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IMMOBILIZATION OF *SACCHAROMYCES CEREVISIAE* IN NOVEL HYDROGELS BASED ON HYBRID NETWORKS OF POLY(ETHYLENE OXIDE), ALGINATE AND CHITOSAN FOR ETHANOL PRODUCTION

Ružica Jovanović-Malinovska[1], Maja Cvetkovska[2], Slobodanka Kuzmanova[1], Christo Tsvetanov[3] , Eleonora Winkelhausen[1]*

[1] *Department of Food Technology and Biotechnology,* [2] *Department of Polymer Science, Faculty of Technology and Metallurgy, Ss. Cyril and Methodius University, Rudjer Bošković 16, 1000 Skopje, Republic of Macedonia*
[3] *Institute of Polymers, Bulgarian Academy of Sciences, Bl. 103-A, Acad. G. Bonchev Str. 1113, Sofia, Bulgaria*
eleonora@tmf.ukim.edu.mk

Hydrogel matrices were designed as hybrid networks of poly(ethylene oxide) (PEO) with natural polymers, alginate or chitosan by UV irradiation. The networks were formulated in the single-stage procedure in which the alginate or chitosan were added to the crosslinking reaction solution of PEO, and two-stage procedure, with additional chemical crosslinking of alginate or chitosan. Double-layer hydrogels composed of PEO hydrogel core with entrapped cells and outer natural hydrogel layer were also synthesized. The hydrogels were characterized by gel fraction yield and degree of equilibrium swelling as well as by rheological measurements. The production of ethanol by immobilized *Saccharomyces cerevisiae* was used to test the suitability of the synthesized hybrid hydrogels to serve as carriers for cell immobilization. The presence of cells affected the mechanical properties and the structure of the polymer networks. The best system for immobilization was found to be the PEO/alginate/Ca, which exhibited high mechanical strength (G', 830; GF, 93; ES^{H_2O}, 15) without affecting the metabolic functions of the cells. The maximum ethanol yield was 0.42 g/g corresponding to 82 % of the theoretical yield.

Key words: hybrid networks; poly(ethylene oxide); alginate; chitosan; cell immobilization; ethanol production

ИМОБИЛИЗАЦИЈА НА *SACCHAROMYCES CEREVISIAE* ВО НОВИ ХИДРОГЕЛОВИ НА БАЗА НА ХИБРИДНИ МРЕЖИ ОД ПОЛИ(ЕТИЛЕН ОКСИД), АЛГИНАТ И ХИТОЗАН ЗА ПРОДУКЦИЈА НА ЕТАНОЛ

Матрици од хидрогелови врз база на хибридни мрежи од PEO со природни полимери, алгинат или хитозан, беа добиени со UV вмрежување. Мрежите беа подготвени со еднократна постапка во која алгинатот или хитозанот беа додадени во растворот од PEO за вмрежување, а со двократна постапка, проследена со дополнително хемиско вмрежување на алгинатот или хитозанот. Исто така беа подготвени двослојни хидрогелови составени од јадро од PEO со заробени клетки и надворешен слој од природен хидрогел. Хидрогеловите беа карактеризирани со определување на гел-фракцијата, степенот на рамнотежно бабрење, како и со мерење на реолошките параметри. Тестирање на погодноста на синтетизираните хибридни хидрогелови како матрици за имобилизација на клетки за продукцијата на етанол беше направено со имобилизирани клетки од *Saccharomyces cerevisiae*. Присуството на клетките влијаеше врз механичките карактеристики и врз структурата на полимерните мрежи. Како најдобар систем за имобилизација беше селектиран PEO/alginate/Ca, кој покажа најголема механичка јачина (G', 830; GF, 93; ES^{H_2O}, 15) без да влијае врз метаболичките функции на клетките. Максималниот принос на етанол беше 0,42 g/g, што одговара на 82 % од теоретскиот принос.

Клучни зборови: хибридни мрежи; поли(етилен оксид); алгинат; хитозан; имобилизација на клетки; продукција на етанол

1. INTRODUCTION

With the inevitable depletion of the world's energy supply, there has been an increasing worldwide interest in alternative sources of energy. Although biofuels offer diverse range of promising alternatives, ethanol constitutes 99 % of all biofuels. Ethanol is a renewable, environmentally friendly fuel that is inherently cleaner than gasoline. Ethanol reduces harmful tailpipe emissions of carbon monoxide, particulate matters, oxides of nitrogen, and other ozone-forming pollutants [1, 2]. Present world bioethanol production is around 50 billion litres per year and it is constantly expanding with a possibility to reach 120 billion litres per year until 2025 [3].

