# IMMOBILISATION OF *SACCHAROMYCES CEREVISIASE* FOR THE PRODUCTION OF BIOETHANOL

Ioan CĂLINESCU[1], Petre CHIPURICI[2], Adrian TRIFAN[3], Corina BĂDOIU[4]

*În acest studiu s-a investigat obținerea de bioetanol din melasă de sfecla de zahăr, prin imobilizarea drojdiei Saccharomyces cerevisiae. Imobilizarea drojdiei pe gel de poliacrilamidă are loc cu o eficiență comparabilă cu cea obținută la imobilizarea drojdiei pe alginat de calciu. Imobilizarea drojdiei pe gelul de poliacrilamidă este avantajoasă deoarece granulele obținute sunt mult mai rezistente şi îşi păstrează forma în timpul procesului de fermentare.*

*In this study, the obtaining of bioethanol from beet molasses, by immobilized Saccharomyces cerevisiae yeast was investigated. The immobilization of yeast on polyacrylamide gel proceeds with an efficiency comparable to the one obtained for the immobilization of the yeast cells on calcium alginate. The advantage of using immobilized yeast on polyacrylamide gel is that the granules are more resistant, and they keep their shape during the fermentation process.*

**Keywords**: *Saccharomyces cerevisiae*, Bioethanol, Immobilized cell, Calcium alginate

## 1. Introduction

In recent years, as response to efforts to reduce carbon dioxide emissions, bioethanol has become one of the most promising biofuels and is considered as the only feasible alternative to fossil fuels in Europe and in the world.

Cane molasses, by-product of sugar industry, a low-cost source of sugar, can be used for the production of ethanol and in contrast to other agricultural by-products, it does not require hydrolysis.

Several microorganisms, such as *Saccharomyces cerevisiae* and *Zymomonas mobilis* are suitable candidates to produce bioethanol [1]. *Saccharomyces cerevisiae* is an important microorganism in bio-industry and its

[1] Prof., Faculty of Applied Chemistry and Material Science, University POLITEHNICA of Bucharest, Romania
[2] Prof., Faculty of Applied Chemistry and Material Science, University POLITEHNICA of Bucharest, Romania
[3] Eng., Faculty of Applied Chemistry and Material Science, University POLITEHNICA of Bucharest, Romania
[4] PhD student, Faculty of Applied Chemistry and Material Science, University POLITEHNICA of Bucharest, Romania, e-mail: anca_corina22@yahoo.com

tolerance to ethanol is one of the main characteristics to decide whether it can be used for alcoholic fermentation.

Various techniques for improving the production of ethanol, including continuous fermentation with cell recycling, vacuum distillation with cell recycling and immobilization of yeast cells have been evaluated. The main objectives of immobilization are to increase the bioreactor productivity with improved cell stability and to have better substrate utilization. Another objective might be to minimize the start-up time by growing the required biomass in a nutrient-rich growth medium [2]. Therefore, in order to be a more desirable alternative, immobilized cells must have a significantly longer working lifetime than free cell systems. Immobilized cells can be applied both for continuous and for discontinuous operation of the reactor.

Immobilized cell systems can be classified according to the physical mechanism of immobilization. Immobilization cell should be carried out under mild conditions in order to maintain the activity of the cells. Methods for immobilization of microbial cells include physical entrapment within porous matrix, encapsulation, adsorption or attachment to a pre-formed carrier and cross-linking.

Entrapment is the most commonly used method of immobilizing cells. This method is preferable for cell immobilization due to several advantages. The preparation exhibits decreased cell leakage, and has high loading capacity [3]. The method is easy, because a wide variety of polymeric material can be used, including synthetic and natural polymers.

As synthetic polymer, polyacrylamide gel has been extensively used for immobilization of many kinds of microbial cells [4]. The natural polymers mostly used for the immobilization of cells are mainly polysaccharides, such as calcium alginate.

The purpose of this research is to obtain high ethanol production with high yield of productivity. A discontinuous reactor was used, with a *Saccharomyces cerevisiae* yeast, Ethanol Red as fermentation agent, using an entrapment technique utilizing both calcium alginate and polyacrylamide gel, as a porous wall to retain the yeast cell.

## 2. Experimental

### 2.1. The immobilization of yeast using calcium alginate

Sodium alginate was prepared from alginic acid, distilled water and sodium hydroxide. The calcium chloride solution was prepared by dissolving 120 g of calcium chloride in 2L distilled water. Sodium alginate and calcium chloride solutions were autoclaved at $121^0$C for 15 minutes, and then were cooled.

