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NOTE

Effect of Temperature on the Accumulation of Pb^{2+} in *Saccharomyces cerevisiae*

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Abstract The accumulation process of Pb^{2+} in an industrial strain of *Saccharomyces cerevisiae* proved to be temperature-dependent, and was quite similar to chemical adsorption at the initial stage of Pb^{2+} accumulation. The initial Pb^{2+} accumulation rate increased from 11.4 to 46.2 mg Pb^{2+}/g cell dry weight/day, in response to the increased temperature from 20°C to 50°C, while the maximal Pb^{2+} accumulation amount (175.8 mg Pb^{2+}/g cell dry weight) was achieved at 30°C. The maximal Pb^{2+} accumulation amount with temperature was independent of ion exchange with K^+ and Mg^{2+}.

Key words: Pb^{2+}, bioaccumulation, *Saccharomyces cerevisiae*

Biological methods for the removal of heavy-metal ions from industrial wastewater may provide an attractive alternative to physico-chemical methods such as chemical precipitation, chemical oxidation and reduction, electrochemical treatment, evaporative recovery, filtration, ion-exchange, and reverse osmosis [17].

Lead present in industrial wastewater is primarily in the form of Pb^{2+} as a hydrolysis product, $PbOH^+$ and/or organic complexes, such as lead tetraethyl. Hundreds of thousands of tons of lead are discharged annually into the atmosphere in the exhausted gases of internal-combustion engines fueled with leaded petroleum. From the atmosphere, the metals, largely as oxides and salts, are washed down by rain to the surface of the earth. The wastewater from the mining, metal, dyestuff, electric, and petroleum industries contains the undesired amounts of Pb^{2+}. In industrial wastewater, the Pb^{2+} concentration reaches 200~250 mg/l; this value is very high in relation to the water-quality standards, and the Pb^{2+} concentrations of wastewater should be reduced to the value of 0.10~0.05 mg/l [9].

Bacteria, algae, fungi, and their microbial products have been used successfully as the removing agents for heavy-metal ions [3, 13]. Interaction between metal ions and microbial cells can occur through the adsorption to cell surfaces, through the metabolically assisted accumulation within the cell, or as the metal complexes with extracellular microbial metabolites [7, 15, 16].

Many microorganisms with surfaces consisting mainly of acidic polysaccharides have the ability to complex heavy-metal ions. The metal uptake process, however, is complex and dependent on the chemistry of the metal ions, the specific surface properties of the organisms, cell physiology, and the physico-chemical influence of the environment, for example, pH, temperature, and metal concentration. However, the effect of temperature on the accumulation/adsorption of heavy-metal ions by microorganisms has not been extensively characterized.

In the present study, we investigated the effect of temperature, which is one of the important environmental factors in the accumulation of heavy-metal ions by microorganisms, on the initial Pb^{2+} accumulation rate and the maximal accumulation amount of Pb^{2+} in *Saccharomyces cerevisiae*.

Microorganism and Growth Condition

Saccharomyces cerevisiae KCTC (Korean Collection for Type Cultures) 1199, obtained from brewery waste, was cultivated at 30°C for 72 h in 300-ml conical flasks with 100 ml medium composed of (as g/l) 100 glucose, 8.5 yeast extract, 1.32 NH_4Cl, 0.11 $MgSO_4$, 0.06 $CaCl_2$ in a rotary-shaker incubator at 150 rpm. Cells were harvested by centrifugation (3,000×g, 10 min) and then washed three times with distilled deionized water, and stored at 4°C in a refrigerator before being used in the Pb^{2+} accumulation experiments.

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Experimental Methods

The prepared cell suspension was mixed with an equal volume of the initial concentration of the aqueous $Pb(NO_3)_2$ solution prepared in the amount two times that of the desired concentration. The pH of the Pb^{2+} solution, cell suspension, and a mixture of cells and Pb^{2+} solution were 3.0~4.0, 5.5~5.7, and 3.5~5.5, respectively. Buffers, acids, and alkalis were not used in adjusting the pH, where no spontaneous Pb^{2+} precipitation was observed in the prepared solutions.

The experiments were carried out by adding 50 ml Pb^{2+} solution and 50 ml cell suspension in 250-ml conical flasks and shaking them in a rotary-shaker incubator at 20, 30, 40, and 50°C (150 rpm), respectively. Samples of 1.8 ml were taken at the proper time period and centrifuged immediately (10,000 × g, 10 min). The Mg^{2+}, K^+, and Pb^{2+} ion concentrations in the supernatant were measured by an atomic absorption spectrometry (Perkin Elmer 3300). The cell dry weight was measured after drying the cells at 105°C for 2 h to a constant weight. All the experiments were repeated three times and the average taken.

