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Cadmium Bioremoval by *Saccharomyces cerevisiae* in Milk

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ABSTRACT

Introduction: The application of biosorbents like bacteria, yeast, and algae is a biotechnological method for eliminating heavy metals from the environment. These microorganisms can also be used for the decontamination of heavy metal in food and water. **Methods**: In this study, we investigated the Cadmium (Cd) biosorption in milk by using *Saccharomyces cerevisiae*. For this purpose, Cd and *S. cerevisiae* were added to milk, and the bioremoval process was monitored for four days. We evaluated six variables, including exposure time, temperature, *S. cerevisiae* concentration, viability yeasts, shaking rate, and initial Cd concentration in the bioremoval process. **Results:** The analysis of ANOVA showed that among the above six variables, *S. cerevisiae* concentration, initial Cd concentration, and exposure time were statistically significantly associated with Cd removal (*P* values ≤0.05). The highest biosorption (70%) was observed after 4 days with 30×10^8 CFU *S. cerevisiae* in milk containing 80 µg/L of Cd. **Conclusion**: Our findings provided further evidence for *S. cerevisiae* as a powerful biosorbent for Cd removal from milk and a potentially safe and green tool for providing safe and healthy food supply.

INTRODUCTION

Heavy metals have adverse effects on human health, posing a threat worldwide [1]. Heavy metals naturally occur in the environment, but anthropogenic activities introduce a large amount of these toxic elements in different environmental compartments resulting in contamination of animals and human food [2]. Worldwide, milk constitutes a central part of the human diet [3, 4]. Different heavy metals may enter the milk supplies through many environmental contaminations like industrial emissions in the air, polluted soil, and contaminated water and plants [5]. Production of heavy metal-free products is one of the primary concerns in the dairy industry, which requires continuous surveillance and adoption of the appropriate method for removing these agents from milk and other dairy product [6]. Cadmium (Cd) is a toxic metal that occurs in industrial wastewaters. The paint, textile, casting, and pharmaceutical industries are significant examples that contribute to heavy metal pollution in the aquatic environment [7]. This heavy metal, even in low concentrations, causes serious health problems to humans, such as hypertension, renal dysfunction, lung damage, teratogenic effects, and hepatic injury [8-10]. Some countries such as Iraq [11], Turkey [12], Nigeria [13], China [14], and Iran [15, 16] have reported Cd contamination in milk.

Various chemical and physical approaches, including precipitation, ultrafiltration, electrolysis, ion exchange, and reverse osmosis, are available for the removal of heavy metals from aqueous solutions [17]. However, in recent years alternative strategies like bioremediation methods have attracted increasing attention. Some studies have indicated using bacteria, yeasts, and fungi such as *Lactobacillus plantarum*, *Lactobacillus* spp., and *Saccharomyces cerevisiae* for bioremoval of heavy metals like Cd and lead (Pb) from foodstuffs. This novel method is cheaper and more suitable for food and water decontamination [18-20].

In the food industry, *S. cerevisiae* is a familiar and promising candidate for heavy metals biosorption from drinks and food [21, 22]. Some studies have reported the bioremoval of heavy metals by this organism [23-26]. This valuable biosorbent is an available, safe, cheap, and suitable agent for the decontamination of foodstuff [27]. Bioremoval is an absorption process in which heavy metals are attached to the cell surface [28]. In general, heavy metals bioremoval occurs through different mechanisms. The functional groups (hydroxyl and carboxyl) in the cell wall of *S. cerevisiae* play an essential role in the bioremoval process. They are responsible for metal ions fixation during the process. Also, the intracellular metal accumulation happens in the cell membrane, and metal ions can bind to other cellular molecules [27].

There is no much data on Cd bioremoval from milk. This

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study, for the first time, reports the use of S. *cerevisiae* for removing low levels (µg/L) of Cd concentration from milk. We also investigated *S. cerevisiae* concentration, temperature, exposure time, and heavy metal concentration as the most crucial variables in the bioremoval process [28].

MATERIAL AND METHODS

The Cd removal was performed by various concentrations of *S. cerevisiae* (10, 20, 30, and 40×10^8 CFU/mL) and Cd (20, 40, 60, 80, 100 mg/L), and exposure times ranging from 1 to 4 days.

Yeast growth. The freeze-dried *S. cerevisiae* PTCC 5020 was provided by the Research and Technology Department of Ministry of Sciences, Tehran, Iran. For yeast culture, a medium containing (g/L) 30 g glucose, 1 g yeast extract, 0.2 K_2HPO_4, 1 g KH_2PO_4, 2.5 g $MgSO_4$, and 9 g $(NH4)_2SO_4$ was prepared and autoclaved for 20 min at 121°C. Once cooled, the medium was inoculated with *S. cerevisiae* followed by incubation (30°C) with shaking at 50 rpm for 20 h. The culture media was stored at 4°C for future use. The activated yeast cells were counted by the dilution method; 1 mL of seed culture was diluted in a ratio of 1/10 with sodium chloride solution, and 10 serial dilutions were obtained. Then 1 mL of each dilution was added to the solid nutrient agar medium by pour plate method [29]. Following the incubation of the plates at 30°C for 72 h, the yeast colonies were counted and showed the mean 30×10^8 CFU/mL. The yeast culture was activated daily for biosorption experiments.

