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Biosorption of Pb^{2+} by *Saccharomyces Cerevisiae* in Static and Dynamic Adsorption Tests

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Abstract This paper demonstrates the Pb^{2+} adsorption capacity and adsorption rate of *Saccharomyces cerevisiae* by both static and dynamic testing to verify its feasibility as a heavy metal bio-absorbent in wastewater treatment. The static testing was divided into two parts. First, we tested *S. cerevisiae* by itself, and then we tested immobilized *S. cerevisiae*. In static testing of the non-immobilized *S. cerevisiae*, the Pb^{2+} adsorption capacity and adsorption rate increased up to 6.52 mg/g and 52.94%, respectively, with time. After immobilization, the Pb^{2+} adsorption capacity and adsorption rate reached 10 mg/g and 80%, respectively. In dynamic testing, the optimal saturated adsorption capacity of immobilized *S. cerevisiae* for Pb^{2+} was 6.64 mg/g. In addition to the static and dynamic testing of adsorption capacity and rate, we used SEM imaging to analyze the mechanics of adsorption, and the images showed that the cell wall played the major roll in Pb^{2+} adsorption.

Keywords *Saccharomyces cerevisiae* · Static test · Dynamic test · Pb^{2+} adsorption

Heavy metal pollution in waste water is difficult to resolve utilizing microorganisms. Some microorganisms have the capacity to accumulate heavy metals through biosorption, forming metal-organic compounds within their cellular structures, but making them toxic as they enter the food chain and doing harm to human health (Wang 2002). Traditional treatment processes for wastewater containing 1–100 ppm of heavy metals are costly. These processes include precipitation, ion exchange, oxidation and deoxidization, membrane filtration, etc. (Ridvan et al. 2003).

Recently, biosorption has attracted worldwide attention (Churchill et al. 1995; Lopez et al. 2000; Savvaidis et al. 2003). Many kinds of adsorbents are capable of adsorbing heavy metals (Volesky and Holan. 1995), such as chitosan (Jansson-Charrier et al. 1996), alga (Laube et al. 1979), grape stalks (Machado et al. 2003), bacteria (Mann 1990) and activated carbon (Gabaldon et al. 1996). In this research, static and dynamic adsorption processes were used to study adsorption of Pb^{2+} by *Saccharomyces cerevisiae.*

Materials and Methods

S. cerevisiae (Dead beer yeast powder) was obtained from Harbin Brewing Group Co. Ltd.

Pretreatment of *S. cerevisiae*: *S. cerevisiae* was washed with deionized water three times to eliminate the nutrimental materials and impurities. It was dried in an oven at 60°C until its weight did not change after vacuum pump treatment. Then it was crushed (smashed) and put into an airer for future use.

Immobilized *S. cerevisiae* was prepared by using sodium alginate. Immobilization increases the stability, cellularity and hydrophilic nature, enhances the adsorption effectiveness of biosorption, and makes it easier to separate the absorbent from the liquid. (Swaminathan and Pakshirajan 2006; Iqbal et al. 2005). Sodium alginate was chosen as the immobilizing material because after comparing

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sodium alginate, glutin, agar and PVA, we found that the difference in adsorbing capacity for Pb^{2+}among them was negligible and that sodium alginate was the most stable for use in our experiment. After centrifuging 1,000 globose units of immobilized *S. cerevisiae* at 5,000 r/min for 20 min at 25°C, only 2 of the globules were broken. Thus 99.8% retained their original shape, which indicated good stability for our experiment.

Preparation of *S. cerevisiae* for immobilization: The pretreated *S. cerevisiae* was comminuted with a muller, then further refined by being put through a 0.25 mm griddle, and put into a cool and dry place.

Procedure for making the immobilized material: First, 30 g *S. cerevisiae* was put into 500 mL deionized water. Then 10 g sodium alginate was added to the mixture, which was churned until it changed into colloid, and left to stand until no air bubbles remained. Then a 100 mL syringe was used to extrude the spherule material into 2% $CaCl_2$ solution. The next day, it was washed with deionized water, dipped into 0.5 mol/L HCL for 24 h, and put into deionized water. The process of dipping it into deionized water was repeated untill the water pH reached 5–6. Then it was kept in deionized water under room temperature. The immobilized *S. cerevisiae* was of globose shape, 1.2–1.9 mm in diameter.

Pb^{2+} solutions: Pb^{2+} concentrations of each of the treated solution samples were determined through three repetitions of dithizone spectrophotometry, the final result being the average of the three.

Apparatus for static experiment: glassware, shaking machine.

Apparatus for dynamic experiment: continuous flow reactor (See Fig. 1).

