Production of bio-ethanol by free and HEC immobilized yeast cells

Velickova E.,[#] Winkelhausen E.[*] and Kuzmanova S.
University SS Cyril and Methodius, Skopje, R. Macedonia
[#] elenavelickova@yahoo.com

INTRODUCTION

In the last few years a new round of interest in biomass and bioenergy has been initiated. The depletion of the oil reserve is going much faster than predicted and the environmental deterioration resulting from the over-consumption of petroleum-derived products is threatening the sustainability of human society (Bai et al. 2008). Hence, the use of bio-ethanol, as an alternative liquid fuel is continuously growing. The most common method for ethanol production is fermentation of sugars by yeasts. Any improvement in the technology of ethanol production will be of a huge economic importance (Hahn-Hägerdal et al. 2006). Immobilized yeast cell systems are expected to have technical and economical advantages compared with the free cell systems.

In this study, immobilized *Saccharomyces cerevisiae* was investigated for ethanol production. A new lyophilization technique for immobilization of the yeast cells in hydroxyethylcellulose (HEC) was used. The immobilization efficiency and cell retention were used to assess the ability of HEC cryogels to serve as immobilization matrices for *S. cerevisiae.*

MATERIALS AND METHODS

Yeast and medium preparation. The yeast used in this study was a commercial grade baker's yeast, *Saccharomyces cerevisiae.* The cells were grown in a medium with following composition (per litre distilled water): 10 g yeast extract, 2 g KH_2PO_4, 1 g NaCl, 0.2 g $CaCl_2 2H_2O$, 1.7 g $MgSO_4 7H_2O$, 0.01 g $FeCl_3 6H_2O$, 2 g NH_4Cl and 50 g glucose. The sugar and salt solutions were autoclaved separately at 121^{o}C for 15 min. Initial pH of both media was 5.

Immobilization of yeast cells in cryogels. Cells of *S. cerevisiae* were immobilized in cryogels of 2-hydroxyethylcellulose (HEC) containing trimethylammoniumchloride (BBTMAC) as photoinitiator. Homogenized cell suspension, containing 10% (w/v) with respect to the polymer solution, was added under stirring at room temperature to the 2% (w/w) aqueous solution of HEC and vortexed to improve homogenization. The resulting homogeneous solution was poured into Teflon dishes (2 cm diameter) forming about 2.5 mm thick layer and frozen at -30^{o}C for 2 h. The dishes were then irradiated with UV light by Dymax 5000-EC curing equipment with 400 W metal halide flood lamp, for 2 minutes on both sides. After immobilization procedure, for revitalization of the cells, the cryogels were placed in 250-ml flasks with 100 ml nutrient medium for 20 hours on a rotary shaker (100 rpm) at 30^{o}C. After that, the gels were used for fermentation of glucose to ethanol.

Fermentation. Fermentations were performed in 250-ml cotton plugged shake flasks containing 100 ml medium. Initial glucose ranged from 50 to 200 g/l. The flasks were placed on a rotary shaker (150 rpm) at 30^{o}C. The batch fermentations with immobilized cells of *S. cerevisiae* were

performed under the same conditions, except that the shaking was 100 rpm. All the fermentations were performed in duplicate.

Analytical methods. Samples of approximately 2 ml were collected periodically for analysis. Free cell concentration was estimated turbidometrically at 620 nm using a calibration curve to relate the optical density units to dry cell concentration. The biomass concentration in the cryogels was estimated using Kjeldahl method. Gels used for this procedure were removed from the fermentation medium, blotted with filter paper and weighed before pyrolysis. Ethanol concentration was measured by gas chromatography using Varian CP 3800 model with a capillary column WCOT fused silica (30m x 0.32mm), equipped with flame ionization detector (FID). The injector and detector temperatures were 250^{o}C, and the column temperature was 200^{o}C. Glucose was determined by Miller method using DNS. Product yield was calculated as mass of the product formed per mass of the substrate used. Cell retention, R_c was determined by dividing the mass of entrapped cells with the mass of the matrix (gel) used for cell immobilization. The immobilization efficiency, η, represents the ratio of the concentration of the immobilized cells to the concentration of the total cells, immobilized plus free cells.

RESULTS AND DISCUSSION

Production of ethanol by free yeast cells. The effect of initial substrate concentration on the biosynthesis of ethanol by *S. cerevisiae* is presented in Table 1. When the initial glucose concentration was increased from 50 to 200 g/l, the yeast accumulated more biomass and ethanol, depleting the glucose. However, ethanol yield decreased.

Glucose concentration (g/l)	Glucose consumption (%)	Cell mass (g/l)	Ethanol concentration (g/l)	Ethanol yield (g/g)	Time (h)
50	99.1	5.4	21.3	0.43	12
100	98.3	8.6	35.4	0.36	24
200	97.1	10.9	66.0	0.34	42

Table 1 : Effect of glucose concentration on growth and product formation of *S. cerevisiae*

Production of ethanol by immobilized *S. cerevisiae* cells. It has been known that during fermentation yeast cells suffer from various stresses. One of them coming from the cell metabolism is ethanol accumulation in the medium. Ethanol inhibits the yeast cell growth and consequently its own biosynthesis since ethanol production is tightly coupled with yeast cell growth (Bai et al. 2008). It has been assumed that the inhibitory effect of ethanol produced will be reduced by entrapment of the yeast cells into a gel because the gel acts as a protector. For this purpose *S. cerevisiae* cells were immobilized in hydroxyethylcellulose cryogels. These cryogels possess macroporous structure with large interconnected pores surrounded by dense walls which impart opacity to the material. HEC is biodegradable and has low cost, properties which are of great interest for large scale production (Petrov et al. 2007).

