

Biotreatment of desized cotton fabric by commercial cellulase and xylanase enzymes

E. Csiszár*, K. Urbánszki, G. Szakács

Technical University of Budapest, P.O. Box 91, H-1521 Budapest, Hungary

Abstract

Desized cotton fabric was subjected to biotreatment with seven commercial cellulase and hemicellulase enzymes in non-agitated and agitated systems at 50°C for 0.5–4 h. The enzymes performed better in agitated bath than in non-agitated ones. All enzymes at 1 g/l concentration in 2 h caused weight loss less than 6%. Those three enzymes (Celluclast 1.5, Cellusoft L and Cellulase (EBT)) which exhibited the highest filter paper activity (FPA) showed the most aggressive action on cotton in agitated system at 1 g/l concentration when time of treatment exceeded 2 h. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Cellulase; Hemicellulase; Cotton; Bioscouring; Degradation

1. Introduction

In the last decade natural biopolymer degrading or modifying enzymes are increasingly used in the textile industry. Cellulases, hemicellulases and pectinases acting on native cellulosic fibres (cotton, flax, hemp, jute, rami, etc.) became the target enzymes in bioprocessing. The production of “aged” denim garments with cellulases is the most successful enzyme process that has emerged in the textile industry in the last 10 years. Each year approx. 1 billion jeans are produced and a big part of it is further finished to stonewashed fashion. The cellulase enzyme removes the cotton fragments from the surface of the garment

and facilitates the abrasion of indigo dye from the fibre surface. Traditional stonewashing process carried out by pumice stones was vastly replaced by enzymatic biostoning and fading or combined procedures [1].

Biofinishing or biopolishing represents another application of cellulases for non-denim cellulosic fabrics and garments. To various extents, all cellulosic materials have a tendency to fuzz formation. Fuzz is the designation for short fibres protruding from the surface of the yarn and fabrics. Traditionally, chemical agents (cationic surface-active compounds) are used to decrease the fuzz formation. Alternatively, cellulosic fabrics can be subjected to treatments with cellulolytic enzymes, and the action of cellulases removes small loose fibre ends that protrude from the fabric surface [2].

Recently published results indicate that enzymes, mainly cellulases and several non-cellulolytic enzymes (lipases, proteases, pectinases) may be used

* Corresponding author. Tel.: +36-1-463-1423; fax: +36-1-463-3648.

E-mail address: emi.oct@chem.bme.hu (E. Csiszár).

effectively in the cleaning processes of cotton [3,4]. Raw cotton contains approx. 10% of non-cellulosics (waxes, pectins, proteins, non-cellulosic polysaccharides, inorganics, lignin-containing impurities, colouring materials, etc.) depending upon variety and cultivation conditions. These impurities are mainly located in the outer layers of the fibre in the cuticle and the primary wall. Traditional cleaning procedure applies concentrated sodium hydroxide solution (alkaline scouring) and additional hydrogen peroxide and/or sodium hypochlorite solutions (bleaching) to eliminate these impurities.

We demonstrated recently [5] that cellulase enzymatic treatment prior to the alkaline scouring process enhanced both the removal and degradation of seed-coat fragment impurities of cotton fabrics. When consecutive cellulase treatment and conventional alkaline scouring were combined, the increase in whiteness of the fabrics was significantly improved. Cellulase pretreatment also allowed the reduction of the hydrogen-peroxide consumption in the consecutive chemical bleaching step [6]. In this study, the effect of seven commercial cellulase and hemicellulase enzymes was tested on fabric weight loss, reducing sugar liberation and change in degree of polymerisation (DP) of desized cotton fabrics. The changes in fibre surface were monitored by scanning electron microscopy.

2. Experimental

Greige cotton print cloth fabric (122 g/m²) obtained from Testfabric, NJ was used for the experi-

ments after amylase enzymatic desizing. Cellusoft L, Viscozyme 120 L, Celluclast 1.5 L, Pulpzyme HC, Denimax L and Denimax Acid L enzymes were supplied by Novo-Nordisk, Copenhagen, Denmark. Cellulase (EBT) was provided by the Environmental BioTechnologies, Santa Rosa, CA. The enzyme activities (Table 1) were measured at 50°C from the products using internationally recognized methods: filter paper cellulase activity (FPA) according to Ghose [7], β -glucosidase (BG) activity according to Kubicek [8], 1,4- β -endoglucanase (EG) activity according to Bailey and Nevalainen [9], xylanase activity according to Bailey et al. [10]. These enzymes exhibit maximal activity at pH 4.5–5.0 values, except Pulpzyme HC and Denimax L, which act preferably around pH 7.

