

PROCESS OF CONTINUOUS PRODUCTION OF OLIGOAMINOSACCHARIDES IN A COLUMN REACTOR

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Abstract

*A continuous production of oligoaminosaccharides from chitosan was achieved with a column reactor packed with chitosanolytic enzymes-containing mycelia of *Mucor circinelloides* and *Mucor racemosus* immobilized in porous carriers. The efficient work of the bed used for chitosan hydrolysis in a column reactor lasted for several or more than 10 days and depended on a type of a carrier and substrate concentration. Because a crucial parameter for large scale applications of enzymes is their operating stability, presented research intended to improve it through stabilization of proteins and elimination of their leakage from the carrier. Cross-linking of mycelial preparations of chitosanolytic enzymes with 1% glutaraldehyde did not increase their stability and further process optimization is necessary.*

Key words: *chitosan, chitosanolytic enzymes, Mucor, chitooligosaccharides, low-molecular weight chitosan, continuous process.*

Abbreviations: *ACH_(RSC) - chitosanolytic activity of immobilized mycelium determined on the basis of a rise in reducing sugars concentration due to chitosan hydrolysis; ACH_(V) - chitosanolytic activity of immobilized mycelium determined on the basis of the decrease in a viscosity average molecular weight of chitosan; CHOS - products with an average molecular weight below 3.9 kDa; GA - glutaraldehyde; LMWCh - products with an average molecular weight ranging between 3.9 kDa and 20 kDa; Mv_η - viscosity average molecular weight of chitosan; PC - concentration of proteins; RSC - reducing sugars concentration.*

1. Introduction

Chitosan is a N-deacetylated derivative of chitin - copolymer built of β -(1,4)-2-amino-2-deoxy- β -D-glucose and β -(1,4)-2-acetamido-2-deoxy- β -D-glucose units. Chemical, physical or enzymatic degradation of chitosan produces oligoaminosaccharides (*CHOS* and *LMWCh*) – that have many interesting properties. They are bioactive, biodegradable, non-toxic and what is advantageous for their industrial applications - fractions with low polymerization degree are very well soluble at neutral pH. These chitosan derivatives display numerous biological activities: antibacterial, antifungal, antiviral, antitumor and antioxidant, exert fat lowering effect and stimulate immune system [1, 2]. Enzymatic method of chitosan hydrolysis (unlike chemical and physical methods) yields products with an intended degree of polymerization and N-deacetylation - parameters which determine their biological activity.

Therefore, many new strategies to produce oligoaminosaccharides with strictly defined molecular weight by using environmentally friendly enzymatic hydrolysis of chitosan have been developed [3 - 6]. Enzymatic processes of chitosan oligomers production were carried out in traditional batch reactors containing free enzymes, in column reactors with immobilized biocatalysts, in ultrafiltration membrane reactors and in other reactor systems which were combinations of aforementioned ones. Continuous production of *CHOS* and *LMWCh* through chitosan hydrolysis with immobilized enzymes is a cost effective method. The immobilized biocatalyst offers a number of technological advantages, such as: its reusability, continuous operation, rapid termination of process and controlled product formation.

Our earlier studies [7 - 9] showed that defatted mycelia of *M. circinelloides* and *M. racemosus* were applicable to industrial production of chitosan oligomers with different polymerization degrees: low-molecular weight chitosan, chitooligosaccharides and D-glucosamine. The chitosanase of *M. circinelloides* has been isolated from fungal cells, partially purified and characterized [9 - 11]. The preliminary results related to continuous hydrolysis of chitosan in a column reactor packed with mycelia of *Mucor* strains immobilized in polyurethane (PU) carriers have been described in the previous paper [9].

An objective of this study was to determine the optimum conditions of fungal mycelium immobilization in PU foams and to improve biocatalyst's stability in continuous process of chitosan hydrolysis through elimination of enzyme leakage from the column reactor.

2. Materials and methods

2.1. Microorganisms and culture conditions

The strains of *Mucor circinelloides* and *Mucor racemosus* from the pure culture collection of the Institute of Technical Biochemistry of TUL were cultivated with agitation at 180 r.p.m. for 72 h at 30 °C in a culture medium containing corn steep liquor powder (3.7% w/v) and olive oil (2.7% v/v). The initial pH of the medium was 4.7.

