APTEFF, 43, X-XXX (2012)
DOI: 10.2298/APT1243325V
UDC: 664.151.2:582.282.23:663.142
BIBLID: 1450-7188 (2012) 43, 325-333
Original scientific paper

# ETHANOL FERMENTATION OF MOLASSES BY *Saccharomyces cerevisiae* CELLS IMMOBILIZED ONTO SUGAR BEET PULP

*Vesna M. Vučurović* and Radojka N. Razmovski*

University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia

*Natural adhesion of Saccharomyces cerevisiae onto sugar beet pulp (SBP) is a very simple and cheap immobilization method for retaining high cells density in the ethanol fermentation system. In the present study, yeast cells were immobilized by adhesion onto SBP suspended in the synthetic culture media under different conditions such as: glucose concentration (100, 120 and 150 g/l), inoculum concentration (5, 10 and 15 g/l dry mass) and temperature (25, 30, 35 and 40 $^{o}$C). In order to estimate the optimal immobilization conditions the yeast cells retention (R), after each immobilization experiment was analyzed. The highest R value of 0.486 g dry mass yeast /g dry mass SBP was obtained at 30$^{o}$C, glucose concentration of 150 g/l, and inoculum concentration of 15 g/l. The yeast immobilized under these conditions was used for ethanol fermentation of sugar beet molasses containing 150.2 g/l of reducing sugar. Efficient ethanol fermentation (ethanol concentration of 70.57 g/l, fermentation efficiency 93.98%) of sugar beet molasses was achieved using S. cerevisiae immobilized by natural adhesion on SBP.*

**KEY WORDS:** immobilization, bioethanol, sugar beet pulp, molasses, *Saccharomyces cerevisiae*

## INTRODUCTION

In the recent years, research on improving ethanol production has been accelerated for both ecological and economic reasons, primarily for its use as an alternative to petroleum-based fuels (1). Currently, the global ethanol supply is originated mainly from sugar and starch feedstocks (2). The development of a fermentation medium based on industrial substrates is economically desirable (3). In the bioethanol production, the composition of the medium affects the physiological state and, consequently, the fermentation performance of the microorganism employed (4). Molasses from the sugar beet processing due to the high content of fermentable sugars, which can be directly used for fermentation without any modification, is a very good raw material which is traditionally used for ethanol production (5). Among many microorganisms that have been exploited for ethanol production, *Saccharomyces cerevisiae* still remains as the prime species (2). Recent-

* Corresponding author: Vučurović M. Vesna, University of Novi Sad, Faculty of Technology, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia, e-mail: vvvesna@uns.ac.rs

ly, yeast cell immobilization techniques have become increasingly important and are being successfully applied in production of ethanol as a method for improving the process productivity (6). Among the different immobilization technologies, entrapment of microbial cells within the polymeric matrices (calcium alginate, agar agar, gelatin, k-carrageenan, etc.) has been studied widely. However, technically it is less suitable for ethanol production because the growth of the yeast cells is restrained and also the slowly growing yeast cells are difficult to be removed from the systems (2). However, the use of yeast immobilized by natural adhesion onto low-cost plant materials such as wood chips (7), apple peaces (8), orange peel (9), sugar cane bagasse (10), sugar cane pieces (11), corn cobs and grape pomace (12) and maize stem ground tissue (13), can effectively overcome these drawbacks. Yeast cells immobilization by adhesion onto the solid supports is attractive in ethanol fermentation, due to the operational easiness and high ethanol productivity, thanking to the effective retention of cells within the bioreactor. Besides, the possibility of recycling cells for inoculum permits the fermentation to be profitably carried out in continuous or repeated-batch mode (14). The sugar beet pulp (SBP) was found to be efficient support for immobilization of *S. cerevisiae* in the ethanol production because of its heterogeneous structure, high porosity, biocompatibility, high water swelling capacity, good mechanical properties, and high cells retention capacity. This immobilization method is cheap, simple and easy (15).

The novelity of this work lies in the investigation of the optimal conditions (inoculum concentration, glucose concentration and temperature) for the *S. cerevisiae* immobilization onto SBP. Further, the efficiency of immobilized yeast for batch ethanol fermentation of sugar beet molasses was investigated with the aim to achieve efficient ethanol production.