As the demand for ethanol has been growing, ethanol industry has to respond with improved, more efficient production technology and greater production capacity. This prospect includes fermentation with immobilized microorganisms. The use of immobilized yeast cell systems for alcoholic fermentation is rapidly expanding research area because of the technical and economical advantages compared with the free cell system. One method that has emerged as successful in the laboratory and promising in commercial applications is the entrapment of cells in polymer hydrogels [4]. Development of the hydrogels is a dynamic field of study wherein there is a continuous search for materials. Polymers are the largest group of materials being exploited. They are used separately and/or as compositions of natural and synthetic materials. Alginate and chitosan are two of the natural polymers with a numerous applications in the immobilization technology due to their non-toxic, biocompatible, biodegradable and antimicrobial properties [5, 6]. Several groups have assessed the encapsulation of cells in double layer alginate or alginate-chitosan, microcapsules [7]. In some studies chitosan was used as the main matrix entrapping the cells [8]. In other studies chitosan or chitosan and alginate was used as the coating membrane of alginate microcapsules with entrapped cells [9, 10].

Recently, the effective syntheses of polymer hydrogels via UV irradiation were reported [11-13]. The advantages of the UV-crosslinking were the extremely short time for efficient gel formation and the simplicity of the technique. In our previous study, UV irradiated high molecular weight poly(ethylene oxide) (PEO) hydrogels with different crosslinkers were used for entrapment of *Candida boidinii* cells [11]. PEO was selected because of its properties: good strength, ability to increase the elasticity, non-toxicity and biocompatibility.

The objective of the present study was to develop novel hydrogels for cell immobilization based on UV irradiated high molecular weight PEO with natural polymers, alginate or chitosan in a single-stage procedure and in a two-stage procedure with additional chemical crosslinking. Double-layer hybrid hydrogels composed of PEO hydrogel core with entrapped cells and outer alginate or chitosan layer were also prepared. The polymer networks were characterized in terms of gel fraction yield, equilibrium swelling and rheological parameters, and tested for immobilization of *Saccharomyces cerevisiae* cells that were subsequently used for ethanol production.

2. EXPERIMENTAL

2. 1. *Materials*

Saccharomyces cerevisiae, a commercial grade baker's yeast, with 32 % dry biomass was used. Poly(ethylene oxide) was from Union Carbide Corp., USA Polyox N12K ($\overline{M}_n = 1 \cdot 10^6$ g/mol), sodium alginate with approximately 70 % G-block content was from Aldrich, and chitosan (medium molecular weight, $\overline{M}_n \sim 4 \cdot 10^5$ g/mol) was from Fluka. The (4-benzoylbenzyl)trimethylammonium chloride (BBTMAC) purchased from Aldrich was used as a photoinitiator, without further purification. Other chemicals used were purchased from commercial sources and were of analytical grade.

2. 2. *Preparation of hydrogels*

2. 2. 1. *Single-stage procedure for hydrogels preparation*

Aqueous solution of PEO (5 mL, 5 % w/v) or PEO with 1 % w/v sodium alginate or 1 %

w/v chitosan containing BBTMAC (5 % of the polymer mass) was poured into a Teflon Petri dish with a diameter of 5 cm, forming a 2 mm thick layer, which was then placed in a freezer at –40 ºC for 2 hours. The frozen solution was irradiated with UV light in a temperature-controlled open chamber connected with cryostat apparatus in a Dimax light curing system 5000-EC equipped with 400 W metal halide flood lamp (input power = 93 mW/cm^2), for 2 minutes (dose = 11.4 J/cm^2).

2. 2. 2. *Two-stage procedure for hydrogels preparation*

PEO/alginate or PEO/chitosan hydrogels (prepared in single-stage procedure as above) were then additionally crosslinked with sterile solution of calcium chloride (1 mol/L) to form PEO/alginate/Ca hydrogels or glutharaldehyde (GA) (3 % w/v) for PEO/chitosan/GA hydrogels.

2. 2. 3. *Double-layer hydrogels*

Aqueous solution of PEO (5 mL, 5 % w/v) containing BBTMAC (5 % of the polymer mass) was irradiated wih UV light. PEO hydrogels were then coated with solutions of sodium alginate (3 % w/v) or chitosan (3 % w/v). Finally, after 30 minutes reaction time, hydrogels were placed in solutions of calcium chloride (1 mol/L) or glutharaldehyde (3 % w/v), as crosslinking agents, to form outer layer.

2. 2. 4. *Immobilization of the cells*

The yeast was immobilized by adding yeast cells (5 or 10 % w/v) to the mixture of polymers, followed by the same crosslinking procedure as already described. The size and shape of prepared hydrogels with yeast cells are as presented in Figure 1.

