

Biosorption of Copper Ions by Caustic Treated Waste Baker's Yeast Biomass

Yekta GÖKSUNGUR, Sibel ÜREN, Ulgar GÜVENÇ*

Department of Food Engineering, Faculty of Engineering, Ege University, 35100 Bornova, İzmir - TURKEY

Received: 02.08.2002

Abstract: Waste baker's yeast (*Saccharomyces cerevisiae*) was used as a biosorbent for Cu^{+2} biosorption. The yeast cells were treated with caustic soda, ethanol and heat to increase their biosorption capacity. Among the treatment methods used, the highest copper uptake (21.1 mg g^{-1}) was obtained with the caustic treatment of baker's yeast. The effect of initial copper concentration and pH on biosorption for caustic treated yeast was studied. The highest Cu^{+2} uptake (120.7 mg g^{-1}) was obtained at pH 4.0, for 198.2 mg l^{-1} initial copper ion concentration. The Langmuir model and Freundlich equation were applied to the experimental data and the Langmuir model was found to be in better correlation with the experimental data. The Langmuir constants were $q_{\text{max}} (\text{mg g}^{-1}) = 181.8$ and $b (\text{l mg}^{-1}) = 0.0312$. In packed bed column studies with calcium alginate immobilized caustic treated yeast, it was found that the real biosorption capacity came from the alginate gel and immobilizing caustic treated yeast particles in the gel only slightly increased the biosorption capacity of the gel.

Key Words: copper, biosorption, *Saccharomyces cerevisiae*, adsorption isotherms, calcium alginate, immobilization

Kostikle Muamele Edilmiş Atık Ekmek Mayası Hücrelerine Bakır İyonlarının Biyosorpsiyonu

Özet: Bu çalışmada atık ekmek mayası (*Saccharomyces cerevisiae*) hücreleri Cu^{+2} geri kazanımı için biyosorbant olarak kullanılmıştır. Maya hücreleri NaOH, etil alkol ve ısı ile muamele edilerek biyosorpsiyon kapasiteleri artırılmaya çalışılmış ve bu yöntemler arasında en yüksek bakır kazanımı (21.1 mg g^{-1}) ile kostikle muamele edilmiş ekmek mayası hücrelerinden elde edilmiştir. Kostikle muamele edilmiş maya hücreleri ile başlangıç Cu^{+2} konsantrasyonu ve pH'nın biyosorpsiyon üzerine etkisi incelenmiştir. En yüksek Cu^{+2} kazanımı (120.7 mg g^{-1}) pH 4.0'da, 198.2 mg l^{-1} başlangıç bakır iyonu konsantrasyonunda elde edilmiştir. Langmuir modeli ve Freundlich denkliği deneysel verilere uygulanmış ve Langmuir modelinin deneysel verilerle daha iyi korelasyon içerisinde olduğu belirlenmiştir. Langmuir sabitleri olan $q_{\text{max}} (\text{mg g}^{-1})$ ve $b (\text{l mg}^{-1})$ sırasıyla 181.8 ve 0.0312 olarak belirlenmiştir. Kostikle muamele edilmiş maya hücreleri kalsiyum aljinat jelinde tutuklanmış ve dolgulu yatak kolonda yapılan denemelerde gerçek biyosorpsiyon kapasitesinin aljinat jelinden kaynaklandığı, immobilize maya hücrelerinin ise jelin biyosorpsiyon kapasitesini sadece bir miktar artırdığı belirlenmiştir.

Anahtar Sözcükler: bakır, biyosorpsiyon, *Saccharomyces cerevisiae*, adsorpsiyon izotermi, kalsiyum aljinat, immobilizasyon

Introduction

Conventional methods for removing dissolved heavy metal ions from waste waters include chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment and evaporative recovery. These techniques have significant disadvantages including incomplete metal removal, the need for expensive equipment and monitoring systems, high reagent or energy requirements or generation of toxic sludge or other waste products that require disposal (1). Biosorption, which is a property of certain types of

inactive, dead microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions, is one of the most promising technologies involved in the removal of toxic metals from industrial waste streams and natural waters (2,3). There are many reports on algae, bacteria, fungi or higher plants that remove and/or accumulate large amounts of heavy metals from their external environment (4-6). The work reported by Volesky and Holan (6) identifies organisms that have potential as adsorbents and also establishes references of adsorbent capacities in these kinds of processes.