## EXPERIMENTAL

Dried sugar beet pulp (SBP) kindly provide by a sugar factory near the city of Senta (Vojvodina province, Serbia) was used as the support for yeast cells. The SBP hydration was carried out by placing an amount of 15 g of dry SBP into 1 l Erlenmeyer flasks containing 500 ml of synthetic culture medium containing different amounts of glucose (100, 120 and 150 g/l) and the same amount of the following constituents: $(NH_4)_2SO_4$ (1 g/l), $KH_2PO_4$ (1 g/l), $MgSO_4$ (5 g/l) and yeast extract (4 g/l) at the pH 5.5, and was sterilized by autoclaving at 121$^oC$ for 30 min. After the sterilization, the flasks were kept at room temperature for 24 h. The working microorganism was a commercial *S. cerevisiae* strain (Alltech-Fermin, Senta, Serbia), commonly used in Serbian baking industry, in the form of pressed blocks (70 % w/w moisture). To immobilize cells onto hydrated SBP, the flasks were inoculated with 5 g/l, 10 g/l and 15 g/l of yeast on dry basis, and placed on a rotary shaker (120 rpm) in a thermostat and kept at 30 $^oC$ for 24 h. After the immobilization of the yeast, the mass of cells immobilized onto the support was quantified gravimetrically according to Santos et al. (10). Cell retention onto the support ($R$, g/g) was calculated as the ratio of dry matter of cells immobilized in the support (g) to the support dry mass (g). Carl Zeiss optical microscope connected to a Cannon S50 camera was used to capture yeast cells immobilized onto SBP. After the immobilization of the cells, the me-

dium was decanted using sterilized gauze. The support without extra medium was then dried at 105°C to constant weight. The identical procedure was conducted by using support particles recovered from the cell-free medium, as a control, in order to avoid any interference in weighing measurements. The selected support containing immobilized yeast, with highest cells retention ($R$), was used for the batch fermentation of 500 ml of the sugar beet molasses in 1 l Erlenmeyer flasks. The reducing sugar content of molasses was 150.2 g/l, the pH was adjusted to 5.5 by addition of 10% (v/v) $H_2SO_4$ and it was sterilized by autoclaving at 121°C for 30 min. The fermentation kinetics was monitored by measuring the weight loss due to $CO_2$ release at various time intervals from the beginning of the fermentation batch until to its end (24 h). Samples of fermented liquids were analyzed for ethanol and reducing sugar. The fermented liquid was centrifuged at 3000 rpm for 15 min. The sample of supernatant was hydrolyzed in 33% HCl at 100°C for 10 min and neutralized with NaOH solution, and reducing sugar content was determined using the 3,5-dinitrosalicylic acid (DNS) method (16). The ethanol concentration in the distillate was determined based on the density of the alcohol distillate at 20°C, by pycnometer method (17). Reducing sugar conversion ($S_u$, %) was calculated as the ratio of utilized reducing sugar to the initial and multiplying by 100. The ethanol yield ($Y_{p/s}$, g/g) was calculated as grams of ethanol produced per gram of utilized reducing sugar. The fermentation efficiency was calculated as the percentage of the maximal theoretical ethanol yield ($E_{p/s}$, %). The volumetric ethanol productivity ($Q_p$, g/lh) was calculated as grams of ethanol produced per liter per hour.

## RESULTS AND DISCUSSION

The cell immobilization and ethanol productivity of immobilized yeast cells depends on the surface characteristics of the support, such as pore size, water content, hydrophilic properties and magnetism (18). The *S. cerevisiae* was found to be immobilized by natural adhesion onto SBP due to the electrostatic interactions between support and the yeast cells surface and due to the capillary forces which hold cells inside the SBP cavities (15). The process of cell adhesion to solid supports by biosorption is believed to occur due to electrostatic or van der Waals interactions between the cell membrane and the support. These adhesion forces are affected by variations in the medium composition and component concentrations, because they can strongly influence the surface energy of the immobilization support (14).

A series of optical microscopic images (Fig. 1) were taken to visually explore the yeast immobilization onto SBP. As is shown in Fig. 1, in the immobilization process, the yeast cells penetrate into the interior of bibulous sugar beet tissue, enabling adsorption onto the surface of the carrier, and meanwhile the SBP cavities are also filled with yeast cells.

