Influence of alginate gel composition on the productivity of an immobilized yeast bioreactor

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Dedicated to Professor E. Kossaczký, CSc., in honour of his 65th birthday

Yeast *Saccharomyces cerevisiae* PZ 43 CHTF was immobilized in beads formed from Ca-alginate gel. In the prepared alginate dispersion, the content of Na-alginate and SiO_2 varied. The influence of the composition of alginate gel on the productivity of a continuous bioreactor was studied. In this bioreactor the maximum productivity was reached for immobilized yeast entrapped in the gel containing 1.5 mass % of Ca-alginate and 4.91 mass % of SiO_2. Experiments in a fixed bed were carried out with various gels at equal conditions (pH = 4.5, temperature 28 °C, residence time 59.24 min, inlet concentration of D-glucose 100 g dm^{-3}). At these conditions, the maximum substrate conversion was 96.13 %, which corresponds to the productivity of 97.36 kg m^{-3} h^{-1}

Дрожжи *Saccharomyces cerevisiae* PZ 43 CHTF были иммобилизированы на перлах, образованных из Ca-альгинатового геля. В полученной альгинатной дисперсии изменялось содержание Na-альгината и SiO_2. Изучалось влияние состава альгинатного геля на производительность непрерывного биореактора. В этом биореакторе наибольшая продуктивность достигалась в случае иммобилизированных дрожжей в геле, содержащем 1,5 % по массе Ca-альгината и 4,91 % по массе SiO_2. Эксперименты в закрепленном слое проводились с различными гелями при равных условиях (pH = 4,5, температура 28 °C, время удерживания 59,24 мин, входящая кон-

центрация D-глюкозы 100 г дм$^{-3}$). При этих условиях максимальная конверсия субстрата составляла 96,13 %, что отвечало продуктивности 97,36 кг м$^{-3}$ час$^{-1}$

Immobilization of cells — biocatalysts is often a precondition for their application in continuous reactors. Production of alcohol by this method is the subject of intensive study from the viewpoint of the product as well as the process as a simple system.

Tyagi and *Ghose* [1] found that in fermentation of cane molasses to ethanol the properties of immobilized cells differed from those of free cells. Immobilized *S. cerevisiae* had a higher specific rate of ethanol formation in comparison with free cells. On the other hand, the specific growth rate of bound cells was lower than in case of free cells. *Richter* and *Becker* [2—4] studied the inhibiting effects in alcohol fermentation. They developed an exponential model for the growth of biomass inhibited by the product, which in the simplest case had a linear form. The authors recognized that the inhibiting influence of substrate on the rate of product formation is manifested above the initial concentration of saccharose in the medium of 100 g dm^{-3} They measured also the dependence of the viability of cells on the osmotic pressure which is a further inhibiting factor in this process.

Both the method of immobilization and the kind of material used have an influence on the function of cells. Many works concerned with ethanol production have focused on the testing of various carriers of yeast cells. *Navarro et al.* [5] immobilized yeast in pectin gel. It was apparent that the rate of ethanol production was negatively influenced by the particle diameter, if the content of cells in the particles was high. The content of pectin only slightly influenced the fermentation activity. *King* and *Zall* [6] immobilized the yeast *Kluyveromyces fragi* in polyacrylamide and kappa-carrageenan gel by occlusion. The investigators fermented lactose to ethanol on a continuous basis and activated a high and stable activity of the yeast. They compared both the above-mentioned types of supports and came to the conclusion that kappa-carrageenan yields better results.

The addition of unsaturated fatty acids and sterols into alginate gel which was used by *Nagashima et al.* [7] for the immobilization of yeast cells *S. cerevisiae* increased the productivity of the continuous bioreactor by more than 50 g EtOH/(dm^3 gel h^{-1}) and prolonged the stability of the entrapped cells by almost half a year. *Fang et al.* [8] investigated the increase of the yield of ethanol by the addition of various amounts of sand into alginate gel *vs.* cells entrapped into pure Ca-alginate. The difference in the yield of ethanol became evident only at high dilution rate when it reached the value of 20 %.

For the immobilization of yeast also other carriers were tested. *Jirků et al.* [9]

immobilized thermally permeabilized cells *Zygosaccharomyces lactis* in a fine-grained hydroxyalkyl methacrylate gel treated by epichlorhydrin by covalent bond formation requiring glutaraldehyde. A number of works are concerned with the immobilization of yeast on the surface of hollow fibres.

Inloes et al. [10] compared the productivity of a membrane bioreactor with bound yeast *S. cerevisiae* using a complete medium and a medium without nitrogeneous source. The utilization of a complete growth medium caused a 13-fold increase of the productivity. *Mehaia* and *Cheryan* [11] conducted experiments with *S. cerevisiae* immobilized on the surface of hollow fibres in a batch and continuous bioreactor. They found that the productivity of a continuous bioreactor is five times higher in comparison with a batch reactor.

