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Characteristics of Zn^{2+} Biosorption by *Saccharomyces cerevisiae*[1]

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Objective To investigate the characteristics of Zn^{2+} biosorption and the release of cations during the process of Zn^{2+} biosorption by intact cells of *Saccharomyces cerevisiae*. **Methods** The batch adsorption test was used to study the biosorption equilibrium and isotherm. Zn^{2+} concentration was measured with atomic adsorption spectrophotometer (AAS) AAS 6·Vario. **Results** When the initial concentration of Zn^{2+} ranged between 0.08 and 0.8 mmol/L, the initial pH was natural (about 5.65) , the sorbent concentration was about 1 g/L and the capacity of Zn^{2+} biosorption was from 74.8 to 654.8 μmol/g. The pH value increased by 0.55-1.28 and the intracellular cations (K^+, Mg^{2+}, Na^+, Ca^{2+}) of the cells were released during the process of Zn^{2+} biosorption. **Conclusion** Ion exchange was one of the mechanisms for Zn^{2+} biosorption. The biomass of *Saccharomyces cerevisiae* is a potential biosorbent for the removal of Zn^{2+} from aqueous solution. More work needs to be done before putting it into practical application.

Key words: *Saccharomyces cerevisiae*; Biosorption; Zinc ion; Cation release

INTRODUCTION

Metallic zinc is one of the most important metals, which due to its relatively low melting point is often found in effluents discharged from acid mine drainage (AMD), electroplating plant and industries involving fabrication of batteries and manufacture of alloys (such as brass), and also in effluents from municipal wastewater treatment plant or from industrial plants that employ roasting or heating of zinc compounds for metallic zinc production[1-3]. Zinc is toxic to animals, plants, and microorganisms when it is present at millimolar concentration levels, although the metal as a trace element is an essential nutrient[2]. Recovering or lowering the levels of zinc ion in (waste) water is of environmental and agricultural importance[2-3].

Biosorption, using biomaterials such as bacteria, fungi, yeast, and algae *etc.*, is regarded as a cost-effective biotechnology for the treatment of high volume and low concentration complex (waste) water containing heavy metal(s) in the order of 1 to 100 mg/L[4]. *Saccharomyces cerevisiae*[5], *Brevibacterium* sp. (zinc-resistant bacterium)[2], biosolids (dewatered waste activated sludge containing non-living microorganisms)[3], natural materials like ground nut shells and sawdust[6], *Oscillatoria anguistissima*[1] and *Rhizopus arrhizus*[7] are used to remove zinc from aqueous solutions. However, most of these researches have focused on the biosorption capacity and influence factors. To date, only a few studies have been carried out on the release of cation from certain biosorbents during biosorption. The release of cellular metal ions (K^+, Mg^{2+}, Ca^{2+}, *etc.*) has been reported during the biosorption of Pb^{2+}[8] and Cu^{2+}[9] by *S. cerevisiae*. The increase in pH value of biosorption system has also been observed during the biosorption of Ag^+ and Ni^{2+} by *Rhizopus arrhizus*[10] and CuP^{2+}, Cd^{2+}, and Pb^{2+} by waste brewery biomass[11]. Understanding the cation changes in the biosorption system is evidently helpful to explore the mechanism of zinc biosorption.

Among the promising biosorbents for the removal of heavy metals identified over the past decades, *Saccharomyces cerevisiae* has received increasing attention due to the unique nature in spite of its mediocre capacity of metal uptake relevant to other fungi. *S. cerevisiae* is widely used in food and beverage manufacturing, easily growing in cheap medium. It is also a by-product and can be obtained in large quantity as a waste of fermentation industry, and easily manipulated at a molecular level[12].

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The present study aims to investigate the characteristics of Zn^{2+} biosorption by cells of *S. cerevisiae*.

MATERIALS AND METHODS

Yeast Strains and Culture Conditions

Saccharomyces cerevisiae 2.606 was obtained from the Institute of Microbiology, Chinese Academy of Sciences. The yeast was routinely maintained and grown on YEPD solid medium containing 10 g/L yeast extract, 10 g/L beef peptone, 10 g/L glucose, 20 g/L agar, 20 g/L deionized water (natural pH). For liquid culture, the yeast strains were grown in YEPD liquid medium of the same composition without the agar. The cells of *S. cerevisiae* were harvested from liquid medium by centrifugation (3000 r/min, 5 min, at room temperature) and then washed twice with deionized water. The washed cells without further treatment were called intact cells. The dead cells were obtained by autoclaving the intact cells at 115℃ for 20 min. Both intact and dead cells were suspended in deionized water without buffer solution and then stored at 4℃. The dry cell weight was determined after drying at 105℃ to a constant weight.

