Hale Bahar Öztürk[1], Anelise Ehrhardt[1,4], Hai Vu-Manh[1,3], Tarja Oksanen[2], Barbora Široká[1], Anna Suurnakki[2], Thomas Bechtold[1*]

[1)]**Christian-Doppler-Laboratory for Textile and Fibre Chemistry in Cellulosics, Research Institute for Textile Chemistry and Textile Physics, University of Innsbruck,**
Hoechsterstrasse 73, A-6850, Dornbirn, Austria,
E-mail: textilchemie@uibk.ac.at
*Corresponding Author

[2)]**VTT Biotechnology,**
P.O. Box 1500, FIN-02044, Espoo, Finland

[3)]**Faculty of Textile-Garment Technology and Fashion Design, Hanoi University of Technology,**
Hanoi, Vietnam

[4)]**Center for Fiber and Textile Science, Kyoto Institute of Technology,**
Matsugasaki, Sakyo-ku, Kyoto-fu, Kyoto 606-8585, Japan

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Ability of a Cellobiohydrolase I+II Mixture and Endoglucanase of the *Trichoderma reesei* Strain to Change Lyocell Fibres

Abstract
Lyocell fibers were treated for 24 h with two different types of cellulase enzymes, endoglucanase (EG) and a mixture of cellobiohydrolases (CBH I+II) from the Trichoderma reesei strain. After treating the fibers with 0.5 and 2 mg/g of EG and 2 mg/g of CBH, the fiber surface was damaged (observed by optical microscope), the fibers became brittle, and hence mechanical properties such as the tensile strength, elongation at break and abrasion resistance decreased. The weight loss (WL) test using 6 % NaOH alone, 4 % urea alone and their mixture showed that the WL of the fibers was not affected by the enzymatic treatments. The moisture regain, degree of polymerisation, crystallinity index, measured by ATR (attenuated total reflectance), and the FTIR of the enzyme treated fibers also did not change.

Key words: *cellulose, enzyme, fibre, lyocell, treatment.*

Introduction

The enzymatic treatment of cellulosic textile materials with cellulases during the finishing process has two major goals, i.e. bio-polishing and bio-stonewashing [1 - 4]. In industrial applications, cellulase treatment is applied to fabrics with an intensive mechanical action. However, the correlation between the effects of cellulase hydrolysis and physical conditions of the processing are still not clear. Although the effects of cellulase treatment on different types of cellulosic materials have been investigated, the effects of EG II alone and a CBH (I+II) mixture prepared from the *Trichoderma reesei* strain on lyocell fibres (cellulose II) have not been investigated thus far. In the current study, lyocell fibre was treated with *T. reesei* EG II alone and a CBH (I+II) mixture. After the enzyme treatments, the weight loss (WL), degree of polymerisation (DP), crystallinity, moisture regain, morphology and mechanical properties of the lyocell fibres were investigated.

Experimental

Materials

Lyocell staple fibre (TENCEL® Standard) without spin finishing was kindly supplied by Lenzing AG Austria. The titer and length of the fibres were 1.3 dtex and 38 mm, respectively. The NaOH (≥ 99%), KCl (≥ 99.5%), KOH (≥ 85%), urea (≥ 99.7%) were from Roth; the K_2CO_3 (≥ 99%) and EWN-Lösungsmittel were from Fluka (Buchs, Switzerland); the $CaCl_2$ (95%) and P_2O_5 (≥ 99%) were from Riedel-de Haen, and the NaCl (≥ 99.5%) and K_2SO_4 (99%) were from Zeller.

The cellulase enzymes used were endoglucanase II (EG II), cellobiohydrolase I (CBH I) and cellobiohydrolase II (CBH II). They were purified from the *Trichoderma reesei* strain at VTT Biotechnology, Finland,as described previously by Suurnäkki et al. [5]. Protein concentrations of the purified cellulase preparations were assayed by the method of Lowry et al. [6].