Desized cotton fabric (3.0 g) was treated with enzymes in non-agitated and agitated systems at pH 5.0 (0.05 M acetate buffer) for the enzymes Cellusoft L, Viscozyme 120 L, Celluclast 1.5 L, EBT and Denimax Acid L, or at pH 7.0 (0.05 M phosphate buffer) for the enzymes Pulpzyme HC and Denimax L; liquor ratio was 1:100; enzyme concentrations were 0.5 and 1 g/l; treatment time 0.5, 1, 2 and 4 h; incubation temperature 50°C; and a non-ionic surfactant concentration of 1 g/l. After enzymatic treatment, the substrate was washed twice in hot distilled water to deactivate the enzyme and then air-dried. Reference treatments were carried out similarly without enzyme addition.

Fabric weight loss was determined by weighing the samples before and after the treatment following 24 h of conditioning at 20°C and 65% rh. Reducing sugar was determined by dinitrosalicylic acid method

Table 1
Activity values of commercial enzymes used throughout the experiments

Enzyme	Cellulase (FPU) [FPU/ml], pH 4.8	1,4- β -endoglucanase [EGU/ml], pH 4.8	Xylanase [IU/ml] pH 4.8	β -glucosidase [IU/ml], pH 4.8
Cellusoft L	100	37,000	5000	76
Viscozyme 120 L	8	12,700	800	5
Celluclast 1.5 L	67	28,000	2300	11
Pulpzyme HC ^a	0.02	33	120,000	0.2
Denimax L ^a	7	2500	16,700	87
Denimax Acid L	18	6300	1000	12
Cellulase (EBT)	108	73,200	12,900	81

^aAt pH 7.0.

[11]. DP was measured by viscometric method [12]. A Hitachi S-570 scanning electron microscope was used for the investigation of the fibre surface morphology.

3. Results and discussion

3.1. Effect of enzymatic treatment on fabric weight loss

Enzymatic degradation of cotton is generally characterised by weight loss. Treatments in buffer

solutions without enzyme resulted in approx. 0.5% weight loss in 4 h. Water-extractable impurities came into the buffer solution. The longer treatments in buffer solution do not cause higher weight loss. The weight loss is independent of the agitation of the buffer solution.

The weight loss values caused by enzymatic treatments are shown in Table 2. At 0.5 g/l enzyme concentration in non-agitated system, enzymes Cellusoft L, Viscozyme 120 L and Denimax Acid L do not cause significant degradation of the fabric even in a 4-h treatment. The weight loss is lower than 1%. Similar values (approx. 1%) are observed for

Table 2
Weight loss of desized cotton fabrics during enzymatic treatment

Enzymes	Concentration [g/l]	Agitation	Weight loss [%]			
			Time of treatment [h]			
			0.5	1	2	4
Control pH = 5	0	–	0.3	0.3	0.3	0.4
		+	0.3	0.5	0.4	0.5
Control pH = 7	0	–	0.2	0.2	0.4	0.4
		+	0.3	0.4	0.4	0.4
Cellusoft L	0.5	–	0.2	0.3	0.3	0.5
		+	1.3	1.5	1.6	2.0
	1	–	0.1	0.2	0.9	1.2
		+	0.8	1.7	4.0	5.2
Viscozyme 120 L	0.5	–	0.1	0.3	0.6	0.9
		+	0.9	1.0	1.1	1.2
	1	–	0.6	1.0	1.2	1.4
		+	1.0	1.0	1.2	2.3
Celluclast 1.5 L	0.5	–	1.0	1.3	1.9	2.6
		+	2.1	2.9	3.2	3.7
	1	–	2.0	2.1	2.3	3.1
		+	2.3	2.8	3.2	3.8
Pulpzyme HC	0.5	–	1.0	1.0	1.0	1.1
		+	1.2	1.2	1.2	1.4
	1	–	1.2	1.5	1.6	1.7
		+	1.4	1.6	1.6	1.6
Denimax L	0.5	–	0.8	0.9	1.0	1.2
		+	0.8	1.4	1.4	1.4
	1	–	0.5	0.6	0.6	0.7
		+	1.6	1.7	1.8	2.0
Denimax acid L	0.5	–	0	0	0.3	0.4
		+	0	0.1	0.7	1.0
	1	–	0.8	0.8	1.0	1.2
		+	0.7	1.1	1.2	1.7
Cellulase (EBT)	0.5	–	1.0	1.6	1.7	2.6
		+	1.8	2.5	4.6	6.3
	1	–	1.3	1.4	1.9	2.9
		+	2.2	2.4	5.7	7.0