2.2. Method of immobilization of *Mucor* strains mycelia

The *Mucor* strains were cultivated under conditions described in section 2.1. Prior to sterilization, one of the following polyurethane foams was added to the culture medium: type A – the foam produced by Corpura B.V., Netherlands, with pore diameter varying between 170 and 220 μm , diced into $1.0 \times 1.0 \times 0.5$ cm cubes, the quantity of this carrier in the culture medium ranged between 1.5 and 3.5% (w/v); type B – the foam produced by Eurofoam, Poland, with pore diameter of ~ 1.96 mm, cut into $1.0 \times 1.5 \times 0.2$ cm cubes and added to the culture medium 0.5 – 2.5% w/v. The mycelia bonded to the polyurethane carriers were carefully washed with water and defatted with acetone, air-dried at room temperature and used in continuous processes of chitosan hydrolysis carried out in a column reactor.

2.3. Treatment of immobilized mycelia with glutaraldehyde

To stabilize the activity of chitosanolytic enzymes, fungal mycelium immobilized in PU carrier was shaken (220 rpm) in 1.0% (v/v) glutaraldehyde (GA) solution at room temperature for 15 or 30 min. Then the samples of biocatalyst were separated, washed with deionized water and shaken (220 rpm) in 1.0% w/v glycine solution at room temperature for 24 hours, washed again with deionized water and dried at room temperature.

2.4. Enzymatic hydrolysis of chitosan in a column reactor

The schematic diagram of the column reactor system used for continuous chitosan hydrolysis (consisting of substrate and product tanks, peristaltic pump and column reactor packed with immobilized mycelium) and connected with a Vivaflow membrane module (Sartorius, Germany) is presented in **Figure 1**. The immobilized mycelium was put into a glass column (2.5 cm \times 18.5 cm) and thoroughly (for 24 hours) washed with deionized water to remove non-adsorbed fragments of mycelium. The reaction procedure consisted in passing of 0.5%

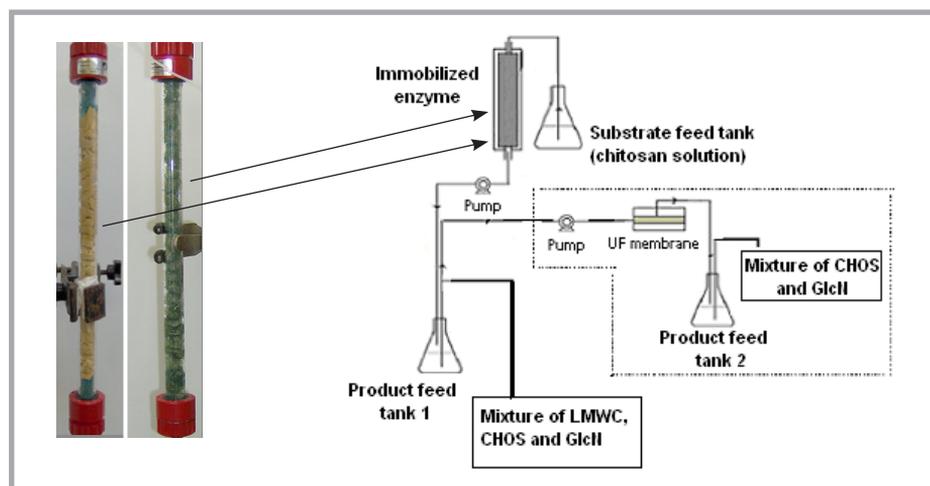


Figure 1. Schematic diagram of a column reactor used for continuous production of oligoaminosaccharides.

chitosan acetate solution (pH 5.5) through the packed column at a flow rate of $1 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$. The obtained mixture of LMWCh, CHOS and some D-glucosamine was supplied to the product tank no. 1 and additionally to UF membrane module (membranes with different cut-off were used) to separate products with different target DP. The process of chitosan hydrolysis was conducted at $24 \text{ }^\circ\text{C}$. The substrate and mixtures of products were kept at $4 \text{ }^\circ\text{C}$.

