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Article

The biosorption capacity of *Saccharomyces cerevisiae* for Cadmium in Milk

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Abstract: This study aimed to evaluate the capacity of *Saccharomyces cerevisiae* for Cadmium absorption in Milk. Nowadays one of the most serious problems of the industrialized world is heavy metals pollution. Applying microorgaisms as a novel biotechnology is so useful especially in foodstuffs. Among the biosorbents used for heavy metals' removal, *Saccharomyces cerevisiae* has got an increasing attention due to its popularity in food industry. In this regard, the effects of some important factors such as the initial metal concentration, biomass concentration and contact time on the biosorption capacity of *Saccharomyces cerevisiae* were studied. The biosorption was analyzed by the inductively coupled plasma mass spectrometer (ICP-MS). The maximum Cd removal (70%) was at 80 μg/L of Cd concentration in milk samples containing 30×10^8 CFU *Saccharomyces cerevisiae* at the end of storage time (the 4th day). There were no significant differences in sensory and physicochemical properties of milk samples during storage ($p < 0.05$). The isotherm studies followed by two popular models; Langmuir and Freundlich and the results showed a better fit to the Langmuir isotherm. Altogether, the results of this study demonstrated that the approach of using this valuable yeast, could be applied for foods' detoxification and producing healthier foodstuffs.

Keywords: *Saccharomyces cerevisiae*; biosorption; milk; ICP-MS; isotherm; sensory evaluation

1. Introduction

Toxic metal contamination is a serious environmental problem all around the world due to the fast development of industries such as fuel, pesticides and mining. Their wastes discharge metals into the environment directly or indirectly [1, 2]. These toxic metals can enter to the food chain and then into our bodies [3]. Cadmium (Cd) is one of the high toxic metals in this regard [4]. Milk is a valuable food source for humans and animals. It has nearly all essential nutrients for growth [5]. According to World Health Organization (WHO), the maximal allowed concentration values for Cd in milk is less than 10 μg/L [6].

Some reports have shown Cd contamination of milk around the world and unfortunately in some places it is more than the permissible level: Turkey [7], China [8], Iraq [9] and Iran [10, 11].

Common techniques for heavy metals removal from aqueous solution like ion exchange, chemical precipitation, membrane technologies, electrochemical treatment and using activated carbon which are expensive and also not effective for using in foodstuffs [12, 13].

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Biosorption as a green technology, is the process of metal binding from aqueous solution to the surface of microorganism. The mechanism occurred through the absorption of metal ions to functional groups which is on the cell wall of the biomass [12]. It is a cheap, eco-friendly and fast technique [14]. Biosorpton is process that the heavy metals trap into the cell wall's active site [12]. The heavy metal's removal takes place through various mechanisms. The functional groups of the cell wall of *S .cerevisiae* such as hydroxyl and carboxyl, are responsible for the biosorption technique. They are the main agents for metals to be attached during the mechanism. Moreover, the heavy metals intracellular accumulation occurs in the cell wall and metals are able to attach to the cell molecules [14-16].

In bosorption method various microorganisms like yeasts, bacteria, algae and fungi are applied. They possess some advantages such as being cheap and practical for foodstuffs [15, 16].

The unique yeast "*Saccharomyces cerevisiae"* is commonly used in bakery and brewery industries. It is an economic available biosorbent [17]. There are some studies about using this yeast for heavy metals biosorption [18- 22].

Our study aims to evaluate the capacity of *S. cerevisiae* for Cd absorption in Milk. So, the effects of three main factors; initial metal concentration, biomass concentration and contact time on the biosorption capacity of *S. cerevisiae* were studied. These factors were chosen through the previous studies of heavy metals bioremoval [20-23] and also based on the results of our research team. This technique would be useful in case of emergency in food and beverage industry.

2. Materials and Methods

2.1. Preparation of the biomass

The *S. cerevisiae* (PTCC-5020) was purchased from the Science Research and Technology Department, Tehran, Iran. Glucose, yeast extract, $(NH_4)_2SO$, K_2HPO_4, MgSO4 and KH_2PO_4 were combined as the yeast culture medium and then autoclaved at 121°C for 20 min. The medium was inoculated with *S. cerevisiae* after cooling followed by 20 h shaking at 70 rpm and then incubated at 30°C. The biomass colonies were counted and the mean of 30×10^8 CFU/mL was obtained through the dilution method; the seed culture (1 mL) was diluted in a ratio of 1:10 with NaCl with serial dilutions (10 times). Then the dilution (1 mL) was added to the nutrient agar medium by pour plate method and incubated for 72 h at 30°C for 72 h [24].

