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Cadmium Biosoption by Immobilized Dead yeast cells From Bioethanol Industries

Gomes, L. H.[1*], Alexandrino, N.[1], Roncato Duarte, K. M.[2] and Andrino, F. G.[1]

[1] USP/ESALQ – Department of Genetics – Yeast Laboratory, Av. Pádua Dias, 11, Piracicaba, SP 13418-900 Brazil

[2] SAA/APTA – Institute of Animal Science and Pastures, Rua Heitor Penteado, 56. Nova Odessa, SP 13460-000. Brazil

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ABSTRACT: Dead yeast cells are an abundant residue of the Brazilian ethanol industry and the industries still don't have a proper or desirable destiny for all these cells, which means tons of vitamins and proteins that have not a major end. In the other hand, heavy metal residues are a problem, especially for leather industry, where Brazil is also a great producer. In this work, dead yeast cells were used to evaluate cadmium biosorption samples. Results have shown that dried dead yeast cells at 20 % (W/V) immobilized in Na-alginate beads 0.5 % can be an efficient alternative for the capture of cadmium. The average biosorption rate was 122.10 mg Cd/ g of dry biomass in comparison to the control using only the Na-alginate beads 0.5 % where the biosorption rate achieved 57.29 mg Cd/g of dry biomass. This research opens wide opportunities to allies two major residues created in Brazilian industries with a noble end for the industries it selves as for the environment.

Key words: Cadmium, Yeast, Biosorption, Dead cells, Residue

INTRODUCTION

There are many reports about effluent discharges that bring hazardous materials and metals poured into aqueous environments and soils that end-up in the food-chain threatening natural and human population health (Tandy *et al.*, 2004). The toxic and carcinogenic cadmium is released by industrial processes at 20,000 tons per year (Volesky, 1990) with no efficient removal or ameliorating system to protect from its negative effects. The first report on cadmium toxicity appeared in 1946 in Japan and was described as the "Itai-Itai Syndrome" related bone problems (Volesky, 1990). Presently cadmium is wide used in the automobile industry, telecommunications, on the dying and ink processes, PVC and other plastics, phosphate fertilizers, batteries, fungicides, leather industry, etc (Jordão *et al.*,1999) and found in large amounts in urban waste and metallurgy industrial discharges.

It is recognized that the major source of human toxicity is food and water contamination. Many attempts have been made to treat cadmium contamination including ion exchange, chemical precipitation and membrane technologies, all showing significant disadvantages and low efficiency. The biosorption method is a promising technology for remediation of contaminated wastewater metal solutions. Biosorption is here understood as a metabolically independent passive metal ion uptake by dead biomass since the use of live biomass is compromised by the metal toxicity. Groups using live yeast cells (Hadi *et al.*, 2003) not immobilized for cadmium biosoption observed that as cells grow older the cadmium biosorption increased in the reactor due to the lower cells viability. The use of *Phanerochaeate chrysosporium* fungi (Pogaku and Kulkarni, 2006) in a study with industrial discharges have shown that dead cells worked better than live cells. The biosorption capability depends on other factors as metal concentration, solution pH, reaction kinetics, equipment, and effluent composition (Sawalha *et al.*, 2008). Recently a research group at Brazil used grape bagasses as biosorbent for cadmium and lead metals from effluent, showing good capacity of biosorption at low costs (Farinella *et al.*, 2008).

As ethanol from sugar-cane fermentation generates high amounts of yeast biomass the alternative use for those cells are object of several studies (Loviey and Coates, 1997). Considering the large scale of cadmium in water solutions from different industry residues this work has the aim to use *Saccharomyces cerevisiae* dead cells from bioethanol

*Corresponding author E-mail: keila@iz.sp.gov.br

fermentation in a process to be adapted for cadmium biosorption.