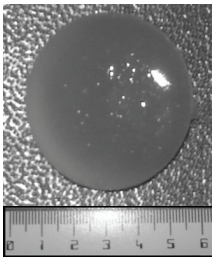

Fig. 1. PEO hydrogel disks loaded with yeast cells

2. 3. *Characterization of the hydrogels*

2. 3. 1. *Measurements of gel fraction yield and equilibrium degree of swelling*

For complete removal of sol fraction, the prepared hydrogels were soaked in water for 48 hours, and then they were taken out and dried to constant mass under vacuum and weighed. The gel fraction yield (GF) was calculated as:

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

For determination of the equilibrium degree of swelling (ES), the hydrogels were equilibrated in distilled water or chloroform for at least 72 h, removed from the solvent, blotted with filter paper and weighed. They were then dried to constant mass under vacuum and weighed again. The ES was calculated as:

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

2. 3. 2. *Rheological measurements*

Dynamic rheological measurements of the hydrogels were performed on a Haake RheoStress 600 rheometer with a cone-plate system (2º) and Peltier temperature controller. Shear storage modulus G' and loss modulus G'' were measured in the frequency range of 0.1-10 Hz at 25 ºC.

The experimental errors of the estimated *GF, ES*, G' and G'' values are ±3 %.

2. 3. 3. *Number average molecular weight between crosslinks*

The number average molecular weight between crosslinks, $\overline{M}_c$ was determined using the equilibrium swelling data and Flory and Rehner equation (Eq. 3) (for PEO) [14, 15] and the data for shear modulus G' (Eq. 4).

$$\frac{1}{\overline{M}_c} = \frac{2}{\overline{M}_n} - \frac{(\bar{\upsilon}/V_1)\left[\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi\upsilon_{2,s}^2\right]}{\left[\left(\upsilon_{2,s}\right)^{1/3} - \frac{\upsilon_{2,s}}{2}\right]} \quad (3)$$

where $\overline{M}_n$ is the number average molecular weight of the polymer before crosslinking

($\overline{M}_n$=1·10^6 g/mol); $\bar{\upsilon}$ is the specific volume of the polymer (for PEO, ρ=1.13 g/cm and $\bar{\upsilon}$ = 0.885 cm^3/g); V_1 is the molar volume of the swelling agent (18 cm^3/mol for water; 80.67 cm^3/mol for chloroform); and χ is the Flory-Huggins constant (for PEO gels and water, 0.45; for PEO gels and chloroform 0.477) [14]. The polymer volume fraction in the equilibrium swollen polymer, $\upsilon_{2,s}$, was calculated as υ_{2s} = 1/ES.

According to [16], $\overline{M}_c$ can be determined as:

$$\overline{M}_c = \frac{cRT}{G'} \tag{4}$$

where R is the gas constant (8.3143 J/mol K), T is the temperature (298 K) at which the modulus G' was measured, c is polymer concentration (5·10^4 g/m^3) in the crosslinking solution.

2. 3. 4. *Mesh size of the PEO polymer network*

The number of links between two crosslinks, n, was calculated as $n = \overline{M}_c / M_r$, where M_r is the average molecular weight of the repeating unit. The mesh size of the polymer network, ξ, was calculated by [17]:

$$\xi = \upsilon_{2,s}^{-1/3} \left(\bar{r}_0^2\right)^{1/2} \tag{5}$$

where the root mean squared end-to-end distance of the polymer chain in the unperturbed state was calculated as $\left(\bar{r}_0^2\right)^{1/2} = \sqrt{C_n}\ell\sqrt{n}$; C_n is the Flory characteristic ratio or rigidity factor of the polymer; l is the carbon-carbon bond length, n is the number of links along the polymer chain.

2. 3. 5. *Scanning electron microscope*

Micrographs of cross-section and the interior of PEO hydrogel loaded with *S. cerevisiae* cells were obtained by using a Jeol JSM-5510 scanning electron microscope (SEM). The composite hydrogels specimens were first dried, then quenched in liquid nitrogen, fixed on a glass substrate and coated with gold in a Jeol JFC-1200 fine coater for 60s.

2. 4. *Cultivation of Saccharomyces cerevisiae*

The medium had the following composition (per litre): 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 glucose. For preculturing the glucose concentration was 20 g/L and for batch production of ethanol, it was increased to 50 g/L. The media were autoclaved at 121ºC for 15 min. The initial pH value was 5. Immobilized cells were cultivated in 50 mL nutrient medium placed in a 250 mL Erlenmeyer flask on a rotary shaker (150 rpm) at 28 ºC. After 20 hours of precultivation, the immobilized cells were washed with sterile distilled water and transferred into the fresh nutrient medium for ethanol production. One gel of about 5 mL volume was placed in each flask providing 1:10 (v/v) ratio of gel to medium.

2. 5. *Analytical methods*

Cell concentration was estimated by UV-VIS spectrometer (Varian) at 620 nm, after diluting the samples within the range of 0.05 to 0.5 units. One optical density unit corresponded to 0.3 mg dry cell mass per mL. Samples were clarified by centrifugation (Heraus) at 2500×g for 15 min for products and residual sugar analysis. Supernatants were stored at –18 ºC before analysis. 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_f= 0.25 μm and a manual injector type 1709. The injector and detector temperatures were 250 ºC, and the column temperature was 200ºC. 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 by a 3,5-dinitrosalicylic acid procedure of Miller. The mean of two individual measurements was used to calculate the glucose, ethanol and cell concentration.