* Corresponding author

Biosorption can be considered a collective term for a number of passive, metabolism independent, accumulation processes and may include physical and/or chemical adsorption, ion exchange, coordination, complexation, chelation and microprecipitation. Biomass cell walls, consisting mainly of polysaccharides, proteins and lipids, offer many functional groups that can bind metal ions such as carboxylate, hydroxyl, sulphate, phosphate and amino groups. In addition to these functional binding groups, polysaccharides often have ion exchange properties (3,7,8). In some cases, adsorption on the external cell surface is a biomass defense system against toxic heavy metals, the microorganism producing an external polymeric layer to avoid metal penetration through the cell wall. In the microprecipitation mechanism, ion removal from solution may also be associated with an active defense system that produces compounds favoring the precipitation process (3).

The biosorption of heavy metal ions using microorganisms is affected by several factors. These factors include the specific surface properties of the organism (biosorbent) and the physicochemical parameters of the solution such as temperature, pH, initial metal ion concentration and biomass concentration (7).

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 (8-10) or crosslinking (11) are known to improve the biosorption capacity of biomass.

For example, the caustic treatment of biomass has the advantages of destroying autolytic enzymes that cause putrefaction of biomass and removing lipids and proteins that mask reactive sites (9). It has also been reported that cell wall soluble proteins, which make complexes with metal ions, can be fixed by some denaturation process such as heat and ethanol treatment. Deactivated yeast cells do not release protein and exhibit higher metal ion removal capacity than live yeast (10).

Saccharomyces cerevisiae is an inexpensive, readily available source of biomass for heavy metal removal from waste water. Investigations conducted by several researchers demonstrated that *S. cerevisiae* is capable of

accumulating heavy metals such as Cu^{+2} , Cd^{+2} , Pb^{+2} , Zn^{+2} , Cr^{+2} , and Ni^{+2} (10-16).

The aim of this study was to test and compare treated (killed) and untreated waste baker's yeast cells (*S. cerevisiae*) for their capacity to adsorb Cu^{+2} , which is one of the most widely distributed heavy metals in water. Two adsorption models, the Langmuir model and the Freundlich equation, were applied to the experimental data obtained for caustic treated yeast biomass and correlations were found for these models. Although calcium alginate gel is a cheap, non-toxic, and abundantly available immobilization matrix, insufficient literature was found on copper removal by alginate immobilized biosorbents. Therefore, calcium alginate immobilized caustic treated yeast biomass was also tested for Cu^{+2} removal in a packed bed bioreactor.

Materials and Methods

Microorganism

Waste dried baker's yeast (*S. cerevisiae*) was obtained from Pakmaya Yeast Company, İzmir. This waste yeast consists of oversieved (>1.5 mm) dried granular yeast particles.

Physical and chemical treatments of yeast cells

Caustic treated cells were prepared by mixing 5 g of yeast cells with 100 ml of 1 mol l⁻¹ NaOH and sterilizing the resultant solution at 121 °C for 15 min. After NaOH treatment, yeast cells were collected by centrifugation (4000 g; 10 min) and washed several times with distilled water to remove excess NaOH. Ethanol treated cells were prepared by embedding 5 g of yeast cells in 100 ml of 700 g l⁻¹ ethanol solution for 20 min. Heat treated cells were prepared by mixing 5 g of yeast cells with 100 ml of distilled water and sterilizing the resultant solution at 121 °C for 15 min. After treatments, yeast cells were collected by centrifugation (4000 g; 10 min). All the treated cells were dried at 70 °C for 12 h and then ground to a gritty consistency to yield granular biosorbent samples. For the untreated control sample, baker's yeast cells were directly used in the biosorption experiments.