Methods

Enzymatic treatment

5 g of lyocell fibre samples were immersed in an enzymatic solution at pH 5 and 45 °C for 24 h. The treatments were carried out in a Linitest using a 5 r.p.m rotation. The enzymatic treatment was stopped by raising the temperature to 90 °C for 10 min, followed by washing with deionized water and drying at room temperature. A reference sample was treated at pH 5 and 45 °C for 24 h in a solution without the enzyme. Two different enzyme dosages of EG II and a 50:50 mixture of CBH I and CBH II were used, aiming at a similar lower and higher hydrolysis rate. The release of sugars in the hydrolysates was analysed by the DNS method [7]. ***Table 1*** shows the enzyme dosages used and the respective hydrolysis rates.

Table 1. *Enzyme dosages and hydrolysis levels of the lyocell fibres.*

Enzyme	Enzyme dosage, mg/g	Solubilised sugars, % of d.w.
Reference	0	0.30
EG II	0.5	0.75
EG II	2.0	1.54
CBH I + CBH II	0.5 + 0.5	0.64
CBH I + CBH II	2.0 + 2.0	1.33

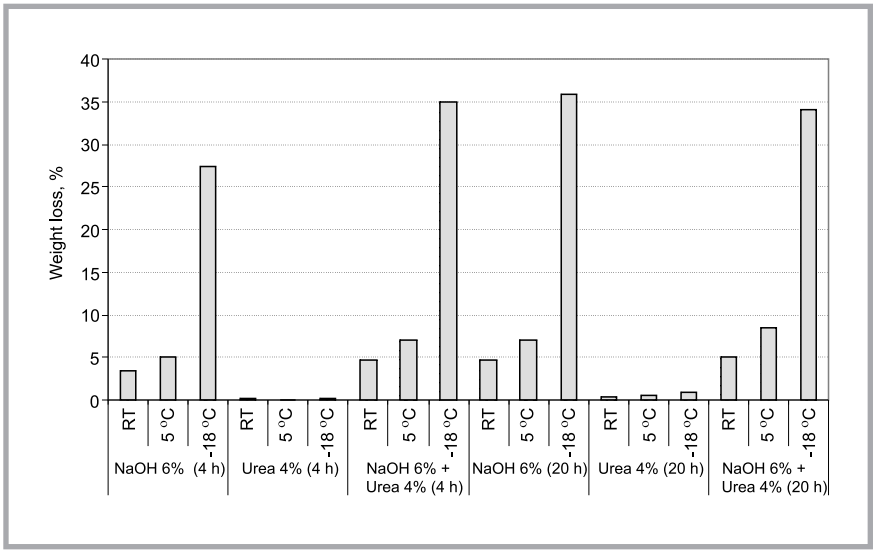

Figure 1. *Weight loss (WL) of lyocell fibres in 6% NaOH alone, 4% urea alone and NaOH/urea solution at room temperature, 5 °C and -18 °C after 4 h and 20 h.*

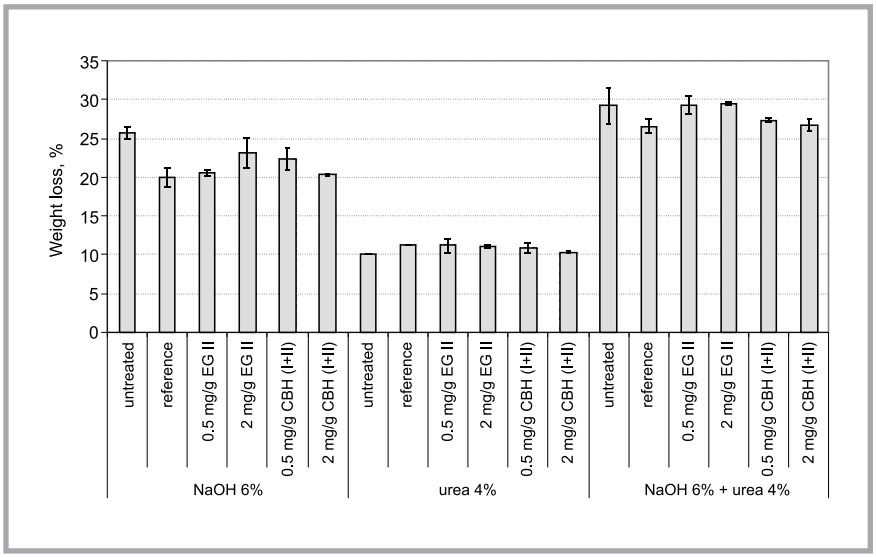

Figure 2. *Weight loss (WL) of the untreated, reference (lyocell fibre treated with solution without the enzyme) and enzyme treated lyocell fibres in 6% NaOH alone, 4% urea alone and NaOH/urea solution at 5 °C after 4 h.*