It is a well-known fact that during the alcohol fermentation CO_2 is evolved which must be drawn off in the course of the process [7]. Various apparatuses which secure CO_2 offtake were suggested. *Navarro et al.* [12] used a horizontal bioreactor which enabled a spontaneous liberation of the formed CO_2.

Only few works are devoted to the influence of the composition of the carrier on the ethanol yield [13—15]. In the present work Ca-alginate gel carriers with various content of SiO_2 have been compared. In the case of experiments performed by a continuous way the results were compared on the basis of bioreactor productivity.

Experimental

Chemicals

Sodium alginate (Lamitex form) (Protan A/S, Norway); yeast extract, pepton for bacteriology, matt agar (Immuna, Šarišské Michaľany); D-glucose, anal. grade (Léčiva, Prague); $CaCl_2$, anal. grade, $Si(OEt)_4$, anal. grade, lactic acid, anal. grade, Bio-La-Test® GLUCOSE (Lachema, Brno).

Microorganism and fermentation medium

Saccharomyces cerevisiae PZ 43 CHTF belonging to the collection of the Institute of Biotechnology of the Slovak Technical University in Bratislava was used during fermentation experiments. The culture was maintained on included agars (wort substrate 8° Bg with 3 % of agar, pH = 6.5) at 4 °C. In the experiments fermentation medium DMA (synthetic substrate according to Pirt) containing D-glucose and yeast extract was used. pH of the substrate was adjusted to 4.5 by means of a 20 % HCl solution. Composition of the fermentation medium is given in Table 1.

Table 1

Composition of the fermentation medium

Component	Content / g
D-Glucose (dry)	100
Yeast extract	3
K_2HPO_4	11.3
$NaH_2PO_4 \cdot 2H_2O$	5.4
$MgSO_4 \cdot 7H_2O$	0.12
NH_4Cl	2.0
$CaCl_2$	0.01
$FeSO_4 \cdot 7H_2O$	0.005
$ZnSO_4 \cdot 7H_2O$	0.0005
$MnSO_4 \cdot 4H_2O$	0.0005
$CuSO_4 \cdot 5H_2O$	0.0001
$CoCl_2 \cdot 6H_2O$	0.0001
$Na_2B_4O_7$	0.0001
$Na_2MoO_4 \cdot 2H_2O$	0.0001
H_2O	to 1000

Analytical methods

For the determination of the D-glucose concentration a colour reaction between D-glucose and *o*-toluidine at elevated temperature was used. The intensity of the colouring corresponding to individual samples was measured by means of a spectrophotometer UV VIS (Zeiss, Jena) at the wavelength of 530 nm.

The concentration of ethanol was measured by gas chromatography on the CHROM-4 chromatograph equipped with a flame ionization detector. The column was packed with poly(ethylene glycol) PEG-400. The details of analysis were described previously [17].

The content of the immobilized yeast was determined analyzing the overall nitrogen content in samples. Before the analysis the samples were treated in the following way: 5 cm^3 of alginate beads was dried in a thermostat at 50 °C for 72 h. Dried alginate beads were weighed. After rubbing in a mortar they were analyzed. This measurement was performed by means of an elemental analyzer CHN + O (model 1102, C. Erba, Milan).

Immobilization technique

Yeast *S. cerevisiae* PZ 43 CHTF was immobilized in calcium alginate gel with an inner source of calcium [16]. Gel particles of bead shape were formed by showering drops of

a water dispersion containing Na-alginate, colloidal SiO_2, and yeast into a 0.2 M-$CaCl_2$ solution. In this solution insoluble calcium alginate and calcium silicate gel which increases the stability of the former gel were formed. The suspension of yeast cells contains 2.06×10^{10} cells/cm^3 The dry matter contained 7.025 mass % of nitrogen. In the prepared gel beads, the content of both alginate and colloidal SiO_2 changed at constant content of cells. The composition of dispersion and properties of the beads are illustrated in Table 2.

Table 2

Properties of the alginate dispersion and gel beads with entrapped yeast

Sample of gel	Dispersion			Gel beads diameter
	Content of Ca-alginate	Content of SiO_2	Viscosity	
	mass %	mass %	η/mPa s	d/mm
1	2.5	—	3.5	3.36±0.75
2	2.0	—	1.5	3.48±0.22
3	1.5	—	1.2	3.55±0.16
4	1.5	4.91	1.5	3.75±0.23
5	1.5	9.82	1.7	3.35±0.54

Bioreactor used in continuous processes

The bioreactor depicted in Fig. 1 was a 30 cm high glass column (diameter 4.5 cm) equipped with a perforated chimney (diameter 1.4 cm) made from brass inside a glass