Biosorption experiments

The stock solution of Cu^{+2} (1 g l⁻¹) was prepared by dissolving a weighed quantity of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in deionized water. Batch biosorption studies were carried out in a

rotary shaker (B. Braun Certomat) operated at 200 rpm. In the first group of experiments, the highest Cu^{+2} uptake was investigated at pH 4.0 for $25 \text{ mg l}^{-1} \text{ Cu}^{+2}$ solution for biosorbents prepared from treated and untreated baker's yeast cells. The effect of pH and initial concentration of Cu^{+2} ions on biosorption together with adsorption isotherms were determined for biosorbent prepared from caustic treated yeast cells with which the highest metal uptake was obtained.

The effect of pH on biosorption was investigated in the pH range 2.0-6.0. pH values higher than 6.0 could not be used due to the rapid precipitation of Cu^{+2} ions. The pH values of Cu^{+2} solutions were adjusted to the desired value with either $2 \text{ mol l}^{-1} \text{ NaOH}$ or $2 \text{ mol l}^{-1} \text{ H}_2\text{SO}_4$ solutions. For biosorption, 0.1 g of granular biosorbent was added to 100 ml of metal solution containing $25 \text{ mg l}^{-1} \text{ Cu}^{+2}$ in erlenmeyer flasks. The flasks were agitated on the shaker at $30 \text{ }^\circ\text{C}$ for 3 h. The effect of the initial Cu^{+2} ion concentration on the biosorption was studied at pH 4.0 as described above except that the concentration of Cu^{+2} ions in the adsorption medium varied between 10 and 250 mg l^{-1} . Samples of 5 ml were taken at the beginning of adsorption and at certain intervals over 3 h. The samples were centrifuged at 4000 g for 5 min and the supernatant liquid was used to determine Cu^{+2} ion concentration. All biosorption experiments were performed in triplicate and mean values reported.

Biosorbent immobilization

For calcium alginate immobilization of biosorbent, 3.33 g of caustic treated biosorbent was suspended in 50 ml of acetate buffer (pH = 4.0) and this suspension was mixed with an equal volume (1:1, v/v) of 4% (w/v) Na-alginate (Sigma, A-2033) solution. A 100 ml aliquot of alginate-biosorbent suspension containing 2% Na-alginate was added dropwise to 1000 ml of 2% CaCl_2 with a peristaltic pump. Alginate drops solidified upon contact with CaCl_2 , forming beads and thus entrapping biosorbent particles. The beads were allowed to harden for 30 min and were then washed with sterile physiological saline solution (0.85% NaCl) to remove excess calcium ions.

Column studies

Continuous biosorption experiments were carried out using jacketed pyrex column reactor packed with 2.0-2.4 mm diameter Ca-alginate beads with entrapped

biosorbent particles. The inner diameter, height and bed volume of column are 1.1 cm, 27.9 cm and 26.5 ml, respectively. Solutions containing 100 mg l^{-1} (pH = 4.0) of Cu^{+2} ions were pumped upward with a peristaltic pump (Chemap AG) through the column packed with Ca-alginate beads. The flow rate of the metal solution was 2.35 ml min^{-1} . Effluent liquid overflowed from an outlet port at the top of the bioreactor, maintaining a constant level inside the column. The temperature of the bioreactor system was maintained at $30 \text{ }^\circ\text{C}$ by circulating constant temperature water from a circulator bath through the jacket of the bioreactor. The residence time of metal ions through the column was 11.3 min. Samples were taken from the effluent at timed intervals and analyzed for Cu^{+2} ions. The experiment was continued until a constant Cu^{+2} ion concentration was obtained.

Copper determination

Concentrations of Cu^{+2} ions in the supernatant fluids were determined by atomic absorption spectrophotometer (PYE UNICAM SP9).