Chemicals

The stock solution of Zn^{2+} (16 mmol/L) was prepared using $Zn(NO_3)_2{\cdot}6H_2O$ (analytical grade) and diluted as appropriate in deionized water.

Biosorption Experiment

The experimental method used in this study was based on the methods recommended by Suh *et al*.[8]. The prepared yeast suspension (50 mL) was mixed with an equal volume of the initial concentration of aqueous zinc ion solution prepared at twice the desired concentration in 250 mL flasks. Final concentration of the biomass was 1 g (dry weight) /L. The pH value of Zn^{2+} solution, cell suspension and mixture of Zn-cells was about 5.0-5.5, 5.9, and 5.6, respectively. The initial pH value of the mixture of Zn-cells without buffer was about 5.6, close to optimum pH 5.8 for zinc biosoprtion by the yeast[5], and no spontaneous zinc precipitation was observed in the prepared solutions. Hence, pH adjustment was not carried out. The flasks were shaken at 30℃, 150 r/min.

Analytical Methods

The concentrations of Zn^{2+}, K^+, Mg^{2+}, Na^+, and Ca^{2+} in supernatant were determined by an atomic absorption spectrometer with flame atomization (AAS 6 Vario). The pH value was measured with a pH meter (WTW pH526).

Data Analysis

The amount of Zn^{2+} adsorbed by cells was calculated as follows:

$$q\ (\mu mol/g) = 1000\ (c_0 - c_t) \times V/W$$

The removal efficiency (η) of Zn^{2+} was calculated as follows:

$$\eta\ (\%) = (c_0 - c_t)\ /c_0 \times 100$$

where t is the contact time (h), c_0 is the initial concentration of Zn^{2+} at t =0 (mmol/L), c_t is the concentration of residual Zn^{2+} in supernatant at the time of t (mmol/L), V is the volume of solution for biosorption (mL), W is the dry weight of yeast (g).

RESULTS

Zn^{2+} Biosorption

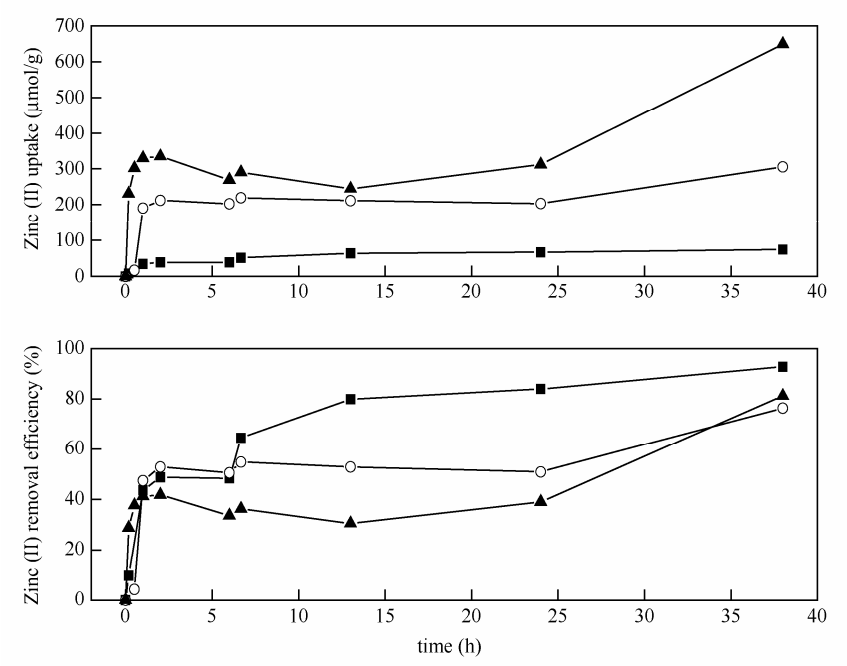
The biosorption of Zn^{2+} by intact cells of *S. cerevisiae* was conducted to investigate the effects of different initial concentrations of the metal ion (0.08 mmol/L, 0.4 mmol/L, 0.8 mmol/L) and contact time (0 to 38 h) at natural pH of 5.65 without buffer. Figure 1 shows the time course of Zn^{2+} uptake and the removal efficiency at various Zn^{2+} concentrations, which suggested that the metal uptake was 74.8-654.8 μmol/g, and the removal efficiency was 76.4%-92.8% when the biomass concentration was 0.992 g (dry cell weight) /L with the contact time of 38 hours.