2.5. Determination of enzymatic activities

The *chitosanolytic activity* $ACH_{(V)}$ of immobilized enzymes was determined on the basis of the decrease in a viscosity average molecular weight (\overline{Mv}_η) of chitosan and was expressed as an amount of enzyme necessary to decrease \overline{Mv}_η of chitosan by 1 kDa in 1 minute under the conditions described below [1 unit = 1 kDa min^{-1}]. Reaction mixtures (pH 5.5) contained 0.5% chitosan solution in 0.5% acetic acid (1 ml) and mycelium immobilized in PU (5 mg of mycelium). pH of reaction mixtures was adjusted by using 2 M CH_3COONa solution. Chitosan hydrolysis was carried out at $24 \text{ }^\circ\text{C}$ for 120 min and was terminated by 5 min incubation in a boiling water bath. Controls (with the same composition as the samples) were first incubated for 5 min in the boiling water bath to inactivate enzymes and then for 120 min at $24 \text{ }^\circ\text{C}$.

The *chitosanolytic activity* $ACH_{(RSC)}$ of immobilized enzymes was determined on the basis of a rise in reducing sugars concentration due to chitosan hydrolysis and was expressed as an amount of enzyme necessary to produce $1 \text{ } \mu\text{mol}$ of reducing sugars in 1 min [$1 \text{ U} = 1 \text{ } \mu\text{mol min}^{-1}$]. Reaction mixtures (pH 5.5) contained 0.5% chitosan solution in 0.5% acetic acid (1 ml) and mycelium immobilized in PU (10 mg of dry mycelium). pH of reaction mixtures was adjusted by using 2M CH_3COONa solution. Chitosan hydrolysis was carried out at $24 \text{ }^\circ\text{C}$ for 24 h and was terminated by 5 min incubation in a boiling water bath. Controls (with the same composition as the samples) were first incubated for 5 min in the boiling water bath to inactivate enzymes and then for 24 h at $24 \text{ }^\circ\text{C}$.

2.6. Analytical methods

The viscosity average molecular weight (Mv_η) of chitosan and its digestion products was determined by the viscometric method by using one of the following solutions:

0.1 M sodium chloride, 0.2 M acetic acid and 4.0 M urea (for chitosan with \overline{Mv}_η between 113 kDa and 492 kDa),	0.30 M sodium chloride and 0.33 M acetic acid (for chitosan with \overline{Mv}_η between 13 kDa and 135 kDa)
and calculated according to the Mark-Houwink's equation [$\eta = k\overline{Mv}_\eta^\alpha$] with	
$k = 8.93 \times 10^{-4}$ and $\alpha = 0.71$	$k = 3.41 \times 10^{-3}$ and $\alpha = 1.02$

respectively [12]. The viscosity measurements were conducted at $25.0 \pm 0.1 \text{ }^\circ\text{C}$ using an Ubbelohde's viscometer (Schott GmbH, type 53110/I). ×

The concentration of reducing sugars (RSC) released from chitosan was determined by Somogyi-Nelson method [13] using D-glucosamine as a standard.

Protein concentration (PC) was determined by the Lowry et al. method [14] using bovine serum albumin as the standard.

Products released from chitosan were analyzed by thin layer chromatography (TLC) on Silica G 60 plates using a mixture n-propanol / 30% ammonia water (2:1 v/v) as the developing solvent. Amino sugars were visualized by spraying with 0.1% (w/v) ninhydrin in 99% ethanol.

3. Results and discussion

3.1. Optimization of conditions of *Mucor* strains mycelia immobilization

Two *Mucor* strains (*M. circinelloides* and *M. racemosus*) were cultivated for 72 h at 30 °C under agitated conditions (180 r.p.m.) in a liquid medium containing corn steep liquor and olive oil as well as various amounts of one of the polyurethane foams described in the section 2.2. The efficiency of immobilization was determined through measurements of defatted and dried mass of mycelium overgrowing the carrier. It depended on the carrier amount in the culture medium and its porosity.

It was found that the highest efficiency of the *Mucor* strains immobilization in polyurethane foams corresponded to their following amounts in the culture medium: 2.5% (w/v) for *type A* and 1.0% (w/v) for *type B* (Table 1).

An increase or a decrease in the carrier content in the culture medium gave rise to the lower immobilization yield; it means less mycelium was associated with the foams.