Metal uptake (q) was determined as follows:

$$q = V \times (C_i - C_f) / S$$

where q (metal uptake, mg g^{-1}) is the amount of Cu^{+2} ions adsorbed on the biosorbent, V (ml) is the volume of metal containing solution in contact with the biosorbent, C_i and C_f (mg l^{-1}) are the initial and equilibrium (residual) concentrations of Cu^{+2} in the solution, respectively and S (g) is the amount of added biosorbent on a dry basis.

Results and Discussion

Effect of pretreatment of baker's yeast on biosorption capacity

In order to investigate the effects of different pretreatments on metal uptake of *S. cerevisiae* cells, the cells were treated by ethanol, heat and caustic soda. Figure 1 shows the metal uptake values obtained by these cells and untreated cells. As seen in Figure 1, physical and chemical treatments of the live yeast cells alter their Cu^{+2} uptake capacities. The highest metal uptake (21.1 mg g^{-1}) was obtained by caustic treated yeast and 7.9 mg g^{-1} of metal uptake was obtained with ethanol treated yeast cells. The same metal uptake values (5.2 mg g^{-1}) were obtained by both heat treated and untreated yeast cells. The high metal uptake obtained by caustic treated yeast cells may be explained by the removal of the protein

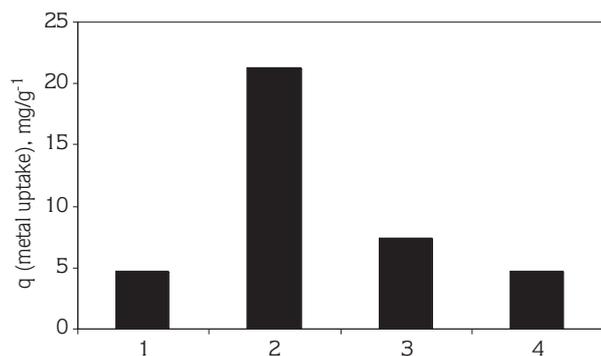


Figure 1. Effect of different pretreatments on the Cu²⁺ uptake of *Saccharomyces cerevisiae* (1. untreated yeast; 2. caustic treated yeast; 3. ethanol treated yeast; 4. heat treated yeast). The biosorbent concentration was 0.1 g/100 ml (dry mass per volume), initial metal concentration was 24.7 mg l⁻¹, pH = 4.0.

groups of the cell wall, which make non-adsorbable protein complexes with Cu²⁺ ions. Huang et al. (10) stated that when heavy metals become tightly bound to acid groups in the side chains of amino acids on the cell surface, salt linkages are thus broken and the proteins are dissolved from the cell wall. Apparently, the decrease in Cu²⁺ removal capacity is affected by the complexation of Cu²⁺ with dissolved protein. The protein molecules in the liquid phase compete for Cu²⁺ ions with the protein molecules on the cell wall. These Cu²⁺-protein complexes are not adsorbable, thereby impeding copper binding. Brady et al. (12) also prepared a granular biosorbent biomass by treating yeast with hot alkali and found that the granular biomass was capable of accumulating a wide range of heavy metal ions. Lu and Wilkins (14) found that caustic treated *S. cerevisiae* cells had high metal removal efficiency for Cu²⁺ and Zn²⁺ ions.

Effect of pH

The effect of pH on copper biosorption was examined in the pH range 2.0-6.0. At pH values higher than 6.0, copper ions precipitated due to the high concentration of OH⁻ ions in the adsorption medium and biosorption studies could not be performed. Caustic treated yeast (0.1 g per 100 ml medium) was used as the biosorbent and a medium containing 24.7 mg l⁻¹ of Cu²⁺ ions was used as the biosorption medium. As seen in Figure 2, the removal of Cu²⁺ ions from aqueous solution was affected by medium pH and the biosorption of copper ions was completed after about 5 min contact with the biosorbent. The short contact time of biosorbent with metal solution