Pulpzyme HC and Denimax L enzymes. The weight loss is practically independent from the time of treatment. Enzymes Celluclast 1.5 L and Cellulase (EBT) cause the most significant weight losses nearly 2.5% in 4 h. There is no significant effect of increase in enzyme concentration from 0.5 to 1 g/l on weight loss of fabric in non-agitated system.

In agitated system at 0.5 g/l enzyme concentration the fabric weight losses are less than 1.5% for enzymes Viscozyme 120 L, Pulpzyme HC and Denimax enzymes. Except for Denimax Acid L, the weight loss is practically independent from the treat-

ment time. Enzymes Celluclast 1.5 L and Cellulase (EBT) caused the highest weight losses. Increase in reaction time resulted in increase in fabric weight loss. In agitated system, Cellulase (EBT) is the most aggressive. Depending on the time of treatment, the weight loss is 1.8–6.3%. These data suggest that agitation has a significant impact on enzymatic degradation of raw cotton fabric. Weight loss values are much higher in agitated solutions than in non-agitated ones. The increase of enzyme concentration from 0.5 to 1 g/l has smaller effect on weight loss than the agitation of the solution. Similar results

Table 3
Reducing sugar liberation from desized cotton fabrics during enzymatic treatment
n. d. = not detectable.

Enzymes	Concentration [g/l]	Agitation	Reducing sugars [mg/g cotton fabric]			
			Time of treatment [h]			
			0.5	1	2	4
Control pH = 5	0	–	n. d.	n. d.	n. d.	n. d.
		+	n. d.	n. d.	n. d.	n. d.
		–	n. d.	n. d.	n. d.	n. d.
Control pH = 7	0	–	n. d.	n. d.	n. d.	n. d.
		+	n. d.	n. d.	n. d.	n. d.
		–	n. d.	n. d.	n. d.	n. d.
Cellusoft L	0.5	–	n. d.	n. d.	n. d.	n. d.
		+	n. d.	n. d.	n. d.	3.7
		–	n. d.	n. d.	5.6	8.6
Viscozyme 120 L	0.5	–	n. d.	n. d.	7.8	13.4
		+	4.9	5.3	5.6	6.1
		–	4.0	4.2	4.6	4.8
Celluclast 1.5 L	1	–	6.2	7.1	7.3	7.6
		+	5.2	5.3	5.4	5.8
		–	6.4	8.2	10.8	12.3
Pulpzyme HC	0.5	–	8.0	9.6	11.6	13.7
		+	n. d.	n. d.	n. d.	n. d.
		–	n. d.	n. d.	n. d.	n. d.
Denimax L	1	–	n. d.	n. d.	n. d.	n. d.
		+	n. d.	n. d.	n. d.	n. d.
		–	n. d.	n. d.	n. d.	n. d.
Denimax acid L	0.5	–	n. d.	n. d.	n. d.	n. d.
		+	4.0	4.0	4.0	5.3
		–	n. d.	n. d.	3.9	4.2
Cellulase (EBT)	0.5	–	4.0	4.0	4.7	6.2
		+	4.1	5.7	8.9	13.2
		–	4.7	6.4	10.0	18.3
Cellulase (EBT)	1	–	4.9	9.7	13.6	22.0
		+	8.7	9.8	21.3	25.8
		–				

were reported by other authors for bleached cotton substrate [13]. Joint effect of agitation and increase in enzyme concentration is especially significant on the activity of Cellusoft L.

Biotreatment of desized cotton fabric with seven commercial cellulases and hemicellulases at 0.5 and 1 g/l enzyme concentration at 50°C for 0.5–4 h does not cause significant weight loss in non-agitated system. The weight losses are less than 3%, and significant cotton cellulose degradation does not occur in these experiments. It is likely that mainly the surface fibrils, small protruding fibres, seed-coat fragments and other natural impurities of cotton fibre are degraded significantly. Agitation of the enzyme solution increases the cellulose degrading effect of the enzymes Cellusoft L, Celluclast 1.5 L and Cellulase (EBT). When the treatment time exceeded 2 h, the degradation of crystalline cellulose components in the primary wall of cotton also starts. Neither Viscozyme 120 L rich in pectinase nor Pulpzyme HC rich in xylanase alters the weight of cotton fabric significantly.