The dry yeast was hydrated by dissolving 100 g of dry yeast in 1L distilled water. The solution thus obtained was stirred slowly, for 15 minutes at $35^0$C. The solution was allowed to settle for 40 minutes, and then it was stirred again for another 5 minutes.

Eight samples were prepared. First, the solution of sodium alginate obtained previously was mixed with the yeast suspension. For the preparation of immobilized cells, 2%, 3% and 4% of alginate was used. Two samples included 0.1, respectively 0.3% gelatin. The beads were prepared by droplet addition of the calcium chloride solution over the 8 samples. The samples were refrigerated for 24 h.

After 24 h, the beads were washed several times with distilled water. The beads were cuted into small pieces. After that, a nutrient solution of 1L was prepared, made up of 10 g glucose, 3 g $KH_2PO_4$, 4.5 g $Na_2PO_4$. The solution was boiled. After the solution was cooled it was added 1 g of dry yeast RedEthanol. Portions of 120 mL of this solution were dropped on the beads. The samples were kept for 24 h at temperature of $36^0$ C.

A glucose solution of 5% was prepared. After 24 h the samples are drained of the nutrient solution, and the beads are introduced each in 120 mL of the glucose solution, and there were kept in a refrigerator until the fermentation mixtures were prepared.

### 2.2. The immobilization of yeast using polyacrylamide gel

Acrylamide (AAm, Merck), N,N'-methylenebisacrylamide (BAAm, Merck), ammonium persulfate (APS, Merck), and N,N,N',N'-tetramethylethylenediamine (TEMED, Merck) were used. Three stock solutions of APS, TEMED and BAAm were prepared by dissolving 0.16 g of APS, 0.5 mL of TEMED and 0.264 g of BAAm in 20 mL of distilled water.

The redox initiator system was the APS and TEMED. Five graduated flasks were prepared. Each of them contain a solutions made up from 0.9736 g of AAm, 2 mL of stock solution of BAAm, 2 mL of stock solution of TEMED and 14 mL of distilled water. The solutions from the graduated flasks were mixed very well.

Different quantities of dry yeast (2, 4, 6 and 8 g) were introduced in the graduated flasks. The solutions from the flasks were cooled to $0^0$C in ice-water bath, without any stirring, and then, nitrogen gas was purged into the hermetically closed graduated flasks for 20 min. After, 2 mL of APS stock solution was added.

The polymerization was carried out for one day, by the immersion of the graduated flasks in a thermostated bath at $-18^0$C. After polymerization, the gels were cuted into specimens of approximately 5 mm in length. The immobilized beads were shared into two, so the quantities of yeast were the same: 2.27 dry

yeast in 100 mL fermentation mixture. The cuted beads were immersed in a large excess of water to wash out any soluble polymers, unreacted monomers and the initiator.

Two types of experiments were carried out with the polyacrylamide beads obtained: the immobilized yeast was used in the fermentation mixture exactly as it was obtained; the immobilized yeast was incubated for 24 h in a nutrient solution (10 g glucose, 3 g $KH_2PO_4$, 4.5 g $Na_2PO_4$ in 1L distilled water and 1 g of dry yeast RedEthanol) at temperature of $36^0$ C.

### 2.3. Batch fermentation

The batch fermentation experiments in order to obtain ethanol were carried out using 16 g / 100 mL of glucose solution as sole carbon source for *Saccharomyces cerevisiae*, and some nutrients, such as: 0.1g $(NH_4)_2SO_4$, 0.1g $KH_2PO_4$ and 0.1g $K_2HPO_4 \times 3\ H_2O$. The glucose is provided by 32 g/ 100 mL solution of molasses. The pH was adjusted at 5.5, using HCl 0.5N. A volume of 100 mL of fermentation mixture was added over the immobilized yeast on calcium alginate or polyacrylamide. The fermentation experiment was carried out at the temperature of $33.5^0$C. The samples for analysis were taken after 2, 4, 6, 8, 24 and 28 h. The purpose of immobilized cell reactor experiment using a batch fermentation system was to compare the amount of yeast concentration, using calcium alginate or polyacrylamide immobilization and bioethanol production.

### 2.4. Bioethanol detection

Bioethanol production in the fermentation process was detected with gas chromatography, Buck Scientific 910 Environmental & BTX, equipped with flame ionisation detector (FID) and a capillary column Stabilwax (MXT-1 0.53 x 60M, I.D. 5.0u, DB1). Helium was used as a carrier gas. Isopropanol was used as an internal standard. The samples from the fermentation medium were taken with a seringe with a Nylon filter of 0.45μm.