Table 1. The effect of amount of PU-carriers in the culture medium on the yield of *M. circinelloides* and *M. racemosus* mycelia immobilization; ¹⁾*type A* – the polyurethane foam produced by *Corpura B.V.*, Netherlands, with pore diameter varying between 170 and 220 μm, diced into 1.0 × 1.0 × 0.5 cm cubes; ²⁾*type B* – the polyurethane foam produced by *Eurofoam*, Poland, with pore diameter of ~ 1.96 mm, cut into 1.0 × 1.5 × 0.2 cm cubes, ³⁾ dry weight of defatted mycelium was measured.

Strain	Type of PU carrier	Content of PU carrier in culture medium, % w/v	Concentration of biomass, g/PUg ³⁾
<i>M. circinelloides</i>	A ¹⁾	1.5	0.52
		2.5	0.56
		3.5	0.16
	B ²⁾	0.5	0.00
		1.0	0.59
		1.5	0.53
<i>M. racemosus</i>	A ¹⁾	2.5	0.09
		1.5	0.48
		2.5	0.59
	B ²⁾	3.5	0.20
		0.5	0.00
		1.0	0.58
		1.5	0.52
		2.5	0.06

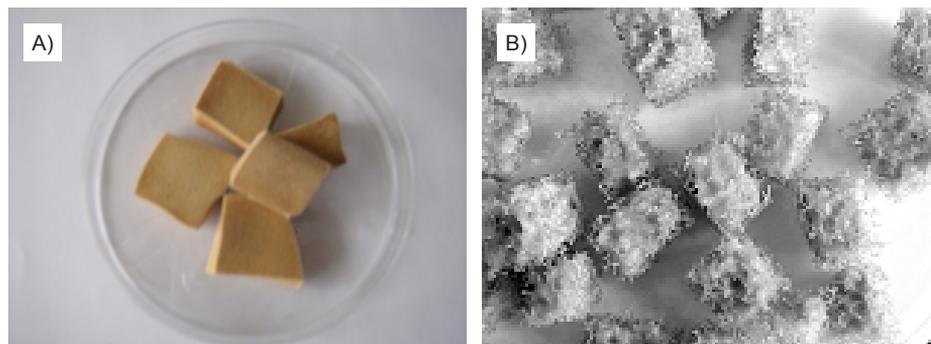


Figure 2. Mycelium of *M. circinelloides* immobilized in polyurethane foams: (A) Corpura B.V., Netherlands, pore diameter varying between 170 and 220 μm and (B) Eurofoam, Poland, pore diameter of ~ 1.96 mm.

When the content of foams *type B* in the culture medium was too low the growing mycelium was not bonded to the carrier.

In these immobilized preparations the mycelia of both *Mucor* strains were firmly attached to the carriers. The immobilized chitosanolytic enzymes obtained through this method have been tested in periodic and continuous processes of chitosan hydrolysis.

3.2. Continuous production of chitosan oligomers

3.2.1. Choice of the carrier

The continuous processes of chitosan hydrolysis have been carried out in a column reactors packed with *M. circinelloides* and *M. racemosus* mycelia immobilized in both the types of PU foams, A and B (see **Figure 2**).

The efficiency of chitosan hydrolysis was estimated based on reducing sugars concentration in eluates from the column collected each day (for 5 days). The results are presented in **Table 2**. It was shown that chitosan hydrolysis efficiency (expressed as the reducing sugars concentration divided per total mass of mycelium contained in the column) was the highest for the column reactor packed with mycelia of both the strains immobilized in the *type B* carrier (pore diameter of 1.96 mm). The amount of mycelium contained in *type A* PU foams was c.a. 4 times higher but the value of the efficiency of chitosan hydrolysis was $\sim 35\%$ lower in comparison to the *type B* foam.

Batch processes of chitosan hydrolysis (section 3.3.) revealed that the activities of the biocatalysts immobilized in *type B* carrier ($ACH_{(V)}$ and $ACH_{(RSC)}$) assayed during 24 h process) were by 30% and 35% higher for *M. circinelloides* and *M. racemosus*, respectively, as compared to the preparations immobilized in the *type A* foam (**Table 3**). This difference can be ascribed to different porosity of the foams. The *type A* foams (circa 10-fold smaller pores) were characterized by higher diffusion resistance (slower exchange of substrates and reaction products) as compared to the *type B* foams. The second reason of different efficiency of chitosan hydrolysis catalyzed by these biocatalysts (A and B) could be different

