P-028	**Double-layered polymer gels intended for entrapment of yeast cells**	

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INTRODUCTION AND OBJECTIVES

The numerous advantages that an immobilization technique, like the cell entrapment, offers over the system with suspended cells are well known, but still there are problems that need to be overcome. Cells located on the periphery of the mono-layered gels multiply and subsequently get loose into medium. In the attempt to prevent the leakage, a mono-layered gel should be coated with an outer layer, shell, that does not contain cells creating in that way double-layered gels (Liuoni 2008).
The objective of this study was to synthesize a double-layered gel that will reduce the cell release from the matrix. We synthesized double-layered gels that consist of a cryogel core and a hydrogel shell. The cryogel core was made from hydroxyethylcellulose (HEC), while the coating was made from poly(ethylene oxide) (PEO). The immobilization efficiency and cell retention were used to assess the ability of double-layered gels to maintain the cells within the matrix.

MATERIAL 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 $CaCl_2 \cdot 2H_2O$, 1.7 g $MgSO_4 \cdot 7H_2O$, 0.01 g $FeCl_3 \cdot 6H_2O$, 2 g NH_4Cl and 50 g glucose. The sugar and salt solutions were autoclaved separately at 121°C for 15 min. Initial pH of both media was 5.

Immobilization of yeast cells in double-layered gels Cells of *S. cerevisiae* were immobilized in cryogels of 2-hydroxyethylcellulose containing trimethylammonium-chloride (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. The resulting solution was poured into Teflon dishes (2 cm diameter) forming about 2.0 mm thick layer and frozen at -30°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. Double-layered gels consisted of HEC core and PEO shell. One millilitre of the homogeneous solution of PEO was poured into polystyrene dishes (35 mm diameter), then the cryogel core was placed and covered with another millilitre of PEO Afterwards the dishes were UV irradiated for 2 minutes on both sides at room temperature, resulting in gels with 2 mm thick core and 1 mm thick outer layer.

Diffusion measurements These measurements were performed in a Plexiglas chamber consisting of two separate cells with double jacketed walls at 28 ± 1°C. One of the cells was filled with distilled water and used as an acceptor cell and the other one was a donor cell filled with the solution examined. Both cells were stirred mechanically. Samples were withdrawn periodically. Before the start of the diffusion measurements the membranes were mounted in the chamber. Plotting the values for the total amount of transferred solutes, glucose and ethanol, through the membrane, Q, against time creates a graph that approaches a straight line, which intercepts the time-axis and can be presented by equation (1):

$$Q = \frac{ADC_0}{l}\left(t_s - \frac{l^2}{6D}\right) \qquad (1)$$

where A is the membrane area (cm^2), C_0 is the initial concentration of the solution (g/cm^3), D represents the diffusion coefficient (cm^2/s), l is the membrane thickness (cm), t_s is the time of sampling (s).

Fermentation After immobilization procedure, for revitalization of the cells, the double-layered gels were placed in 250-ml flasks with 100 ml nutrient medium for 20 hours on a rotary shaker (100 rpm) at 28°C. After that, the gels were used for fermentation of glucose. Batch fermentation was carried out in 100 ml nutrient medium placed in a 250 ml Erlenmeyer flask on a rotary shaker (100 rpm) at 28 °C.

Analytical methods Samples of approximately 2 ml were collected for analysis. Free cell concentration was estimated turbidometrically at 620 nm. The biomass concentration in the double-layered gels was estimated using Kjeldahl method. Gels used for this procedure were removed from the fermentation medium, blotted with filter paper and weighed before pyrolysis. 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

The synthesized double-layered gel consisting of hydroxyethylcellulose cryogel core with immobilized cells and a second layer of poly(ethylene oxide) hydrogel is shown in Fig. 1.