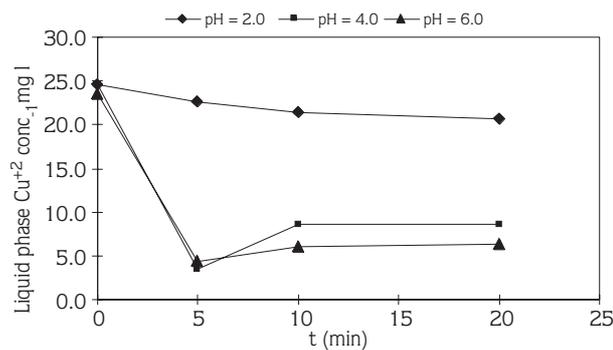


Figure 2. Effect of pH on biosorption of Cu²⁺ by caustic treated baker's yeast cells. The biosorbent concentration was 0.1 g/100 ml (dry mass per volume), initial metal concentration was 24.7 mg l⁻¹.

for biosorption suggests that adsorption onto the biosorbent surface is the main mechanism of uptake. Similar metal uptake values were observed at pH 4.0 and 6.0 (21.2 and 19.2 mg g⁻¹, respectively) while low uptake (4.0 mg g⁻¹) was obtained at pH 2.0. At pH values above the isoelectric point, there is a net negative charge on the cell wall components and the ionic state of ligands such as carboxyl, phosphate and amino groups will be such as to promote a reaction with metal cations. As the pH is lowered, however, the overall surface charge on the cells will be positive, which will inhibit the approach of positively charged metal cations. It is likely that protons will then combine with metal ions for the ligands and thereby decrease the interaction of metal ions with cell components (7). Many other researchers also observed low copper uptake values at low pH values for different microorganisms (7,8,17-20). Zouboulis et al. (8) stated that copper cations begin to be bound at approximately pH 4, reaching maximum sorption at a pH of around 6. Aksu and Acikel (17) studied the biosorption of copper with *C. vulgaris* and determined the optimum adsorption pH to be 4.0. Kuyucak and Volesky (18) found the optimum pH for the biosorption of Cu²⁺ as 4-5 in *Sargassum natans*. They could not conduct the experiments at higher pH values because of the precipitation of copper hydroxide above pH 5.0. Sag and Kutsal (7) studied the biosorption of copper ions with *R. arrhizus* cells and determined the initial pH for biosorption of copper ions to be 4.0.

Effect of initial concentrations of copper ions

Biosorption experiments with caustic treated yeast cells were conducted for solutions containing 10-250 mg

l^{-1} copper ions. As seen in Figure 3, at lower concentrations of Cu^{+2} ($10-50 \text{ mg } l^{-1}$) biosorption was complete in about 5 min but at higher concentrations it took 30-60 min.

After biosorption, residual copper concentrations were 1.6, 3.6, 8.8, 23.8, 43.5, 77.5 and $135.9 \text{ mg } l^{-1}$ and metal uptake values were 8.4, 21.1, 39.5, 72.4, 102.2, 120.7, and $109.3 \text{ mg } g^{-1}$ for 10.0, 24.7, 48.3, 96.1, 145.7, 198.2, and 245.2 initial copper ion concentrations, respectively.

Adsorption isotherm of copper

The copper sorption isotherm is shown in Figure 4. C_e ($\text{mg } l^{-1}$) is the final equilibrium concentration of copper remaining in the solution and q ($\text{mg } g^{-1}$) is the metal uptake.

Two adsorption models, the Langmuir model and Freundlich equation, were applied to the experimental data. The Langmuir model, which was initially developed to study physical adsorption, is a useful tool for the interpretation of biosorption profiles. The Langmuir model can be explained as follows:

$$q = (q_{\max} \times b \times C_e) \times (1 + b \times C_e)^{-1}$$

where q_{\max} ($\text{mg } g^{-1}$) is the maximum sorbate (Cu^{+2}) uptake under the given conditions and b ($l \text{ mg}^{-1}$) is the Langmuir constant related to the affinity between the sorbent and sorbate. For the fitting of experimental data, the model was linearized as follows:

$$1 / q = (q_{\max} \times b)^{-1} \times (C_{eq})^{-1} + (q_{\max})^{-1}$$

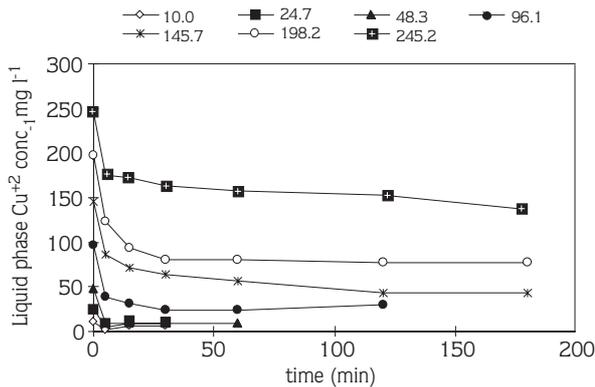


Figure 3. Biosorption of Cu^{+2} by caustic treated baker's yeast cells at different initial Cu^{+2} concentrations. The biosorbent concentration was $0.1 \text{ g}/100 \text{ ml}$ (dry mass per volume), $\text{pH} = 4.0$.

The Freundlich equation deals with heterogenous surface adsorption and can be explained as follows:

$$q = k \times C_e^{(1/n)}$$

where k and n are Freundlich constants. This equation is easily linearized by plotting it in a log-log format.

$$\log q = \log k + n^{-1} \times \log C_e$$

The correlation coefficients obtained from the Langmuir model and Freundlich equation were 0.9932 and 0.9308, respectively. As seen in Figure 5, the Langmuir equation was more in correlation with the experimental data. Langmuir model and Freundlich equation parameters found from the fitting of experimental points from Figure 5 are shown in the Table.

The Langmuir model makes several assumptions, such as monolayer adsorption and constant adsorption energy, while the Freundlich equation deals with heterogeneous surface adsorption (20). The agreement of the experimental data with the Langmuir model implied that monolayer adsorption existed for the experimental conditions used.

Column studies using calcium alginate immobilized biosorbent

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, Ca-alginate gel was chosen for the immobilization experiments as it is cheaply and

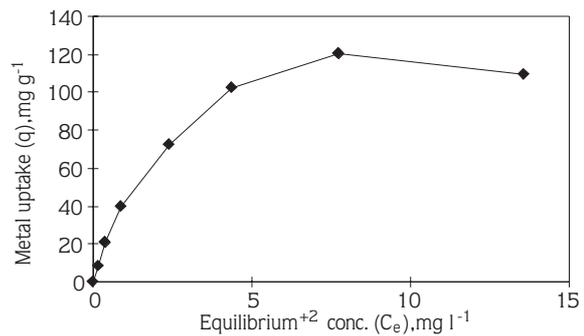


Figure 4. Adsorption isotherm of Cu^{+2} by caustic treated baker's yeast at $\text{pH} 4.0$.

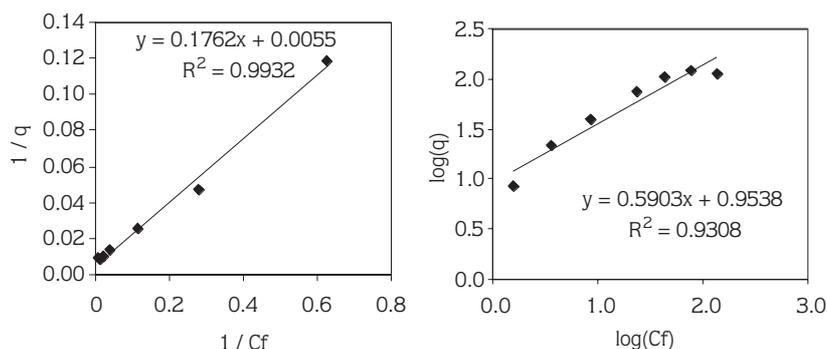


Figure 5. Application of adsorption models to experimental data. (a) Langmuir model, (b) Freundlich equation.

Table. Langmuir model and Freundlich equation parameters estimated from the fitting of experimental points of copper biosorption.