3. RESULTS AND DISCUSSION

3. 1. *Physical and mechanical properties of hydrogels*

Hydrogel matrices were designed as hybrid networks of PEO and natural polymers, alginate or chitosan, by UV irradiation, with additional chemical crosslinking. Double-layer hydrogels composed of PEO hydrogel core with entrapped cells and outer natural hydrogel layer were also synthesized. The properties of the formed hydrogels, as shown in Figure 2, were affected by the hydrogel preparation technique. Gel fraction yields between 53 and 93 % and equilibrium degrees of swelling between 5 and 65 in chloroform were determined. Two-stage preparation procedure contributed to the higher values of gel fraction yield, 93 % for PEO/alginate/Ca and 87 % for PEO/chitosan/GA, and to the smaller equilibrium degree of swelling, ES^{CHCl_3}=11 for PEO/alginate/Ca, and ES^{CHCl_3}=5 for PEO/chitosan/GA, respectively. As expected, additional crosslinking of natural polymers resulted in the networks that allow less swelling. The phenomena of adding a crosslinking agent to decrease water uptake has also been noted by other authors [18].

The physico-mechanical properties of the hydrogels are of crucial importance. The presence of cells during the preparation of the matrices can affect the physical properties and the structure of the formed polymeric matrix. Therefore, all

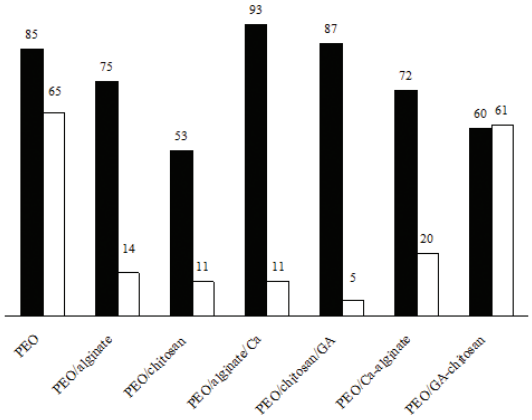

Fig. 2. Hydrogels prepared by UV irradiation. Gel fraction yield (%) (■), equilibrium swelling degree in chloroform (□).

studies were performed on hydrogels without and with incorporated yeast cells and the results are presented in Figure 3. The apparent values of G' and G'' were independent of the frequency and $G' > G''$ over the entire frequency range explored. The observed results were consistent with the typical behaviour of a polymer gel. For the control sample of PEO hydrogels, irradiated at room temperature, the values of G' and G'' were 255 Pa and 10 Pa. The hybrid hydrogels exhibited quite a different behaviour. The values of G', as shown in Figure 3a, are orders of magnitude higher than the values for the PEO hydrogels. The highest increase of G' was observed with PEO/alginate/Ca (3.3-fold) and PEO/chitosan/GA (2.8-fold) as compared to the control sample of PEO hydrogels. Our study evidently showed that the two-stage procedure for hydrogel preparations resulted in strong and stable matrices.

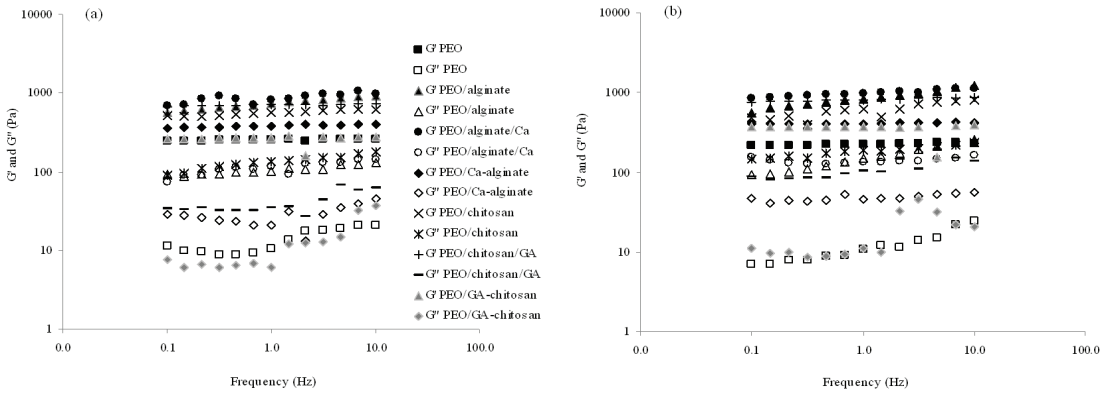

Fig. 3. Mechanical spectra of differently prepared PEO based hydrogels. Elastic modulus (G') and loss modulus (G'') of hydrogels without cells (a) and loaded with cells (b)