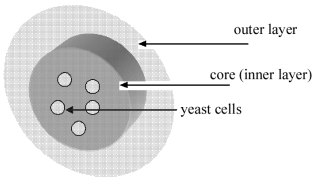

Figure 1. Drawing of a double-layered gel

Diffusion coefficient The main feature of both hydrogels and cryogels is their ability to absorb and hold in their structure an amount of the solvent. The transport of the solute molecules is an important aspect of gels because of their wide applicability in different technologies (Khare 1995). Other researchers such as Perez (1995) and Jang (2007) already determined that the diffusion coefficient for glucose through PEO were $1.73 \cdot 10^{-6}$ cm^2/s and $2 \cdot 10^{-6}$ cm^2/s. In this study the focus was on the diffusivity of HEC cryogel. The diffusion coefficients for glucose and ethanol through the HEC cryogel were $3.9 \cdot 10^{-6}$ cm^2/s and $0.97 \cdot 10^{-5}$ cm^2/s, respectively. The diffusion coefficient of glucose through the gel with immobilized cells had a value of $6.9 \cdot 10^{-6}$ cm^2/s, proving that the cells inside the matrix are not a barrier for the transport of the solute molecules. The obtained diffusion coefficients for glucose and ethanol in the gel are similar to the diffusion rate of these solutes in liquids (Hannoun 1986), but also within the comparable range of other solutes: ascorbic acid ($1.82 \cdot 10^{-6}$ cm^2/s), vitamin B_{12} ($3.6 \cdot 10^{-6}$ cm^2/s), dextran ($0.89 \cdot 10^{-6}$ cm^2/s) and theophylline ($6 \cdot 10^{-6}$ cm^2/s) (Peppas 1996, Russell 2001).

Entrapment efficiency Two important parameters characterizing the efficiency of cell immobilization, cell retention (Rc) and immobilization efficiency (η) are presented in Fig. 2. It was obvious that the cells followed different behavior in the mono and in the double-layered gels. In the mono-layered gels they doubled in the first 24 hours but then the cells started leaking from the matrix corresponding to the lower immobilization efficiency of this matrix. On the other hand, in the double-layered gels, cell concentration doubled in the first 6 hours and continued to grow slowly till the end of the fermentation, proving that double-layered gels had better cell retention. The immobilization efficiency for the double-layered gels was around 0.97±0.02, which is 1.16 times higher than the immobilization efficiency for the single layered gels indicating that the second layer played significant role in preventing the cell leakage of the entrapped cells.

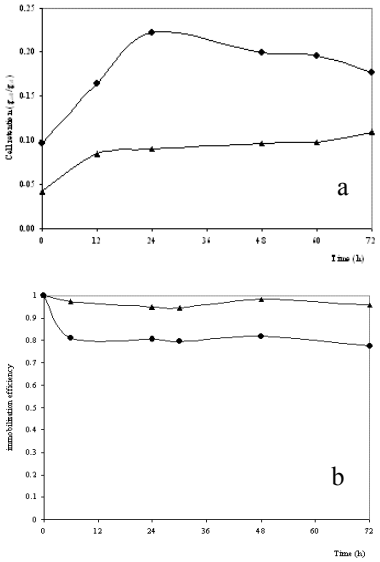

Figure 2. a) Cell retention during 72 h fermentation and b) immobilization efficiency of single-layered (-●-) and double-layered (-▲-) gels.

CONCLUSION

It was proven that double-layered gels synthesized with UV-curing of different polymer solutions (HEC and PEO) are suitable for whole cell immobilization. The diffusion coefficients for glucose and ethanol through the hydroxyethylcellulose cryogel were $3.9 \cdot 10^{-6}$ cm^2/s and $0.97 \cdot 10^{-5}$ cm^2/s. The immobilization efficiency of the entrapped cells into the double-layered gels was higher than the efficiency of mono-layered gels.

REFERENCES

• Hannoun B.J.M. et al. (1986) *Diffusion coefficients of glucose and ethanol in cell-free and cell-occupied calcium alginate membranes*. Biotechnology and Bioengineering 28 (6) 829-835

• Jang S.S. et al. (2007) *Mechanical and transport properties of the poly(ethylene oxide)-poly(acrylic acid) double network hydrogel from molecular dynamic simulations*. Journal of Physical Chemistry B 111(7) 1729-1737

• Khare A.R. et al. (1995) *Swelling/deswelling of anionic copolymer gels*. Biomaterials 16(7) 559–567

• Liouni M. et al. (2008) *Studies of the mechanical properties and the fermentation behavior of double layer alginate-chitosan beads, using* Saccharomyces cerevisiae *entrapped cells*. World Journal of Microbiology and Biotechnology 24(2) 281-288

• Peppas N.A. et al. (1996) *Diffusion of small molecular weight drugs in radiation-crosslinked poly(ethylene oxide) hydrogels*. Journal of Controlled Release 42, 195-202

• Perez E. P. (1995) *PhD thesis*, Massachusetts Institute of Technology

• Russell R.J. et al. (2001) *Mass transfer in rapidly photopolymerized poly(ethylene glycol) hydrogels used for chemical sensing*. Polymer 42 (11) 4893- 4901