Table 2. Comparison of chitosan hydrolysis efficiencies in continuous processes catalyzed by *Mucor mycelia* immobilized in two different polyurethane carriers; 1) type A – the polyurethane foam produced by Corpura B.V., Netherlands, with pore diameter varying between 170 and 220 μm , diced into $1.0 \times 1.0 \times 0.5$ cm cubes, 1 g of immobilized biocatalyst contained in g: 0.56 or 0.58 of *M. circinelloides* or *M. racemosus* dry mycelium, respectively; 2) type B – the polyurethane foam produced by Eurofoam, Poland, with pore diameter of ~ 1.96 mm, cut into $1.0 \times 1.5 \times 0.2$ cm cubes, 1 g of immobilized biocatalyst contained in g: 0.56 or 0.51 of *M. circinelloides* or *M. racemosus* dry mycelium, respectively; Explanation: column dimensions – 2.5×18.5 cm, the flow rate of chitosan solution (0.5% chitosan acetate, pH 5.5) – $1 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$, temperature of chitosan hydrolysis of 24°C . Samples of chitosan degradation products were taken from product feed tank no 1 (see Figure 1) on the first five days of column work and the mean value of reducing sugars concentration (RSC) was determined.

Type of immobilized biocatalyst		Content of dry mycelium in a column reactor, g	RSC, $\mu\text{mol}/\text{cm}^3$	Efficiency, $\mu\text{mol cm}^{-3}\text{g}^{-1}$
Strain	Carrier			
<i>M. circinelloides</i>	Type A ¹⁾	6.70	0.88 ± 0.03	0.131
	Type B ²⁾	1.61	0.32 ± 0.025	0.199
<i>M. racemosus</i>	Type A ¹⁾	7.33	0.75 ± 0.03	0.102
	Type B ²⁾	1.64	0.26 ± 0.01	0.159

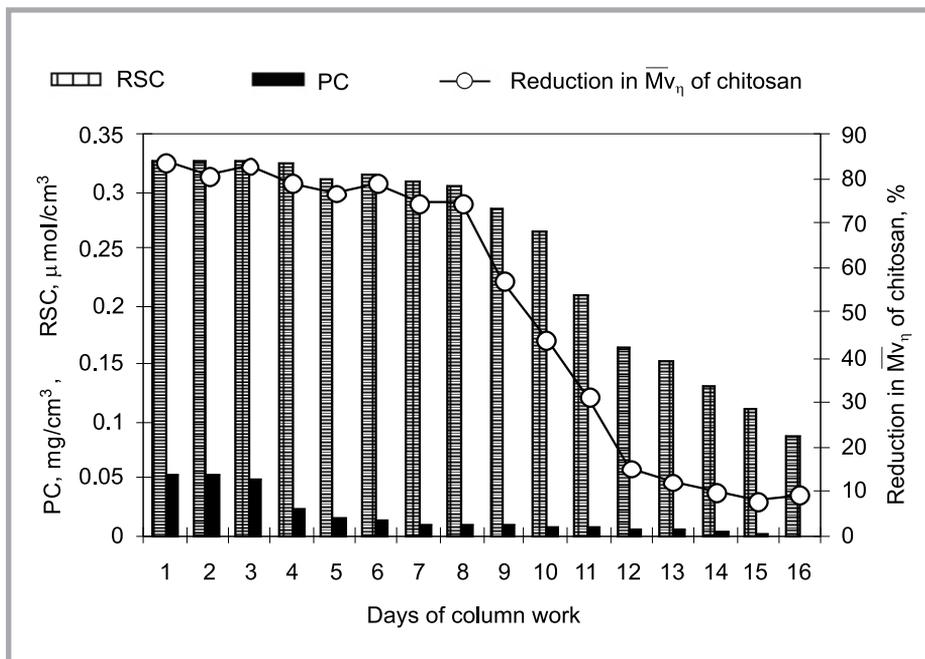


Figure 3. Dynamics of continuous process of chitosan hydrolysis catalyzed by *M. circinelloides* mycelium immobilized in type B PU carrier with pore diameter of ~ 1.96 mm. Samples of chitosan degradation products were taken from product feed tank no 1 (see Figure 1). **Explanation:** column dimensions – 2.5×18.5 cm, flow rate of chitosan solution (0.5 % chitosan acetate, pH 5.5) – $1 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$, mass of immobilized biocatalyst – 2.98 g (PU containing approximately 1.66 g of dried mycelium), temperature of chitosan hydrolysis of 24°C . Reduction in \overline{M}_{v_n} of chitosan was determined under standard assay conditions (section 2.6.) taking the initial \overline{M}_{v_n} of biopolymer (421 kDa) as 100%.

activity of immobilized enzymes. Unfortunately, liberation of the mycelium from the carrier A (to determine the chitosanolytic activity) is impossible.