Reagents. All chemicals used in this project were prepared from Merck (Darmstadt, Germany). Cd standard solution was used as the stock solution (Accu Trace, New Haven, USA). All containers were acid-washed by 15% (v/v) HNO_3 overnight, and then autoclaved at 121°C, 1.4 atm pressure, for 20 min.

Bioremoval condition. Amounts of 50 mL of milk with different metal Cd concentrations (20, 40, 60, 80, 100 mg/L) were prepared. The effects of six variables including exposure time (1-4 days), temperature (4 and 40°C), *S. cerevisiae* concentrations (10×10^8 to 40×10^8 CFU/mL), the viability (living and non-living), shaking rate (5-20 rpm) and initial Cd concentration (20, 40, 60, 80, 100 mg/L) on the biosorption efficiency of Cd by *S. cerevisiae* were evaluated [30, 31].

Measurement of Cadmium by ICP- MS. The inductively coupled plasma mass spectrometer (ICP-MS) was used for measuring the Cd amount. First, 5 ml of milk samples were digested by using the microwave procedure at 1200 W for 10 min and then were injected to the ICP- MS spectrometer [32].

The sample mixtures containing Cd and *S. cerevisiae* were centrifuged at 2000×g for 15 min, and the supernatant recovered. Finally, Cd concentration in the supernatant was measured by using the ICP-MS. All the trials were repeated three times.

The Cd removal by *S. cerevisiae* was calculated by the following equation [33]:

$$R = \frac{C0 - Ce}{C0} \times 100$$

Where R is % Cd removal, C0 is the initial concentration of Cd in milk in (mg/L), and Ce is the concentration of Cd in milk after biosorption (mg/L).

RESULTS

The effect of six variables was analyzed by the analysis of variance (ANOVA). Our analysis revealed that the Cd bioremoval was significantly affected by three variables; Cd concentration, *S. cerevisiae* concentration, and exposure time. *P*-values of less than 0.05 represent the significant variables in this project (Table 1).

Table 1. ANOVA results for the effect of parameters in Cd biosorption by *S. cerevisiae*.

Source	Sum of Squares	df	Mean Square	F-value	*P*-value
Shaking rate	0.2057	1	0.2057	1.92	0.3995
***S. cerevisiae* viability**	0.3426	1	0.3426	3.41	0.3034
Cd concentration	8.86	1	8.86	89.62	0.0365
***S. cerevisiae* concentration**	8.35	1	8.35	76.93	0.0698
Exposure time	8.76	1	8.76	81.40	0.0665
Temperature	0.3858	1	0.3858	3.23	0.3025

Effect of *S. cerevisiae* concentration on Cd bioremoval. The Cd biosorption efficiency enhanced by increasing *S. cerevisiae* concentrations from 10×10^8 to 30×10^8 CFU/mL (Fig. 1A). The maximum Cd removal (70%) was achieved at the 30×10^8 CFU/mL. By increasing the *S. cerevisiae* concentrations from 30×10^8 to 40×10^8 CFU/mL, the removal efficiency decreased.

Effect of exposure time on Cd bioremoval. During the four days of the experiment, the Cd removal by *S. cerevisiae* increased with the extended exposure time, but it showed a faster rate on the second day and a slower trend from the second to the fourth day (Fig. 1B).

Effect of Cd concentration on the Cd bioremoval. The Cd biosorption efficiency was evaluated in different Cd concentrations (20, 40, 60, 80, and 100 mg/L). The bioremoval increased with Cd concentration from 20 to 100 mg/L. The highest biosorption (70%) was observed at the 100 mg/L of Cd (Fig. 1C).