pH Changes During Zn^{2+} Biosorption Process

pH value is one of the most important environmental factors that influence not only the site dissociation of biomass, but also the solution chemistry of heavy metals[13].

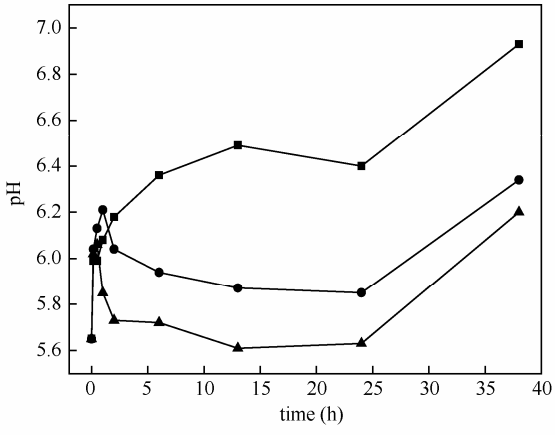
In this study, the natural pH value of Zn^{2+}-cell mixed solution without buffer was about 5.65. During the process of Zn^{2+} biosorption, the pH value profile at three different stages corresponded to the Zn^{2+} uptake process (Fig. 2).

The pH profile of the suspension of intact and dead cells (stored at 4℃ for 2 days) was measured during incubation (0-72 h), in the presence and absence of metal ion Zn^{2+}. The results were shown in Fig. 3. The pH value of intact or dead cell suspension without addition of Zn^{2+} increased with the incubation time. However, the presence of Zn^{2+} would reduce H^+ uptake by yeast cells. The higher the initial Zn^{2+} concentration, the lower the pH value of the Zn^{2+}-yeast system, and the narrower the final pH shift range. The pH shift range was 1.28 (initial Zn^{2+} concentration was 0.08 mmol/L), 0.74 (0.4 mmol/L), and 0.55 (0.8 mmol/L), respectively.



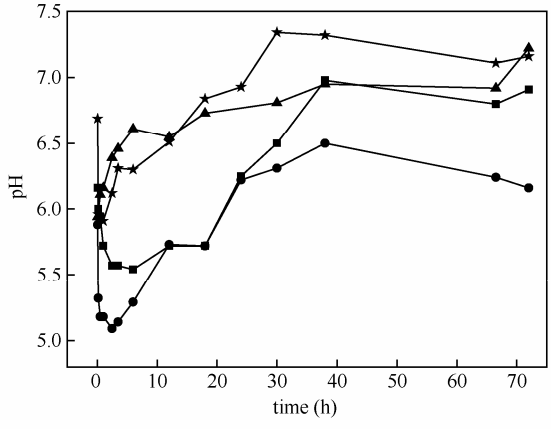
(■) Initial Zn^{2+} concentration, c_0= 0.08 mmol/L; (○) c_0=0.4 mmol/L; (▲) c_0=0.8 mmol /L. Initial cell concentration was 0.992 g/L and stored at 4℃ for 45 days.

FIG. 1. Effect of initial Zn^{2+} concentration and contact time on Zn^{2+} biosorption and removal efficiency.



(■) Initial Zn^{2+} concentration, c_0= 0.08 mmol/L; (●) c_0=0.4 mmol/L; (▲) c_0=0.8 mmol/L. Initial cell concentration was 0.992 g/L and stored at 4℃ for 45 days.

FIG. 2. pH value of Zn-yeast system at different initial Zn^{2+} concentrations.



(★) Dead cells in the absence of Zn^{2+}; (▲) Intact cells in the absence of Zn^{2+}; (■) Intact cells in the presence of Zn^{2+}; (●) Dead cells in the presence of Zn^{2+}. The initial Zn^{2+} concentration was 0.75 mmol/L, the yeast was stored at 4℃ for 2 days.

