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# Entrapment of *Saccharomyces cerevisiae* cells in u.v. crosslinked hydroxyethylcellulose/poly(ethylene oxide) double-layered gels

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ABSTRACT

Double-layered gels, consisting of hydroxyethylcellulose cryogel core and poly(ethylene oxide) hydrogel shell, were synthesized with u.v. irradiation, using the same photoinitiator, (4-benzoylbenzyl) trimethylammoniumchloride (BBTMAC) for the both layers. The gels were characterized by measuring their rheological parameters, gel fraction yield, the degree of equilibrium swelling and diffusion coefficient. The diffusion coefficients for glucose and ethanol through the hydroxyethylcellulose cryogel were $3.9 \times 10^{-6}$ cm$^2$/s and $0.97 \times 10^{-5}$ cm$^2$/s, respectively. The applicability of these double-layered gels as carriers for immobilization was investigated by entrapment of *Saccharomyces cerevisiae* cells. The immobilization efficiency and cell retention were determined in batch fermentation for ethanol production from glucose. The operational stability of the gels was evaluated in batch fermentation with three consecutive runs. The ethanol yield was in the range from 60% to 77% of the theoretical yield.


## 1. Introduction

Hydrogels are three-dimensional, cross-linked networks of water-soluble polymers. They can be synthesized from any water-soluble polymer, encompassing a wide range of chemical compositions and bulk physical properties. Their highly porous structure can easily be tuned by controlling the density of cross-links in the gel matrix and the affinity of the hydrogels for the aqueous environment in which they are swollen [1]. Keeping in mind these characteristics, researchers turned to the synthesis of hydrogels, based on natural or synthetic precursors [2–5]. The good properties of the hydrogels provide them wide industrial applications, such as the controlled release of drugs, modern soft contact lenses or superabsorbents for personal hygiene products [6]. Their broad application has pushed multinational groups toward development and synthesis of new generation of hydrogels, also known as cryogels [7]. Cryogels are synthesized in semi-frozen aqueous medium where ice crystals act as porogens and templates for the large interconnected pore that become available after melting the ice [8]. As an alternative technology in the production process, irradiation techniques have been used. The irradiation method is determined only by the absorbed dose, which means by the irradiation time [9] and therefore it is being used increasingly around the world because of its processing speed and simplicity in synthesis [10]. Both, hydrogels and cryogels, with this simple and gentle procedure can be produced from practically any gel-forming system with a broad range of porosity thus providing a broad range of applications in cell immobilization, bioseparations, chromatography and biomedical application [11].

Modification of the biotechnological processes by using immobilized biocatalysts, over the years has gained attention of many biotechnologists, because such biocatalysts display better operational stability and higher efficiency. The selection of a proper support matrix is of a great importance for successful performance of the immobilized cell system. The cell carrier should posses good permeability, chemical, mechanical and thermal stability, suitable shape and insolubility [12]. Each of the carriers have certain advantages and disadvantages in relation to their influence on the entrapped cells as well as on the physico-chemical and biological stability of the resultant matrix, simplicity of preparation and the availability and cost of raw materials [13].

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. Hence, a mixed system is formed consisting of immobilized and free cells. In the attempt to prevent the leakage, a mono-layered gel should be coated with an outer layer, shell, that does not contain cells

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creating in that way double-layered gels. Therefore, the aim of this study was to synthesize a double-layered gel that will preserve the viability of the cells and reduce their leakage from the matrix. We have managed to synthesize double-layered gels that consist of a cryogel core and a hydrogel shell. Both, the hydrogels and the cryogels, were synthesized by simple and mild procedure through the u.v. irradiation in the presence of the same photoinitiator, (4-benzoylbenzyl) trimethylammoniumchloride. The natural polymer, hydroxyethylcellulose, was chosen as an inexpensive and nontoxic to microorganism, for the cell entrapment [14]. For the coating poly(ethylene oxide) was used due to its non-toxicity, biocompatibility, strength, absorbency and flexibility, additionally modified with different crosslinkers. The obtained double-layered gels, without and with cells, were characterized by gel fraction yield, equilibrium swelling, rheology and diffusion properties, while the viability of the entrapped cells was tested by their ability to produce ethanol in an alcoholic batch fermentation.

## 2. Experimental

### 2.1. Materials

Natrosol (2-hydroxyethylcellulose) (Mn = $1.3 \times 10^6$ g/mol) and polyethylene oxide (Mn = $1 \times 10^6$ g/mol) were obtained from Union Carbide Chemicals and Plastics Co. The (4-benzoylbenzyl) trimethylammoniumchloride (BBTMAC) purchased from Aldrich was used as a photoinitiator, without further purification. The chemicals used as crosslinkers, N,N′-methylenediacrylamide (bisAA) and tetraethyleneglycoldimethacrylate (TEGDMA), were bought from Merck and Fluka, respectfully, and were used without purification. All other chemicals used were purchased from commercial sources and were of analytical grade.

### 2.2. Microorganism and media

Glucose medium with commercial grade baker's yeast, *Saccharomyces cerevisiae*, with 29.4% dry biomass, was used for ethanol production. Prior to batch fermentation, the cells were cultured for 20 h in a nutrient medium with the following composition (per litre of 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 20 g glucose. In the batch production of ethanol the glucose concentration was increased to 100 g/l. The sugar and salt solutions were autoclaved separately at 121 °C for 15 min. The initial pH values of both media were 5.