Figure 1 shows a plexiglass reactor, 3 cm in diameter and 30 cm in height. Water was pumped into the system from a water tank by a peristaltic pump and controlled by a flowmeter. Then the water entered the column in an up-flow style. The water-distributing board brought the water and immobilized *S. cerevisiae* into contact with each other.

All glass ware for static and dynamic experiments was put into 0.1 mol/L HNO_3 for 24 h, then washed with deionized water and dried to decrease the disturbance of heavy metals.

Static analysis: Adsorption capacity and rate were calculated according to the following formulas:

$$Q(mg/g) = \frac{(C_0 - C) \times V}{M} \quad (1)$$

$$R(\%) = \frac{C_0 - C}{C_0} \times 100\% \quad (2)$$

In the formula, Q, adsorption capacity; V, solution volume (L); C, Pb^{2+} concentration after adsorption (mg/L); C_0, Pb^{2+} concentration before adsorption (mg/L); M, adsorbent quantity (g); R, adsorption rate (%).

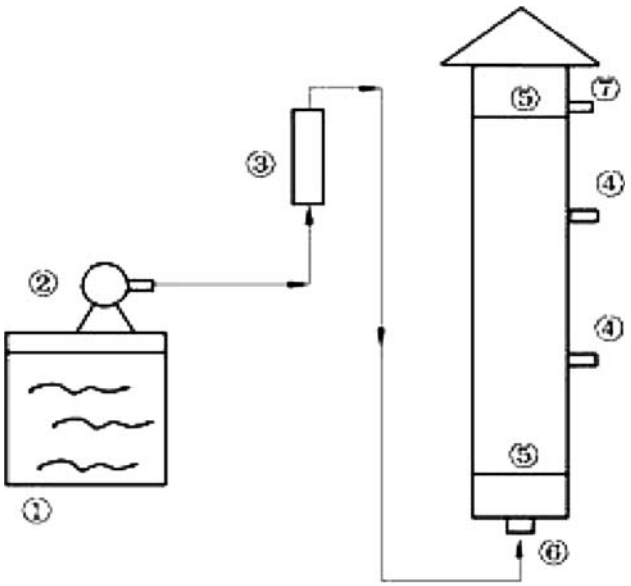

Fig. 1 Schematic diagram of a continuous flow reactor. *1* Water tank, *2* peristaltic pump, *3* flowmeter, *4* sampling site, *5* water-distributing board, *6* input water site, *7* output water site

Dynamic analysis: The Thomas model, the most widely used model of column adsorption, was used to study the dynamic adsorption process. It was expressed as:

$$\frac{c}{c_0} = \frac{1}{1 + \exp\frac{k_{th}(q_0 x - c_o v_{eff})}{Q\prime}} \quad (3)$$

In the formula, K_{th}, Thomas constant (mL/min/mg); q_0 ,saturated adsorption capacity by a unit mass of adsorbent (mg/g); x, adsorbent mass (g); v_{eff}, output solution volume(mL); c_0, initial Pb^{2+} concentration (mg/L); Q', flow rate (mL/min).

Its linearity form was

$$\ln\left(\frac{c_0}{c} - 1\right) = \frac{k_{th} q_0 x}{Q\prime} - \frac{k_{th} c_0}{Q\prime} v_{eff} \quad (4)$$

In this linear equation, the Thomas constant and saturated adsorption capacity could be calculated through the slope and intercept of the line.

Results and Discussion

Our research involved static adsorption and dynamic adsorption experiments.

Static adsorption experiment (Part 1):

We put 25 mL solution of $Pb(NO_3)_2$(40 mg/L) into an 100 mL centrifuge tube, and 0.08 g *S. cerevisiae* was added. The tube was shaken continuously for 8 h at a constant temperature (25°C ± 3, 150 r/min). The sample

was filtered with 0.45 μm filter paper. Finally, the concentration of Pb^{2+} was determined, and Q and R were calculated with relative equations. The procedure for using immobilized *S. cerevisiae* is the same as above but with immobilized *S. cerevisiae*.

As is shown in Fig. 2, the Q (adsorption capacity) and R (adsorption rate) of Pb^{2+} by *S. cerevisiae* tended to increase with time: during the first 5 min, Q and R were 3.51 mg/g and 28.54%, respectively; at 180 min, Q and R had reached 6.52 mg/g and 52.94%; during the next 180–480 min, Q did not make obvious changes. Therefore, it could be concluded that at 180 min, the adsorption capacity of *S. cerevisiae* for Pb^{2+} was saturated.

As Fig. 3 shows, in contrast with the non-immobilized *S. cerevisiae*, after immobilization Q and R both apparently increased, with their maximum values reaching as high as 10 mg/g and 80%. In this case, duration of adsorption before saturation became longer: for about 3 h.