FIG. 3. Influence of Zn^{2+} on pH value of the biosorption system.

Release of Cellular Metal Ions During Zn^{2+} Biosorption

As the biosorption of Zn^{2+} proceeded, cellular metal ions (K^+, Mg^{2+}, Na^+, Ca^{2+}) were concomitantly released (Fig. 4, initial Zn^{2+} concentration was 0.4 mmol/L). The released amount of K^+ was sharply increased in the first hour (399.9 μmol/g), then slowly increased after 6 h (507.1 μmol/g)and finally reached 548.3 μmol (K^+) /g after 38 h. Mg^{2+} and Na^+ were released continuously, but their maximal level (35.4 μmol Mg^{2+}/g cell dry weight, 61.1 μmol Na^+/g cell dry weight) was much lower than that of K^+, while the amount of released Ca^{2+} was very small (only 2.5 μmol Ca^{2+}/g cell dry weight at 38 h). In brief, potassium ion

was first released rapidly in the order of hundreds of μmol/g, then magnesium and sodium ions were released slowly with several to less than a hundred of μmol/g, and the release of calcium ion was rare.

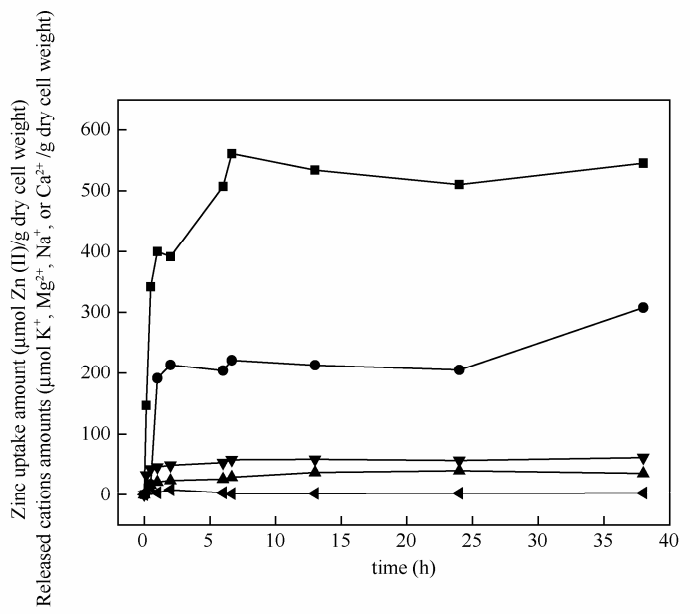


FIG. 4. Relationship between Zn^{2+} (●) uptake and release of K^+ (■), Mg^{2+} (▲), Na^+ (▼), Ca^{2+} (◀). The initial Zn^{2+} concentration, c_0=0.4 mmol/L, the yeast was stored at 4℃ at for 45 days.

DISCUSSION

As indicated by Fig. 1, the time course of Zn^{2+} uptake could be basically divided into 3 stages within 38 hours. (1) From 0 to 2 hours, Zn^{2+} was removed from solution rapidly. For example, when the concentration of Zn^{2+} was 0.4 mmol/L, Zn^{2+} uptake reached 213.4 μmol/g after 2 hours of biosorption. Accordingly, the removal efficiency was 52.9%, which was 70% of the maximal removal efficiency (76.4% after 38 hours). (2) Desorption of Zn^{2+} occurred. Zn^{2+} uptake and the removal efficiency decreased. The duration and degree of desorption were different at different initial Zn^{2+} concentrations. It occurred within 2 to 6 hours (at an initial concentration of 0.08 mmol/L); 2 to 22 hours (0.4 mmol/L) or 2 to 13 hours (0.8 mmol/L), respectively. The higher the initial Zn^{2+} concentration, the more serious the desopriton. Whether it is related to reinforced repulsion force of Zn^{2+} at high concentrations is not clear and needs to be further explored. The fact that desorption of Zn^{2+} occurred also implied that Zn^{2+} biosorption reached a relative equilibrium state at 2 hours of contact time. (3) Zn^{2+} uptake entered a new stage, in which Zn^{2+} uptake capacity (q) and the removal efficiency (η) increased. The degree of Zn^{2+} uptake increase depended upon the initial Zn^{2+} concentration. At 38 hours, when the initial Zn^{2+} concentration was 0.08, 0.4, and 0.8 mmol/L, Zn^{2+} uptake reached 74.8, 307.9, and 654.8 μmol/g, respectively, increasing by 9.5%, 33.4%, and 52.0% in comparison with the results of contact time (24 hours). The removal efficiency reached 92.8%, 76.4%, and 81.2% at the end of experiment.