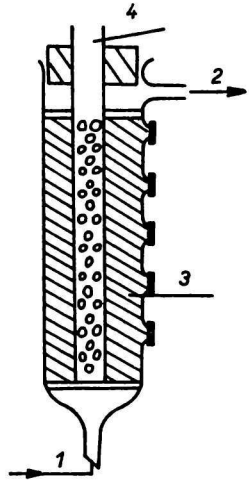

Fig. 1. Schematic diagram of the bioreactor used in continuous experiments.
1. Substrate; *2.* product; *3.* biocatalyst; *4.* chimney.
Diameter of column: 4.5 cm; diameter of perforated chimney: 1.4 cm; height of bed: 15 cm.

pipe. This perforated pipe ensured the CO_2 offtake along the whole bioreactor. The head of the apparatus was covered by gauze to prevent contamination and formation of various impurities from the air. The packing on the bottom of the bioreactor was fixed by a glass frit in order to prevent the passage of released cells from the gel into the storage reservoir for the fermentation medium. The packing in the bed of the reactor was pressed by a perforated brass ring. The height and the volume of the packing was 15 cm and 150 cm^3, respectively.

Method of measurement of ethanol production in an immobilized yeast bioreactor

At the beginning of the experiments the bioreactor was filled with 150 cm^3 of alginate beads and the packing was pressed into the column by a brass ring in order to ensure a 15 cm height of the immobilized yeast. At the beginning of the experiments, the content of proteins was determined in alginate beads.

Yeast immobilized in the alginate gel continuously fermented D-glucose to ethanol at 28 °C and pH = 4.5 in DMA medium containing a yeast extract in the amount of 3 $kg\,m^{-3}$ The medium was transported from the bottom to the top of the bioreactor. Experiments with five samples of gels were carried out. In these the conversion of the substrate to the product at a constant flow of the medium (86 $cm^3\,h^{-1}$) was compared. The concentration of D-glucose in the medium was in all cases 100 $kg\,m^{-3}$. At certain time intervals the concentration of both D-glucose and ethanol in the outlet from the immobilized cell bed was examined. Samples were taken until a steady state was reached. This state was reached after about 70 h for all cases investigated.

Results and discussion

In continuous experiments the bioreactor was working for 70 h until a steady state was reached. The dependences of the residual D-glucose concentration *vs.* time and ethanol concentration in the outlet medium from the bioreactor at certain time intervals are illustrated in Figs. 2 and 3. The achieved various ethanol concentrations and residual D-glucose concentrations for the steady state of the bioreactor and individual gel samples indicate a certain relation between the composition of the carrier and the kinetics of the process. Since the experiments were performed at equal conditions, the most suitable gel could be chosen for continuous experiments. The residence time of the fermentation in experiments performed on a continuous basis was 59.2 min. Substrate conversion and bioreactor productivity were calculated from the measured values of substrate concentration at certain times. Dependences of bioreactor productivities on time for individual gels are plotted in Fig. 4.

In Table 3 the comparison of the substrate conversion and bioreactor productivity at a steady state for individual gels is presented. It is evident that

the highest productivity is achieved with the system containing immobilized cells entrapped in gel *4*.

Since in the immobilization of yeast in gels we did not succeed in achieving an equal concentration of the biomass (expressed by the concentration of

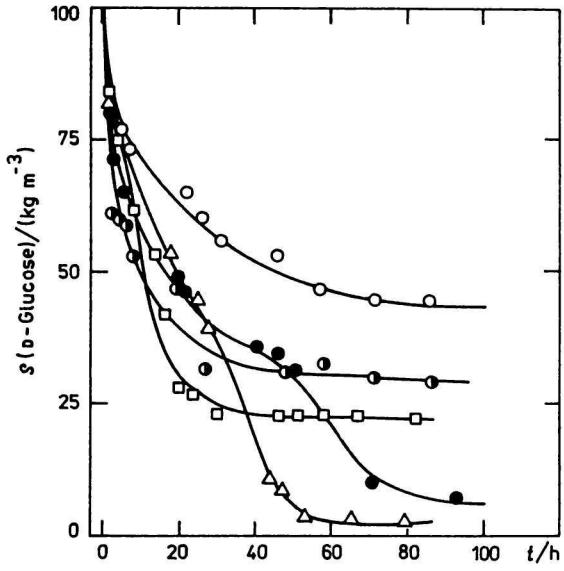

Fig. 2. Outlet D-glucose concentration from packed bed bioreactor with immobilized *S. cerevisiae vs.* time. Sample of gel: ◑ *1*, ● *2*, ○ *3*, △ *4*, □ *5*.