Dynamic adsorption experiment (Part 2):

Temperature: 25°C;

Pb^{2+} concentration: 20 and 15 mg/L, respectively;

Immobilized materials: sodium alginate;

Flow rates: 1, 2, 4, 8 and 15 mL/min, respectively.

The reactor was run for 8 h, the treated solution was sampled at different times, and the concentration of Pb^{2+} was determined.

Table 1 shows how the Thomas equation applied to the adsorption process. R varied from 0.8476 to 0.9547. Retention time of water in the column decreased with increasing flow rate, thus better adsorption results could be achieved by reducing C_0. Generally speaking, K_{th} and q_0 decreased as Q' increased and C_0 decreased. As a result,

Table 1 Saturated adsorption capacity and correlative constants of Thomas model

Q' (mL/min)	C_0 (mg/L)	K_{th} (mL/min/mg)	q_0 (mg/g)	r
1	20	1.95×10^{-3}	6.05	0.9252
2	20	2.19×10^{-3}	6.64	0.9547
4	15	3.21×10^{-4}	5.23	0.8981
8	15	7.65×10^{-4}	4.87	0.9034
15	15	8.77×10^{-4}	4.01	0.8476

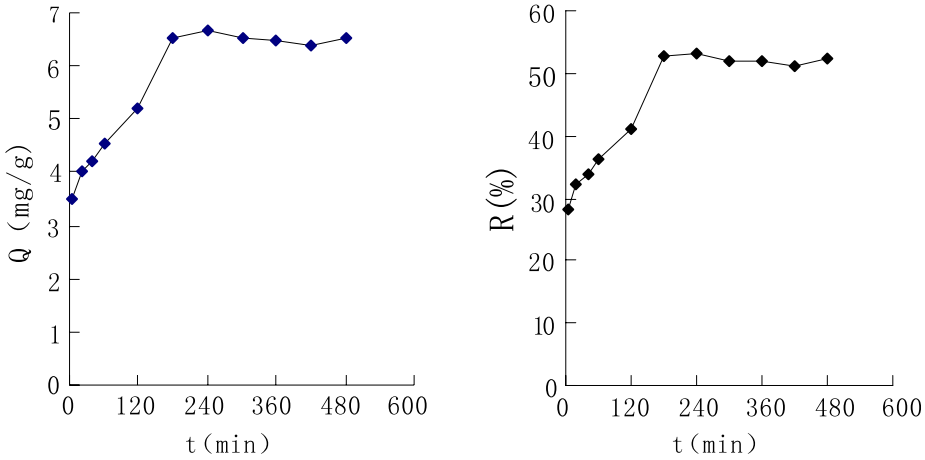

Fig. 2 Effect of reaction time on Pb^{2+} adsorption capacity and adsorption rate

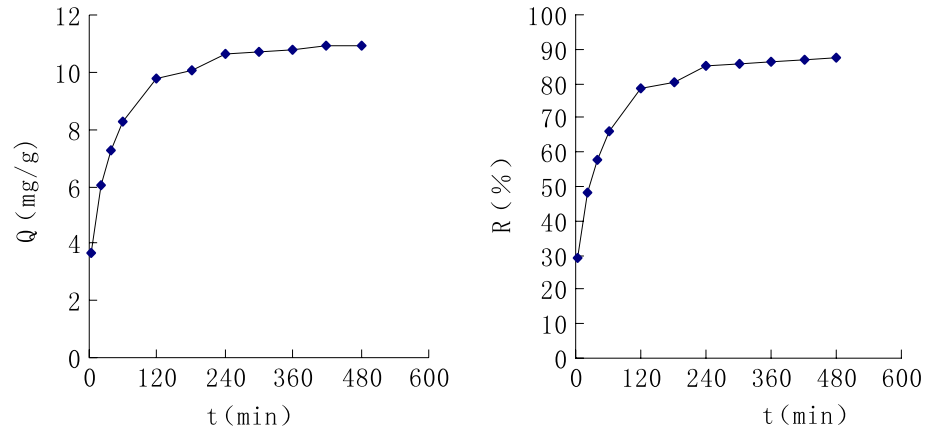

Fig. 3 Effect of reaction time on Pb^{2+} adsorption capacity and adsorption rate by immobilized *S. cerevisiae*