Weight loss of cellulosic fibres (%)

A certain amount of fibres (w_1), 6% NaOH alone, 4% urea alone, and 6% NaOH/4% urea mixture aqueous solutions were kept either at room temperature or in a refrigerator for 6 h to cool down approximately to the temperature desired (5 °C or -18 °C). The fibres were treated with the solutions under the action of stirring for 5 min at room temperature. The samples were washed three times under tap water and immersed in water for 5 min at each interval of the washing stage. The fibres were neutralised with acetate buffer solution at pH 5 for 5 min and washed with distilled water for 5 min. Afterwards the samples were oven-dried for 24 h at 60 °C and kept in a desiccator containing phosphorous pentoxide for 12 h and then weighed (w_2). The WL of the samples was calculated according to ***Equation 1***. Two repetitions were conducted for each sample to obtain a mean value.

$$WL\ (\%) = [(w_1-w_2)/w_2]\times 100 \qquad (1)$$

Attenuated total reflectance Fourier-transform infrared (ATR FTIR) spectroscopy

ATR FTIR measurements were conducted according to literature [8]. The Calculation of the crystallinity ratios was done using the 1377/2901 absorbance ratio (TCI = total crystallinity index) and 1423/893 absorbance ratio (LOI = lateral order index) [9].

Degree of polymerisation (DP)

The DP of the fibres was assessed by the viscosimeter method using ferric sodium tartrate complex solvent (FeTNa) (EWN-Lösungsmittel). Prior to the DP analyses, the fibres were conditioned at 65 ± 2% relative humidity and 20 ± 2 °C for at least 24 h. Approximately 0.020 g of the fibre sample was dissolved in 25 ml FeTNa solution for 16 h at room temperature after filling the bottles with an inert gas (Ar). The viscosity of the solutions was measured by a KPG®-Ubbeholde Viscosimeter Nr Ic (Schott Geräte) at 20±1°C of the water bath according to literature [10]. Two repetitions were conducted to determine the mean value.

Moisture regain (MR)

Approximately 0.3 g of the fibre sample was placed in a weighting glass and set into a dessicator with powdered P_2O_5 at 23.4 ± 1.9 °C. After equilibrating the samples with P_2O_5, the P_2O_5 was replaced with a saturated solution of salt. The samples were kept in the dessicator until equilibrium was reached, and subsequently the weights of the samples were recorded (W_w). Salt solutions with a given relative humidity (RH) for sorption were used:

- KOH (RH = 13.6%),
- $CaCl_2$ (RH = 25.1%),
- K_2CO_3 (RH = 47.4%),
- NaCl (RH = 79.5%), and
- KCl (RH = 98.2%) to
- K_2SO_4 (RH=99.7%).

After allowing the samples to equilibrate in the different atmospheres, the samples were dried for 4 hours at 105 °C, and the dry weights (W_d) were recorded. The MR was calculated using the following equation:

$$MR\ (\%) = 100 \times [(W_w-W_d) / W_d].$$

Three repetitions were conducted to determine the mean value.

A tensile test and of the fibres was performed and the abrasion resistance evalu-

Table 2. *Upper limit of the DP (degree of polymerisation) of cellulose I that is dissolvable in differing solvents [13, 14].*

Degree of polymerisation (DP)	Solution
200	9% NaOH
425	6% NaOH/4% urea
500	6% NaOH/5% thiourea
700	7% NaOH/12% urea

ated according to literature [11, 12]. Fibre images were obtained using an OLYMPUS CX41 optical microscope.