Several authors reported on a decrease in the mechanical properties of the gel matrices as a result of the presence of microbial cells [11, 19-21]. Our findings in these experiments indicated that the incorporation of the yeast cells had an impact on the viscoelastic properties of all gels studied and the resultant hydrogel networks were of high mechanical strength, causing increase of G' values (Figure 3b). PEO/alginate/Ca and PEO/chitosan/GA with entrapped cells, exhibited the highest values of storage modulus, 977 and 812 Pa, respectively, recorded at 1 Hz, compared with 830 Pa and 710 Pa for the same hydrogels without cells. When cells were incorporated in the polymer networks a decrease of G' values was observed only with the control sample of PEO hydrogel (225 Pa).

The approximate values of the number average molecular weights between crosslinks, $\overline{M}_c$, was calculated for PEO networks, thus characterizing the extent of crosslinking.

The results for $\overline{M}_c$ and mesh size, derived from two experimental methods including swelling (in water and chloroform) and dynamic mechanical analysis for PEO hydrogel are presented in Table 1. It can be noted that value for $\overline{M}_c$ calculated according to Eq. 3 for swelling in chloroform (434,000 g/mol) were comparable with the value for $\overline{M}_c$ calculated from dynamic rheological measurements (486,000 g/mol), since chloroform is a better swelling agent for this type of hydrogel. The equivalent mesh sizes were similar as well, 173 and 161 nm, respectively. Scanning electron microscopy revealed the presence of *S. cerevisiae* in the inner region of PEO hydrogel loaded with cells. In Figure 4 the relative size of both cells and matrix porosity are seen. The size of the pores gives enough space for the growth of gel-entrapped cells. Morphology of *S. cerevisiae* cells does not seem to be affected by the UV-crosslinking entrapment procedure.

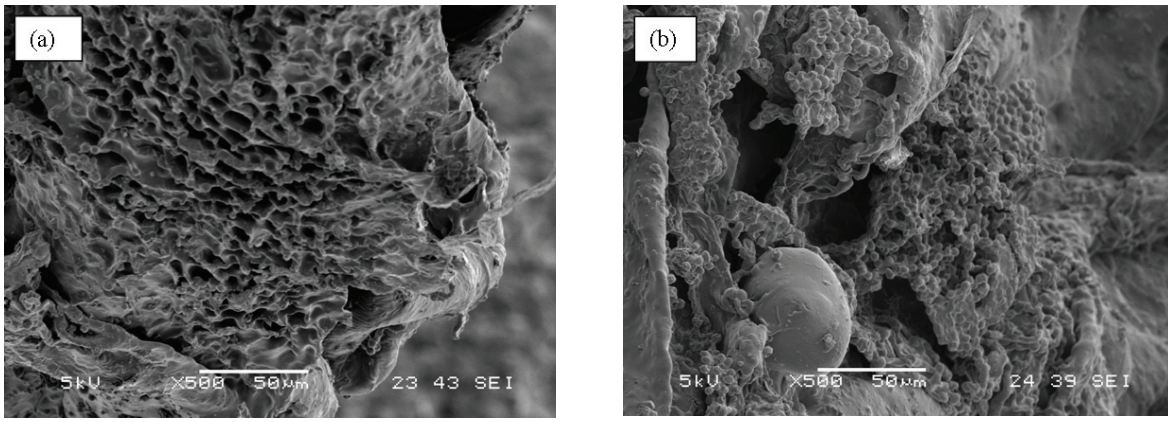

Fig. 4. Scanning electron micrographs (SEM) of the cross-section of PEO hydrogel loaded with *S. cerevisiae* cells after immobilization (a) and after one fermentation cycle of 24 hours (b)

T a b l e 1

Number average molecular weight between crosslinks and mesh size for PEO hydrogels

	ES^{H_2O}	$\overline{M}_c$ [a] (g/mol)	ξ (nm)	ES^{CHCL_3}	$\overline{M}_c$ [b] (g/mol)	ξ (nm)	G' (Pa)	$\overline{M}_c$ [c] (g/mol)	ξ (nm)
PEO	44	135,000	85	65	434,000	173	255	486,000	161

Calculated according to [a] Eq. 3 swelling in water; [b] Eq. 3 swelling in chloroform; [c] Eq. 4; G' recorded at 1Hz.

The apparent values for $\overline{M}_c$ for the hybrid hydrogels, calculated by dynamic rheological measurements, are summarized in Table 2. It can be seen that increasing the crosslinking density resulted in an increase of G', and consequently a decrease of $\overline{M}_c$ values. The values for $\overline{M}_c$ varied from 149,000 to 221,000 g/mol.

T a b l e 2

Number average molecular weight between crosslinks for hybrid networks based on PEO.