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MATERIAL & METHODS

Yeast cells from sugar-cane bioethanol fermentation process 80% concentrated were obtained from COSAN Group, at Piracicaba, SP, Brazil. The yeast cell suspension was spread on trays, frozen at –80 ^{0}C, 24 h and lyophilized for 48 h and then killed by heat and pressure at 120 ^{0}C 30 minutes, 1 atm. Na-alginate 0.5 % and 20% (W/V) of dead yeast cells were added to 100 mL of deionized water at 9.5 pH. This suspension was mixed homogenized and droplets poured into Calcium Chloride 4% using a peristaltic pump for the micro spheres production. The same procedure was done with alginate solution without adding dead yeast cells. A scanning electron microscope was used to exemplify the microspheres in the alginate net. Two double draft tube fluidized acrylic bioreactors were built (15 cm height) with 40 mL of internal volume and 100 mL of external volume dimensions. Each internal column was filled with 28 g of micro spheres connected to a peristaltic pump calibrated to sustain a flow of 2 mL per minute of cadmium synthetic solution at a concentration of $CdCl_2$ of 250 mg Cd/L. Micro spheres used for the biosorption assays in the filled Columns were washed 2 x bed volume (mL) water for the cadmium release and then washed with 1 L of nitric acid solution 0.5 N at a flow system of 6 mL per minute. Samples were collected each hour and analyzed in atomic absorption spectrophotometer Varian AA-175. The discharge solution was also analyzed for the cadmium presence after passed through out the columns.

The standard procedures were established on a wave length of 228.8 nm, flame type acetilene/air (Oxidant) spectrophotometer Varian Model AA-175. The sample readings were diluted at a rate 125µL to 25 mL, using an integration three which expresses the average of six readings in 30 seconds. The standard cadmium curve was prepared from a commercial standard solution of 1000±3 mg/L Cd in 2% HNO_3 (High-Purity Standards Cat.#10008-1) diluted in purified water.

RESULTS & DISCUSSION

Prior to the biosorption assays, the concentration of dead yeast cells, Na-alginate and cadmium concentration were determined to optimize the results obtained. Higher levels of Na-alginate (around 2%) had very small difference between columns with alginate and the control on the copper biosorption (Göksungur *et al.*, 2003). As the Na-alginate is considered a good biosorbent for heavy metals removal, this work aimed to achieve the highest dead yeast cells concentration and the lowest Na- alguinate concentration. Low concentration of Na- alginate leads to micro spheres deterioration that clogged all the system in the columns. Alginate polymerization is highly dependent on basic pHs which improve polymerization due to the chemical properties of hydroxyls anions free in the solution that do not compete for the calcium linkage (Sawalha *et al.*, 2008) what occur at neutral and acid pH solutions where the hydrogen ions are cations that compete with the calcium. To avoid such effect all the micro spheres were prepared at pH 9.5 with Na-alginate 0.5 % and dead yeast cells 20% (w/v) for the assays called AY and, only Na-alginate 0.5 % for the control assay (A). Cadmium solution was always pH 7.0 to avoid precipitation of cadmium salts that happens at pH higher or lower than 7.0 reducing the solubility of the metal (Sekhar *et al.*, 2008). In order to visualize the spheres structures, micrograph images were generated (Fig.1).