Results and discussion

Weight loss (WL)

Figure 1 shows the WL of lyocell fibres in 6% NaOH alone, in 4% urea alone and in 6% NaOH/4% urea solution at room temperature, at 5 °C and at -18 °C after 4 h and 20 h of swelling. The effect of the swelling time (4 h, 20 h) on the WL of the fibres was found not to be distinct. Thus 4 h was chosen for further WL analyses.

According to ***Figure 1***, lyocell showed the least WL in 4% urea. 6% NaOH solution caused a slightly lower WL than that of the effect of the NaOH/urea mixture. As the temperature decreased, the WL of the fibres increased. However, due to the partial freezing of the solutions at -18 °C, this temperature was found not to be appropriate for measurements. Thus 5 °C was chosen for further dissolution analyses.

It was found that Lyocell fibres do not dissolve totally but lose weight after treatments in either 6% NaOH alone or in a NaOH/urea mixture (***Figure 1***). This is consistent with the literature (***Table 2***), which mentioned that 6% NaOH solution alone is able to dissolve cellulose with a degree of polymerization (DP) of up to 200, while 6% NaOH/4% urea solution can dissolve cellulose with a DP of up to 425 [13, 14].

Figure 2 shows the WL of lyocell fibres treated in 6% NaOH, 4% urea and NaOH/urea solutions at 5 °C after 4 h. The WL was the highest for the NaOH/urea mixture. The 4% urea solution alone resulted in the least WL (10%). The samples showed a comparable WL for a chosen solution.

FTIR ATR spectra and crystallinity

Figure 3 illustrates the ATR FTIR spectra of the untreated and enzyme treated lyocell fibres. The band near 1160 cm^{-1}, representing the anti-symmetric bridge stretching of the C-O-C groups, the band near 1318 cm^{-1},representing CH_2 wagging vibrations, and the 895 cm^{-1}, being characteristic of ß linkages, did not alter after enzymatic hydrolysis. The bands near 3400 cm^{-1} represent OH vibrations, which were wider for the untreated sample but got narrower after the enzymatic

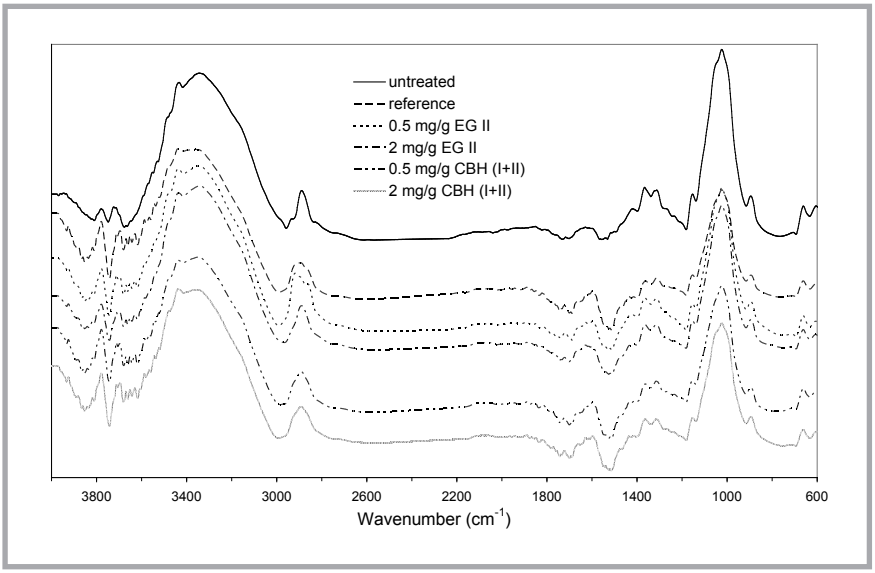

Figure 3. *ATR FTIR spectra of the untreated, reference (lyocell fibre treated with solution without the enzyme) and enzyme treated lyocell fibres.*