System	Concentration of polymer (w/w %)	G' (Pa)	$\overline{M}_c$ * (g/mol)
PEO/ alginate	5/1	725	171,000
PEO/chitosan	5/1	562	221,000
PEO/ alginate/Ca	5/1	830	149,000
PEO/chitosan/GA	5/1	710	174,000

*Calculated according to Eq. 4; G' recorded at 1 Hz.

3. 2. *Activity of the immobilized yeast cells*

3. 2. 1. *Effect of the initial yeast cell loading on the fermentation behaviour of the yeast*

The effect of the initial cell loading in hydrogels on cell proliferation, metabolic activity and product formation by the immobilized cells was investigated. It has been generally observed that it is difficult to predict the type and magnitude of the metabolic changes in the cells due to the immobilization procedure.The first experment with PEO immobilized *S. cerevisiae* cells demonstrated that immobilization procedure did not affect cell activity. Figure 5 showed the distinctive profile of glucose consumption and ethanol formation in immobilized cell culture with different initial cell loadings of 5 and 10 % w/v. It was clear that both culture systems had a good metabolic activity. The ethanol production started at 3rd hour, and reached a maximum level at 24th hour (15.3 g/L) with 5 % entrapped cells (Figure 5a), while with 10 % entrapped cells (Figure 5b) ethanol concentration reached its maximum level (14.7 g/L) at 12th hours.

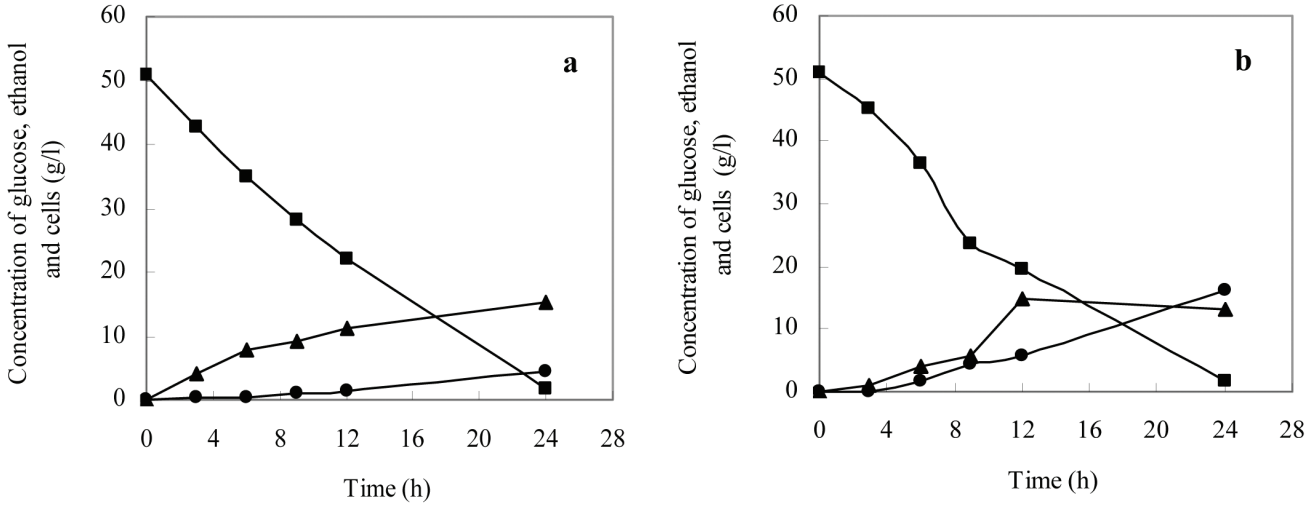

Fig. 5. Glucose (■), ethanol (▲), and biomass (●) concentration during batch culture of immobilized *S. cerevisiae* in synthetic PEO hydrogel with cell loading (a) 5 % w/v and (b) 10 % w/v

However, the concentration of the free cells in the medium was very high, 16.04 g/L at 24th hour in the case with 10 % entrapped cells, thus suggesting that the cell load of 5 % w/v was a better choice for further immobilization studies. Lower initial cell density in microcapsules of alginate-chitosan-alginate was also reported to be better for proliferation, metabolism and product formation by *S. cerevisiae* [9].

One of the most common problem to overcome in using entrapped yeast cells for fermentation is the leakage of cells from the entrapment matrix into the medium. In the next experiments, our ultimate goal was to create hydrogel/cell systems where the core PEO hydrogel will contain the immobilized cells and the additional polymer will ensure the mechanical strength and will act as barrier membrane against cell leakage as well. For this purpose, yeast cells were cultivated in the hybrid hydrogels and double-layer carriers based on PEO hydrogels with natural polymers, alginate or chitosan. The size of the hydrogel samples and yeast cell content (5 % w/v) were prepared to be identical for the three techniques of preparation. The effects of these types of polymer matrices on the ethanol production by immobilized *S. cerevisiae* are demonstrated in Figures 6 and 7.