Langmuir model	Freundlich equation
$q_{max} \text{ (mg g}^{-1}\text{)} = 181.8$	$k \text{ (l g}^{-1}\text{)} = 8.99$
$b \text{ (l mg}^{-1}\text{)} = 0.0312$	$n = 1.69$
$R^2 = 0.9932$	$R^2 = 0.9308$

abundantly available, nontoxic and highly selective for certain ion species. The flow rate in the packed bed bioreactor was 2.35 ml min⁻¹ and the inlet copper concentration in the feed was held constant at 95.5 mg l⁻¹ (pH = 4.0). The residence time of metal ions through the column was 11.3 min. Ca-alginate gel without caustic treated yeast biomass was also used for biosorption in the same experimental conditions. As can be seen from Figure 6, the outflow concentration profiles show that copper removal is fast and highly effective during the initial phase; subsequently, metal removal decreases, as a consequence of the progressive saturation of the binding sites. Such a profile depicts a breakthrough curve similar to those observed by other authors for other immobilizing materials (1,10,12,21). At the initial phase of biosorption, calcium alginate gel with immobilized biosorbent removed 98.3% of copper, and calcium alginate gel alone removed 91.7% of copper ions. After collecting 1200 ml of effluent from the column, the column came to saturation. It can be concluded that Ca-alginate gel itself is a good biosorbent of copper and immobilizing caustic treated yeast particles in the gel only slightly increases the biosorption capacity of the gel. Yang and Volesky (22) also found that in the brown alga *Sargassum* biomass, alginate was the main component responsible for cadmium biosorption. They stated that

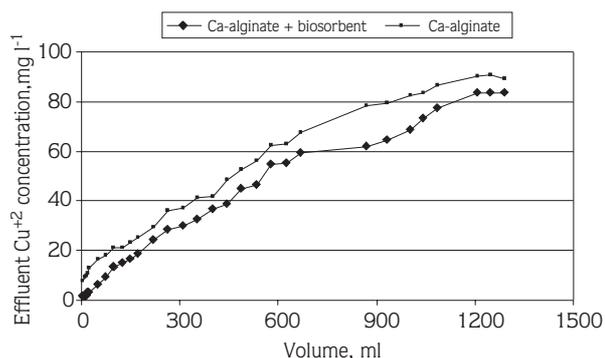


Figure 6. Effluent copper concentration after biosorption with Ca-alginate beads and Ca-alginate beads immobilized with caustic treated yeast biomass in a packed bed bioreactor (pH = 4.0, initial copper concentration = 95.5 mg l⁻¹, flow rate = 2.35 ml min⁻¹, T = 30 °C).

alginate was present in a gel form in the cell wall, which appears very porous and easily permeable to small ionic species.

It was thought that the results obtained in this study would fulfill the lack of scientific information on copper biosorption by calcium alginate immobilized biosorbent particles.

Conclusion

In this study, caustic treated waste baker's yeast (*S. cerevisiae*) has been successfully used as a biosorbing agent for the removal of copper ions from artificial waste water. The Langmuir adsorption model and Freundlich equation were used for the mathematical description of the biosorption of Cu⁺² ions onto caustic treated yeast biomass. It was seen that the adsorption equilibrium data conformed well with the Langmuir model. Calcium alginate gels with and without biosorbent particles were

used as an immobilization matrix and it was found that calcium alginate gel alone was also a good biosorbent for copper biosorption. Immobilizing caustic treated yeast biomass in calcium alginate only slightly increased biosorption capacity. It seems that the use of readily available waste *S. cerevisiae* biomass from fermentation industries offers an alternative for the removal of copper from industrial waste waters. Further research will focus

on the use of waste baker's yeast as a biosorbent for the removal of cadmium and lead from model solutions.

Acknowledgments

The authors gratefully acknowledge Ege University's Scientific Research Project Fund for providing financial support.