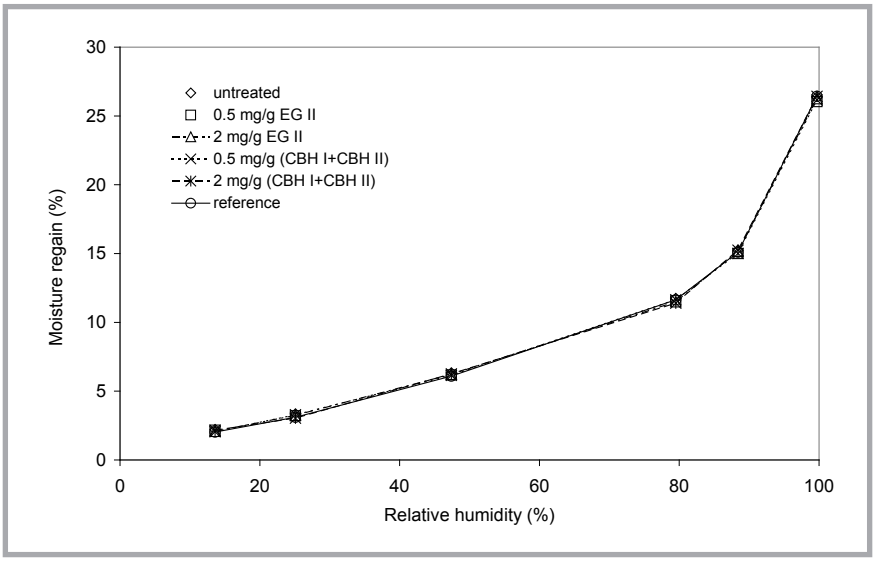

Figure 4. *Moisture regain of the untreated, reference (lyocell fibre treated with solution without the enzyme) and enzyme treated lyocell fibres (All samples showed comparable moisture regain within the whole range of relative humidity).*

treatments, which was also the case for the reference sample (***Figure 3***). This shows that a portion of the hydrogen bonds were broken and reorganised during the enzymatic hydrolysis, which was also due to treatment conditions (pH 5, 45 °C) without enzyme action.

Table 3 presents the total crystallinity index (TCI) and lateral order index (LOI) values of the untreated and enzyme treated lyocell fibres obtained from ATR FTIR measurements. Enzyme treatment was found not to have changed the crystallinity index of the fibres significantly compared to that of the untreated sample. The degree of crystallinity of Tencel fabrics does not change in a range of WL up to 5% [15 - 17]. Only after 55.2% WL did the crystallinity of Tencel fabric increase by 3.0% [18], which indicates that the re-

Table 3. *ATR FTIR crystallinity indexes of the untreated, reference (lyocell fibre treated with solution without the enzyme) and enzyme treated lyocell fibres.*

Sample	A1377/2901 (TCI)	A1423/893 (LOI)
untreated	0.93±0.08	0.96±0.02
0.5 mg/g EG II	0.92±0.07	0.95±0.02
2 mg/g EG II	0.93±0.04	0.96±0.01
0.5 mg/g CBH (I+II)	0.92±0.08	0.95±0.02
2 mg/g CBH (I+II)	0.93±0.04	0.96±0.01
reference	0.90±0.06	0.94±0.02

Table 4. *Degree of polymerisation (DP) of the untreated, reference (lyocell fibre treated with solution without the enzyme) and enzyme treated lyocell fibres.*

Treatment	Degree of polymerisation (DP)
untreated	594.2 ± 62.6
0.5 mg/g EG II	563.9 ± 40.7
2 mg/g EG II	609.4 ± 14.5
0.5 mg/g CBH (I+II)	550.7 ± 52.2
2 mg/g CBH (I+II)	613.7 ± 25.9
reference	537.2 ± 51.1

moval of less ordered parts in amorphous zones of the fibre is favoured.

Degree of polymerisation (DP)

Table 4 shows the DP of lyocell samples, which were found not to be significantly different after enzymatic treatments.