2.2. Chemicals

All chemicals were provided from Merck company (Germany) and Cd standard solution from Accu Trace company (USA). All the containers were acid-washed by HNO_3 (15% v/v) overnight and then then rinsed with distilled water.

2.3. Sample Preparation

Each sample was prepared of milk (50 mL) with levels of *S. cerevisiae* (10×10^8 to 50×10^8 CFU/mL) and different initial Cd concentration (40, 50, 60, 70, 80 μg/L) and stored in fridge for 4 days. Then the effect of 3 variables; initial metal concentration (40-80 μg/L), biomass concentration (10 - 50×10^8 CFU/mL) and contact time (1-4 days), on the biosorption capacity of *S. cerevisiae* were studied.

2.4. Physicochemical Analysis

The pH, acidity and density of milk samples were determined according to AOAC methods [24]. The pH value of milk samples was evaluated with a pH meter (Metrom, Switzerland) at room temperature. The titratable acidity was determined by titration method; milk sample (10 ml) was titrated by NaOH solution (0.1 N) and adding phenolphthalein as an indicator. The Lactodensimeter (Alla, France) was used to measure the density of milk samples [24].

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2.5. Sensory Analysis

The sensory analysis was evaluated during storage time (1st to 4th day) by 10 trained panelists [25]. Milk samples were analyzed for consistency, color, odor and overall acceptability. The samples were scored in a 9-point hedonic scale. The scores were from 1(extremely dislike) to 9 (extremely like). Mean values (± SD) were calculated from the panelists scores of each sample.

2.6. Central Composite Design (CCD)

The 3 variables; initial Cd concentration, *S. cerevisiae* biomass and contact time, having significant effects on Cd removal. In this study, CCD was used to find the optimal conditions of Cd biosorption with the experimental factors levels as shown in the Table 1.

Table 1. Levels of the main variables for the central composite design.

Main Variable	**Range and level**				
	− α (−1.6)	**−1**	**0**	**+1**	**+α (+1.6)**
***S. cerevisiae* biomass dosage (× 10^8 CFU)**	10	20	30	40	50
Initial Cd concentration (µg/L)	40	50	60	70	80
Contact time (day)	0	1	2	3	4

2.7. ICP-MS Analysis

The inductively coupled plasma mass spectrometer (ICP- MS, England) applied in this study, with a standard torch, a cross flow nebulizer and a quartz spray chamber. It was tuned before each experiment started. All the samples were put in microwave 1200W (Milestone Micro oven) to be digested with segmented rotor MPR-600 [26].

2.8. Removal Evaluation

The milk sample containing *S. cerevisiae* and Cd were digested in the microwave and then centrifuged (at 2000×g) for 15 min. The supernatant was injected to the ICP- MS for Cd residual determination. measured by using the ICP-MS. All the trials were repeated triple.

The Cd removal efficiency (%) was calculated by Eq. (1) [27]:

$$\%\text{Removal} = 100(Co\text{-}C_f) / Co \quad (1)$$

where Co (µg/L): is the initial Cd concentration in solution; C_f (µg/L): is the final Cd concentration in solution.

2.9. Absorption Isotherm

The biosorption isotherm were evaluated by adding the biosorbent (*S. cerevisiae*) to the milk samples with initial Cd concentrations (20 - 100 µg/L). After biosorption, the remained Cd was determined by ICP-MS. The biosorption experiments were repeated three times.

2.10. Statistical Analysis

The statistical analysis was done by MINITAB statistical software (version 14). The statistics data was provided by analysis of variance (ANOVA). The data are presented as the mean value ± SD during storage days. The *P*-values below 0.05 were statistically significant.

3. Results

3.1. The effect of initial metal concentration

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The effect of initial Cd concentration (40, 50, 60, 70, 80 µg/L) on the bioremoval efficiency was investigated (Figure 1a). The results showed that by increasing the Cd concentrations, the absorption improved. The highest Cd removal (70%) was observed at the initial metal concentration of 80 µg/L.

3.2. The effect of contact time

In this study, the Cd biosorption was evaluated during the contact times from 1 to 4 days. Figure 1(b) shows that Cd removal by *S. cerevisiae* increased as the time passed. As it shows the maximum removal of Cd was occurred in the 4th day. By increasing time up to 8 days, the yeast count was enhanced as the removal was nearly constant. Table 2 shows the yeast count and the bioremoval levels during 8 days of storage.

