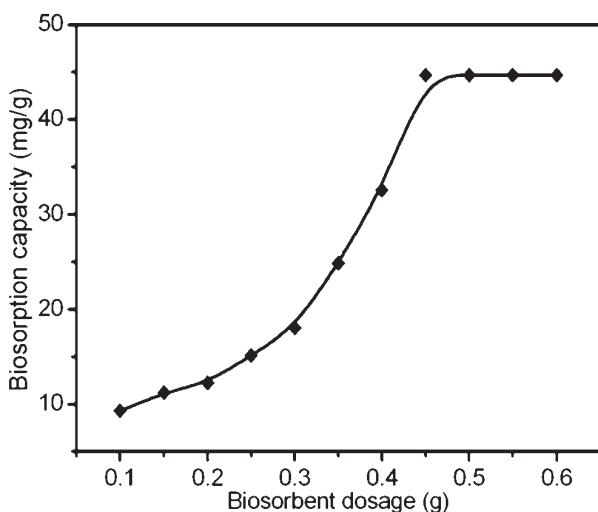


Fig. 4. Effect of biosorbent dosage on thallium(I) biosorption by *Pseudomonas fluorescens* (V: 100 mL, Tl: 50  $\mu$ g/mL, time: 60 min, 25°C).

capacity was increased from 9.78 to 44.97 mg/g, which can be explained by the fact that the increased biomass can provide large biosorption surface area and the available solute was sufficient to entirely cover the available exchangeable sites on strain surface and result in metal biosorption at high biomass concentration. The maximum biosorption capacity of Tl(I) was observed at 0.5 g/L. After reaching the maximum, there was no obvious increase in biosorption capacity, which is because the biomass reached saturation. Increased dosage can bring a surface effect to protect the active sites from being occupied by Tl(I), as documented by Nasrin Masoudzadeha et al. [31]. Meanwhile, no increase in biosorption as the result of the mutual interference between adsorption sites. This result is in line nicely with those published in other research papers [32].

#### Effect of Contact Time on Biosorption

Biosorption of Tl(I) by the *Pseudomonas fluorescens* strain as a function of time is described in Fig. 5. The biosorption capacity of Tl(I) for the dead biomass reached equilibrium within 60 min. In the beginning, the biosorption capacity of biosorbents increased quickly owing to sufficient exploitable active adsorption sites on the biomass surface, and Tl(I) ions can easily interact with the binding sites. Afterward, these active binding sites were gradually occupied with Tl(I) ions and with gradual occupancy of these sites. After 60 min, the biosorption capacity was almost constant due to the reduction of very few active sites being available in successful collisions of the Tl(I) ions on the surface of the strain *Pseudomonas fluorescens*, and due to a reduction in concentration gradient. Therefore, biosorption efficiency remains unchanged in the later stages [33]. Maximum biosorption capacity of Tl(I) by *Pseudomonas fluorescens* was found to be 44.91 mg/g under this condition in batch mode.

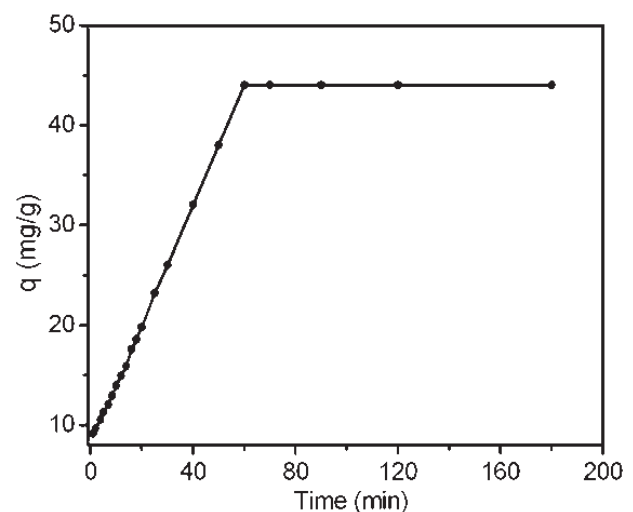


Fig. 5. Effect of contact time on thallium(I) biosorption by *Pseudomonas fluorescens* (m: 0.45g, V: 100 mL, Tl: 50  $\mu$ g/mL, 25°C).

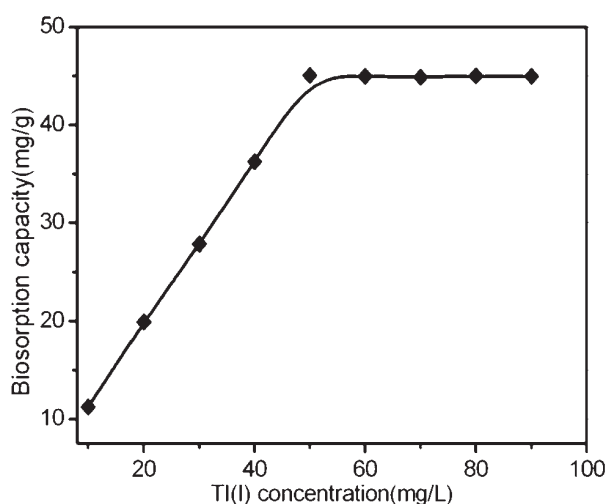


Fig. 6. Initial concentration of thallium (I) on biosorption by *Pseudomonas fluorescens* (m: 0.1g, V: 100 mL, 25°C).