3.2.2. Determination of stability of chitosanolytic enzymes immobilized in PU carriers in a column reactor

Mycelia of *M. circinelloides* and *M. racemosus* immobilized in the carrier B (superior to preparations immobilized in the carrier A) were used in continuous chitosan hydrolysis in order to determine their operational stability. The eluates collected during this process were analyzed for concentration of proteins (PC) leaking from the columns, apart from assays of reducing sugars concentration (RSC) and Mv_{η} of chitosan. Our earlier investigations showed that the leakage of enzyme was the principal reason of a decrease in the activity of the immobilized biocatalysts [9]. Results presented in **Figures 3** and **5** and TLC profiles of chitosan hydrolysis products contained in the eluates (shown in **Figures 4** and **6**) revealed that RSC and the degree of chitosan degradation (specified by Mv_{η} in % and oligoaminosaccharides profiles) remained unchanged for 6 (*M. racemosus*) and 8 days (*M. circinelloides*) of the column operation.

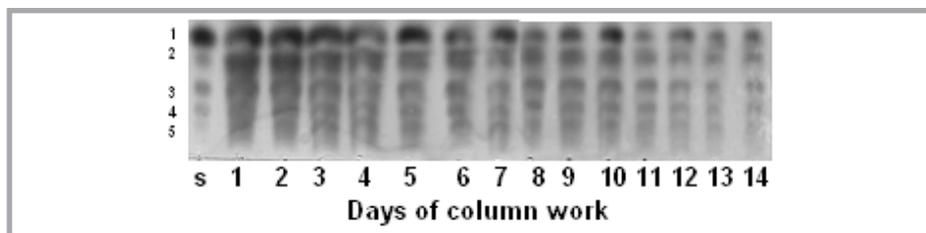


Figure 4. TLC profiles of products obtained during continuous hydrolysis of chitosan catalyzed by immobilized *M. circinelloides* mycelium in a column reactor. Samples of chitosan degradation products were taken from product feed tank no 2 (see Figure 1). Products dialyzable through UF membrane with 5 kDa cut off were analyzed by TLC.; Lane S – standards of: GlcN (1), (GlcN)₂ (2), (GlcN)₃ (3), (GlcN)₄ (4) and (GlcN)₅ (5). Lanes from 1 to 14 - mixtures of chitooligosaccharides obtained as described under Figure 3.

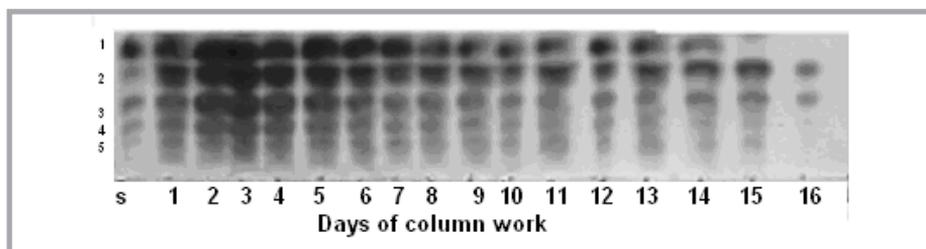


Figure 6. TLC profiles of products obtained during continuous hydrolysis of chitosan catalyzed by immobilized *M. racemosus* mycelium in a column reactor. Samples of chitosan degradation products were taken from product feed tank no 2 (see Figure 1). Products dialyzable through UF membrane with 5 kDa cut off were analyzed by TLC; Lane S – standards of: GlcN (1), (GlcN)₂ (2), (GlcN)₃ (3), (GlcN)₄ (4) and (GlcN)₅ (5). Lanes from 1 to 16 - mixtures of chitooligosaccharides obtained as described under Figure 5.