APTEFF, 43, X-XXX (2012)
DOI: 10.2298/APT1243325V
UDC: 664.151.2:582.282.23:663.142
BIBLID: 1450-7188 (2012) 43, 325-333
Original scientific paper

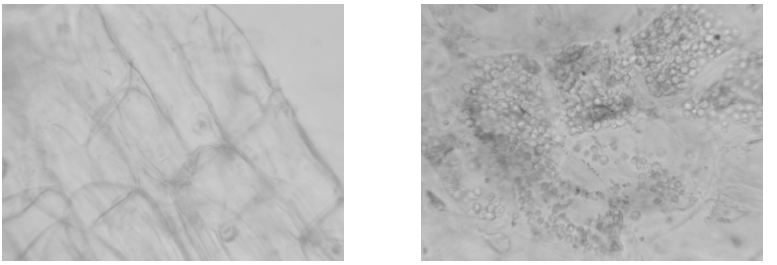

**Figure 1.** Optical microphotograph of a) SBP and b) *Saccharomyces cerevisiae* cells (400×) immobilized onto the SBP

Cells retention capacities obtained after 24 h of yeast immobilization at temperatures of 25, 30, 35, 40°C are shown in Figures 2-5.

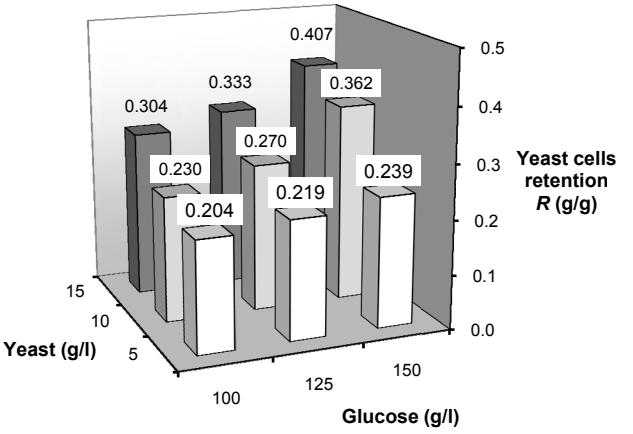


**Figure 2.** Retention of *S. cerevisiae* cells onto SBP after immobilization at 25°C

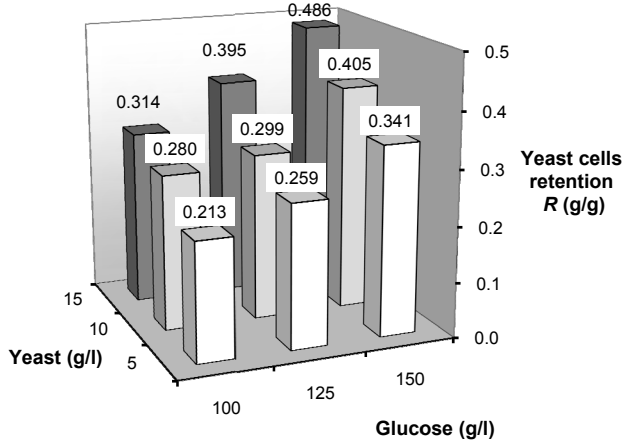


**Figure 3.** Retention of *S. cerevisiae* cells onto SBP after immobilization at 30°C

UDC: 664.151.2:582.282.23:663.142
BIBLID: 1450-7188 (2012) 43, 325-333
Original scientific paper

