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Biosorption of Lead (Pb^{2+}) from Aqueous Solution by *Rhodotorula aurantiaca*

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Abstract The aim of this work was to investigate the adsorption isotherm and kinetic model for the biosorption of lead (Pb^{2+}) by *Rhodotorula aurantiaca* and to examine the environmental factors for this metal removal. Within five minutes of contact, Pb^{2+} sorption reached nearly 86% of the total Pb^{2+} sorption. The optimum initial pH value for removal of Pb^{2+} was 5.0. The percentage sorption increased steeply with the biomass concentration up to 2 g/l and thereafter remained more or less constant. The Langmuir sorption model provided a good fit throughout the concentration range. The conformity of these data to the Langmuir model indicated that biosorption of Pb^{2+} by *R. aurantiaca* could be characterized as a monolayer, single-site type phenomenon with no interaction between ions adsorbed in neighboring sites. The maximum Pb^{2+} sorption capacity (q_{max}) and Langmuir constant (b) were 46.08 mg/g of biomass and 0.04 l/mg, respectively. The pseudo second-order equation was well fitted to the experimental data. The correlation coefficients for the linear plots of t/q against t for the second-order equation were 0.999 for all the initial concentrations of biosorbent for contact times of 180 min. The theoretical q_{eq} value was very close to the experimental q_{eq} value.

Key words: *Rhodotorula aurantiaca*, biosorption, Pb^{2+}, pH, Langmuir, pseudo second-order kinetics

The accumulation of heavy metal contaminants in the environment has become a concern due to growing health risks to the public. The metals of most immediate concern are Pb, Cd, Cr, Mn, Fe, Cu, Zn, and Hg. For humans, poisoning by most of these metals causes severe dysfunction of the kidneys, reproductive system, liver, brain, and central nervous system. Hundreds and thousands of tons of lead are discharged from electric battery manufacturing, lead smelting, and mining activities, as well as internal-combustion engines fueled with leaded petroleum. Lead acts on the central nervous system, on blood pressure, and on reproduction [15].

Conventional methods for heavy metal removal are precipitation, coagulation, reduction, ion exchange, evaporation, and membrane processes. These methods have several disadvantages, such as less effective metal-ion removal, high regent requirements, high costs, the generation of toxic sludges, and the problem of the safe disposal of the materials [10]. The biosorption (biological metal removal) process has distinct advantages over conventional methods such as being highly selective, more efficient, easy to operate, and cost effective. The potential for using microorganisms in the treatment of metal-bearing wastewater has been intensively studied and many microorganisms including bacteria, fungi, and algae have been found to remove metals from solutions [8, 14, 17, 18].

Numerous studies have investigated the nature and the ability to sequester heavy metals of yeast and these have shown the potential of yeast for metal recovery and removal [2, 24]. Among yeasts, *Saccharomyces cerevisiae* has been the most studied with respect to heavy metal biosorption [5, 29]. It was also reported that *Rhodotorula* sp., including *R. rubra*, *R. mucilaginosa*, *R. glutinois*, and *R. minuta* were able to uptake cadmium, lead, and copper [7, 11, 20, 26]. *Rhodotorula* sp. was frequently isolated from lagoons polluted by industrial and municipal sewage and mine drainage effluent [3]. *Rhodotorula* sp. also has an aptitude for the degradation of cyano-metals and the bioleaching of minerals containing metals [9, 25]. These studies demonstrate that *Rhodotorula* sp. has a potential for the bioremediation of soil and the treatment of wastewater containing toxic metals. However, only a limited number of studies have so far been focused on the use of *Rhodotorula* sp. for the removal of heavy metal from wastewater. *R. aurantiaca* newly isolated from soil was chosen as the biosorbent because of the relative lack of information about its sorption.

The aim of this study was to investigate the adsorption isotherm and kinetic model for the biosorption of lead (Pb^{2+}) by *R. aurantiaca* and to examine the environmental factors for this metal removal.

