# REMOVAL OF Zn<sup>2+</sup> FROM SOME SYNTHETIC WASTEWATERS BY IMMOBILIZED SACCHAROMYCES CEREVISIAE CELLS

# CORNELIA MAJDIK<sup>a</sup>, CERASELLA INDOLEAN<sup>a</sup>, TONK SZENDE<sup>b</sup>, ANDRADA MĂICĂNEANU<sup>a</sup>, PERNZESYI TIMEA<sup>c</sup>, TOTHMERESZ BELA<sup>d</sup>

**ABSTRACT.** Biosorption of heavy metal ion  $(Zn^{2+})$  by immobilized *Saccharomyces cerevisiae* cells was studied. The biosorbent that we studied was made from fresh Bakers' yeast commercially available under beads form. We used three different initial concentrations of  $Zn^{2+}$  in solution, 129.60 mg  $Zn^{2+}/L$ , 213.41 mg  $Zn^{2+}/L$  and 304.88 mg  $Zn^{2+}/L$ , for biosorption study in dynamic regime, at 25°C and neutral pH. Adsorption yields,  $\eta$ , and retention capacity,  $Q_s$ , were calculated and compared. From all the experimental data it can be concluded that using yeast as a biological filter the concentration of  $Zn^{2+}$  from synthetic samples was considerably reduced. UV/VIS spectroscopy was used for determination of the adsorption degree of  $Zn^{2+}$  from synthetic wastewater samples.

**Keywords:** Saccharomyces cerevisiae, heavy metals, biosorption, immobilization, molecular adsorption method.

### INTRODUCTION

Biosorption of heavy metals by microbial cells has been recognized as a potential alternative to existing technologies for recovery of heavy metals from industrial waste streams [1]. Most studies of biosorption for metal removal involved the use of either laboratory-grown microorganism or biomass generated by the pharmacology and food processing industries or wastewater treatment units. The biosorption of heavy metal ions using microorganisms is affected by several factors. These factors include the specific surface

<sup>&</sup>lt;sup>a</sup> Universitatea Babeş-Bolyai, Facultatea de Chimie şi Inginerie Chimică, Str. Kogălniceanu, Nr. 1, RO-400084 Cluj-Napoca, România, <u>majdik@chem.ubbcluj.ro</u>

<sup>&</sup>lt;sup>b</sup> Universitatea Sapientia, Facultatea Stiinte și Arte, Str. Matei Corvin, Nr. 4, RO-400112 Cluj-Napoca, România, <u>tonk.szende@sapientia.ro</u>

<sup>&</sup>lt;sup>c</sup> Pécsi Tudományegyetem, Természettudományi Kar, Ifjuság utca 6, HU-7624 Pécs, Magyarország, ptimea@ttk.pte.hu

Debreceni Egyetem, Természettudományi Kar, Egyetem tér 1, HU-4032 Debrecen, Magyarország, tothmerb@delfin.klte.hu

properties of the organism (biosorbent) and the physicochemical parameters of the solution such as temperature, pH, initial metal ion concentration and biomass concentration [2]. Non-living biomass appears to present specific advantages in comparison to the use of living microorganisms. Killed cells may be stored or used for extended periods at room temperature, they are not subject to metal toxicity and nutrient supply is not necessary. Moreover, the pretreatment and killing of biomass either by physical or chemical treatments [3, 4] or crosslinking [5] are known to improve the biosorption capacity of biomass. For example, the immobilization of biomass has the advantages of using an adsorption column in a multi-cycle biosorption process.

Saccharomyces cerevisiae is an inexpensive, readily available source of biomass for heavy metal removal from wastewaters and possesses good metal-binding potential [6-8]. Yeasts are a growth form of eukaryotic microorganisms classified in the kingdom Fungi, with about 1,500 species described [9]. Investigations conducted by several researchers demonstrated that *S. cerevisiae* is capable of accumulating heavy metals such as Cu<sup>2+</sup>, Cd<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>,Cr<sup>2+</sup> and Ni<sup>2+</sup> [10-14].

The main application of zinc is as a coating for the protection of steel against corrosion. Zinc itself forms an impervious coating of its oxide on exposure to the atmosphere, and, hence, the metal is more resistant to ordinary atmospheres than iron, and it corrodes at a much lower rate.

The toxicity of the metals increases sharply in the order Zn < Cd < Hg. The free zinc ion in solution is highly toxic to plants, invertebrates, and even vertebrate fish. The free zinc ion is also a powerful Lewis acid up to the point of being corrosive [15].

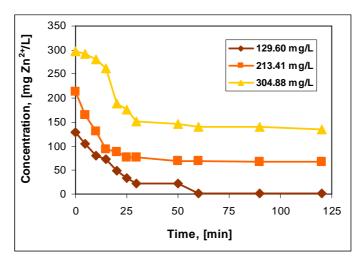
Metal uptake by microorganisms occurs in two stages: first stage consisting in passive adsorption of metal ions to the external cell surface, and second stage in which metal ions are subsequently transported through the cell membrane into the cell itself [16, 17].