### 2.3. Preparation of gels, without and with yeast cells

#### 2.3.1. Synthesis of mono-layered cryogels

Hydroxyethylcellulose was dissolved in an appropriate amount of distilled water to obtain 2% aqueous solution at 40 °C. Then the solution was kept for 24 h at room temperature to ensure complete dissolution and homogeneity. Given amount of photoinitiator, BBTMAC, (2 wt.% of the polymer mass) dissolved in 1 ml distilled water was added under stirring at room temperature. The homogeneous solution was poured into Teflon dishes (20 mm diameter) forming a 2–3 mm thick layer, followed by freezing step at −30 °C for 2 h. Afterward the dishes were quickly placed in a temperature-controlled chamber and the polymer was u.v. irradiated in a Dimax 5000-EC u.v. light curing system for 2 min. The gels in the Teflon dishes were left at room temperature for few minutes, turned over the other side and placed back in the freezer for additional 2 h, when they were u.v. irradiated again on the other side for 2 min under same conditions.

For the formation of the immobilized biocatalysts, homogenized cell suspension, containing 15% (w/v) cells 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 gelation process followed the same procedure used for preparation of pure cryogels.

#### 2.3.2. Synthesis of double-layered gels

Double-layered gels consisted of hydroxyethylcellulose core and poly(ethylene oxide) shell. The core, without and with cells, was synthesized according to the previously described procedure. One millilitre of the homogeneous solution of poly(ethylene oxide) was poured into polystyrene dishes (35 mm diameter), then the cryogel core was placed and covered with another millilitre of poly(ethylene oxide). Afterwards the dishes were quickly placed in a temperature-controlled chamber and the polymer was u.v. irradiated in a Dimax 5000-EC u.v. light curing system for 2 min on both sides at room temperature, resulting in gels with 2 mm thick core and 1 mm thick outer layer. Several different concentrations of the polymer solution with and without cross-linking agent were tested.

### 2.4. Measurements of gel fraction yield and equilibrium degree of swelling

Gel fraction (GF) yield and equilibrium degree of swelling (ES) of double-layered gels were determined gravimetrically. The gel fraction content in the dried sample was estimated by weighing the insoluble part after extraction in distilled water for 6 days at room temperature:

$$\text{Gel fraction} = \frac{\text{Mass of the dried sample after extraction}}{\text{Initial mass of the polymer}} \cdot 100\ (\%) \quad (1)$$

The equilibrium degree of swelling (ES) was determined at room temperature. Disks of dried gel were equilibrated in distilled water for 48 h, removed from the water, blotted with filter paper and weighed. They were then dried to constant mass under vacuum and weighed again. The ES is presented as grams of swollen gel sample per gram of dried gel sample:

$$\text{Equilibrium swelling} = \frac{\text{Mass of equilibrated swollen sample}}{\text{Mass of dry sample}} \quad (2)$$

### 2.5. Dynamic rheological measurements

Dynamic rheological measurements of the cellulose cryogels were performed on a ThermoHaake Rheostress 600 rheometer with a parallel plate sensor system (20 mm diameter) and Peltier temperature controller. Disks of the gels were extracted for 6 days in distilled water, then removed from water and blotted with filter paper before the dynamic storage ($G'$) and loss ($G''$) moduli were measured in a frequency range of 0.1–10 Hz at 25 °C.

### 2.6. Scanning electron microscopy

For scanning electron microscopy (SEM) micrographs, samples were taken from fresh cryogels, without and with yeast cells, as well as from cultivated gels with immobilized cells. Composite cryogels were quenched in liquid nitrogen, freeze dried, and coated with gold in a Jeol JFC-1200 fine coater. At the end samples were examined by SEM using the model Jeol JSM-5510.

### 2.7. Diffusion measurements

These measurements were performed in a Plexiglas chamber consisting of two separate cells with double jacketed walls (Fig. 1). Each cell represented a cylinder of 200 ml volume. The temperature was kept at 28 ± 1 °C. The cells were held together with clams and the area around the junction was sealed with parafilm. 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. The membranes, tested for diffusion of glucose and ethanol, were made in special dishes with a diameter of 36 mm by the same procedure, previously described, for synthesis of mono-layered cryogels. Before the start of the diffusion measurements the membranes were mounted in the chamber and pre-swollen in distilled water for 24 h at room temperature.

The total amount of solute transferred increases linearly with time. Plotting the values for this amount of glucose and ethanol transferred through the membrane, $Q$, against $t$ creates a graph that approaches a straight line, which intercepts the time-axis (Fig. 2) and can be presented by:

$$Q = \frac{ADC_0}{l}\left(t_s - \frac{l^2}{6D}\right) \tag{3}$$

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 ($s$). The intercept of the linear part of the curve is referred to as the lag time. Rearranging the Eq. (3) the diffusion coefficient could be calculated using the following equation:

$$D = \frac{l^2}{6t} \tag{4}$$

### 2.8. Determination of entrapment efficiency

Characterization of the suitability of carriers for cell immobilization is done by determination of the cell retention and immobilization efficiency. Cell retention, $R_c$, was calculated by dividing the mass of the entrapped cells with the mass of the matrix used for cell immobilization. The immobilization efficiency, $\eta$, represents the ratio of the concentration of the immobilized cells to the concentration of the total cells, immobilized plus free cells.