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MATERIALS AND METHODS

Isolation and Cultivation of Microorganism

Rhodotorula aurantiaca was isolated from soil and identified by its morphological characteristics, biochemical properties, and the carbon assimilation test. The cells were maintained on yeast malt agar plates at 4°C and transferred monthly. The growth medium consisted of 10 g/l of glucose, 3.0 g/l of yeast extract, 3.0 g/l of malt extract, and 5.0 g/l of peptone. The cells were grown in 500-ml Erlenmeyer flasks, each containing 100 ml of the growth medium. Cultures were incubated at 25°C on an orbital shaker at 150 rpm.

Biomass Preparation

At the end of the exponential growth phase, i.e., after 30 h incubation, the cells were harvested by centrifugation at 10,000 ×g for 5 min at 4°C. Once harvested, the cells were washed twice with deionized distilled water. After washing, the cells were dried at 70°C for 24 h and powdered in a mortar and pestle. As a resut, the powdered biomass was obtained and used in Pb^{2+} biosorption studies.

Biosorption Experiments

Every metal used in this experiment has nitrate as its counter anion to eliminate any effects of anion on the sorption. Stock Pb^{2+} solutions (1,000 mg/l) were prepared using $Pb(NO_3)_2$. The desired concentrations of Pb^{2+} were prepared by dilution of stock solution in deionized distilled water. For all metal binding equilibrium experiments, 0.1 g of biomass was added to 50 ml of Pb^{2+} solution in a 250-ml Erlenmeyer flask shaken at 150 rpm in an orbital shaker at 25°C. To determine the effect of biomass concentration on Pb^{2+} biosorption, the amount of dissolved Pb^{2+} concentration was kept constant while the biomass concentration was varied from 0.1 g/l to 6 g/l. Before adding biomass, the pH of the metal solutions was adjusted to the desired values using 0.1 N NaOH and 0.1 N HNO_3. To examine the effect of pH, experiments were carried out at a pH range of 2.0–6.0 and not conducted beyond pH 6.0 in order to avoid Pb^{2+} precipitation.

Analytical Methods

After biosorption, the biomass was then separated by centrifugation at 10,000 ×g for 5 min and the concentration of lead in the supernatant was determined by using a atomic absorption spectrophotometer (Varian AA-220FS, U.S.A.). All the biosorption experiments were repeated twice to confirm the results. The data were the mean values of both replicate determinations.

Biosorption Isotherm and Kinetics

The specific metal sorption value was calculated using the following equation:

$$q_{eq}=V(C_i-C_{eq})/1000M \quad (1)$$

where q_{eq} is the specific metal sorption (mg metal/g of biomass), V is the volume of metal solution (ml), C_i and C_{eq} are the initial and equilibrium concentration of metal (mg metal/l), respectively, and M is the dry weight of the biomass (g).

The biosorption equilibrium isotherm was obtained by the Langmuir sorption model and Freundlich sorption model [13, 21]. The mathematical description of the Langmuir model for a single component adsorption is

$$q_{eq}=q_{max}bC_{eq}/(1+bC_{eq}) \quad (2)$$

where q_{max} is the maximum metal sorption (mg metal/g of biomass) and b is the Langmuir isotherm constant (l/mg metal). q_{max} and b can be obtained from the linear plot of $1/q_{eq}$ versus $1/C_{eq}$.

The Freundlich model takes the following form for a single component adsorption:

$$q_{eq}=KC_f^{1/n} \quad (3)$$

where K and n are the Freundlich constants related to the adsorption capacity and adsorption intensity of the sorbent, respectively. Equation (3) can be linearized in logarithmic form and the Freundlich constants can be determined.