## 3. Results and discusions

Table 1 shows the results of the bioethanol concetration using immobilised yeast on calcium alginate at 8 and 28 h. The suitable alginate concentration based on activity of the beads for ethanol production was sample number 8, with 2% alginate, although the reaction was started a little bit later then the other samples.
As it is shown in Fig. 1, the yeast content influences the ethanol concentration. Even if the final concentrations of bioethanol are not so different, the sample with the smaller quantity of yeast has started the reaction slower then the other two.

The influence of the addition of gelatin on samples 3, 6 and 7 are shown in Fig. 2. The three samples have the same alginate concentration, the same yeast content, but the samples 6 and 7 have also a quantity of gelatin, 0.1, respectively 0.3%.

*Table 1*

**The bioethanol concentration using immobilization of yeast on calcium alginate**

| | Experimental conditions | | | Bioethanol concentration, g/L | |
|---|---|---|---|---|---|
| Sample | Yeast content, g | Alginate concentration, % | Gelatin content, g | 8 h | 28 h |
| 1. | 2.27 | 3.00 | 0 | 38.41 | 58.22 |
| 2. | 0.91 | 3.00 | 0 | 27.01 | 55.09 |
| 3. | 0.18 | 3.00 | 0 | 2.04 | 47.95 |
| 4 | 1.82 | 2.00 | 0 | 29.15 | 58.87 |
| 5. | 0.73 | 4.09 | 0 | 15.78 | 59.66 |
| 6. | 0.22 | 2.96 | 1 | 6.37 | 51.76 |
| 7. | 0.22 | 2.96 | 3 | 2.97 | 52.2 |
| 8. | 0.68 | 2.00 | 0 | 10.99 | 65.04 |

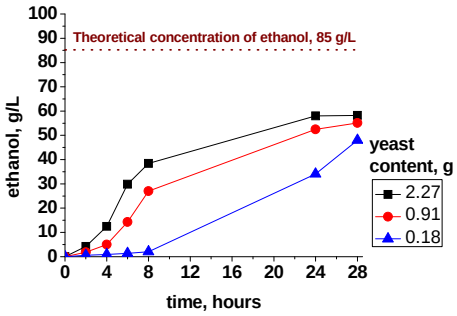


Fig. 1. The influence of the yeast content on ethanol concentration (3% alginate concentration, without gelatin)

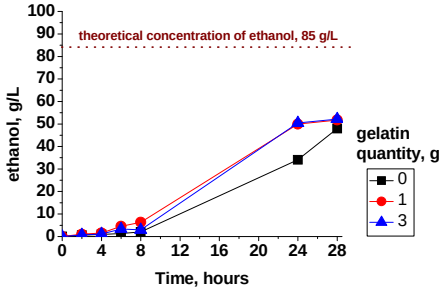


Fig. 2. The influence of the gelatin addition on ethanol concentration (3% alginate concentration and 0.2 g yeast content)

Fig. 2 shows that a low quantity of gelatin filled into the calcium alginate beads improves the properties of alginate and allows increasing the speed of reaction on lower reaction times. Similar concentrations of bioethanol are obtained at longer reaction times.

The influence of the different alginate concentration on the bioethanol obtained for the samples 2, 5 and 8 is presented in Fig. 3.

The samples have almost the same yeast content, about 0.7 g, but have different alginate concentrations. The samples don't have gelatin in their content. The best ethanol productivity has the sample containing 2% alginate. As a

consequence, a higher quantity of alginate in the immobilized yeast doesn't increase the amount of the ethanol obtained.

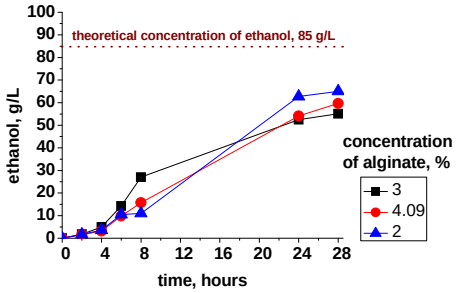


Fig. 3. The influence of the alginate concentration on ethanol concentration (0.7 g yeast, without gelatin)

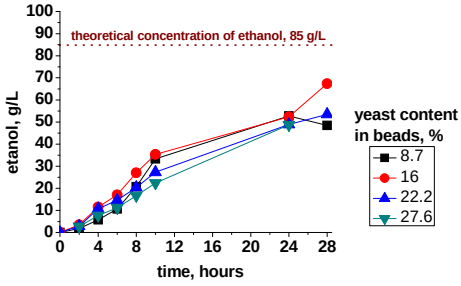


Fig. 4. The influence of yeast concentration from the polyacrylamide gel beads on ethanol concentration (without nutrient solution)

Table 2 shows the results of the bioethanol concetration using immobilised yeast on polyacrylamide gel at 8 and 28 h. The samples that were grown in nutrient solution were noted from 1 B to 5 B, and the samples containing the immobilized yeast exactly as it was obtained were noted from 2A to 5 A.