3.2. Reducing sugars released by enzymatic treatment

None of the two buffer solutions and the enzymes Pulpzyme HC and Denimax L yield measurable reducing end groups from cotton carbohydrates. Table 3 shows the time dependence of reducing sugar liberation during the enzymatic treatments in agitated and non-agitated system at 0.5 and 1 g/l enzyme concentrations. The data suggest that there is a close correlation between the weight loss of cotton fabric and the amount of liberated reducing sugars measured in the bath. In non-agitated system, enzymes Cellusoft L, Pulpzyme HC, Denimax L and Denimax Acid L do not produce reducing end groups in the solutions. Enzymes Celluclast 1.5 L and Cellulase (EBT), having high FPA (Table 1), cause the highest fabric weight loss and liberate the most reducing sugars. The amount of released reducing sugar by these two enzymes increases with the time of treatment. At higher enzyme concentration (1 g/l) when the treatment time is longer than 1 h, the enzymes Cellusoft L and Denimax Acid L also liberate reducing sugars. Increase in reaction time also results in increase in reducing sugar production.

The amount of reducing sugars liberated by the different enzymes is higher in agitated system than those of in non-agitated one. Cellulase (EBT) produces the highest level of reducing sugars of all enzymes tested. The amount of reducing sugars formed by Celluclast 1.5 L increases almost linearly with the time of treatment. Both the agitation and the enzyme concentration have a strong influence on action of Cellusoft L. Effect of Viscozyme 120 L cannot be characterised by production of reducing sugars. The reducing sugar values are around 6 mg/g regardless of the time of treatment.

Enzymatic treatment with four commercial enzymes (Viscozyme 120 L, Pulpzyme HC, Denimax L, Denimax Acid L) in non-agitated and agitated solutions at 0.5 and 1 g/l enzyme concentrations for 0.5–4 h does not cause significant degradation in raw cotton fabric. The maximum weight loss is approx. 1.5%, and the reducing sugar liberation is not considerable.

Enzymes Cellulase (EBT), Celluclast 1.5 L and Cellusoft L exhibiting high FPA, in non-agitated solution at 0.5 and 1 g/l enzyme concentrations, when the treatment time is less than 2 h, do not cause significant cotton cellulose degradation. The maximum weight loss values are near 2%, and the reducing sugar liberation is also not significant. It is likely that mainly the surface fibrils, small protruding fibres, seed-coat fragments and certain constituents of cuticle are degraded. At longer duration (e.g. 4 h), these enzymes, especially Cellulase (EBT), liberate significant amount of reducing sugars. Due to the damage of the outer layer of cotton fibre, cellulase can reach the primary cell wall and start to degrade the less crystalline cellulose constituents, releasing reducing sugars into the solution. Agitation of the enzyme solution increases the cellulose degrading effect for all enzymes. The degradation of crystalline cellulose components in the cotton primary wall is significant when the treatment time exceeds 1 h.

3.3. Effect of enzymatic treatment on the DP

The DP values of the enzyme treated and control fabrics are shown in Table 4. Treatment time was 4

Table 4
DP of enzyme treated and control cotton fabrics^a

Sample	Agitation	Enzyme concentration [g/l]		
		0	0.5	1
Control (pH = 5)	–	1870	–	–
	+	1890	–	–
Control (pH = 7)	–	1890	–	–
	+	1950	–	–
Cellusoft L	–	–	2100	1950
	+	–	2000	1880
Viscozyme 120 L	–	–	2100	1970
	+	–	1900	1950
Celluclast 1.5 L	–	–	2050	2030
	+	–	2030	1940
Pulpzyme HC	–	–	2070	2040
	+	–	2030	1990
Denimax L	–	–	2020	2030
	+	–	2040	1980
Denimax acid L	–	–	1980	1950
	+	–	1990	1910
Cellulase (EBT)	–	–	2010	2000
	+	–	2050	2020

^aTime of treatment: 4 h.

h. None of the treatments causes chain fragmentation to a measurable extent. The data suggest that none of

the enzymatic treatments, even the most drastic ones, lowers the DP values of cotton fabrics.