Accumulated Pb^{2+} amount per gram of dried microorganism (q) was calculated from a Pb^{2+} mass balance equation outlined by Volesky [18]: q (mg Pb^{2+}/g cell dry weight) = $(C_i - C_e)$/m. Where C_i and C_e are the concentrations of Pb^{2+} (mg/l) in the initial and the equilibrium state, respectively, and m is the concentration of dried cells (mg/l).

Initial Pb^{2+} Accumulation Rate

The initial Pb^{2+} accumulation rate was measured by calculating the slope of a plot of the accumulated Pb^{2+} amount per gram of dried microorganism (mg Pb^{2+}/g cell dry weight) vs. time (min).

Effect of Temperature on the Accumulation of Pb^{2+} in *S. cerevisiae*

As shown in Fig. 1A, the accumulation process of Pb^{2+} in *S. cerevisiae* was obviously temperature-dependent. The initial Pb^{2+} accumulation rate in *S. cerevisiae* increased from 11.4 to 46.2 mg Pb^{2+}/g cell dry weight/day within the temperature ranges from 20°C to 50°C (Fig. 1B). A similar result using UO_2^{2+} by *S. cerevisiae* between 20°C and 50°C had been reported [14]. It had also been pointed out that the initial adsorption rates of Pb^{2+} by *Zoogloea ramigera* and *Rhizopus arrhizus* were increased with temperature from 15°C to 45°C [10], and the Co^{2+} and Cd^{2+} accumulation rates in *S. cerevisiae* at 25°C were higher than those at 5°C [8].

In general, the reaction rates depend on the temperature and the physical adsorption reactions are normally an exothermic process [11]. The adsorption of Cu^{2+} by *R. arrhizus* was a physical adsorption because the adsorption rate of Cu^{2+} decreased with increasing temperature above 20°C [11]. However, in this work, the initial Pb^{2+} accumulation rate was increased according to the increase of temperature. Thus, it could be concluded that the Pb^{2+} accumulation in *S. cerevisiae* was endothermic and its process was quite similar to the chemical adsorption at the initial time period within the range of the experiment.

Maximal Pb^{2+} accumulation amount (175.8 mg Pb^{2+}/g cell dry weight) was achieved at 30°C. A few workers