The aim of this study was to test and compare immobilized *Saccharomyces cerevisiae* cells for their capacity to adsorb Zn<sup>2+</sup>, which is a widely distributed heavy metal in water. Although calcium alginate is a cheap, non-toxic, and abundantly available immobilization matrix, insufficient literature was found about Zn<sup>2+</sup> removal by alginate immobilized biosorbents.

## **RESULTS AND DISCUSSION**

In order to investigate the effects of different  $Zn^{2+}$  concentration on metal uptake of immobilized *S. cerevisiae* cells, the biosorption experiments were conducted increasing the heavy metal ion initial concentration (129.60 mg/L; 213.41 mg/L; 304.88 mg/L). Figure 1 shows the variation of  $Zn^{2+}$  concentrations during the biosorption process.

As it can be seen in figure 1, the initial  $Zn^{2+}$  concentration decreases in every biosorption experiment. In the first 25 minutes from the beginning of the experiment,  $Zn^{2+}$  concentration drops significantly (exponential decrease). This trend is followed, in all cases, by a slowly decrease until a constant value (equilibrium concentration) was reached. For the initial concentration  $C_1$ =129.60 mg  $Zn^{2+}/L$ , after approximately 55 – 60 minutes the heavy metal ion content from analyzed samples goes to zero, which means that  $Zn^{2+}$  ions are totally retained on the immobilized *S. cerevisiae*.



**Figure 1.** The biosorption of Zn<sup>2+</sup> dependence of time for immobilized *S. cerevisiae* cells, at different initial Zn<sup>2+</sup> concentrations. The biosorbent concentration was 2.75 g/150 ml (dry mass/volume).

Our results are in agreement with literature data regarding biosorption mechanism that is considered to take place in two stages [18]. In a first stage (dynamic regime) a pseudo-equilibrium is reached, while in a second stage (that takes place in some cases in static regime, on longer time intervals) a slowly decrease of metal concentration takes place. This decrease may be explained as a metal crossing through the cell wall, when intracellular accumulation takes place [18].

The adsorption yield, n [%], was calculated as follows:

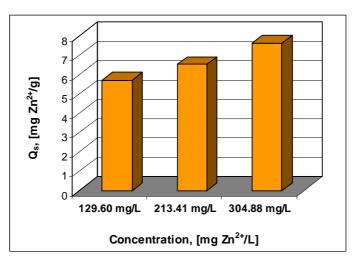
$$\eta = \frac{C_i - C_f}{C_i} \times 100$$

where,

 $C_i$  is the initial concentration of solution ( $C_1$ ,  $C_2$ ,  $C_3$ ), mg/L  $C_f$  is the final concentration of  $Zn^{2+}$  in solution, mg/L.

For this parameter, the calculated values are ranging between 98.80% (for  $C_1 = 129.60$  mg  $Zn^{2+}/L$ ) and 56% (for  $C_3 = 304.88$  mg  $Zn^{2+}/L$ ).

The metal uptake is influenced by the initial  $Zn^{2+}$  concentration. The retention capacity,  $Q_s$ , of yeast adsorbent increases from 5.1 mg  $Zn^{2+}/g$  adsorbent for  $C_1$ =129.60 mg  $Zn^{2+}/L$  to 7.1 mg  $Zn^{2+}/g$  adsorbent for  $C_3$ =304.88 mg  $Zn^{2+}/L$  (figure 2).



**Figure 2.** The influence of the initial concentration over the Zn<sup>2+</sup> uptake, Q<sub>s</sub>, by the immobilized *S. cerevisiae*. The biosorbent concentration was 2.75 g/150 ml (dry mass/volume).

## **CONCLUSIONS**

In this study, immobilized *S. cerevisiae* cells have been successfully used as a biosorbent for the removal of Zn<sup>2+</sup> ions from synthetic wastewaters.

For the successful application of biosorption, biomass needs to be immobilized to increase its mechanical strength, density, reusability and resistance to mechanical environments. In this study, Calcium alginate gel was chosen for the immobilization experiments as it is cheaply and abundantly available, nontoxic and highly selective for certain ion species. Calcium alginate proved to be a suitable material for immobilization of *Saccharomyces cerevisiae* cells.

The initial  $Zn^{2+}$  concentration decreases in every biosorption experiment. For the initial concentration  $C_1 = 129.60$  mg  $Zn^{2+}/L$ , after approximately 55-60 minutes the heavy metal ion content from analyzed samples goes to zero, which means that  $Zn^{2+}$  ions are totally removed from the water sample. The maximum retention capacity,  $Q_s$ , was calculated to be 7.1 mg  $Zn^{2+}/g$  adsorbent for the biggest initial concentration ( $C_3$ =304.88 mg  $Zn^{2+}/L$ ).