3.4. Surface appearance of cotton fibres after enzymatic treatment

Morphological changes in the cellulase-treated samples have been studied with scanning electron microscope. Fig. 1 shows the surface morphology of the untreated (control) fibres from desized cotton fabrics. There are small impurities and flaking compounds on the fibre surface. Celluclast 1.5 L enzyme (1 g/l, 2 h, agitation, weight loss: 3.2%) removes all the protruding compounds from the fibre and produces a typical smooth clean surface (Fig. 2). The fibre is free from small fuzz and any damage cannot be noticed on the surface. Cellulase (EBT) treatment (1 g/l, 2 h, agitation, weight loss: 5.7%) breaks up and peels off the outer layer of the fibre (Fig. 3), allowing the enzyme to reach and attack the cellulose molecules in the primary wall. This process produces significant weight loss and reducing end groups in agitated system. However, development of

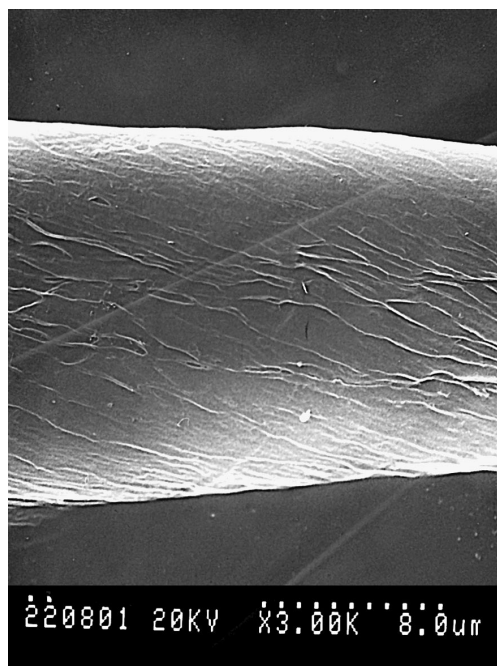


Fig. 1. Scanning electron micrograph of the fibre from desized cotton fabric.

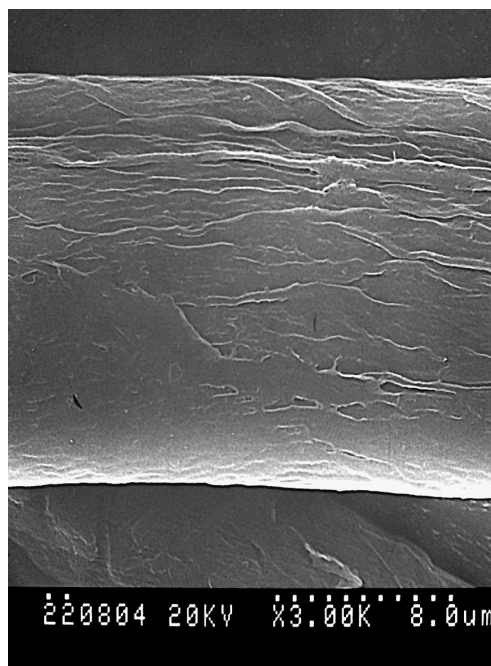


Fig. 2. Scanning electron micrograph of a fibre from cotton fabric treated with Celluclast 1.5 L enzyme (1 g/l, 2 h, agitated bath).

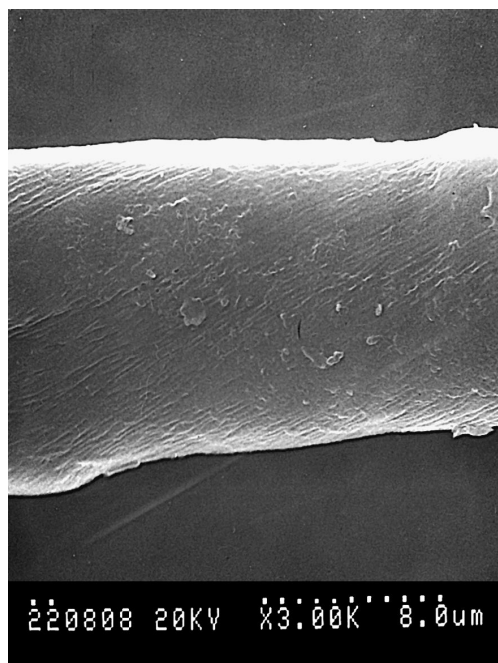


Fig. 3. Scanning electron micrograph of a fibre from cotton fabric treated with Cellulase (EBT) enzyme (1 g/l, 2 h, agitated bath).

cracks and cavities, which might be the consequence of a serious damage in the main body of cotton fibre, cannot be seen on the photomicrograph. The fibre surface variations are consistent with the results of the previous chapters and with the activity nature of the applied enzymes.