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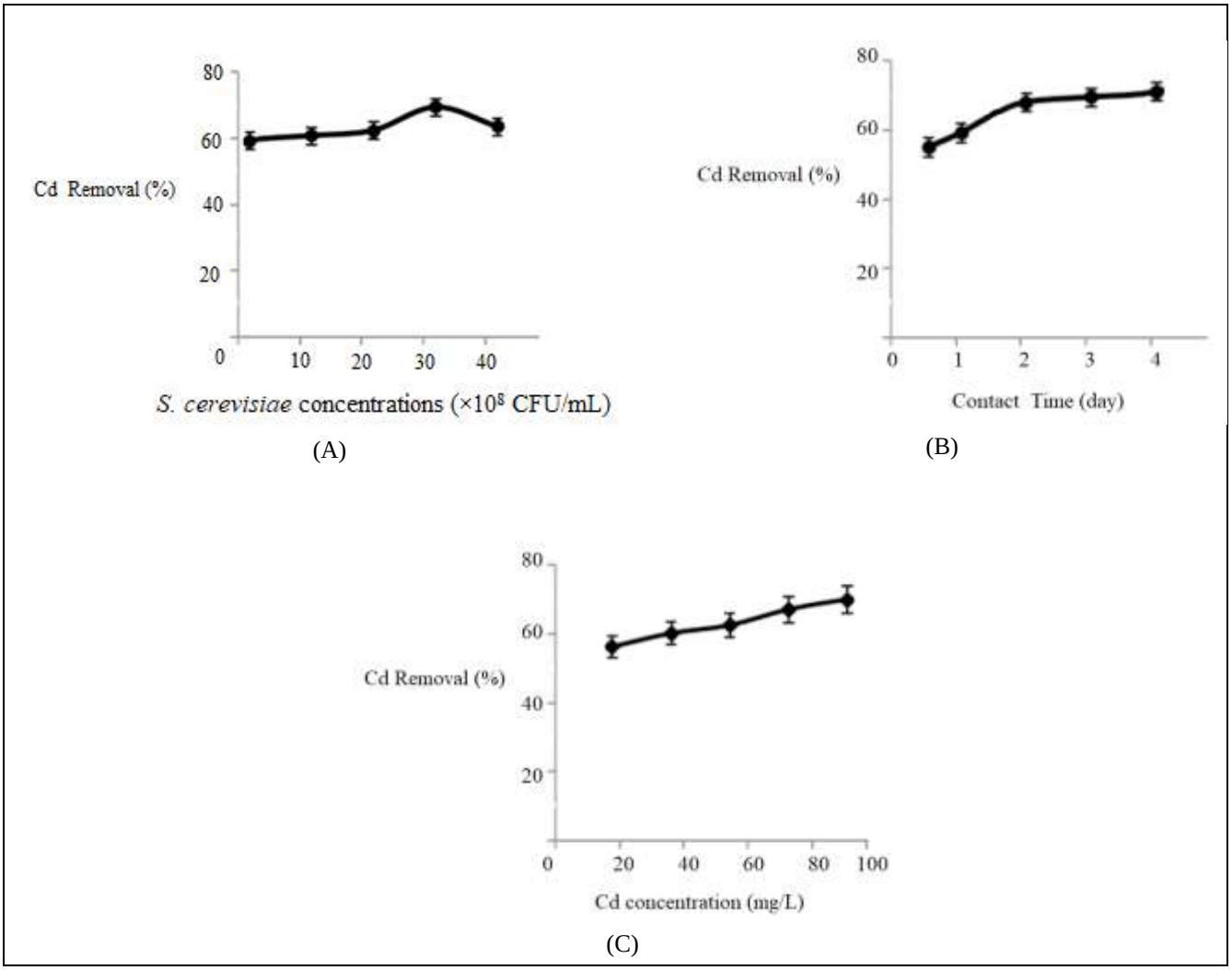


Fig. 1. The effect of *S. cerevisiae* concentration (A), Exposure time (B) and Cd concentration (C) on Cd bioremoval

DISCUSSION

Using microorganisms as environmentally friendly biosorbents for toxic metals bioremediation from food and water resources is a novel method. In the food industry, *S. cerevisiae* is one of the potential cost-effective biosorbents for the decontamination of heavy metals from foodstuff. The biosorption occurs through a surface binding process that relates to the functional groups like hydroxyl, carboxyl, and amide groups of *S. cerevisiae* [34, 35]. In this study, we used *S. cerevisiae* for Cd removal from milk, and the effects of variables, including exposure time, *S. cerevisiae* concentration, and initial Cd concentration on the Cd bioremoval by *S. cerevisiae*, were evaluated.

The biosorption increased with *S. cerevisiae* concentration in milk samples as the active sites on the cell wall of *S. cerevisiae* became more available for attaching to the metal ions. Our results showed agreement with those of similar studies that used *S. cerevisiae* for bioremoval of heavy metals like Cd, Zink (Zn), and Mercury (Hg) [24, 36, 37].

The Cd uptake enhanced by the increase in the exposure time from one to four days, which led to the attachment of more Cd ions to *S. cerevisiae* receptor sites resulting in more Cd removal [38]. Our findings are in agreement with the study of Hadiani *et al.* (2018) that reported enhanced mercury bioremoval by *S. cerevisiae* when exposure time extended from 24 h to 48 h [24]. Also, Massoud *et al.* (2019) and Hatamifard *et al.* (2016) reported an increase in Pb and Hg biosorption over extended exposure time [25, 39].

The present study also assessed the bioremoval in different initial Cd concentrations, 20, 40, 60, 80, 100 mg/L with constant *S. cerevisiae* concentration (30×10^8 CFU/mL), and over the day exposure time. Clearly, by increasing the number of metal ions, their adsorption to *S. cerevisiae* receptors would increase as a result of the higher driving force for metal ions to interact with *S. cerevisiae* membrane binding sites [35, 40, 41].

The maximum Cd bioremoval (70%) with 30×10^8 CFU/mL of *S. cerevisiae* and 80 mg/L Cd concentration in milk was achieved on the 4th day. Our study provided further evidence for the suitability of *S. cerevisiae* for bioremediation of heavy metals in food industry, opening a new window for providing safe and healthy food supply.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

ETHICAL APPROVAL

This article contains no assay on humans or animals by the contributing authors.

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