Moisture regain

Figure 4 (see page 25) shows the moisture content of samples, which was not significantly affected after the enzymatic treatments, being consistent with the literature. The moisture sorption and dye adsorption (K/S value) give an estimation of material accessibility in dry and wet states, respectively, and reflect changes in the crystallinity, pore structure and accessible internal surface area [15]. Upon the attack of cellulase, no changes in moisture regain, i.e. accessible less-ordered regions, occurred, despite the fact cellulase activity is the highest in amorphous cellulose. This shows that the hydrolysis of crystalline cellulose is the rate determining step in the total degradation of cotton cellulose [2]. The dyeability and moisture content of cotton (cellulose I) after enzyme treatment did not change since no changes in the crystallinity occurred [19].

Fibre mechanical properties

Table 5 shows the fibre tensile properties of samples in a conditioned state and in a wet state, and abrasion resistance in a wet state. 0.5 mg/g and 2 mg/g EG treatments were found to make the fibres brittle, as did the mixture of 2 mg/g CBH (I+II). The tensile properties of the reference samples in a conditioned and wet state were found to be comparable to that of the untreated sample. Treatment with a mixture of 0.5 mg/g CBH (I+II) also resulted in comparable tensile test results to those of the untreated sample. A slight decrease in the tensile and abrasion properties of fibre samples in a wet state was also observed after 0.5 mg/g CBH (I+II) treatment. Although no significant WL of lyocell fibres (***Figure 2***) was observed, a loss in the tensile and abrasion resistance of the fibres was observed, which can be attributed to a certain amount of hydrogen bond loss during enzyme treatment, as discovered by ATR FTIR (***Figure 3***). This explains why enzyme treatment needs additional mechanical treatment in order to shear off the fibre/fabric surface for polishing and stone-washing as used commercially. Enzyme treatment without mechanical action is not effective enough to achieve these goals. Similar results were found in study [20], which analysed cellulase hydrolysis using the pad-batch technique, where no agitation

Table 5. *Tensile strength in cN/tex, elongation at break in % and abrasion resistance in counts of the untreated, reference (lyocell fibre treated with solution without the enzyme) and enzyme treated lyocell fibres in a conditioned and a wet states.*

Treatment	Conditioned state		Wet state		
	Tensile strength, cN/tex	Elongation at break, %	Tensile strength, cN/tex	Elongation at break, %	Abrasion resistance, counts
untreated lyocell fibre	29.25 ± 4.39	12.85 ± 0.96	27.97 ± 3.65	14.65 ± 1.87	41.09 ± 11.05
0.5mg/g EG II	brittle	brittle	brittle	brittle	brittle
2 mg/g EG II	brittle	brittle	brittle	brittle	brittle
0.5 mg/g CBH (I+II)	29.55 ± 4.04	10.18 ± 0.77	24.38 ± 4.33	12.02 ± 1.84	35.42 ± 9.18
2 mg/g CBH (I+II)	brittle	brittle	brittle	brittle	brittle
reference	29.23 ± 2.62	9.73 ± 0.88	27.75 ± 2.75	14.36 ± 1.83	41.21 ± 9.42