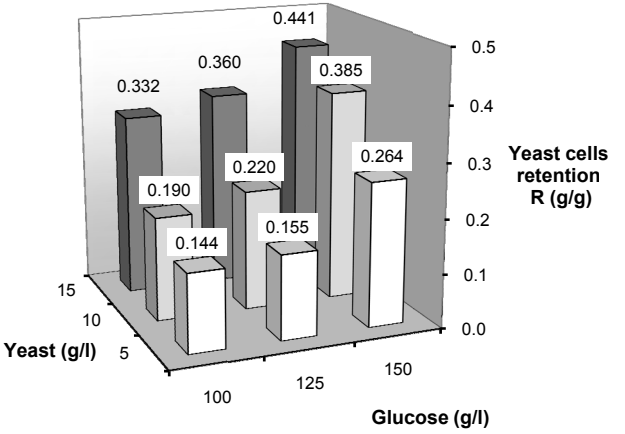


**Figure 4.** Retention of *S. cerevisiae* cells onto SBP after immobilization at 35°C

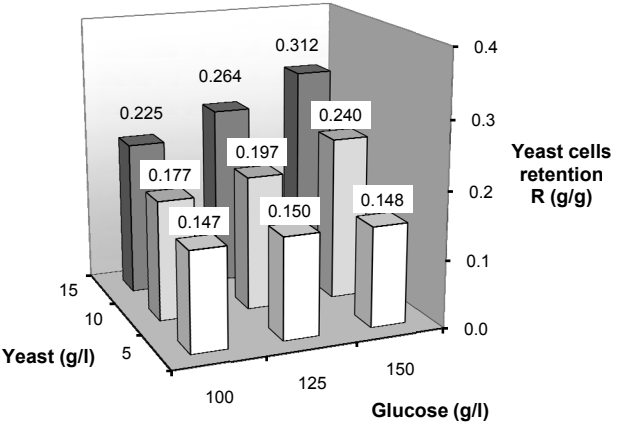


**Figure 5.** Retention of *S. cerevisiae* cells onto SBP after immobilization at 40°C

By comparing retention capacities obtained for the different yeast concentrations and glucose concentration obtained at each immobilization temperature (25, 30, 35, 40°C) (Fig. 2-5) it can be concluded that the cells retention increased along with the increase of the initial yeast concentration from 5 g/l to 15 g/l, and with the increase of glucose concentration in the medium from 100 g/l to 150 g/l. Maximum cells retention capacity of 0.407 g/g, 0.486 g/g, 0.441 g/g and 0.312 g/g for the respective immobilization temperatures of 25, 30, 35, 40°C, were obtained at the yeast concentration of 15 g/l and glucose concentration of 150 g/l. On the basis of the yeast immobilization results it can be concluded that for all inoculums and glucose concentrations, maximal values of cells retention capacity ($R$) were obtained at the immobilization temperature of 30°C. After 24 h of yeast immobilization at 30 °C the cells retention capacity ranged from 0.213 g/g for yeast concentration of 5 g/l and glucose concentration of 100 g/l to 0.486 g/g for yeast concentration of 15 g/l and glucose concentration of 150 g/l. These results imply that the most

appropriate temperature for yeast cells growth and, consequently, for immobilization is 30°C, while the temperatures of 25, 35 and 40°C were less effective. These results are in accordance with the influence of temperature on yeast growth. Considering firstly temperature, this is one of the most important physical parameters which influence yeast growth. In most laboratories and industries, yeast is generally grown in the range of 20-30°C (19).

Due to the highest yeast retention capacity of 0.486 g/g, the yeast immobilized onto the support by natural cell adhesion at 30 °C, glucose concentration of 150 g/l and inoculum concentration of 15 g/l was used for sugar beet molasses fermentation. Table 1 summarizes the fermentation parameters such as reducing sugar utilization, ethanol productivity, ethanol yield and fermentation efficiency obtained at the end of the fermentation batch of sugar beet molasses by immobilized *S. cerevisiae* obtained at 30°C. Since, 146.93 g/l reducing sugar was utilized, the fermentation efficiency was 97.83 %. This indicates that the cells immobilized onto SBP utilized almost all available reducing sugar from the molasses, suggesting efficient exploitation of this raw material for ethanol production. The final ethanol concentration of 70.57 g/l and ethanol productivity of 1.47 g/lh were achieved in the batch fermentation of molasses. The ethanol yield per consumed reducing sugar of 0.480 g/g was achieved, equal to 93.98% of its theoretical value expressed as the fermentation efficiency, indicating that almost all utilized reducing sugar was converted to ethanol. On the basis of these results it can be concluded that yeast cells immobilized onto SBP showed high fermentative activity and may be recommended for the further use in repeated batch or continuous process.