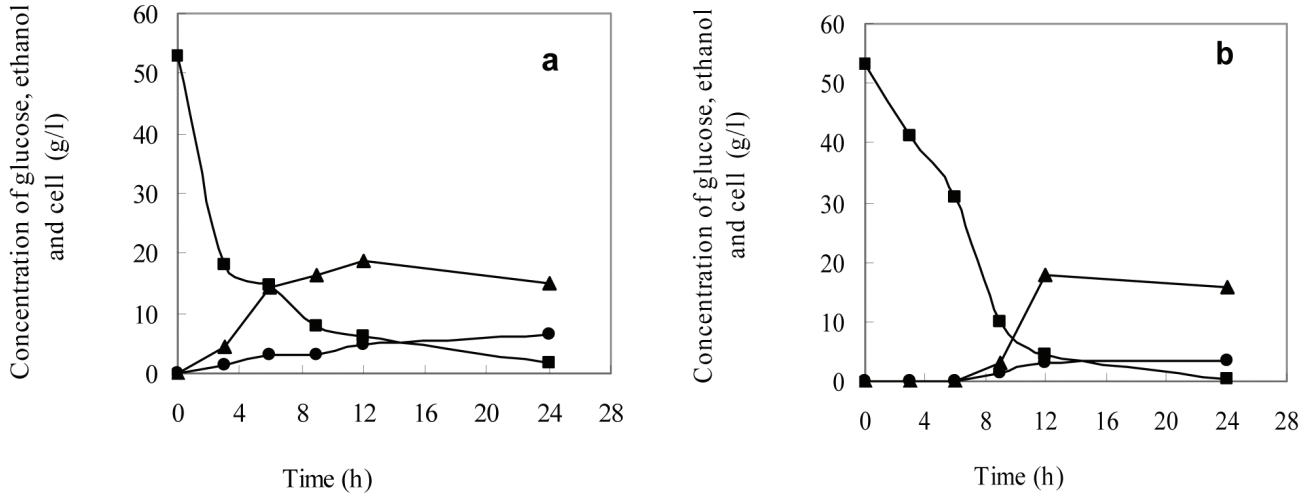

Fig. 6. Time course of glucose (■), ethanol (▲) and cell mass (●) concentration in batch cultivation with entrapped *S. cerevisiae* cells carriers with alginate: (a) PEO/alginate and (b) PEO/alginate/Ca

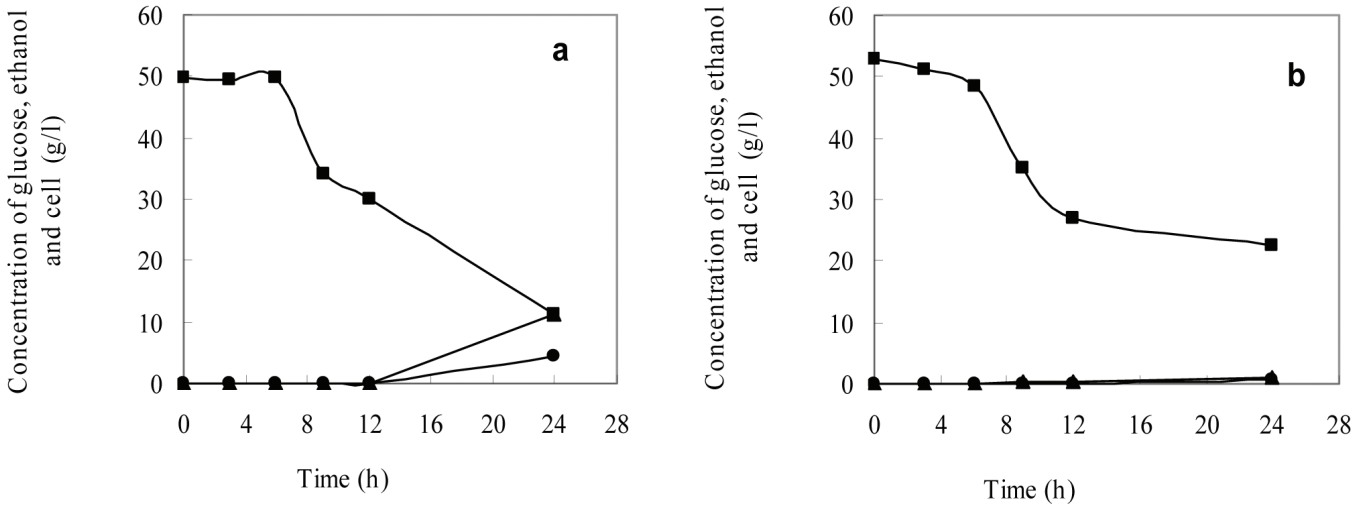

Fig. 7. Time course of glucose (■), ethanol (▲) and cell mass (●) concentration in batch cultivation with entrapped *S. cerevisiae* cells carriers with chitosan: (a) PEO/chitosan and (b) PEO/chitosan/GA.