The pseudo second-order kinetic model based on the sorption capacity of the solid phase can be used in this case assuming that measured concentrations are equal to cell surface concentrations [16]. If the rate of sorption is a second-order mechanism, it may be represented as follows:

$$dq/dt=k_2(q_{eq}-q)^2 \quad (4)$$

where k_2 is the second-order biosorption rate constant. For the boundary conditions t=0 to t=t and q=0 to $q=q_{eq}$, the integrated and linear form of Eq. (4) becomes

$$t/q=1/(k_2q_{eq}^2)+(1/q_{eq})t \quad (5)$$

RESULTS AND DISCUSSION

To choose the appropriate metal species for the biosorption by *R. aurantiaca*, Pb^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+}, and Ni^{2+} biosorption experiments were carried out. Figure 1 shows that *R. aurantiaca* exhibited sorption capacities for tested metals in the following decreasing order of $Pb^{2+}>Cd^{2+}>Cu^{2+}\geq Mn^{2+}\geq Ni^{2+}$. Thus, Pb^{2+} ion was chosen for biosorption experiments by *R. aurantiaca*.

Time Course of Pb^{2+} Biosorption

Figure 2 shows that Pb^{2+} sorption by *R. aurantiaca* is very rapid. Within five minutes of contact, Pb^{2+} sorption reached nearly 86% of the total Pb^{2+} sorption. After a very rapid sorption, the amount of adsorbed Pb^{2+} did not significantly change with time. This behavior suggests that the sorption

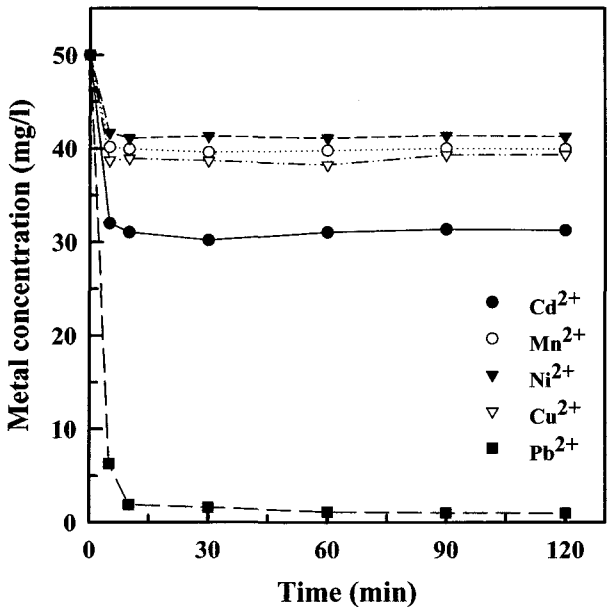

Fig. 1. Biosorption of various metal cations by *R. aurantiaca*.

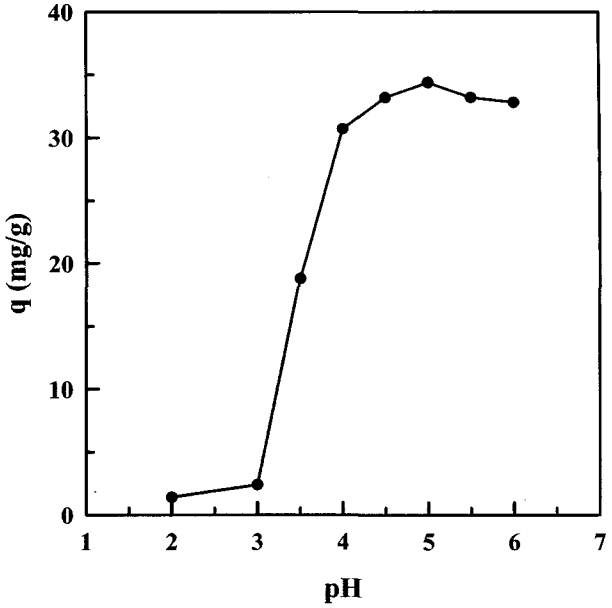

Fig. 3. Effect of pH on Pb^{2+} biosorption by *R. aurantiaca*.

of Pb^{2+} by *R. aurantiaca* is typical for the biosorption of metals involving no energy mediated cell surface binding.