**Table 1.** Parameters of sugar beet molasses fermentation by *S. cerevisiae* immobilized on SBP

| **Parameter** | **Value** |
|---|---|
| Initial reducing sugar, $S_o$ (g/l) | 150.20 ± 0.36 |
| Utilized reducing sugar, $S_u$ (g/l) | 146.93 ± 0.65 |
| Reducing sugar utilization, $S_u$ (%) | 97.83 ± 0.63 |
| Ethanol concentration, $P$ (g/l) | 70.57 ± 0.83 |
| Ethanol productivity, $Q_p$ (g/lh) | 1.47 ± 0.02 |
| Ethanol yield, $Y_{p/s}$ (g/g) | 0.480 ± 0.004 |
| Fermentation efficiency, $E_{p/s}$(%) | 93.98 ± 0.84 |

## CONCLUSION

The work demonstrated the potential use of SBP as a support for *S.cerevisiae* immobilization under different immobilization conditions such as: glucose (100, 120 and 150 g/l), inoculum concentration (5, 10 and 15 g/l dry mass) and temperature (25, 30, 35 and 40°C). Efficient cells immobilization was confirmed by optical microscopy. The support was effective for yeast immobilization for each examined combination of conditions. Pre-

sented results show that the cells retention increases along with the increase of initial yeast concentration from 5 g/l to 15 g/l, and with the increase of glucose concentration in the medium from 100 g/l to 150 g/l. Also, it was found that the most appropriate temperature for yeast cells growth and consequently immobilization is 30°C. The highest yeast cells retention (0.486 g dry mass yeast /g dry mass SBP) was obtained at 30°C, glucose concentration of 150 g/l, and inoculum concentration of 15 g/l. Efficient bioethanol production from the molasses containing 150.2 g/l of reducing sugar, was achieved using thus immobilized yeast (ethanol concentration of 70.57 g/l, fermentation efficiency 93.98%).

**Aknowledgements**

Financial support of the Project **TR-31002** from the Ministry of Science and Technological Development of the Republic of Serbia is highly acknowledged.

## REFERENCES

1. Alfenore, S., Cameleyre, X., Benbadis, L., Bideaux, C., Uribelarrea, J.L., Goma, G., Molina-Jouve, C. and Guillouet, S.E.: Aeration strategy: a need for very high ethanol performance in *Saccharomyces cerevisiae* fed-batch process. Appl. Microbiol. Biotechnol. **63** (2004) 537-542.
2. Bai, F.W., Anderson, W.A. and Moo-Young, M.: Ethanol fermentation technologies from sugar and starch feedstocks. Biotechnology Advances **26** (2008) 89-105.
3. Pereira, F.B., Gumirães, P.M.R., Taxieira, J.A. and Domingues, L.: Optimization of low-cost medium for very high gravity ethanol fermentations by *S. cerevisiae* using statistical experimental disings. Bioresour. Technol. **101** (2010) 7856-7863.
4. Hahn-Hägerdal, B., Karhumaa, K., Larsson, C.U., Gorwa-Grauslund, M., Gorgens, J. and van Zyl, W.H.: Role of cultivation media in the development of yeast strains for large scale industrial use. Microb. Cell Fact. **4** (2005) 31.
5. Krajnc, D. and Glavič, P.: Assesment of different strategies for the co-production of bioethanol and beet sugar. Chem. Eng. Res. Des. **87** (2009) 1217-1231.
6. Reddy, L.V., Reddy, Y.H.K., Reddy, L.P.A. and Reddy, O.V.S.: Wine production by novel yeast biocatalyst prepared by immobilization on watermelon (*Citrullus vulgaris*) rind pieces and characterization of volatile compounds. Process Biochem. **43** (2008) 748-752.
7. Razmovski, R. and Pejin, D.: Immobilization of *Saccharomyces diastaticus* on wood chips for ethanol production. Folia Microbiol. **41** (1996) 201-207.
8. Kourkoutas, Y., Kanellaki, M. and Koutinas, A.A.: Apple peaces as immobilization support of various microorganisms. LWT **39** (2006) 980-986.
9. Plessas, S., Bekatorou, A., Koutinas, A.A., Soupioni, M., Banat, I.M. and Marchant, R.: Use of *Saccharomyces cerevisiae* cells immobilized on orange peel as biocatalyst for alcoholic fermentation. Bioresour. Technol. **98** (2007) 860-865.