According to the trend of time course of Zn^{2+} uptake, and the fact that high removal efficiency (92.8%) was reached at a low initial concentration (0.08 mmol/L), the time to reach the real adsorption equilibrium state may be longer than 38 hours at a higher initial concentration. Our experimental results showed that when the intact cells (stored for 2 d at 4℃) were used for biosorption of Zn^{2+} (0.70 mmol/L), the equilibrium time was about 72 hours (data not shown). Suh *et al.*[8] found that the equilibrium time for Pb^{2+} accumulation by intact cells of *S. cerevisiae* decreased with the increase of the storage time of cells.

Figure 1 indicates that Zn^{2+} uptake capacity (q, μmol/g) decreased and the removal efficiency increased when the initial Zn^{2+} concentration was raised at a constant of biomass concentration.

As shown by Fig. 2, the pH value increased rapidly from 5.65 to 6.04 in the first 2 hours, then decreased to 5.85 from 2 to 24 hours, and finally rose to 6.39 at 38 hours, when initial Zn^{2+} concentration was 0.4 mmol/L. pH increased by 0.74 after 38 hours of biosorption. The same phenomenon was observed during the biosorption of Ag^+ and Ni^{2+}, which was regarded to reflect an intrinsic ability of the microbial biomass to shift the pH towards a favorable value,

usually closer to neutrality[10]. Marques *et al.*[11] also confirmed that measured pH shift was not related to metal uptake, but was caused by the microbial biomass itself. The transfer of this biomass to a low salt medium causes osmotic unbalance, leading to spontaneous H^+ uptake and/or a release of alkaline intracellular products into the medium[11].

Although the pH profile of Zn^{2+}-yeast cell suspension during the process of Zn^{2+} biosorption was various at different initial Zn^{2+} concentrations, it was almost consistent with the Zn^{2+} uptake profile of corresponding initial Zn^{2+} concentration. This indirectly reflected the competition between Zn^{2+} and H^+.

Does the increase of pH value lead to precipitation of Zn^{2+}? The hydrolyzed species of Zn^{2+} depends on pH value and Zn^{2+} concentration. The dominant species of Zn^{2+} complex is $[Zn(OH_2)_6]^{2+}$ at pH<7, $Zn(OH)_2$ (precipitate) at pH 7-9, and $[Zn(OH)_4]^{2-}$ at pH>9[2]. In this study, $[Zn(OH_2)_6]^{2+}$ predominantly existed because the pH ranged between 5.65 and 6.93. Of course, formation of hydrolyzed complexes is dependent upon the concentration[14]. Taniguchi *et al.*[2] have suggested that Zn^{2+} adsorbed by lyophilized cells of *Brevibacteria* sp. strain may exist as a divalent cation rather than a deposit of metal crystalloid.

Brady and Duncan[9] have also observed the similar phenomenon during the biosorption of Cu^{2+} caused by *S. cerevisiae*. The biosorption of Cu^{2+} by yeast resulted in rapid release of 70% of cellular K^+, followed by a slower release of approximately 60% of cellular Mg^{2+}, but a very small amount of Ca^{2+}. The release of these light metal ions indicates that ion exchange mechanism may play an important role in Zn^{2+} biosorption by yeast cells.

CONCLUSIONS

The Zn^{2+} biosorption capacity is 74.8-654.8 μmol/g, and the removal efficiency is 76.38%-92.80% when the sorbent concentration is about 1 g/L and the initial Zn^{2+} concentration is 0.08-0.8 mmol/L. The pH value increases by 0.55-1.28 and the intracellular cations (K^+, Mg^{2+}, Na^+, Ca^{2+}) of the cells are released during the process of Zn^{2+} biosorption. The ion exchange is one of the mechanisms for Zn^{2+} biosorption. The biomass of *Saccharomyces cerevisiae* is a potential biosorbent for the removal of Zn^{2+} from aqueous solution. More work needs to be done before it is applied for the treatment of Zn^{2+}-containing wastewater.

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