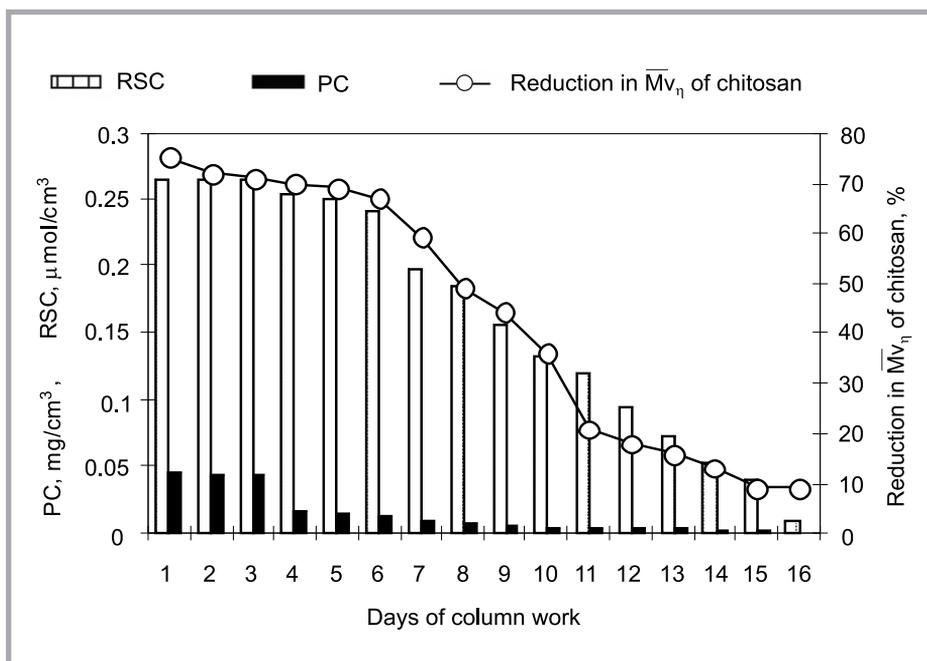


Figure 5. Dynamics of continuous process of chitosan hydrolysis by *M. racemosus* mycelium immobilized in type B PU carrier (with pore diameter of ~ 1.96 mm). Samples of chitosan degradation products were taken from product feed tank no 1 (see Fig. 1). **Explanation:** column dimension – 2.5×18.5 cm, flow rate of chitosan solution (0.5% chitosan acetate, pH 5.5) – $1 \text{ cm}^3 \text{ cm}^{-2} \text{ h}^{-1}$, mass of immobilized biocatalyst – 3 g (PU containing approximately 1.6 g of dried mycelium), temperature of chitosan hydrolysis of 24°C . Reduction in $\overline{M}_{v\eta}$ of chitosan was determined under standard assay conditions (section 2.6.) taking the initial $\overline{M}_{v\eta}$ of biopolymer (421 kDa) as 100%.

The highest concentration of proteins released from the immobilized preparations was observed during the first 3 days (the eluates contained approximately $50 \mu\text{g}$ protein in 1 cm^3). In the next days this concentration considerably decreased. We think that the proteins liberated during the first days were either not involved in chitosan hydrolysis or they did not decide of its dynamics because the efficiency of the process catalyzed by the immobilized biocatalysts was almost the same. However, proteins were present in all samples of chitosan hydrolyzates collected from the columns (protein concentration varied between 20 and $10 \mu\text{g}/\text{cm}^3$ on the 4th and 7th day of the process) and their slow, continuous leakage could be the main reason of gradual inactivation of the biocatalysts. Therefore, we tried to improve their stability through the traditional cross-linking with glutaraldehyde.

3.3. PU-foam-immobilized mycelia stabilization by cross-linking with glutaraldehyde

Cross-linking with bifunctional compounds (e.g. glutaraldehyde) is frequently used to stabilize proteins [15, 16] and therefore *Mucor* mycelia (*M. circinelloides* and *M. racemosus*) immobilized in PU (type A and B) were treated with 1% solution of glutaraldehyde (as described in section 2.3). The stability of the cross-linked biocatalysts in chitosan

hydrolysis (repeated batch process) was compared with the stability of the original preparations. The repeated batch process consisted in putting the biocatalysts to fresh portions of the substrate (prepared as described in section 2.5 for $ACH_{(RSC)}$ activity determination) after each 24 h batch (carried out with intensive agitation at 220 r.p.m.).