4. Conclusions

The effect of seven commercial cellulase and hemicellulase enzymes was investigated on the weight loss, reducing sugar liberation and change in DP of desized cotton fabrics. The fibre surface was characterised by scanning electron microscopy. Treatment in buffer solutions without enzyme addition caused only 0.5% weight loss. Neither treatment time nor agitation had any effect on the measured weight loss. Biotreatment in non-agitated system at 0.5–1.0 g/l enzyme concentration for 0.5–4 h did not cause significant degradation in cotton fibre. The weight loss was less than 3% and only a small amount of reducing sugar was produced. These re-

sults suggest that mainly the surface fibrils, small fibres, seed-coat fragments, water-extractable materials and other natural impurities have been degraded significantly. Increase in enzyme concentration from 0.5 to 1 g/l did not show significant effect on the fabric weight loss when agitation was not used. In agitated system, however, when the treatment time exceeded 2 h, enzymes Cellusoft L, Celluclast 1.5 L, Cellulase (EBT) exhibiting high FPA started to decompose even the cellulose components in the cotton primary wall. The agitation itself had a more remarkable effect on weight loss caused by enzymes than the increase of enzyme concentration from 0.5 to 1 g/l. A good correlation was found between the weight loss of cotton and the quantity of liberated reducing sugars measured in the solution. Longer treatment time (e.g. 4 h) enhanced the cotton degradation, especially for the three enzymes: Celluclast 1.5 L, Denimax L and Denimax Acid L. None of the enzymatic treatments caused a detectable change in viscometric DP of cotton cellulose. Partial degradation of the outer layer of cotton fibre was observed on the photomicrograph when high FPA dosage was used for biotreatment in agitated system. These data suggest that commercial cellulase and hemicellulase enzymes may be used at appropriate dosage (concentration) and conditions for modification of the cotton fibre, without a remarkable harmful effect.

Acknowledgements

This research was partly supported by the Hungarian National Science Foundation (OTKA T 026403 and OTKA T 029387) and by the Hungarian Ministry of Education (FKFP 0516/99).

References

- [1] K. Lange, in: P. Suominen, T. Reinikainen (Eds.), Proceedings of the second TRICEL symposium on *Trichoderma reesei* cellulases and other hydrolyses, Espoo, Finland 1993, Foundation for Biotechnical and Industrial Fermentation Research vol. 8, 1993, pp. 263–272.
- [2] A. Cavaco-Paulo, in: K-E.L. Eriksson, A. Cavaco-Paulo (Eds.), Enzyme Applications in Fiber Processing, ACS Sym-

- posium Series 687, American Chemical Society, Washington, DC, 1998, p. 180, Chap. 15.
- [3] M.M. Hartzell, Y.L. Hsieh, *Text. Res. J.* 68 (1998) 233.
- [4] Y. Li, I.R. Hardin, *Text. Res. J.* 68 (1998) 671.
- [5] E. Csiszár, G. Szakács, I. Rusznák, *Text. Res. J.* 68 (1998) 163.
- [6] E. Csiszár, G. Szakács, I. Rusznák, in: K-E.L. Eriksson, A. Cavaco-Paulo (Eds.), *Enzyme Applications in Fiber Processing*, ACS Symposium Series 687, American Chemical Society, Washington, DC, 1998, p. 204, Chap. 17.
- [7] T.K. Ghose, *Pure Appl. Chem.* 59 (1987) 257.
- [8] C.P. Kubicek, *Arch. Microbiol.* 132 (1982) 349.
- [9] M.J. Bailey, K.M.H. Nevalainen, *Enzyme Microb. Technol.* 3 (1981) 153.
- [10] M.J. Bailey, P. Biely, K.J. Poutanen, *J. Biotechnol.* 23 (1992) 257.
- [11] G.L. Miller, *Anal. Chem.* 31 (1959) 426.
- [12] I. Rusznák, J. Frankl, *Cellul. Chem. Technol.* 23 (1989) 3.
- [13] A. Cavaco-Paulo, L. Almeida, *Text. Res. J.* 66 (1996) 287.