References

1. Aksu Z., Egretli G., Kutsal, T. A comparative study for the biosorption characteristics of chromium(VI) on Ca-alginate, agarose and immobilized *C. vulgaris* in a continuous packed bed column. *J Environ Sci Health A* (34)2: 295-316, 1999.
2. Volesky, B. Biosorption for the next century. International Biohydrometallurgy Symposium, El Escorial, Spain, 1999.
3. Veglio, F., Beolchini F., Gasbarro, A. Biosorption of toxic metals: an equilibrium study using free cells of *Arthrobacter sp.* *Process Biochem* 32: 99-105, 1997.
4. Gadd, G.M., White, C. Microbial treatment of metal pollution - a working biotechnology? *Trends Biotechnol* 11: 353-359, 1993.
5. Volesky, B. Biosorbents for metal recovery. *Trends Biotechnol* 5: 96-101, 1987.
6. Volesky, B., Holan Z.R. Biosorption of heavy metals. *Biotechnol Progr* 11: 235-250, 1995.
7. Sag, Y., Kutsal, T. The selective biosorption of chromium(VI) and copper(II) ions from binary metal mixtures by *R. arrhizus*. *Process Biochem* 31: 561-72, 1996.
8. Zouboulis, A.I., Rousou, E.G., Matis, K.A., Hancock, I.C. Removal of toxic metals from aqueous mixtures. Part 1: Biosorption. *J Chem Technol Biot* 74: 429-36, 1999.
9. Brady, D., Stoll, A., Duncan, J.R. Biosorption of heavy metal cations by non-viable yeast biomass. *Environmental Technology* 15: 429-38, 1994.
10. Huang, C.P., Huang, C.P., Morehart, A. The removal of Cu (II) from dilute aqueous solutions by *Saccharomyces cerevisiae*. *Wat Res* 4: 433-39, 1990.
11. Leusch, A., Holan, Z.R., Volesky, B. Biosorption of heavy metals (Cd, Cu, Ni, Pb, Zn) by chemically-reinforced biomass of marine algae. *J Chem Technol Biot* 62: 279-288, 1995.
12. Brady, D., Duncan, J.R. Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* 34: 149-154, 1994.
13. Engl, A., Kunz, B. Biosorption of heavy metals by *Saccharomyces cerevisiae*: Effects of nutrient conditions. *J Chem Technol Biot* 63: 257-61, 1995.
14. Lu, Y., Wilkins, E. Heavy metal removal by caustic-treated yeast immobilized in alginate. *Journal of Hazardous Materials* 49: 165-179, 1996.
15. Volesky, B.A., Mayphillips, H.A. Biosorption of heavy metals by *Saccharomyces cerevisiae*. *Appl Microbiol Biotechnol* 42: 797-806, 1995.
16. Wilhelmi, B.S., Duncan, J.R. Metal recovery from *Saccharomyces cerevisiae* columns. *Biotech Lett* 17: 1007-1012, 1995.
17. Aksu, Z., Acikel, U. A single-staged bioseparation process for simultaneous removal of copper(II) and chromium(VI) by using *C. vulgaris*. *Process Biochem* 34: 589-599, 1999.
18. Kuyucak, N., Volesky, B. Accumulation of cobalt by marine alga. *Biotechnol Bioeng* 33: 809-814, 1989.
19. Say, R., Denizli, A., Arica, M.Y. Biosorption of cadmium(II), lead(II) and copper(II) with the filamentous fungus *Phanerochaete chrysosporium*. *Bioresource Tech* 76: 67-70, 2001.
20. Zhou, J.L., Kiff, R.J. The uptake of copper from aqueous solution by immobilized fungal biomass. *J Chem Technol Biot* 52: 317-30, 1991.
21. Vecchio, A., Finoli, C., Di Simone, D., Andreoni, V. Heavy metal biosorption by bacterial cells. *Fresenius J Ana. Chem* 36: 338-42, 1998.
22. Yang, J.K., Volesky, B. Cadmium biosorption rate in protonated Sargassum biomass. *Environ Sci Technol* 33: 751-757, 1998.