### 2.9. Determination of the cell viability

The outer shell of the gel (PEO layer) was mechanically removed by cutting it off with sharp blades, while the inside core was degraded by cellulolytic enzymes as described elsewhere [8]. About 10 mg of freeze dried gel with entrapped cells were immersed in 10 ml of the enzyme solution for a given time. The enzyme concentration was 0.2 mg/ml. Tests were carried out at 30 °C, without shaking. After incubation, the samples were immersed in an excess of distilled water and extracted for 24 h to wash away the degraded polymer. Afterwards, the viability of the cells in the suspension was assessed by two methods, plate counting and dye exclusion method using Evans Blue. One drop of Evans Blue (0.25% w/v) was added to one drop of cell suspension on a glass slide. After 5 min the slide was washed twice with distilled water and then observed by a light microscope at 100×. Living cells should exclude the dye, whereas dead cells should be stained blue. All tests were repeated three times.

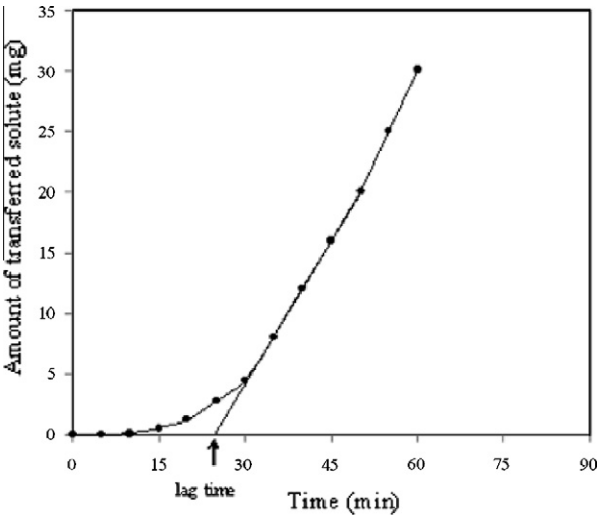


**Fig. 2.** Graphic presentation of lag time method for diffusion of solutes.

### 2.10. Fermentations

Batch fermentations with immobilized cells of *S. cerevisiae* in the single and double layered matrices were carried out in 100 ml nutrient medium placed in a 250 ml Erlenmeyer flask on a rotary shaker (100 rpm) at 28 °C. After 20 h of preculturing, the gels with immobilized cells were washed in sterile distilled water and transferred into fresh nutrient medium for ethanol fermentation, under same conditions. The same number of gel pieces, 40, was placed in each flask providing 40 ml:100 ml volume ratio of gel to medium.

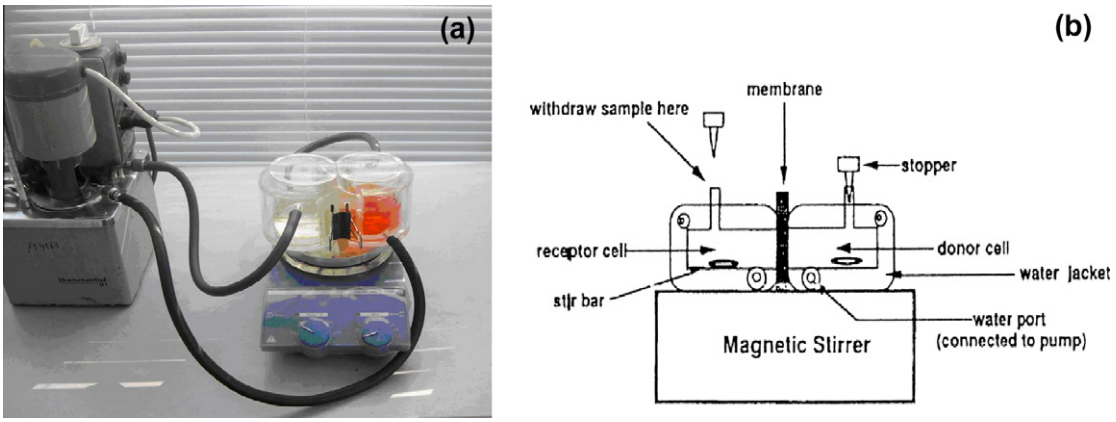


**Fig. 1.** Two compartment plexiglass chamber for diffusion measurements: (a) original setup and (b) sketch of the setup.

For repeated batch fermentation after every cycle the immobilized biocatalysts were recovered from the flasks and were immersed in fresh medium to start a new batch cycle.

### 2.11. Analytical methods

In batch fermentation, approximately 2 ml samples were collected for analysis. Dry weight of yeast cells in the gels was estimated by the Kjeldahl method. Gels used for this procedure were removed from the fermentation medium, blotted with filter paper and weighed before pyrolysis. A double-layered gel without cells was used as a blank sample. To determine the degree of cell leakage from the matrices, after the immobilization procedure, the cell concentration in the fermentation broth was calculated by measuring the absorbance of each sample withdrawn from the medium at 620 nm using spectrophotometer, Varian, Cary 50.