As a general conclusion we can say that using yeast as a biological filter it's a good method in reducing the concentration of heavy metals ions from wastewaters.

#### **EXPERIMENTAL SECTION**

**Microorganism.** The microorganisms were obtained from commercial type *Saccharomyces cerevisiae* cells (Pakmaya).

**Biosorbent immobilization.** For calcium alginate immobilization of yeast, 2.5 g biosorbent (baker yeast's) was suspended in 30 ml alginate solution (3g Na-alginate mixed with 1 ml ethanol was added to 100 ml distillated water and incubate for 30 minutes). A 100 ml aliquot of alginate - biosorbent suspension containing 2% Na-alginate was added drop by drop to 1000 ml of 2% CaCl<sub>2</sub> solution with a peristaltic pump. Alginate drops solidified upon contact with CaCl<sub>2</sub>, forming beads and thus entrapping biosorbent particles. The beads were allowed to harden for 30 min and then were washed with distillated water in order to remove excess of calcium ions.

**Metal uptake.** The retention capacity of biosorbent,  $Q_s$ , was calculated as follows:

$$Q_s = \frac{m_i - m_f}{g}$$

where,

 $m_{\rm i}$  is the initial mass of metal from ion solution, mg  $m_{\rm f}$  is the final mass of metal in the solution after biosorption, mg. g is the mass of the dry *S. cerevisiaie*, mg

**Biosorption experiments.** The stock solution of  $Zn^{2+}$  was prepared by dissolving a weighed quantity of  $ZnSO_4 \times 7H_2O$  in deionized water. The immobilized biosorbent, 2.75 g, was added in a flask over 150 ml  $Zn^{2+}$  solution (129.60 mg  $Zn^{2+}/L$ , 213.41 mg  $Zn^{2+}/L$  and 304.88 mg  $Zn^{2+}/L$ ) under continuous magnetic stirring at 200 rot/min for 2 h. The experiment was continued until a constant  $Zn^{2+}$  ion concentration was obtained. 1 ml samples were taken at different intervals of time and analyzed in order to determine  $Zn^{2+}$  concentration.

**Zn determination.**  $Zn^{2+}$  ions concentration was determined in the supernatant according to STAS 6327-81 using spectrophotometric method (potassium ferricyanide,  $\lambda$  = 420 nm, UV/VIS JENWAY 6305 spectrophotometer).

#### REFERENCES

- 1. B. Volesky, *Tibtech.*, **1987**, *5*, 96.
- A. I Zouboulis, E. G. Rousou, K. A. Matis, I. C. Hancock, Chem. Technol. Biot., 1999, 74, 429.
- 3. D. Brady, A. Stoll, J. R. Duncan, Environmental Technology, 1994, 15, 229.
- 4. C. P. Huang, A. Morehart, Water Res., 1990, 4, 433.
- 5. A. Leusch, Z.R. Holan, B. Volesky, J. Chem. Technol. Biot., 1995, 62, 279.
- 6. G. M. Gadd, "Fungi in Bioremediation", Cambridge University Press, Cambridge, **2001**, chapter 5.
- 7. C. P. Kurtzman, J. W. Fell, "The Yeast Handbook", Germany: Springer Verlag Berlin Heidelberg, **2006**, chapter 1.
- 8. P. R. Norris, D. P. Kelly, J. Gen. Microbiol., 1977, 99, 317.
- 9. B. Volesky, H. May, Z. R. Holan, Biotechnol. Bioeng., 1993, 41, 826.
- 10. D. Brady, J. R. Duncan, Appl. Microbiol. Biotechnol., 1994, 34, 149.
- 11. A. Engl, B. Kunz, J. Chem. Technol. Biot., 1995, 63, 257.
- 12. Y. Lu, E. Wilkins, J. Hazardous Materials, 1996, 49, 165.
- 13. B. A. Volensky, H. A. Mayphillips, Appl. Microbiol. Biotechnol., 1995, 42, 165.
- 14. B. S. Wilhelmi, J. R. Duncan, Biotech. Lett., 1995, 42, 1007.
- 15. C. White, G. M. Gadd, FEMS Microbiol. Ecol., 1986, 38, 277.
- 16. G. M Gadd, J. A. Sayer, "Environmental Microbe–Metal Interactions", ASM Press, Washington DC, **2000**, chapter 3.
- 17. K. J. Blackwell, I. Singleton, J. M. Tobin, *Appl. Microbiol. Biotechnol.*, **1995**, *43*, 579.
- 18. S. Li, J-I. Xia, H. Huan, N. Zhen-Yoan, *J. Central South University of Technology*, **2007**, *14*, 41.