Effect of pH

The effect of initial pH on Pb^{2+} sorption by *R. aurantiaca* is shown in Fig. 3. The Pb^{2+} sorption capacity increased sharply with an increase in pH from 3.0 to 4.0. No adsorption of Pb^{2+} occurred at extreme acidic condition (pH 2.0). The greatest Pb^{2+} sorption capacity was obtained at the initial pH value of 5.0. Metal biosorption depends on the protonation or deprotonation of the cell wall functional groups [12]. At low pH, the concentration of protons is so high that metal binding sites become positively charged and metal cations and protons compete for binding sites, which results in lower sorption of metal. With an increase in pH, the functional groups on the cell wall with a negative charge increase due to deprotonation of the metal binding sites, which promotes the metal sorption.

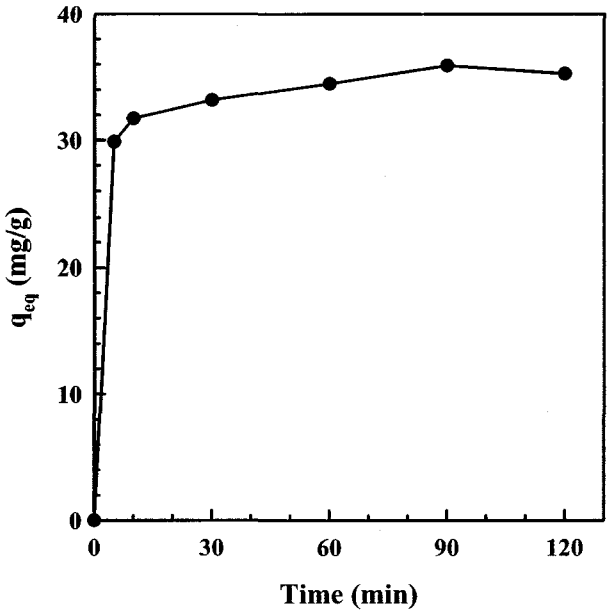

Fig. 2. Time course profile for Pb^{2+} biosorption by *R. aurantiaca*.

Effect of Biomass Concentration

The biomass concentration is an important factor that determines the extent of metal sorption from solution. The effect of biomass concentration in percentage sorption and specific sorption is depicted in Fig. 4(a) and 4(b). The percentage sorption increased steeply with the biomass concentration up to 2 g/l. This proved that Pb^{2+} sorption yield decreased when the biomass concentration increased. The 75% of dissolved Pb^{2+} was removed with 2 g/l biomass concentration. As shown in Fig. 4(b), the specific sorption value decreased with an increase in biomass concentration. It was reported that higher specific sorption at lower biomass concentrations could be due to an increased metal to biosorbent ratio [1, 23]. It was suggested that with increasing biomass concentration there is an increase in electrostatic interactions between cells, causing the cells to agglomerate, which contributes to a decrease in the number of binding sites available.

Biosorption Isotherm

In order to determine if *R. aurantiaca* could be modeled using adsorption isotherms, the two most commonly used adsorption isotherms for biosorption of Pb^{2+} (the Langmuir