Ethanol was analyzed by gas chromatography using a Varian CP 3800 with a capillary column WCOT fused silica (30 m × 0.32 mm), CP WAX 52 CB, $d_i$ = 0.25 μm, equipped with flame ionization detector (FID) and a manual injector type 1709. The injector and detector temperatures were 250 °C, and the column temperature was 200 °C. Nitrogen was used as a carrier gas with a flow rate of 30 ml/min. Isopropanol was used as an internal standard.

Glucose concentration was determined using a reducing chemical reagent, 3,5-dinitrosalycilic acid solution following the procedure described by Miller.

Ethanol yield was calculated as mass of the ethanol formed per mass of the glucose consumed, while the volumetric productivity was calculated as mass of the ethanol formed per litre medium per hour. The experimental error of the measurements was ±3%. In some figures experimental error bars overlap the chart symbols.

## 3. Results and discussion

The cell leakage is one of the most serious problems that appear in cell entrapment and some researchers studied various methods to eliminate and prevent the cell loss, such as changing the cross-linking agent and preparing more concentrated polymer networks [15]. In this study, we tried to overcome the cell leakage by synthesizing double-layered gels. The synthesized double-layered gels consisting of hydroxyethylcellulose cryogel core with immobilized cells and a second layer of poly(ethylene oxide) hydrogel is shown in Fig. 3.

### 3.1. Characterization of double-layered gels

The effect of the concentration of the poly(ethylene oxide) in outer layer as well as the effect of different cross-linking agents and their concentration was investigated. The characterization of the obtained double-layered gels is presented in Table 1 and in Fig. 4. The properties of the single hydroxyethylcellulose cryogels were previously tested [14].

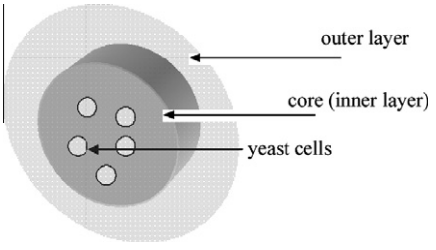


**Fig. 3.** Drawing of a double-layered gel.

**Table 1**
Effect of poly(ethylene oxide) and cross-linker concentration in the coating on the gel fraction yield and the degree of swelling of double-layered gel with hydroxyethylcellulose cryogel core.

| Sample | Second layer composition PEO:crosslinker% (w/v) | GF (%) | ES |
|---|---|---|---|
| PEO/bisAA | 3/5 | 58 | 39 |
| PEO/bisAA | 3/10 | 59 | 39 |
| PEO/TEGDMA | 3/5 | 81 | 53 |
| PEO/bisAA | 5/10 | 39 | 32 |

The results confirmed that the increase of the concentration of the cross-linking agent, N,N′-methylenediacrylamide, from 5% to 10% in respect to the polymer mass, in the systems 3/5% and 3/10% (w/v), gave same values for the gel fraction yield and the degree of swelling, but did have stronger influence on the storage modulus, $G'$. Twofold concentration increase of the cross-linking agent into the aqueous polymer solution of poly(ethylene oxide) brought to fourfold mechanical strengthening of the gels. Using the tetraethyleneglycoldimethacrylate as a cross-linking agent on the other hand, although having higher values for the gel fraction yield and the degree of swelling, unfortunately gave very pour mechanical properties. The same increase of the concentration of this agent resulted in no gelation of the outer layer and was therefore excluded from further experiments.

The rheological behaviour of double-layered gels was studied to identify the relationship between gels' composition and their performance under stress. The variation of both storage ($G'$) and loss modulus ($G''$) in the gels as a function of the different coating was also observed (Fig. 4). The rheological curves for the double-layered gels prepared from pure HEC cryogel core which outer layer consisted of 3% PEO additionally crosslinked with TEGDMA mechanically did not correspond to the increased gel fraction and suggested formation of inadequate gels for further testing. The rest of the double-layered gels prepared with hydroxyethylcellulose cryogel core and poly(ethylene oxide) shell additionally crosslinked with bisAA exhibited the behaviour of a true gel, with high $G'$ values and independence of the frequency of oscillation. Furthermore the increase of the concentration of PEO for just 2%, from 3% to 5%, resulted in ten times stronger gels, which is evident from the rheology measurements depicted in Fig. 4.

Usually the research is focused on the influence of cell entrapment in gels on cell viability and physiology, and relatively low attention is paid to the effect of immobilized microbial cells on properties of gel carriers [7,16]. In this study attention was paid to all factors affecting the functionality of the immobilized system. In our previous work [14] the best cell loading was already established and therefore the double-layered gels were prepared from core loaded with 15% cells coated with a second layer that did not have any entrapped cells. The values for the gel fraction yield and the swelling degree, as well as the behaviour of the gels under stress are given in Table 2 and in Fig. 5.