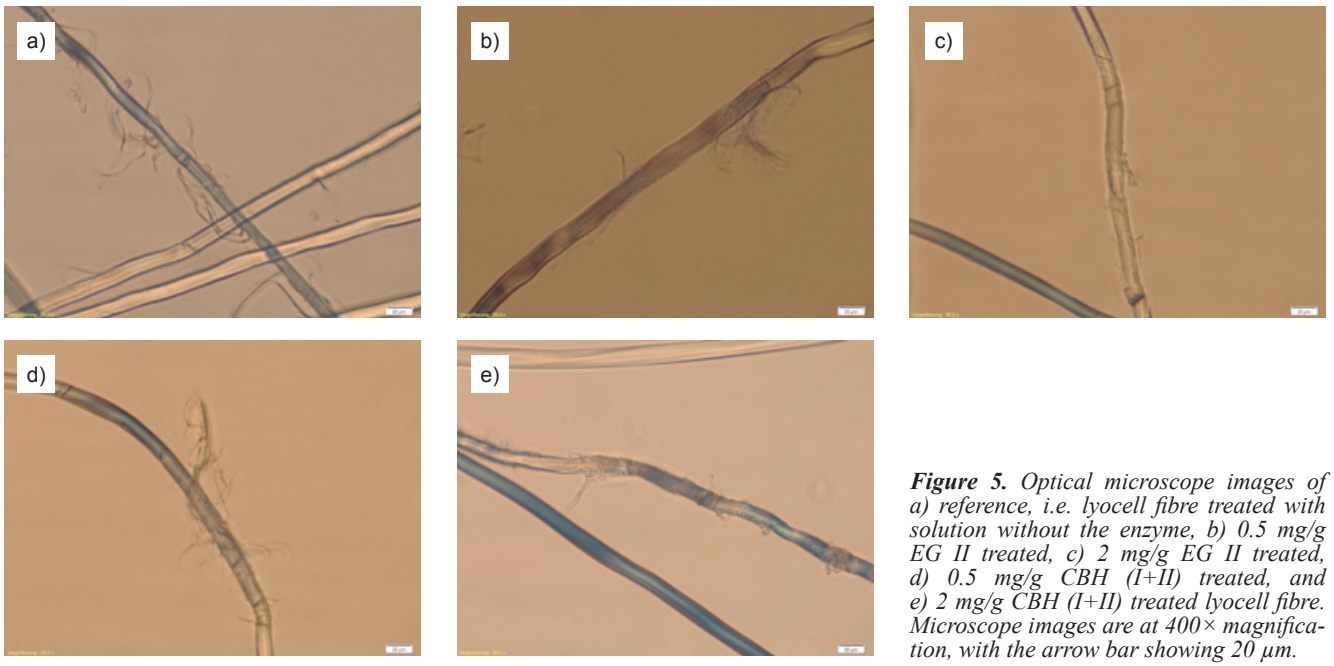

Figure 5. *Optical microscope images of a) reference, i.e. lyocell fibre treated with solution without the enzyme, b) 0.5 mg/g EG II treated, c) 2 mg/g EG II treated, d) 0.5 mg/g CBH (I+II) treated, and e) 2 mg/g CBH (I+II) treated lyocell fibre. Microscope images are at 400× magnification, with the arrow bar showing 20 μm.*

is used. For example, static cellulase hydrolysis caused a 1% WL, while agitated hydrolysis caused a 24% WL for cotton fabric at 22 °C.

Morphology change

Figure 5 presents optical microscope images of the reference and enzyme treated lyocell fibres, which were found to show fibrillation. It is consistent with the literature, which mentioned that the fibrillation (peeling of fibrils from the fibre surface) of lyocell fibre occurs in the swollen state of the fibre caused by the application of mechanical stress [21].

Conclusions

The enzymatic treatments were found not to affect the WL, DP, moisture regain and crystallinity of lyocell fibres distinctly. However, the mechanical properties of the fibres decreased, a change in the hydrogen bonding was found by ATR FTIR, and the fibrillation of lyocell fibre was observed by optical microscope.

The effect of EG and CBH treatment on fibre brittleness differed depending on the hydrolysis level. EG treatment was found to make lyocell fibres brittle at hydrolysis levels of 0.8 and 1.5% of the dry weight, whereas CBH treatment resulted in brittle fibres only after a hydrolysis of 1.3% of the fibre material.

The changes in mechanical properties (tensile, abrasion) of the fibres were not reflected by the DP, which shows that DP is the bulk property of a polymer; however, mechanical properties show the local weakest point of the fibre, where the fibre breaks. Mechanical properties are affected by linkages (hydrogen bonds) between macromolecules but not by the macromolecule length (DP).

In the current study, enzymatic treatment without *intensive* mechanical agitation of neither EG nor CBH gives a basic understanding of the weakening of the physical properties of the fibre without significant changes in the fundamental fibre properties, which explains the finding that the brittleness of cellulase treated fibres makes them sensitive to mechanical stress. In industrial applications, both the location and intensity of enzymatic treatment on fabric can be controlled by additional *mechanical action*. For example, the bio-stonewashing of jeans requires a tumbling movement of the washing machine together with enzymatic solution. As a result, *intensive* mechanical movement is used in all standard processes of cellulase application.

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