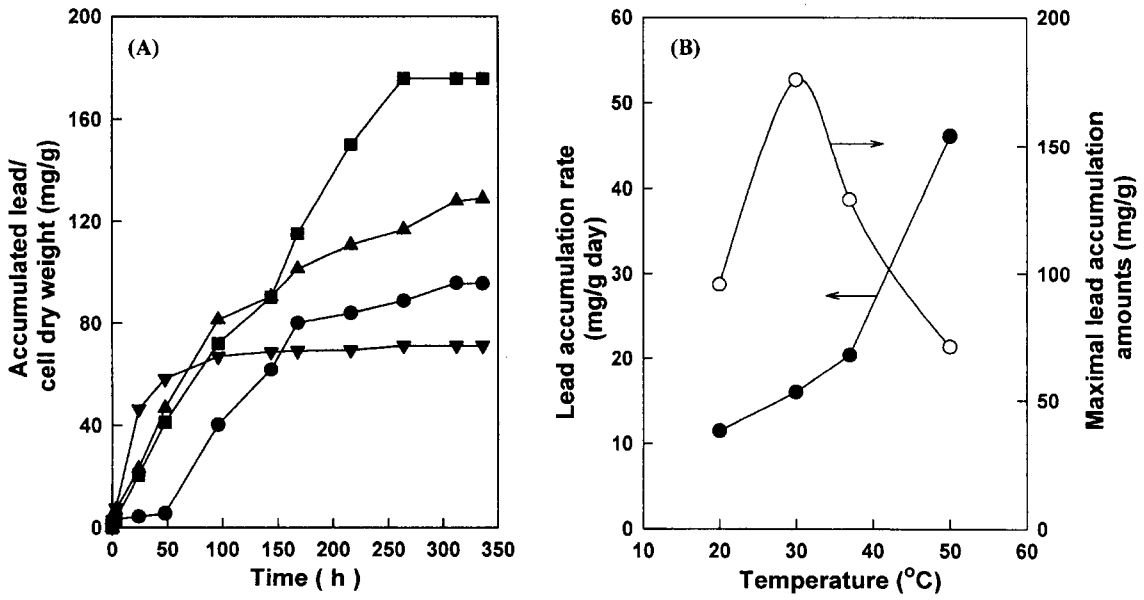

Fig. 1. (A) Time course of Pb^{2+} accumulation in *S. cerevisiae* at various temperatures; (●) 20°C, (■) 30°C, (▲) 40°C, (▼) 50°C. (B) Effect of temperature on the initial Pb^{2+} accumulation rate (●) and maximum Pb^{2+} accumulation amount (○) in *S. cerevisiae*. Initial concentrations of cells and Pb^{2+} were 1.0 and 0.2 g/l, respectively.

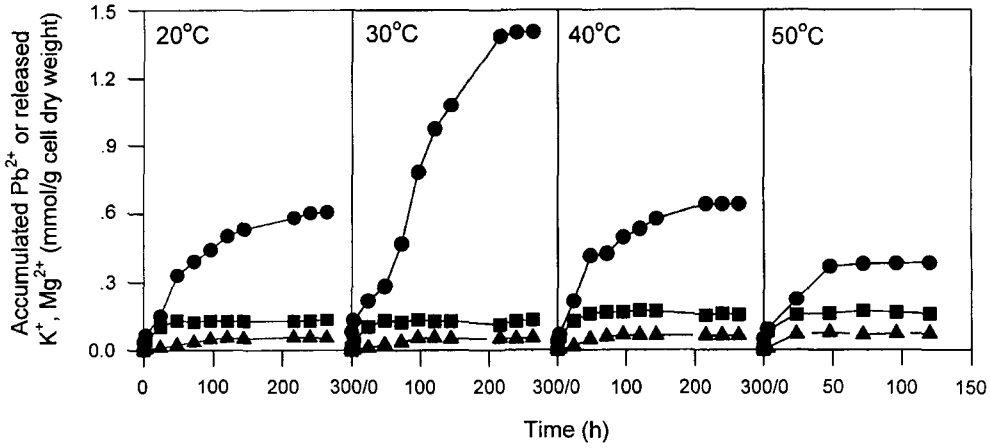

Fig. 2. Effect of temperature on the accumulation of Pb^{2+} (●) and the release of K^+ (■) and Mg^{2+} (▲) in *S. cerevisiae*. Initial concentrations of cells and Pb^{2+} were 0.6 and 0.2 g/l, respectively.

had described the effect of temperature on the uptake of heavy-metal ions by microorganisms. The maximal accumulation of Cu^{2+} by *S. cerevisiae* had been shown to be at 25°C~30°C [1]. Contradictory results had been reported that the temperature had little effect on silver biosorption by freeze-dried *S. cerevisiae* within the temperature ranges from 4°C to 55°C [12] and on the uptake of CH_3Hg and Hg^{2+} in *S. cerevisiae* from 5°C to 60°C [4].

Accumulation processes that depend on the cellular metabolism would be those that are the most likely to be inhibited by low temperature, where high temperature could affect the integrity of cell membranes and hinder compartmentalization of Pb^{2+}, also leading to a low uptake level. At high temperatures (30°C~70°C), a significant reduction in the adsorption of Zn^{2+} in *Penicillium* sp. has been reported [5]. In this study, the deformation of the cell wall and membrane might be one of the most important reasons for the decrease in Pb^{2+} accumulation at high temperatures (40°C and 50°C).

Release of K^+ and Mg^{2+} in the Course of Pb^{2+} Accumulation

The amount of Pb^{2+} accumulation and K^+-Mg^{2+} release with temperature were investigated in order to elucidate the effect of temperature on the Pb^{2+} accumulation process. K^+ release has been associated with cell membrane disruption and the loss of viability [2], and Mowll and Gadd [6] suggested that K^+ efflux occurred to maintain the ionic balance across the cell membrane. As illustrated in Fig. 2, the amount of Pb^{2+} accumulation decreased from 1.40 to 0.38 mmol Pb^{2+}/g cell dry weight at elevated temperatures within the ranges from 30°C to 50°C. Meanwhile, the release amounts of K^+ (0.13~0.16 mmol K^+/g cell dry weight) and Mg^{2+} (0.05~0.07 mmol Mg^{2+}/g cell dry weight) were nearly constant irrespective of the temperature variation. In conclusion, the difference in maximal Pb^{2+} accumulation amount with temperature did not result from the action of ion exchange with K^+ and Mg^{2+} within the temperature ranges examined.

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