10. Santos, D.T, Sarrouh, B.F., Rivaldi, J.D., Converti, A. and Silva, S.S.: Use of sugarcane bagasse as biomaterial for cell immobilization for xylitol production. J. Food Eng. **86** (2008) 542-548.
11. Liang, L., Zhang, Y., Zhang, L., Zhu, M., Liang, S. and Huang, Y.: Study of sugarcane pieces as yeast supports for ethanol production from sugarcane juice and molasses. J. Ind. Microbiol. Biotechnol. **35** (2008) 1605-1613.
12. Genisheva, Z., Mussatto, S.I., Oliveira, J.M., Teixeria, J.A.: Evaluating the potential of wine-making residues and corn cobs as support materials for cells immobilization for ethanol production. Ind. Crops Prod. **34** (2010) 979-985.
13. Razmovski, R. and Vučurović**,** V.: Bioethanol production from sugar beet molasses and thick juice using *Saccharomyces cerevisiae* immobilized on maize stem ground tissue. Fuel **92** (2012) 1-8.
14. Yu, J., Zhang, X. and Tan, T.: A novel immobilization method of Saccharomyces cerevisiae to sorghum bagasse for ethanol production. J. Biotechnol. **129** (2007) 415-420.
15. Vučurović, V. and Razmovski, R.: Sugar beet pulp as support for *Saccharomyces cerevisiae* immobilization in bioethanol production. Ind. Crops Prod. **39** (2012) 128-134.
16. Miller, G.L.: Use of dinitrosalycilic acid for determining reducing sugar. Anal. Chem. **31** (1959) 426–428.
17. Associattion of official Analitical Chemists International, 17th edition. Gaithersburg, AOAC, (2000) Official Method 942.06.
18. Fujii, N., Sakurai, A., Onjoh, K. and Sakakibara, M.: Influence of surface characteristics of cellulose carriers on ethanol production by immobilized yeast cells. Process Biochem. **34** (1999)147-152.
19. Walker, G.: Yeast physiology and biotechnology. John Wiley and Sons, Chichester, England (1998) p.146.

## АЛКОХОЛНА ФЕРМЕНТАЦИЈА МЕЛАСЕ ПОМОЋУ ЋЕЛИЈА *Saccharomyces cerevisiae* ИМОБИЛИСАНИХ НА РЕЗАНЦИМА ШЕЋЕРНЕ РЕПЕ

*Весна М. Вучуровић* и Радојка Н. Размовски*

Универзитет у Новом Саду, Технолошки факултет, 21000 Нови Сад, Булевар Цара Лазара 1, Србија

Природна адхезија *Saccharomyces cerevisiae* на резанцима шећерне репе (SBP) је једноставан и јефтин метод имобилизације којим се одржава висока густина квасца у ферментационом систему. У овом раду су ћелије квасца имобилисане адхезијом у синтетском медијуму под различитим условима имобилизације: концентрација глукозе (100, 120 и 150 g/l), концентрација инокулума (5, 10 и 15 g/l) и температура (25, 30, 35 и 40$^{o}$C). У циљу процене оптималних услова имобилизације након сваког поступка имобилизације анализиран је остварени степен имобилизације (*R*). Највиша вредност степена имобилизације биокатализатора од 0,486 g суве масе

APTEFF, 43, X-XXX (2012) UDC: 664.151.2:582.282.23:663.142
DOI: 10.2298/APT1243325V BIBLID: 1450-7188 (2012) 43, 325-333
Original scientific paper

квасца по g суве масе SBP је остварена при температури 30$^{o}$C, концентрацији глукозе од 150 g/l и концентрацији инокулума 15 g/l. Овако имобилисани квасац је примењен за ферментацију меласе шећерне репе почетне концентрације редукујућих шећера 150,2 g/l. У овом раду је приказана ефикасна алкохолна ферментација меласе (концентрација етанола од 70.57 g/l, ефикасност ферментације од 93.98%) применом ћелија *S. cerevisiae* имобилисаних природном адхезијом на SBP.

**Кључне речи:** имобилизација, биоетанол, резанци шећерне репе, меласа, *Saccharomyces cerevisiae*

Received:04 June 2012
Accepted: 12 September 2012