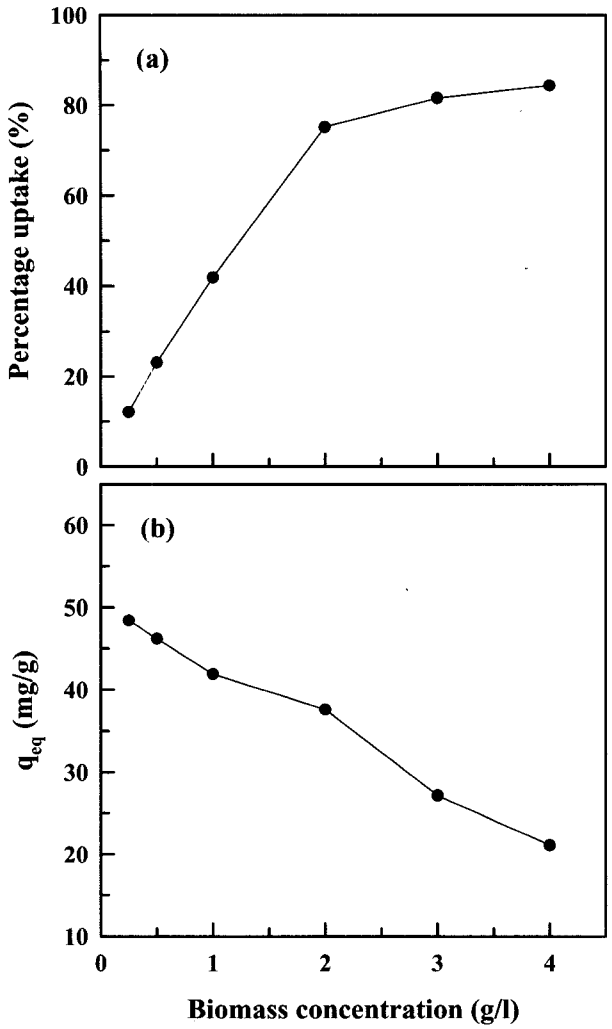

Fig. 4. Effect of biomass concentration on Pb^{2+} biosorption by *R. aurantiaca*.
(a) Percentage uptake; (b) specific uptake.

and Freundlich adsorption isotherms) were investigated. The sorption isotherm study was carried out at varying initial Pb^{2+} concentrations as shown in Fig. 5. The specific metal sorption of the biomass increased with an increase in the initial concentrations of metal ions and reached a saturation value. This result showed that Pb^{2+} sorption by *R. aurantiaca* was a chemically equilibrated and saturable mechanism whose sorption capacity increased as long as binding sites were available. Figure 6 shows Langmuir and Freundlich plots for Pb^{2+} sorption by *R. aurantiaca*. *The* Langmuir constants (q_{max} and b) and Freundlich parameters (K and n) are presented in Table 1. It is clear from Fig. 6 that the Langmuir sorption model provided a good fit throughout the concentration range, with correlation coefficients of 0.99. The conformity of these data to the Langmuir model indicated that biosorption of Pb^{2+} by *R. aurantiaca* could be characterized as a monolayer, single-site type

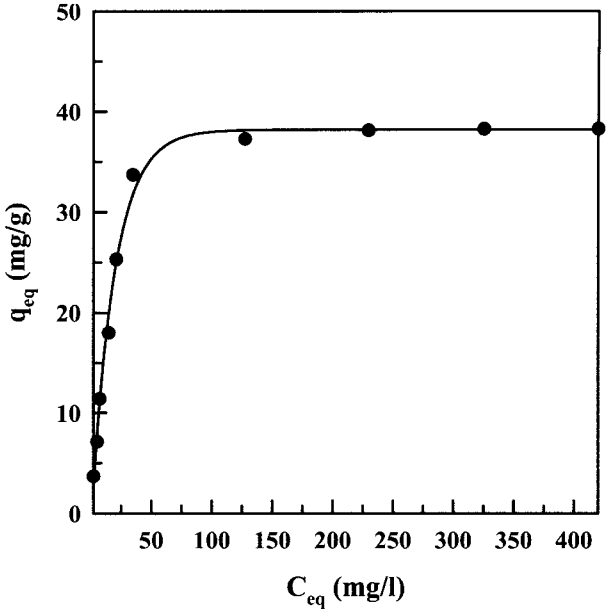

Fig. 5. Effect of initial Pb^{2+} concentration on biosorption of Pb^{2+} by *R. aurantiaca*.

phenomenon with no interaction between ions adsorbed in neighboring sites [21]. The maximum adsorption capacity (q_{max}) and Langmuir constant (b) for *R. aurantiaca* on Pb^{2+} sorption were 46.08 mg/g and 0.04 l/mg, respectively. The q_{max} value was higher than that of *R. rubra* (9.09 mg/g) [26].