The first experiment with the immobilized *S. cerevisiae* in HEC was performed with initial glucose concentration of 50 g/l and 10 pieces of cryogels in 100 ml medium during 24 hours. As can be seen from Fig. 1, after 24 h, immobilized *S. cerevisiae* consumed 63.7% of the glucose and

produced 10.2 g ethanol/l with yield of 0.31 g ethanol/g consumed glucose. These values are lower than the corresponding values of the free cell system (Table 1). The final concentration of cells released in the medium from the cryogels was 1.8 g/l.

The second experiment with the immobilized *S. cerevisiae* cells in HEC cryogels was performed with initial glucose concentration of 100 g/l and 40 gels in 100 ml medium during 72 hours. Two important parameters which characterize this immobilized system, cell retention and immobilization efficiency are presented in Table 2. The values of the immobilization efficiency and cell retention confirmed the ability of HEC cryogels to serve as immobilization matrices for *S. cerevisiae.* Both parameters indicated that the cryogels are stable, suggesting possible use of the gels for continuous or repeated batch production of ethanol. When sorghum bagasse without pre-treatment was used for immobilization of *S. cerevisiae*, R_c was 0.6 g dry cell mass/g dry bagasse (Jianliang et al. 2007).

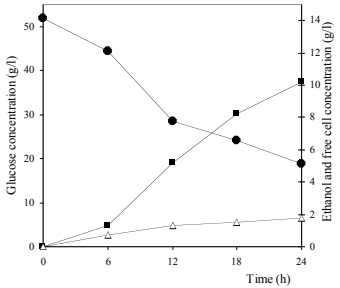

Figure 1: Glucose (•), ethanol (■) and free cell (△) concentration during fermentation with immobilized *S. cerevisiae* cells

Time (h)	0	12	24	48	60	72
R_c (g cells/g gel)	0.11	0.17	0.23	0.20	0.20	0.18
η	1.00	0.94	0.94	0.92	0.92	0.91

Table 2 : Cell retention (R_c) and efficiency of immobilization (η) performed with *S. cerevisiae* cells

Kinetic profiles of glucose consumption, ethanol formation and cell growth are presented in Fig. 2.

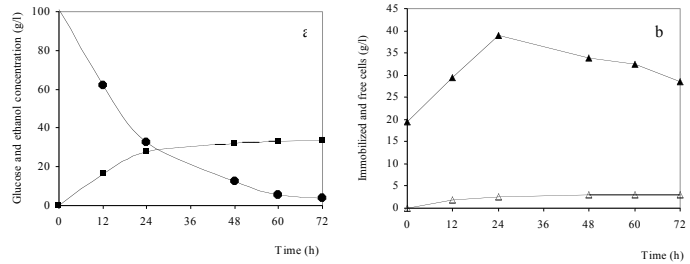

Figure 2: Fermentation profile of immobilized *S. cerevisiae* cells during ethanol production. Concentration of (•) glucose, (■) ethanol, (▲) immobilized cells and (△) free cells

During the first 24 h, fast consumption of glucose was observed (68%) which coincided with fast ethanol production and cell growth in the polymer carrier. The concentration of the immobilized cells (38.9 g immobilized cells/l medium) peaked at the 24th hour. The cell free concentration gradually increased but did not surpassed 3 g free cells/l medium which was 10% of the average concentration of the immobilized cells. The maximum ethanol yield was 0.40 g ethanol/g glucose (79% of the theoretical yield) while the corresponding productivity was 1.15 g ethanol/lh. It is important to note that the HEC cryogels can be successfully used for immobilization of the yeast cells, and consequently for ethanol production.

CONCLUSION

The initial substrate concentration affected ethanol production by *S. cerevisiae.* A new lyophilization technique for immobilization of *S. cerevisiae* cells in hydroxyethylcellulose was investigated and selected glucose concentrations were used to evaluate the ability of immobilized cells for ethanol production. The immobilization efficiency and cell retention values obtained confirmed the ability of the HEC cryogels to serve as immobilization matrices indicating that the cryogels are stable and can be reused in repeated batch cultures for ethanol production. In the batch ethanol production by immobilized *S. cerevisiae* cells with initial glucose concentration of 100 g/l, ethanol yield was 79% of the theoretical yield, and the productivity was 1.15 g ethanol/lh.

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REFERENCES

Bai F.W. et al. (2008) *Ethanol fermentation technologies from sugar and starch feedstocks.* Biotechnology Advances 26 (1) 89-105

Hahn-Hägerdal B. at al. (2006) *Bio-ethanol – the fuel of tomorrow from the residues today.* Trends in Biotechnology 24 (12) 549-556

Jianliang Y. et al. (2007) *A novel immobilization method of* Saccharomyces cerevisiae *to sorghum bagasse for ethanol production.* Journal of Biotechnology 129 (3) 415-420

Petrov P. et al. (2007) *Synthesis of biodegradable hydroxyethylcellulose cryogels by UV irradiation.* Polymer 48 (17) 4943- 4949