### Influence of Initial Tl(I) Concentration on Biosorption

The influence of initial Tl(I) concentration on biosorption by *Pseudomonas fluorescens* was investigated under varying initial Tl(I) concentrations ranging from 10 to 90 mg/L (Fig. 6). From 10 to 50 mg/L it is clear that the adsorption capacity of Tl(I) increased with the increasing amount of initial Tl(I) concentration, due to the fact that the ratio of the initial molecules of solute to the available surface area is low, and the initial concentration generates an important driving force to overcome all mass transfer resistance of Tl(I) between the aqueous and solid phases, and the maximum amount of Tl(I) adsorbed could reach 44.83 mg/g at an initial Tl(I) ion concentration of 50 mg/L. When the initial Tl(I) ions concentration increased from 50 to 90 mg/L, biosorption showed saturation at higher metal ion concentrations. Because cells offer a finite number of surface binding sites, similar results have been referred to by other authors according to Anindita et al. [34].

### Biosorption Isotherms

Biosorption isotherms play an important role in the exploration and description of an adsorption model system. Thus, in our research the biosorption of Tl was evaluated by Langmuir and Freundlich isotherm models with Tl(I) ion concentration ranging from 10 to 90 mg/L at 25°C (Figs 7a-b). The Langmuir adsorption isotherm can fit the equilibrium data very well, with a high value of correlation coefficient ( $R^2 = 0.9967$ ) and a maximum biosorption capacity of 93.76 mg/g. Compared with the Langmuir isotherm, the Freundlich model described the adsorption process for less accuracy with a lower coefficient value of 0.9166. This result implies that Tl(I) biosorption by the *Pseudomonas fluorescens* strain seems more likely to be a monolayer surface adsorption than heterogeneous adsorption.

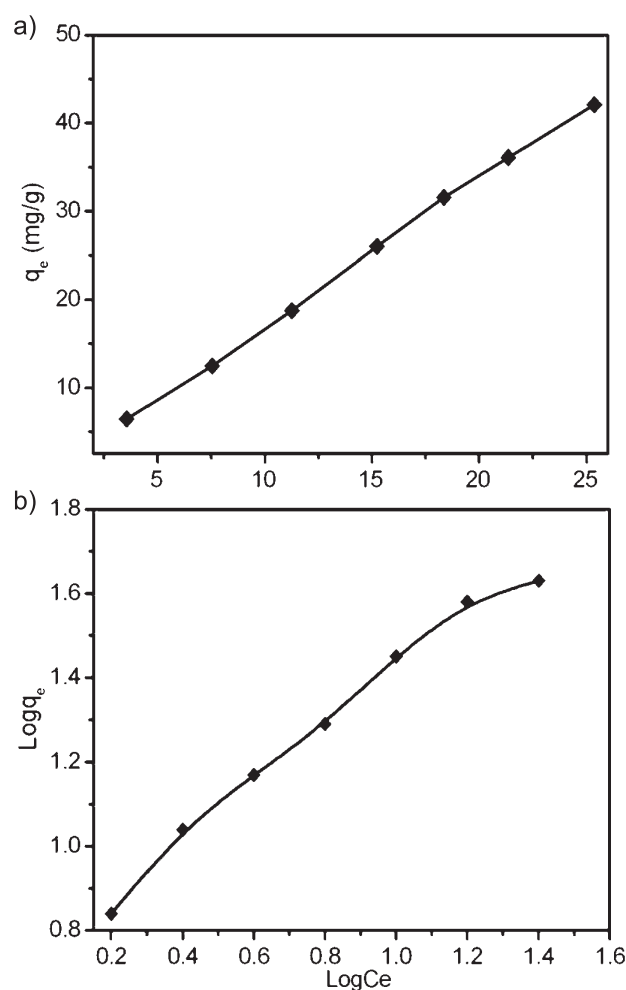


Fig. 7. a) Langmuir isotherm, b) Freundlich isotherm of sorption thallium on pseudomonas (m: 0.1g, V: 100 mL, Tl: 50  $\mu$ g/mL, time: 60 min, pH: 5.0).

### Biosorption Kinetic

We used the explicit adsorption mechanisms of equilibrium processes such as functional groups transfer, chemical exchange, two kinetic models (including pseudo-first-order [35] and pseudo-second-order [36]) to depict the kinetic characteristics of Tl(I) on the strain of *Pseudomonas fluorescens*.

The pseudo first-order kinetic model in linear equation can be expressed as:

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

...where  $q_e$  is equilibrium adsorption (mg/g),  $q_t$  is the adsorption capacity at time  $t$  (mg/g) on the adsorbents, and  $k_1$  means the equilibrium rate constant of pseudo first-order adsorption ( $\text{min}^{-1}$ ).