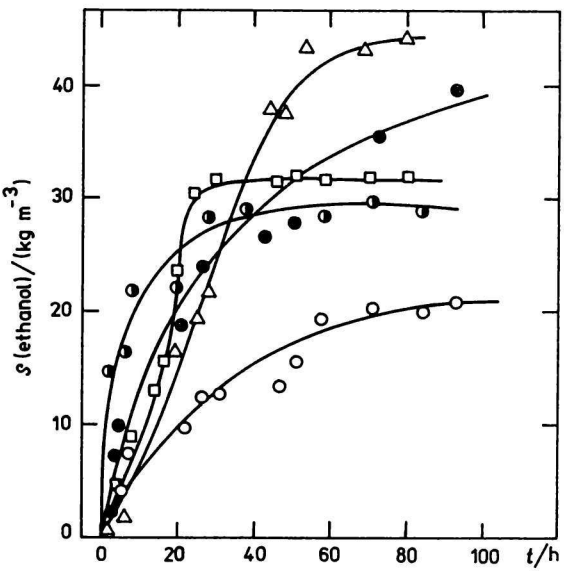

Fig. 3. Time dependence of outlet ethanol concentration from packed bed reactor with immobilized *S. cerevisiae*. Sample of gel: ◑ *1*, ● *2*, ○ *3*, △ *4*, □ *5*.

proteins), we expressed the productivity of the bioreactor on the amount of the immobilized biomass (in g of proteins). Table 3 reveals that an increase in the alginate concentration unfavourably influences the ethanol production. The addition of SiO_2 proved to be favourable, especially for the sample *4*.

Besides the investigation of the influence of alginate gel on the bioreactor productivity, the growth of biomass in gel beads was studied during continuous

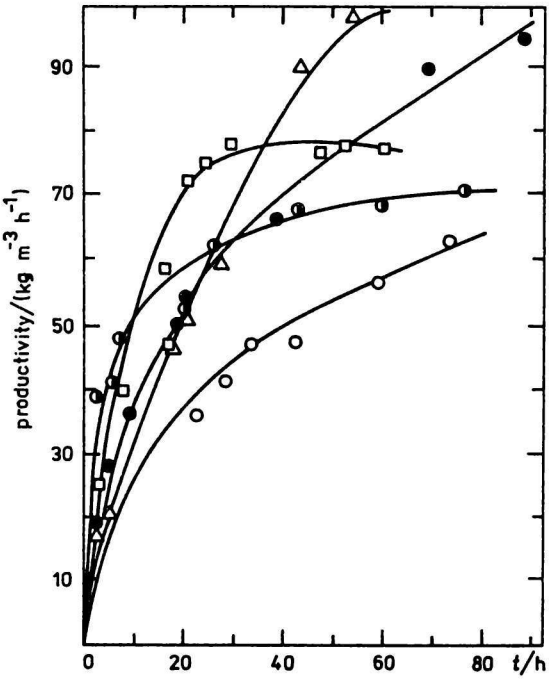

Fig. 4. Effect of alginate gels composition on the productivity of immobilized *S. cerevisiae* packed bed reactor.
Sample of gel: ◑ *1*, ● *2*, ○ *3*, △ *4*, □ *5*.

Table 3

Comparison of the substrate conversions and bioreactor productivities

Sample of gel	Conversion	Productivity $P/(\mathrm{kg\,m^{-3}\,h^{-1}})$	Total content of protein in bioreactor m/g	Productivity of reactor on g protein $P/(\mathrm{kg\,m^{-3}\,h^{-1}\,g^{-1}})$
1	0.7097	71.69	0.66	107.4
2	0.9346	94.66	0.69	136.6
3	0.5574	56.46	0.40	138.9
4	0.9613	97.36	0.45	215.6
5	0.7680	77.79	0.44	176.4

Table 4

Protein concentration at the beginning and the end of experiments

Sample of gel	Protein concentration at the beginning $\varrho/(g\,dm^{-3})$	Protein concentration at the end $\varrho/(g\,dm^{-3})$	Increase
1	4.45	19.8	4.4
2	4.62	20.3	4.4
3	2.71	13.6	5.0
4	3.01	14.8	4.9
5	2.94	15.1	5.1

experiments. For this reason the amount of proteins bound in alginate beads was determined at the beginning and the end of the experiments. In Table 4 the measured values are given of protein concentration in the carrier at the beginning and the end of experiment.

In studying the influence of the alginate gel composition on the productivity of a bioreactor with immobilized cells *S. cerevisiae* PZ 43 CHTF the following conclusions were drawn:

1. For continuous ethanol production in a fixed bed, the highest productivity ($97.36\,kg\,m^{-3}\,h^{-1}$) was attained in the case of gel *4*. Comparing these values one can see that the influence of the carrier composition on the productivity is not negligible in a continuous process.

2. In continuous experiments it was recognized that the amount of biomass bound in alginate beads, which was determined on the basis of the overall nitrogen, was five times higher at the end of the experiments.

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