From the results presented in Table 2 and in Fig. 5 it was evident that the cell load did not have negative effect on the synthesis of double-layered gels and although there were no significant changes in the fraction yield and the swelling degree, the physical characteristics of the double-layered gels were notably improved. Previously, Jovanovic-Malinovska et al. [17] and Winkelhausen et al. [18] reported that entrapped *Candida boidinii* cells decreased the mechanical strength of the gels, whereas Velickova et al. [14] reported that *S. cerevisiae* cells acted as porous fillers and increased the mechanical properties of the gels.

The viability of the yeast cells was checked by plate counting and by staining the cells. Plate counting indicated that the yeast cells were viable. Microscopic evaluation of the cells using Evans Blue showed that the majority of the cells excluded the dye con-

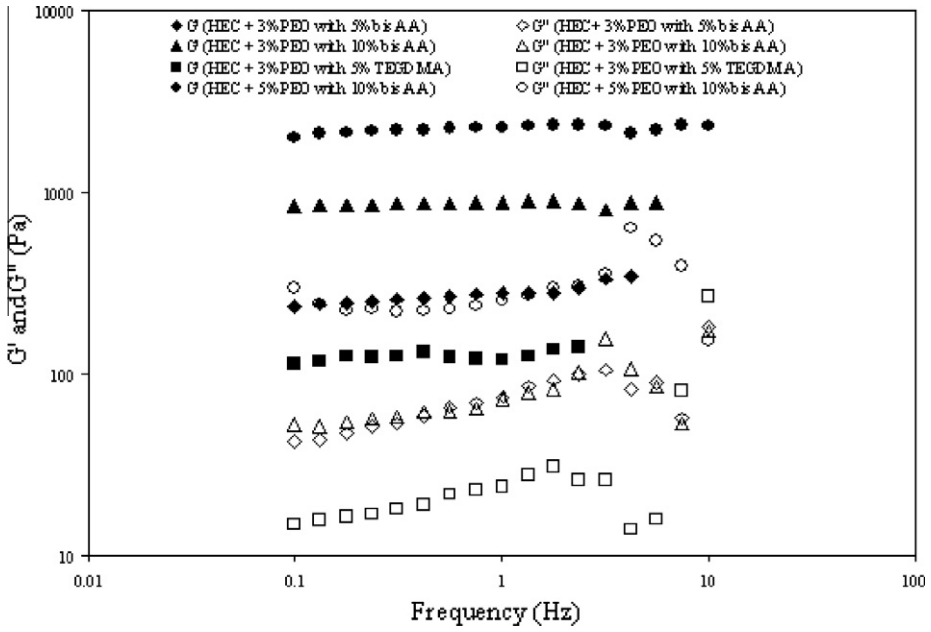


**Fig. 4.** Rheogram of pure double-layered gels with 2% hydroxyethylcellulose cryogel core and hydrogel shell synthesized with different concentration of poly(ethylene oxide) and use of different crosslinkers. Elastic modulus ($G'$) and storage modulus ($G''$) versus oscillating frequency.

**Table 2**
Characteristics of the synthesized double-layered gels with 15% entrapped cells in respect to the polymer solution.

| Sample | Second layer composition PEO:crosslinker% (w/v) | GF (%) | ES |
|---|---|---|---|
| PEO/bisAA | 3/5 | 39 | 42 |
| PEO/bisAA | 3/10 | 44 | 36 |
| PEO/bisAA | 5/10 | 36 | 31 |

firming again their viability. At each observation only few dead cells stained blue were spotted.

The scanning electron microscopy was used to determine the network structure of the double-layered gels, since it is suitable technique for characterizing the fine structure of porous materials. Fig. 6 presents the inner region of the double-layered gels without and with entrapped cells. This micrograph (Fig. 6a) clearly indicated that there is a difference between the centre part and the surface part of the double-layered gel. It can be seen that the structure and the pore size of the hydrogel and cryogel layer are different. The cryogel core had larger pores of approximately 200 μm, while the outer layer made of hydrogel had pores in the range between 50 and 100 μm. The size of the yeast cells was around 5–10 μm (Fig. 6b). It was obvious that the micropore structure of the carrier might favourably keep the microbial growth in internal pore and prevent cell leaking which leads to nice stability of the immobilized cells. The thickness of the pore walls along with their