*Table 2*

**The bioethanol concentration using immobilization of yeast on polyacrylamide gel**

| Immobilization of yeast on polyacrylamide gel | | Ethanol concentration, g/L | |
|---|---|---|---|
| Sample | % yeast in polyacrylamide gel | 8h | 28h |
| 2A | 8.7 | 20.7 | 48.5 |
| 3A | 16 | 27.1 | 67.4 |
| 4A | 22.2 | 20.4 | 53.5 |
| 5A | 27.6 | 16.7 | 53.3 |
| 1B | 0.1 | 1.7 | 13.2 |
| 2B | 8.7 | 35.2 | 59.7 |
| 3B | 16 | 34.8 | 72.5 |
| 4B | 22.2 | 24.5 | 66 |
| 5B | 27.6 | 19 | 65.6 |

Fig. 4 shows the influence of yeast concentration from the polyacrylamide gel beads on the ethanol concentration, without using a nutrient solution.

Fig. 5 shows the influence of yeast concentration from the polyacrylamide gel beads on the bioethanol concentration, using a nutrient solution.

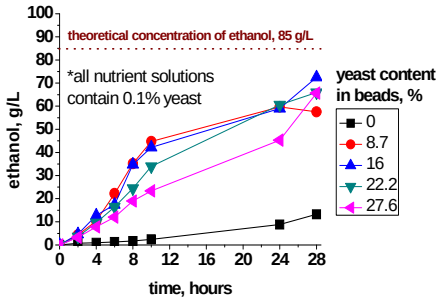


Fig. 5. The influence of yeast concentration from the polyacrylamide gel beads on bioethanol concentration (with nutrient solution)

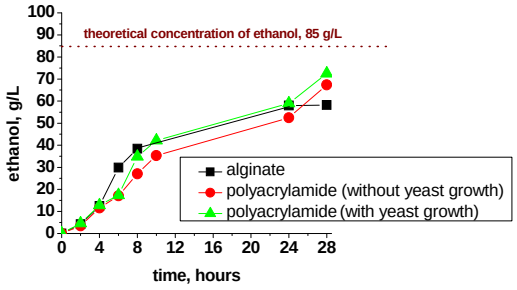


Fig. 6. The influence of the of polymeric material used for the immobilization of yeast on ethanol concentration

It can be noticed that the difference of the bioethanol concentration between the two kinds of experiments, for samples number 3A and 3B, using the same quantity of yeast in polyacrylamide gel beads with and without a nutrient solutin is not so representative. Although, for the sample with nutrient was obtained a slightly higher quantity of bioethanol.

A correlation between the ethanol concentration and the type of polymeric material used on the immobilisation of cells, using 2.27 % EthanolRed yeast in a solution containing 16 g/L glucose is shown in Fig. 6.

## 4. Concusions

The immobilization of yeast on polyacrylamide gel leads to an efficient immobilization of the yeast cells, the results of the experiment being comparable with the ones obtained for the immobilization of yeast on calcium alginate.

The advantage of using immobilized yeast on polyacrylamide gel is that the granules are more resistant, and they keep their shape during the fermentation process. The immobilization of yeast on polyacrylamide gel is more efficient in a continuous fermentation process.

The addition of gelatin in the calcium alginate beads increase the alginate stability, but decrease the efficiency of the fermentation process.

## REFERENCES

1. *M.C. Flickinger and S.W. Drew,* "Energy metabolism, microbial and animal cells, Encycl. Bioprocess. Technol.: Ferment., Biocatal., Biosep.", **2**, pp. 929, 1999
2. *F. Ghorbani, H. Younesi, Abbas E. Sari and G. Najafpour* , Renewable Energy, **36**, pp. 503-509, 2011

3. *C.A. Cardona and O.J. Sanchez,* Bioresource Technology, **98**, pp. 2415-2457, 2007
4. *M.V. Dinu , M. . Ozmen, E.S. Dragan and O. Okay*, Freezing as a path to build macroporous structures: Superfast responsive polyacrylamide hydrogels, Polymer, **48**, pp. 195 – 204, 2007
5. *M. Jones* (Union Carbide), Brit. Pat. 601238 Sept 2, 1959: cf. *Chem .Abstr.,* **54**, pp.12808, 1960
6. *D. Sima and J. Oliniev*, Novel Materials for Efficient Energy Conversion, First International Electronic Conference on Synthetic Materials, Varna, **1**, pp. 18-24, 1-3 September 1997.