The pseudo second-order model is based on solid phase adsorption ability and is used to describe the whole biosorption process as shown by the following linear equation:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

...where  $k_2$  (g/mg/h) is the second-order rate constant of adsorption process,  $q_t$  (mg/g) is the quantity of biosorption at time (min)  $t$ , and  $q_e$  (mg/g) is the quantity of biosorption equilibrium.

Using the above-mentioned formula, a linear equation with the vertical coordinates of  $\log(q_e - q_t)$  vs. the horizontal coordinates of  $t$  is plotted in Fig. 8. The  $k_2$ ,  $q_e$ , and the value of correlation coefficient ( $R^2$ ) at 25°C could be calculated

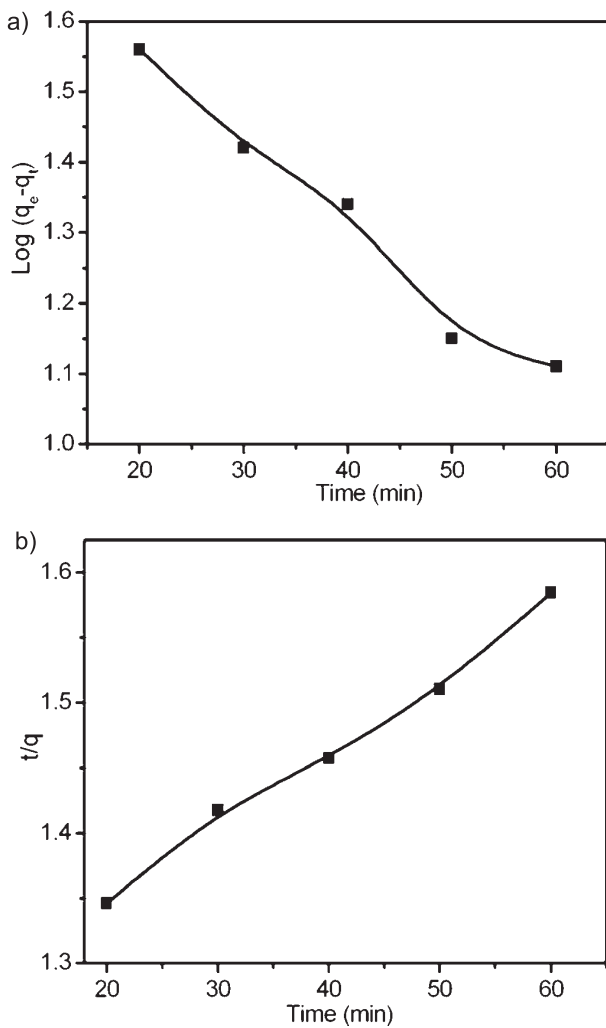


Fig. 8. Pseudo a) first-order kinetic, b) second-order kinetic of sorption thallium on pseudomonas (m: 0.1g, V: 100 mL, TI: 50 µg/mL, pH: 5.0).

Table 1. Langmuir and Freundlich biosorption isotherm constants for Thallium *Pseudomonas fluorescens* biosorbents.

Langmuir model			Freundlich model		
$q_{max}$ (mg/g)	b (l/g)	$R^2$	$K_f$	n	$R^2$
93.76	0.0275	0.9967	6.9231	1.9214	0.9166

Table 2. Parameters of the pseudo first- and second-order kinetic models for the biosorption of Thallium on *Pseudomonas fluorescens*.

Pseudo first-order kinetic model			Pseudo second-order kinetic model		
$Q_1$ (mg/g)	$K_1$ (min <sup>-1</sup> )	$R^2$	q (mg/g)	$K_2$ (gmg <sup>-1</sup> min <sup>-1</sup> )	$R^2$
93.76	0.2336	0.9892	43.64	0.00081618	0.9950

through the slope and intercept of the fitted linear equation (Table 1). It can be seen that the correlation coefficients ( $R^2 = 0.995$ ) for the pseudo second-order was higher than that of pseudo first-order, which could be used to deduce that the pseudo second-order kinetic model can better describe the adsorption of Tl(I) by the strain *Pseudomonas fluorescens* than the pseudo first-order one.

### Conclusions

The results obtained demonstrate that dead cells have the ability to uptake quantities of thallium. The maximum adsorption capacity of thallium by dead biomass was 93.76 mg/g with an initial Tl(I) ion concentration of 50 mg/L at pH 5 and 25°C. The batch adsorption isotherm can to be described using the Langmuir isotherm and Freundlich model. The equilibrium process fit the Langmuir model well, suggesting that monolayer adsorption is stronger than the heterogeneous adsorption for this process. Kinetic analysis shows that much higher  $R^2$ -values and a more accurate value of  $q_e$  for the pseudo second-order than for the pseudo first-order one.

The mechanism of adsorption includes mainly ion exchange and generation of complexes between heavy metal ions and binding sites onto the cell wall. The results of FT-IR spectrum analysis confirmed the existence of carboxyl, hydroxyl, and amino groups in the biomass of *Pseudomonas fluorescens* and their possible participation in Tl(I) ion adsorption. These show that the strain of *Pseudomonas fluorescens* may be a potential and low-cost biosorbent for the removal of Tl(I) ion form aqueous solutions.

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