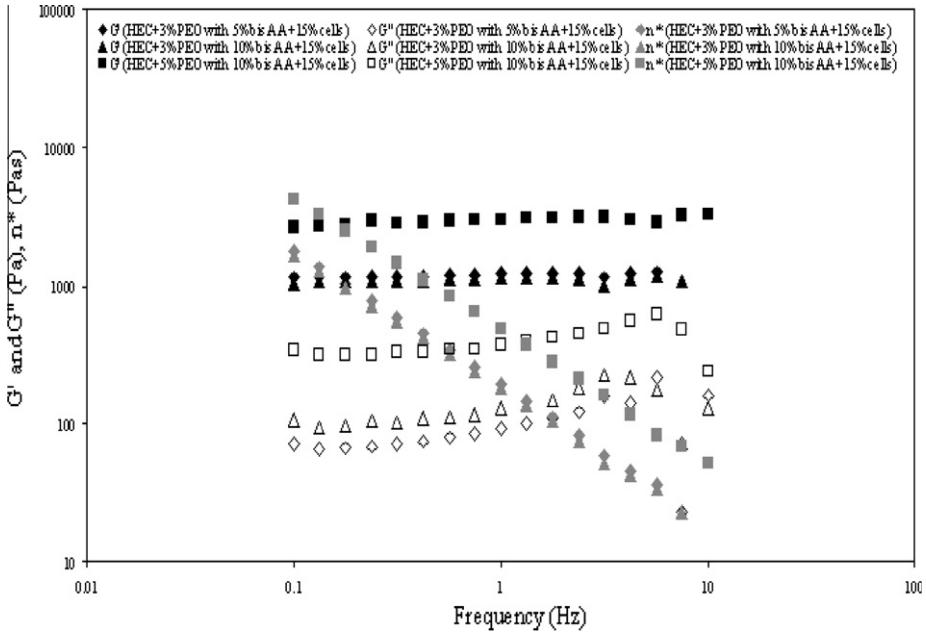


**Fig. 5.** Rheogram of double-layered gels with entrapped cells. Elastic modulus ($G'$), storage modulus ($G''$) and complex viscosity ($n^*$) versus oscillating frequency.

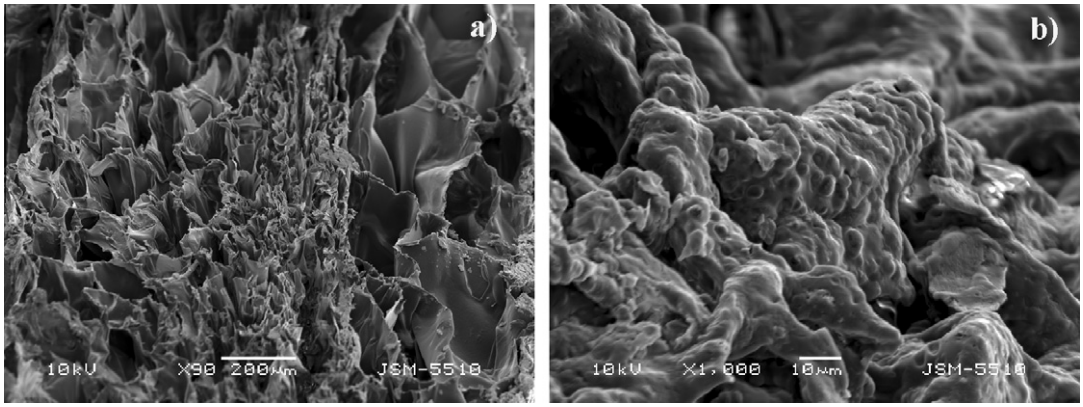


**Fig. 6.** SEM micrographs of double-layered gels with 15% entrapped cells to the polymer mass: (a) the structure of different layers and (b) inner region.