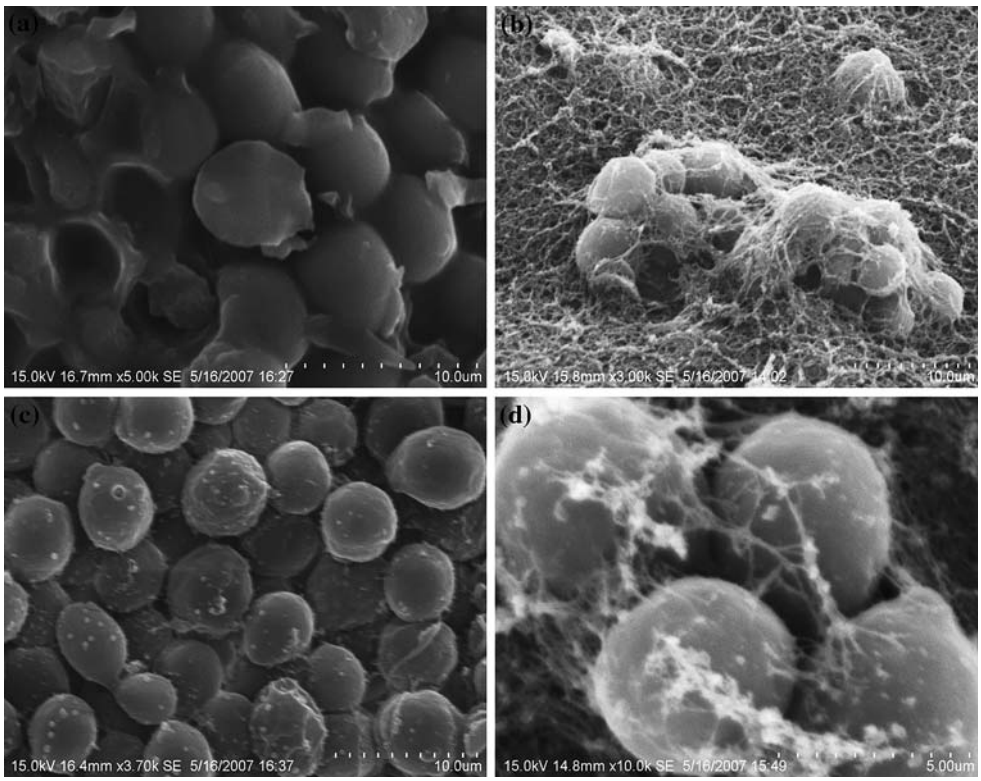

Fig. 4 *S. cerevisiae* and immobilized *S. cerevisiae* SEM photos on Pb^{2+} biosorption. **a** *S. cerevisiae* SEM photo (×5000). **b** immobilized *S. cerevisiae* SEM photo (×3000). **c** *S. cerevisiae* SEM photo on Pb^{2+} biosorption (×3700). **d** immobilized *S. cerevisiae* SEM photo on Pb^{2+} biosorption (×10000)

adsorbent and heavy metal ions were not able to have adequate contact. High flow rate would reduce the adsorption effect, but the adsorption effect would also be affected by other differences in adsorbent and reactor characteristics. Therefore, C_0, Q' and q_0 were regarded as optimal at 20 mg/L, 2 mL/min and 6.64 mg/g, respectively. In practical application, all factors must be taken into account to achieve the optimal adsorption result.

Adsorption mechanism analysis by Scanning Electron Microscope (SEM) test on Pb^{2+} biosorption:

S. cerevisiae was shaped like an egg or slightly elongated spheroid, and measured 1–5 μm in diameter and 5–30 μm in length. Its composition was found to be quite complex, in that the cell wall was composed of 40% β-polysaccharide, 40% α-amrita polysaccharide, 8% protein, 7% fat, 3% mineral and so on (Hu and Zhou 1988). This kind of composition and structure would expose many active radical sites of *S. cerevisiae*'s cell wall. This would make the cell wall highly advantageous for adsorbing heavy metal ions.

Figure 4 shows SEM photos of cells before and after Pb^{2+} adsorption by *S. cerevisiae* and immobilized *S. cerevisiae*. Figure 4a shows the *S. cerevisiae* cells, presenting spheroidal or slightly elongated spheroidal shapes, with regular and smooth surfaces. The regular surface area appeared very large and advantageous to the adsorption process. After immobilization by sodium alginate, many fibers extended from and among the surfaces of the cells, as Fig. 4b shows. This web-like structure caused cells to connect with each other closely, creating mechanical strength for dynamic adsorption. At the same time, the web-like structure also held the individual cells far enough apart from each other so that the surface area of their cell walls was not obstructed by adjacent cells and was thus open to sufficient contact with the heavy metal ions to allow efficient adsorption. Figure 4c, d show the changes between *S. cerevisiae* and immobilized *S. cerevisiae* after adsorption on Pb^{2+}. The photos clearly show that the shape of cells did not change much. The photos also show the heavy metal ions as spot-like particles distributed on the surface of the cells. This demonstrates that the cell wall was the main part of the cell to adsorb Pb^{2+}.

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