Results shown in **Table 3** demonstrate that the original biocatalysts (immobilized in *type A* and *B* foams) were unstable in the repeated batch processes because in the first round they lost more than 85% of $ACH_{(RSC)}$ activity (as much as 2 mg of protein leaked from 100 mg of mycelium). We believe that it was a result of intensive agitation.

The leakage of proteins from all the cross-linked immobilized preparations of *Mucor* strains was lower during the first round of these processes, but unfortunately, the chitosanolytic activity was drastically lower (see **Table 4**). Besides, the mechanical durability of GA-modified preparations was weaker. Fragments of the GA-treated mycelia were released from PU carriers (in particular, from *type B* foams).

Our trial aimed at stabilization of the immobilized in PU preparations of chitinases through 15 - 30 minute treatment with 1% glutaraldehyde was unsuccessful. We plan either to optimize cross-linking conditions (type and concentration of cross-linking agent, temperature and duration of treatment) or to use another method for this purpose.

We would like to mention that the same mycelia of *Mucor* strains immobilized in the foam with pore diameter of 1.96 mm (*type B*) have been successfully used as rich sources of lipase in continuous processes of plant oils transesterification with ethanol in systems con-

Table 3. Chitosanolytic activities ($ACH_{(RSC)}$ and $ACH_{(V)}$) and protein retention in mycelia of *Mucor* strains immobilized in 2 different PU carriers tested during four-round repeated batch processes of chitosan hydrolysis. ¹⁾, ²⁾ – information shown in Table 2. **Explanations:** mycelium immobilized in PU carrier was shaken (220 r.p.m.) with 0.5% chitosan acetate solution (pH 5.5) for 24 h (one reaction cycle), filtered from the reaction mixture and repeatedly used for the next batch. Other details related to reaction mixture composition are described in section 2.5.

Type of immobilized biocatalyst		Cycle of hydrolysis	$ACH_{(RSC)}$, U/g of biomass ($\times 10^{-2}$)	PC, mg/cm ³ ($\times 10^{-2}$)	$ACH_{(V)}$, U/g of biomass
Strain	Carrier				
<i>M. circinelloides</i>	Type A ¹⁾	1	5.48	38.27	324.00
		2	0.76	4.44	-
		4	0.26	1.50	-
	Type B ²⁾	1	7.72	29.23	448.20
		2	1.22	3.21	-
		4	0.24	1.20	-
<i>M. racemosus</i>	Type A ¹⁾	1	2.34	13.84	156.60
		2	0.78	2.10	-
		4	0.04	0.80	-
	Type B ²⁾	1	3.68	15.89	246.94
		2	0.50	1.15	-
		4	0.26	0.41	-

Table 4. The effect of PU-foam-immobilized-mycelia treatment with GA on stability of chitosanolytic activity ($ACH_{(RSC)}$) and protein retention; 1), 2) – information shown in Table 2. **Explanation:** Conditions of glutaraldehyde (GA) treatment of immobilized mycelium were described in section 2.3.

Type of immobilized biocatalyst		Cycle of hydrolysis	Time of mycelium treatment with GA, min.	$ACH_{(RSC)}$ [U/g of biomass] ($\times 10^{-2}$)	PC [mg/cm ³] ($\times 10^{-2}$)
Strain	Carrier				
M. circinelloides	Type A ¹⁾	1	15	0.92	1.04
			30	1.44	0.19
		4	15	0.08	0.21
			30	0.13	0.05
	Type B ²⁾	1	15	0.81	1.95
			30	1.60	0.60
		4	15	0.29	0.11
			30	0.31	0.02
M. racemosus	Type A ¹⁾	1	15	0.49	1.40
			30	0.47	0.35
		4	15	0.02	0.16
			30	0.01	0.13
	Type B ²⁾	1	15	0.80	0.98
			30	0.83	0.26
		4	15	0.04	0.18
			30	0.08	0.13

taining petroleum ether [17]. More hydrophobic environment of these processes rendered the biocatalyst very stable.

4. Conclusions

This study showed that mycelia of Mucor filamentous fungi immobilized in PU carriers efficiently catalyzed the continuous hydrolysis of chitosan. This process is a potential, cost effective method of production of oligoaminosaccharides with different polymerization degrees in industrial scale. Therefore, we intend to increase the stability of immobilized biocatalysts used in column reactors.

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