Biosorption Kinetics

In order to analyze the biosorption kinetics of Pb^{2+} ions, the pseudo second-order kinetic model was applied to the data. The pseudo second-order equation was well fitted to the experimental data. The comparison of experimental biosorption capacities and the theoretical values estimated from the equation (4) are presented in Table 2. The correlation coefficients for the linear plots of t/q against t for the second-order equation were 0.999 for all the initial concentrations of biosorbent for contact times of 180 min (Fig. 7). The theoretical q_{eq} value was very close to the experimental q_{eq} value. All these results suggest that *R. aurantiaca* biosorbent systems are described by the pseudo second-order kinetic model, based on the assumption that the rate-limiting step may be chemical sorption involving valence forces through the sharing or exchange of electrons between biosorbent and sorbate which provides the best correlation of the data.

A variety of ligands located on the cell wall are known to be involved in metal biosorption. The main chemical groups of biomass surfaces that are capable of participating in the sorption and chelation of a number of bivalent metal cations are polar or anionic in nature, such as hydroxyl, sulfhydryl, carboxyl, and phosphate, and are mainly composed of polysaccharidic materials which constitute

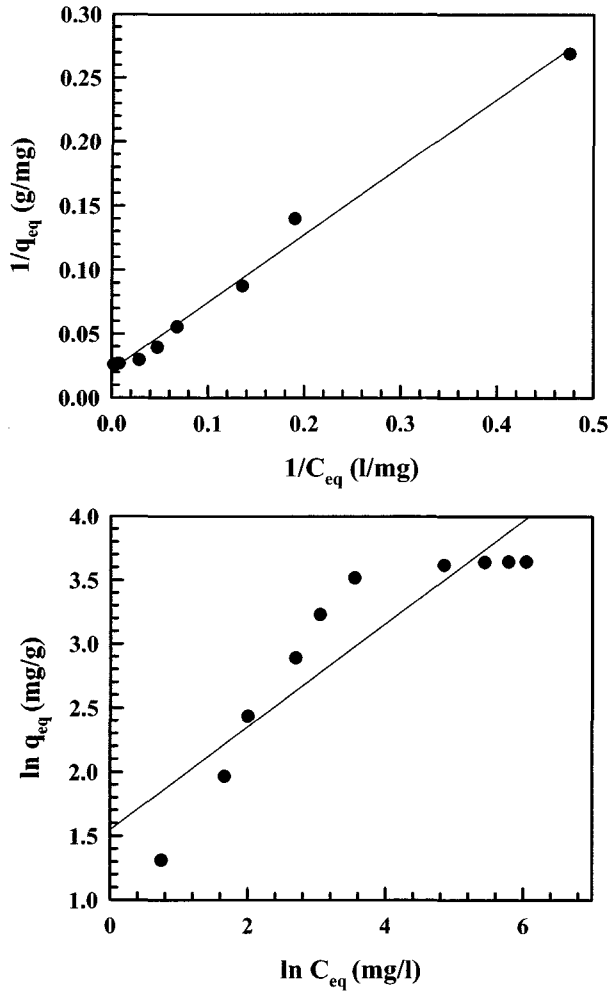

Fig. 6. Langmuir and Freundlich adsorption isotherms for Pb^{2+} biosorption by *R. aurantiaca*.
(a) Langmuir isotherm; (b) Freundlich isotherm.

most of the cell wall. The nature of the specific interactions between metal ions and biomass is quite controversial due to their complex nature and the significant number of different available binding sites for metal ions. The role of phosphomannans and carboxyl groups of cell wall protein of *S. serevisiae* for metal binding has been identified [28].