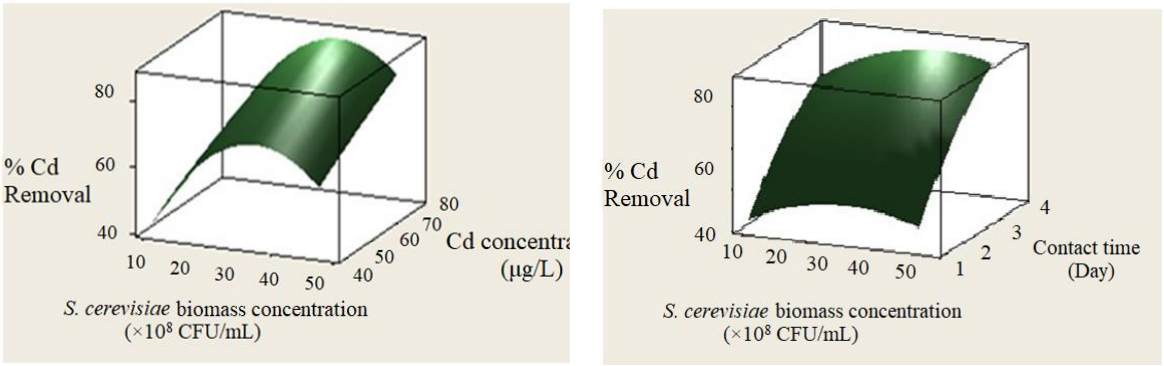
Table 2. The bioremoval level of Cd in milk samples during storage.

	Storage Time (day)		
	1	**4**	**8**
***S. cerevisiae* biomass (CFU/mL)**	10^8	10^8	10^9
Total count (CFU/mL)	10^{12}	10^{12}	10^{15}
Cd bioremoval (%)	45.51^a	70.10^b	70.21^b

Different letters are significantly different ($p < 0.05$).

3.3. The effect of biomass concentration

As shown in Figures 1 (a and b), by increasing *S. cerevisiae* biomass concentration from 10 up to 50×10⁸ CFU/mL, the removal efficiency enhanced. The optimum level of *S. cerevisiae* biomass concentration was 30×10^8 CFU with the highest removal amount of 70%.



(a) (b)

Figure 1. The effect of initial Cd concentration on bioremoval (**a**), the effect of biomass concentration initial on bioremoval (**b**).

3.4. Physicochemical evaluation

There was a slight reduction in pH values and a rise in titratable acidity of the milk samples. Also the density level was nearly constant. However, the differences were not significant in the milk samples ($p>0.05$) (Table 2).

3.5. Sensory evaluation

Table 3 also represents the results of sensory analysis during the storage of milk samples. There were no significant differences in consistency, smell and color of these milk samples during time

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intervals with control samples ($p < 0.05$). Also the overall acceptance of milk samples had no significant difference through storage period ($p < 0.05$).

Table 3. Physicochemical and sensory properties of milk samples during storage.

	***S. cerevisiae* biomass concentration (CFU/mL)**					
	Control		**10 ×108**		**50 ×108**	
Storage time (day)	**1**	**4**	**1**	**4**	**1**	**4**
Physicochemical properties						
pH	6.70±0.01[a]	6.67±0.05[a]	6.78±0.01[a]	6.67±0.07[a]	6.80±0.07[a]	6.71±0.07[a]
Acidity (% lactic acid)	0.14±0.01[a]	0.15±0.05[a]	0.14±0.07[a]	0.15±0.07[a]	0.14±0.07[a]	0.16±0.07[a]
Density (g/cm3)	1.01±0.01[a]	1.01±0.05[a]	1.02±0.01[a]	1.03±0.05[a]	1.02±0.07[a]	1.02±0.05[a]
Sensory property						
Color	7.99±0.07[a]	7.97±0.05[a]	7.97±0.07[a]	7.89±0.07[a]	7.90±0.05[a]	7.85±0.05[a]
Smell	7.99±0.05[a]	7.96±0.05[a]	7.95±0.05[a]	7.66±0.05[a]	7.95±0.07[a]	7.52±0.05[a]
Consistency	7.98±0.05[a]	7.98±0.07[a]	7.97±0.05[a]	7.94±0.07[a]	7.90±0.05[a]	7.88±0.05[a]
Overall acceptance	7.98±0.07[a]	7.95±0.05[a]	7.98±0.07[a]	7.95±0.05[a]	7.98±0.07[a]	7.90±0.07[a]

Different letters are significantly different ($p < 0.05$).

3.6. Isotherm studies

The capacity of *S. cerevisiae* biomass concentration (10^8 CFU/mL) for Cd biosorption was determined at Cd initial concentrations (20, 40, 60, 80 and 100 µg/L) via two popular biosorption isotherms; Langmuir and Freundlich models. The regression coefficient (R^2) represent the better isotherm model for Cd biosorption by *S. cerevisiae*.