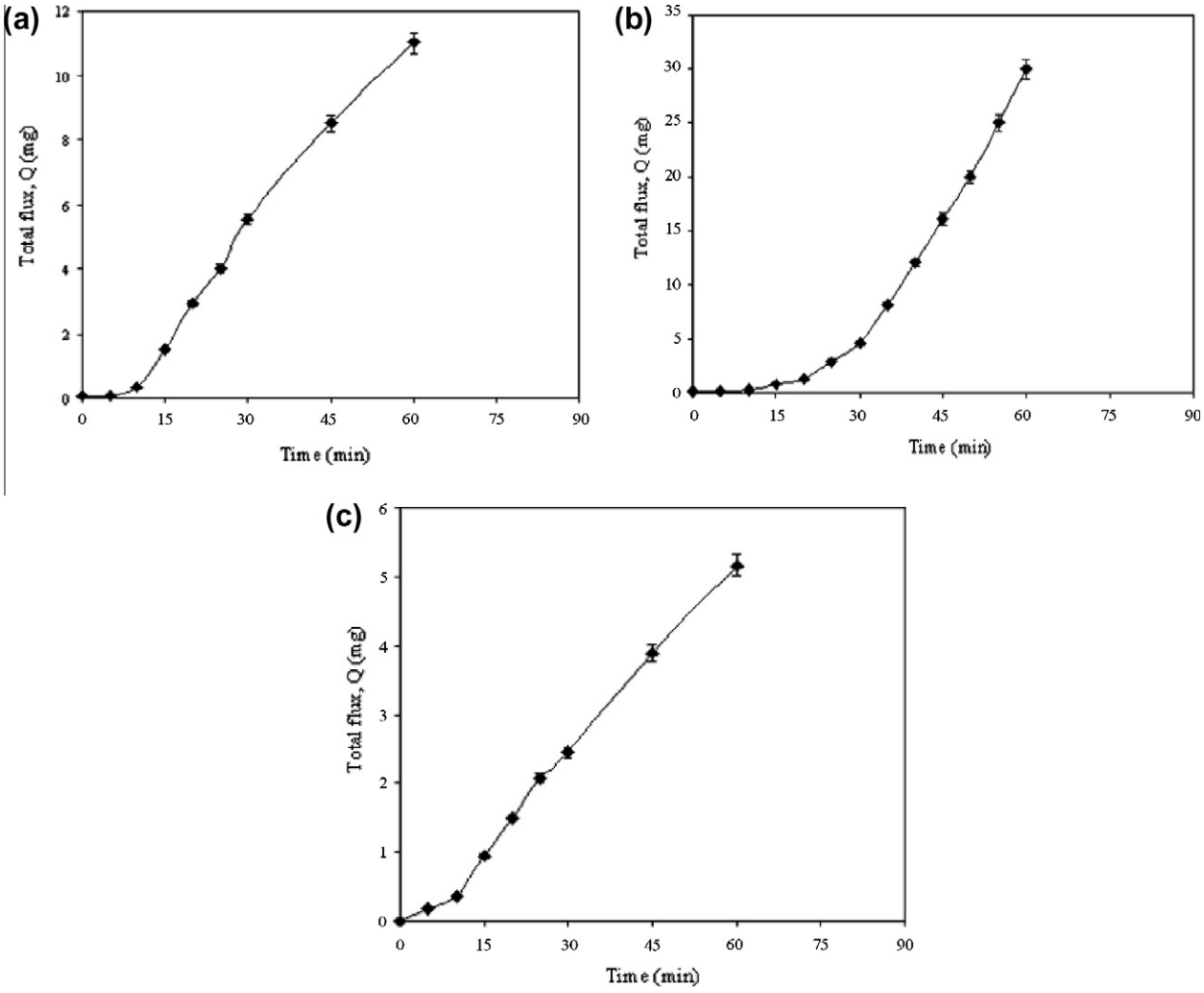


**Fig. 7.** Lag time for solute diffusion in 2% hydroxyethylcellulose cryogel: (a) glucose diffusion through pure gels, (b) glucose diffusion through gels with entrapped cells and (c) ethanol diffusion through pure gels (glucose concentration, $C_0$ = 100 g/l, ethanol concentration, $C_0$ = 81 g/l, $A$ = 5.3 cm$^2$, $l$ = 0.15 cm, $t_s$ = 60 min).

density determined the macroscopic mechanical properties of the gels.

### 3.2. 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 applicability for biomedical, pharmaceutical and other technology. From the numerous studies focusing on the variables affecting the solute transport, it is understood that the transport depends on mesh size, swelling ratio, ionization, ionic strength, pH values and temperature [19–21]. Other researchers such as Perez [22] and Jang et al. [23] already determined that

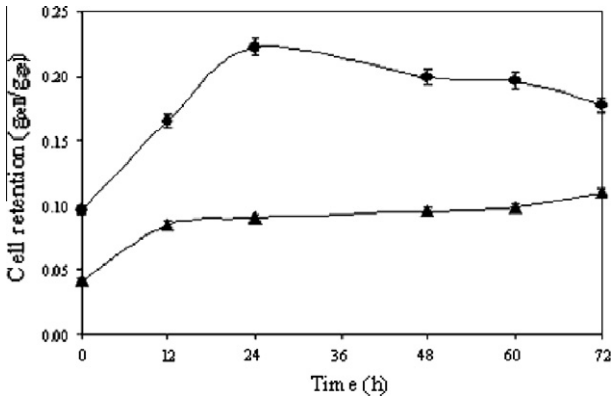


**Fig. 8.** Cell retention during 72 h fermentation within single-layered (-●-) and double-layered (-▲-) gels.

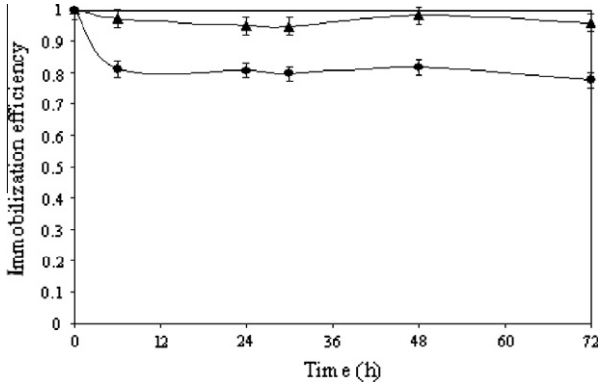


**Fig. 9.** Immobilization efficiency of single-layered (-●-) and double-layered (-▲-) gels.

the diffusion coefficient for glucose through poly(ethylene oxide) were $1.73 \times 10^{-6}$ cm$^2$/s and $2 \times 10^{-6}$ cm$^2$/s. In this study the focus was on the diffusivity of hydroxyethylcellulose cryogel. Fig. 7a and 7c represents the experimental graphs of the total amount of transferred solute versus the time for the diffusion of glucose and ethanol, in 2% hydroxyethylcellulose cryogel. Fig. 7b represents the diffusion of glucose through the gel loaded with yeast cells.

The diffusion coefficients for glucose and ethanol through the hydroxyethylcellulose cryogel were $3.9 \times 10^{-6}$ cm$^2$/s and $0.97 \times 10^{-5}$ cm$^2$/s, respectively. The diffusion coefficient of glucose through the gel with immobilized cells had a value of $6.9 \times 10^{-6}$ cm$^2$/s, proving that the cells inside the matrix are not a barrier for the transport of the solute molecules. It is evident that solutes such as glucose and ethanol with diameters of 7.2 and 4.5 Å, can smoothly pass through the both hydrogel (poly(ethylene oxide)) and cryogel (hydroxyethylcellulose) with pore size of 50 μm and 200 μm, respectively. The obtained diffusion coefficients for glucose and ethanol in the gel are similar to the diffusion rate of these solutes in liquids [24], but also within the comparable range of other solutes: ascorbic acid ($1.82 \times 10^{-6}$ cm$^2$/s), vitamin $B_{12}$ ($3.6 \times 10^{-6}$ cm$^2$/s), dextran ($0.89 \times 10^{-6}$ cm$^2$/s) and theophylline ($6 \times 10^{-6}$ cm$^2$/s) [20,21,25].