Yeasts are used in a variety of industrial fermentation processes and can be easily cultivated using unsophisticated fermentation techniques and inexpensive growth media. Yeasts cultures are also amenable to genetic and morphological

Table 1. Langmuir and Freundlich parameters for biosorption of Pb^{2+} by *R. aurantiaca.*

Langmuir parameters			Freundlich parameters		
q_{max} (mg/g)	b (l/mg)	R^2	K	n	R^2
46.08	0.04	0.999	4.69	2.48	0.823

Table 2. The second-order kinetic constants for biosorption of Pb^{2+} by *R. aurantiaca.*

	Experimetal	Second-order kinetics		
C_i (mg/l)	q_{eq} (mg/g)	q_{eq} (mg/g)	$k_2 \times 10^2$ (g/mg min)	R^2
50	19.25	19.46	3.58	0.999
100	31.89	32.05	2.07	0.999
200	34.85	35.09	1.74	0.999
300	36.95	37.17	1.49	0.999

manipulations, which may result in better raw biosorbent material. Among yeasts, heavy metal biosorption by *S. cerevisiae* has been most studied. However *S. cereviseae* biomass has been referred to as a mediocre biosorbent. Also, the potential of other yeasts for heavy metal biosorption has been less reported.

The interactions between *Rhodotorula* sp. and heavy metals have focused on trace metal tolerance and the accumulation of heavy metals by metabolically active biomass. Although the removal of cadmium and lead by viable and non-viable biomass of *R. rubra* was investigated, the metal uptake value was significantly lower than that of other biosorbents [26]. *Rhodotorula* sp. is soil yeast. Therefore, the *R. aurantiaca* studied in this work could be used for the bioremediation of soil contaminated by toxic heavy metals.

The composition of the cell wall of basidiomycetous yeast *Rhodotorula* sp. is characterized by a cell wall with a different composition and structure from those of the cell walls of ascomycetous yeasts such as *S. cerevisiae.* One important characteristic of red yeast cell wall is the presence of a mannan-type polymer containing fucose,

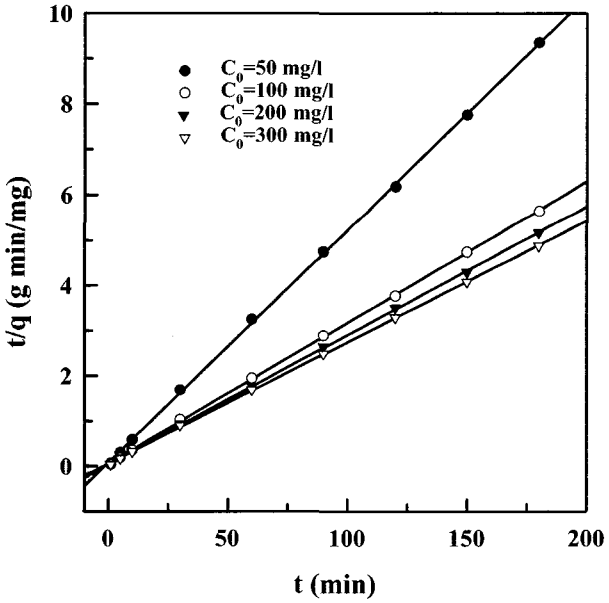

Fig. 7. t/q vs. t plot for Pb^{2+} biosorption by *R. autantiaca.*

galactose, and glucose. This polysaccharide has a highly branched structure but has not been detected in the cell wall of *Saccharomyces* [22]. This structure could contribute to the higher adsorption capacity of *R. aurantiaca.*

It is also important to bear in mind that *Rhodotorula* sp. produces capsular and extracellular polysaccharide. It is known that *R. glutinis* KCTC 7989 produces acidic heteropolysaccharide composed of mannose, fucose, glucose, galactose, and uronic acid [6]. EPS has been described as a trap for dissolved species and noted for its tendency to avidly bind metals [19, 27]. It is also known that bacterial colonies can form sticky EPS layers, which can cause the deposition of dissolved metal species [4]. The role played by structural polysaccharides is not fully understood and needs to be studied in greater detail.

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