3. 2. 2. *Ethanol production by immobilized cells in PEO-based hybrid hydrogels*

The behaviour of the immobilized *Saccharomyces cerevisiae* in PEO/alginate and PEO/alginate/Ca hybrid hydrogels is presented in Figure 6. The rapid glucose consumption in the first 12 h of the fermentation coincided with the maximum ethanol production of 18.6 g/L (78 % from the theoretical yield) for the PEO/alginate and 19.0 g/L ethanol (82 % from the theoretical yield) for the PEO/alginate/Ca. These results suggested that both PEO/alginate and PEO/alginate/Ca could serve as matrices for yeast cell immobilization and could be used for ethanol production. However, the cell leakage in the medium with PEO/alginate (4.9 g/L) was higher than the cell leakage with PEO/alginate/Ca (3.0 g/L). Obviously, the PEO/alginate could not retain the proliferated cells due to its lower crosslinking density (GF, 75 % and ES^{H_2O}, 33) compared to PEO/alginate/Ca (GF, 93 and ES^{H_2O}, 15). These results suggested that the PEO/alginate/Ca could be used as a yeast cell carrier for ethanol production in scaled-up experiments. These experiments should employ higher initial glucose concentration and should be carried out for a longer time so that the operational stability of the system could be also investigated.

In the fermentation with entrapped cells in PEO/chitosan and PEO/chitosan/GA, the cells did not start consuming glucose before 6th hour (Figure 7). The cells might be inactivated not only due to the immobilization procedure, but also due to the composition of the hydrogels. Additionally, ethanol was not detected before 24th hour. It was assumed that ethanol was produced only by *S. cerevisiae* cells immobilized in PEO/chitosan (11.1 g/L) due to the more expressed swelling in water media (ES^{H_2O}=19) compared to PEO/chitosan/GA (ES^{H_2O}=9). In addition, the absence of the ethanol in the PEO/chitosan/GA was most probably a result of the use of glutharaldehyde as a crosslinker. Similar behaviour of the immobilized cells has been also reported [22]. Because of the toxic effect of the glutharaldehyde, on the one hand, and the solid physico-mechanical properties of the PEO/chitosan/GA, on the other hand, a substitution of glutharaldehyde by sodium tripolyphosphate will be investigated as alternative.

3.2.3. *Ethanol production by immobilized cells in PEO-based double-layer hydrogels*

Immobilized *S. cerevisiae* cells in double-layer hydrogels of PEO/Ca-alginate and PEO/GA-chitosan did not produce ethanol (data not shown). Besides, the double-layer polymer carriers did not fulfil our expectations of good mechanical properties. The outer layer of these carriers was fragile. As shown in Figure 3b, the G' values were the lowest of all hybrid hydrogels, 404 Pa for PEO/Ca-alginate and 376 Pa for PEO/GA-chitosan.

4. CONCLUSIONS

Hydrogel matrices were designed as hybrid networks of poly(ethylene oxide) with natural polymers, alginate or chitosan, by UV irradiation and chemical crosslinking. We explored the feasibility of these hybrid hydrogels as matrices for immobilization of *Saccharomyces cerevisiae* cells. The immobilized yeast cells were used for ethanol production from glucose. The best system for immobilization was found to be PEO/alginate/Ca, which exhibited high mechanical strength and did not negatively affect the metabolic activity of the cells. In addition, the PEO/alginate/Ca system reduced significantly the leakage of the entrapped cells into the medium. Having selected the proper carrier, the further experiments should focus on optimizing the conditions for ethanol production.

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NOMENCLATURE

BBTMAC	(4-benzoylbenzyl)trimethylammonium chloride
ES	equilibrium swelling degree
ES^{CHCl_3}	equilibrium swelling degree in chloroform
ES^{H_2O}	equilibrium swelling degree in water
G′	elastic modulus, Pa
G″	loss modulus, Pa
GA	glutharaldehyde
GF	gel fraction yield, %
PEO	poly(ethylene oxide)
PEO/alginate	hybrid network of poly(ethylene oxide) and sodium alginate
PEO/alginate/Ca	hybrid network of poly(ethylene oxide) and calcium alginate
PEO/Ca-alginate	double-layer carrier of poly(ethylene oxide) and calcium alginate
PEO/chitosan	hybrid network of poly(ethylene oxide) and sodium alginate
PEO/chitosan/GA	hybrid network of poly(ethylene oxide) and chitosan-glutharaldehyde
PEO/GA-chitosan	double-layer carrier of poly(ethylene oxide) and glutharaldehyde-chitosan
SEM	scanning electron microscope

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