The Langmuir equation is as the Eq. (2) [28]:

$$C_e/Q_e = 1/(K * Q_{max}) + C_e/Q_{max} \quad \text{Eq. (2)}$$

Where Qe (µg/L) is the Cd amount in absorbing process, Ce (µg/L) is the Cd equilibrium concentration in milk, Qmax (µg/L) is the maximum Cd absorption level. KL (L/µg) is the Langmuir constant. The Freundlich equation is as the following the Eq. (3) [29]:

$$LnQ_e = LnK_f + 1/n\ LnC_e \quad \text{Eq. (3)}$$

Where n and K_f are the Freundlich constants. The Langmuir and Freundlich parameters are shown in Table 4.

Table 4. Langmuir and Freundlich isotherm parameters for Cd removal.

Cd initial concentration (µg/L)	**Langmuir model†**			**Freundlich model§**	
	Ce	**Qe**	**Ce/Qe**	**Ln Qe**	**Ln Ce**
20	13.4	6.6	2.033	1.887	2.595
40	23.2	16.8	1.381	2.821	3.144
60	27	33	0.818	3.256	3.269
80	28	51	0.522	3.889	3.263
100	28	70	0.389	4.254	3.321

†R^2 for Langmuir model was obtained 0.9186
§ R^2 for Freundlich model was obtained 0.8587

As Table 3 shows, both correlation coefficients were high in Langmuir and Freundlich isotherm models. By the comparison of calculated R^2 values, it was revealed that the Langmuir isotherm model showed better fit than Freundlich model.

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4. Discussion

As shown in figure 1 (a and b) by rising the biomass concentration up to 30 × 10^8 CFU, the absorption rate increased. The yeast of *S. cerevisiae* has a high biosorption affinity for heavy metals [30, 31]. This trend is due to the carboxyl, hydroxyl and amino groups of the cell wall as the main responsible for the heavy metals' absorption [32-34]. As the amount of metal ions increased, their absorption to the surface of the *S. cerevisiae* increases so, the higher biosorption would be observed [21, 35 and 36]. By enhancing the *S. cerevisiae* biomass concentrations, the biosorption increases that is because of the more available binding sites for metal ions and therefore more binding combinations [37].

Also by increasing Cd concentration from 40 to 100 µg/L, the biosorption yield increased (Figure 1a). Similar to our studys' results; Hadiani et al. [21] reported that Cd removal by *S. cerevisiae* increased with rising the Cd level (25 to 80 µg/L). Ghorbani et al. [38] observed the Cd bioremoval by *S. cerevisiae* (2.13 g/L) at the concentration of 26.46 mg/L. Also Peng et al. [39] showed that Cu absorption by *S. cerevisiae* increased by increasing the metal from 40 to 120 mg/L. As shown in Figure 1(b), Cd absorption enhanced by rising contact time from 1- 4 day. With time passing, more Cd ions would attach to *S. cerevisiae* receptor sites in the surface [40]. The findings of this study is in accordance with Hadiani et al. [21] observed the increasing Mercury biosorption by *S. cerevisiae* from 24- 48 h and Hatami Fard and Mehrnia [35] reported more mercury absorption during 4 days. Like the above studies, in this study, the highest Cd removal efficiency (70%) was observed at the Cd concentration of 80 µg/L and the biomass of 30 × 10^8 CFU in the 4th day of storage. Prolongation of the experiment is recommended to evaluate more removal of heavy metal in longer exposure. Also, it should be taken into account that in the case of spoilage of milk with bacterial cells, or high initial microbial loading of milk the rate of bio-decontamination could be quite different with this report.

Also Table 3 shows that the absorption increased by increasing the initial concentration of Cd, as more initial concentration prepared more contact sites for absorbent and Cd [35]. Comparing both R^2 values in Langmuir and Freundlich isotherm models, it shows that Langmuir model has a better fit, which confirms that Langmuir equation is correct for monolayer absorption on surface with similar sites. The higher R^2 in Langmuir model confirm the Cd absorption by *S. cerevisiae* in our study obey this model.

5. Conclusions

In this study, three important variables; Cd and biomass concentration and the contact time for Cd bioremoval by *S. cerevisiae* were evaluated. Our findings showed the highest level of Cd biosorption (70%) observed in the *S. cerevisiae* concentration of 30 × 10^8 CFU and Cd amount of 80 µg/L in the 4th day. The ability of *S. cerevisiae* had been studied in high levels (ppm) of Cd and other heavy metals in effluents not in foodstuffs. This study shows the ability of this valuable yeast for Cd remediation in very low concentrations (ppb) from milk with no changes in physiochemical and sensorial acceptability. *S. cerevisiae* is a desirable and eco-friendly biosorbent for toxic metals bioremediation from food and water resources. These findings open the window for evaluating the capacity of heavy metals' binding by S. cerevisiae in milk. There is a need for more studies in this field to reduce the toxic effects of the heavy metals in food and drinks.

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