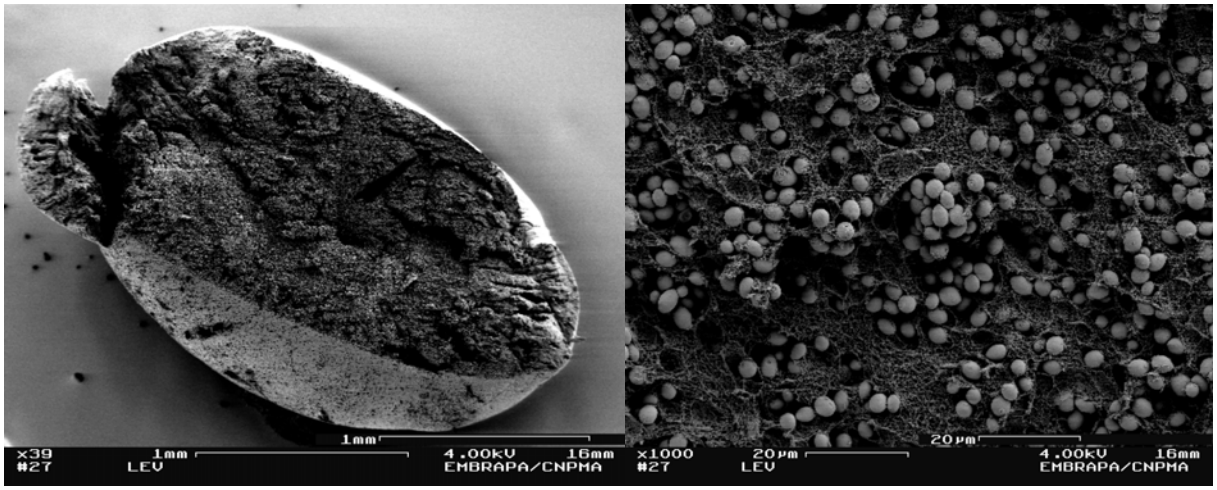

Fig. 1 . (A) Micro sphere (Yeast 20% X Alginate 0.5%) cut in half, 39X increased; (B) Micro sphere (yeast 20% X Alginate 0.5%) , 1000X increased, showing yeast cells attached to a net.Images from a scanning electron microscope

Comparative results of cadmium biosorption are presented in Table 1 and Fig.2. where for the first 60 minutes solution flow no significant differences are observed for both treatments (AY and A). It can be observed that cadmium biosorption from 120 minutes and on show high significant differences with faster saturation on the biosorption ability of a micro spheres in comparison to AY micro spheres (Fig. 2). This behavior could be explained by the time necessary to the yeast cells in the inner part of the micro sphere to start cadmium capture. For the first 60 minutes cadmium capture is done mainly by the micro sphere surface and then the yeast dead cells in the inner micro sphere start to capture cadmium. As can be seen in Table 2 the biosoption of cadmium at 120 minutes for AY is 44.21 % higher than A and after 600 minutes the value is 97.78 % superior than A. After this time AY is able to bio-sorbs 37.6 % of cadmium the solution. Similar results were obtained (Hadi *et al.*, 2003) using live yeast cells and using live and dead P*haneroyate chrysosporium* fungi (Pogaku and Kulkarni, 2006) although different results have been obtained (Göksungur *et al.*, 2005) using high Na-alginate concentrations where the Yeast dead cells biosorption capacity could not be observed.

The removal of cadmium from the columns after biosorption assays were made by washing with nitric acid solution 0.5N which data are presented in Table 1. The removal of cadmium from column A reached 114.58 mgCd/L and from AY 341.90 mgCd/L. It is to be observed that the discharge solution presents the real amount of cadmium biosorved in the process and that the ratio mgCd/L/dry weight of the micro spheres is an efficiency measurement expressed in mg metal/g of dry biomass (Table 2).

Table 1. Cadmium (mg) released after desorption

	A	AY
MINUTES	mgCd/L	mgCd/L
10	1182,78	1158,75
60	133,06	*425,06
120	0,00	*70,23
180	0,00	0,00
Discharge	114,58	341,90

* F test significant at P <0.01

Any process for heavy metals recovery shall consider the efficiency of metal removal per unit of substrate and costs (Nakajima and Sakaguchi, 1986). Other additional factors are important such as speed, the suitability to different processes and reactors and the separation of the metal from the support must be easy. Studies on biosorption must be always linked to desorption studies (Ferraz *et al.*, 2004), which means the metal captured have to be removed from the column, to clean the system and to allow the reuse of the support, when it is possible. All these requirements were observed in the process developed in the present work except the reuse of the yeast biomass since after the acid treatment for desorption the biosorption ability is lost. Otherwise the organic residue of dead yeast cells and Na-alginate free from the metal can be easily discharged with no environmental harm and fast biodegradation speed.