### 3.3. Entrapment efficiency

Two important parameters characterizing the efficiency of cell immobilization, cell retention ($R_c$) and immobilization efficiency ($\eta$) are presented in Figs. 8 and 9. It was obvious that the cells followed different behaviour in the mono and in the double-layered gels. In the mono-layered gels they doubled in the first 24 h but then the cells started to leak 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 h 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 immobili-

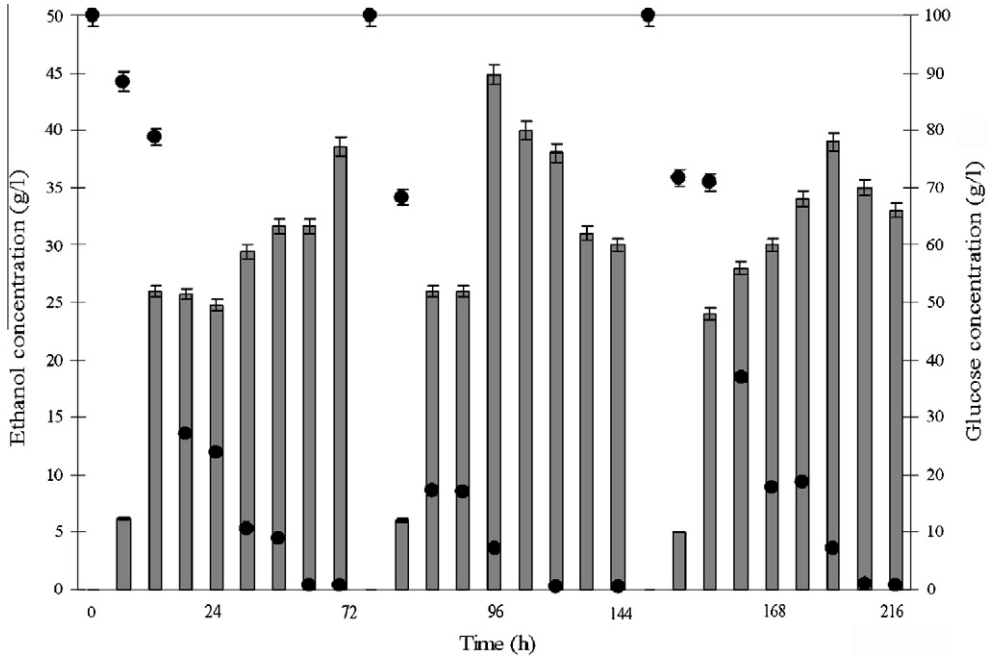


**Fig. 10.** Fermentation profile during ethanol production in three consecutive runs with 17.64 g/l initial biomass. Concentration of glucose (-●-) and ethanol (vertical bars) in the medium.

zation efficiency for the single-layered gels indicating that the second layer played significant role in preventing the cell leakage of the entrapped cells.

### 3.4. Cultivation of immobilized cells in single and double-layered gels

Mixing cells with solutions that have a high viscosity and that require high shear forces to effectively mix, can damage the cell membranes and retard their recover, leading to high death rates for the cells and low productivity [2]. But the highly elastic and porous u.v. cross-linked gels were shown to be promising matrices for whole-cell immobilization. The high porosity of the gels provided favourable conditions for the non-hindered mass transfer of substrates and metabolites. Their excellent rheological properties allow them to be used even in well mixed reactors [11]. The application of the double-layered gels as matrices for cell immobilization was tested in three consecutive batch fermentation cycles (Fig. 10). From the results it was evident that the coating with a second layer did not have any effect on the diffusion of glucose into the gels due to the very high glucose consumption rate of 99% in all batches. The ethanol concentration at the end of the fermentations induced by the cells entrapped in the double-layered gels was 38.6, 30 and 33 g/l for the first, second and third batch respectively, while the average ethanol yield reached through the batches was 0.39, 0.30 and 0.33 $g_{ethanol}/g_{glucose}$, corresponding to 76.45%, 59.42% and 65.36%, of the theoretical yield.

## 4. Conclusion

It was proven that double-layered gels that can be synthesized with u.v. curing of different polymer solutions (HEC and PEO) in the presence of the same photoinitiator are suitable for whole-cell immobilization. The diffusion coefficients for glucose and ethanol through the hydroxyethylcellulose cryogel were $3.9 \times 10^{-6}$ $cm^2/s$ and $0.97 \times 10^{-5}$ $cm^2/s$. The efficiency of the entrapped cells into the double-layered gels was determined by two important parameters, cell retention and immobilization efficiency. The suitability of the gels for ethanol production was investigated during repeated batch fermentation and the immobilized yeast cells were able to produce ethanol in relatively good yields.

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