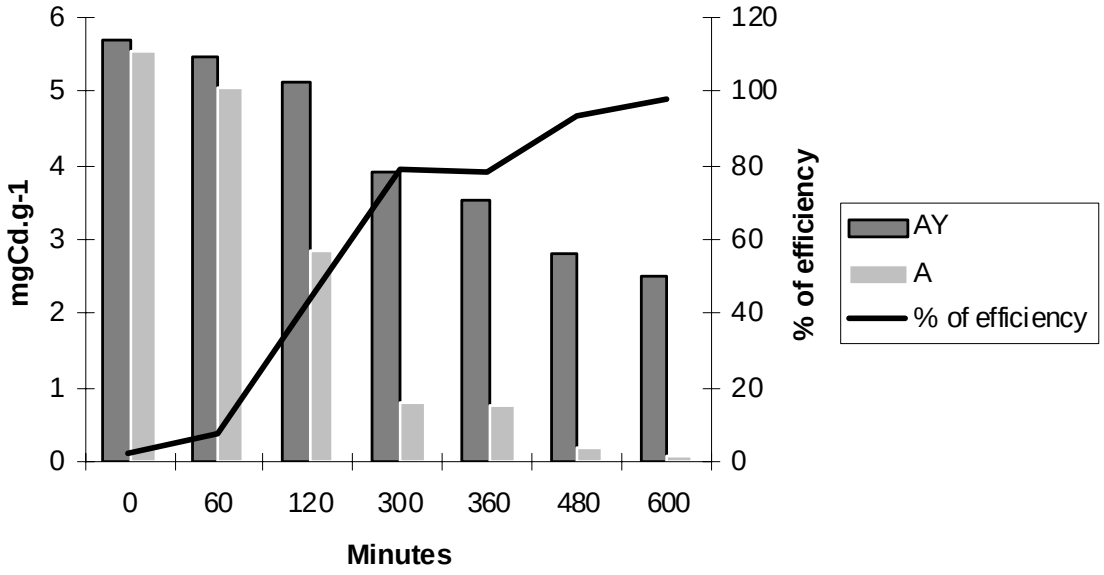

Fig. 2. Biosorption of Cd, expressed in mg per g of support (mgCd/g) and % of efficiency biosorption among AY and A assays

Table 2 . Ratio of mg Cd biosorved and biomass dry weight

Assay	Wet weight (g)	Dry weight (g)	mgCd/L in the discharged solution	mgCd/g of dry biomass
AY	28.00	2.80	341.90	122.11
A	28.00	2.00	114.58	57.29

CONCLUSION

The cadmium capture from solutions using immobilized dead yeast cells from the bioethanol industry indicates the possibility to devise an efficient process for metal removal. High yields were achieved using 20 % yeast cells (W/V) immobilized in Na-alginate 0.5 % that double the yield of the control (122.10 mg Cd/g of dry biomass compared to 57.29 mg Cd/g of dry biomass Na-alginate 0.5 % alone. Machado and collaborators (Machado *et al.*, 2008) used flocculating yeast cells to remove heavy metals form effluents, trying to avoid immobilization procedures, as Na-alginate support. Such investigations are due to the fact Na-alginate support at 2% is inefficient, which is not the case in our work, once NA-alginate concentrations was established at 0.5 %. Probably the use of flocculating yeast cells plus Na-alginate at low concentrations can be a base for a future study in this removal metal from effluent discharges.

Alternative technology for the use of biomass, particularly Yeast cells is a challenge for many sectors, as the work done with Baker´s yeast residue (Göksungur *et al.*, 2005), to remove copper from aqueous effluents and on the removal of chrome from industrial effluents (Parvati and Nagendran, 2007). For countries as Brazil, the yeast cells residue from fuel industry represents large amounts of such biomass.

The research represented by the use of yeast cells from ethanol industry in a stable and efficient process demands future studies with the purpose of effluent treatment other than cadmium and other heavy metals to develop large scale processes using immobilized yeast cells.

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