Enzyme Technology

Cellulase Activities and Finishing Effects

By *Artur Cavaco-Paulo* and *Luis Almeida*, University of Minho, Guimarães, Portugal, and *David Bishop*, DeMontfort University, Leicester, U.K.

Cellulases are increasingly being used in cotton finishing. The most widely used application is the replacement of the stone washing process to produce the fashionable aged appearance of denims. Other cellulase treatments are used to improve the appearance of cotton fabrics by removing fuzz-fiber and pills from the surface.[1] Such processes also modify the fabric's mechanical properties in ways that lead to the perception of improved handle, particularly softness.[2]

Cellulases are secreted by various fungi as complex mixtures of three major types of enzymes—endoglucanases (EGs, EC 3.2.1.4), cellobiohydrolases (CBHs, EC 3.2.1.91), and cellobiases (EC 3.2.1.21).[a] A general model for the action of these different enzyme components in the hydrolysis of cellulose is that EGs cause random hydrolytic chain scission at the most accessible parts of cellulose chains, CBHs split cellobiose from the ends of cellulose chains in a stepwise manner along cellulose chains, and cellobiase hydrolyzes cellobiose to glucose. Efficient hydrolysis of crystalline cellulose by cellulases requires the synergistic action of the EGs and CBHs.[3]

ABSTRACT

Cellulase enzymes are usually characterized by evaluation of their activities towards different forms of cellulose and their derivatives. For textile applications, it was suggested that the comparative evaluation should also include the measurement of the activities in tests that simulate the large scale treatments with different degrees of mechanical agitation.

Comparisons were made between three cellulase mixtures with known compositions and two commercial cellulases by the proposed methodologies. The relations between cellulase activities of these enzyme mixtures and finishing effects obtained for different processes with various degree of mechanical agitation are shown.

KEY TERMS

Cellulases
Cotton
Enzymes
Finishes
Trichoderma reesei

Advances in biotechnology have lead to the development of recombinant DNA techniques and have made possible the manipulation of the genes of cellulase-secreting fungi. Using these techniques, new strains can be developed enabling industrial production of novel cellulase compositions.[4,5] These may provide the opportunity for achieving radically new cellulase finishing effects, as well as improving the existing ones.

Cellulolytic enzymes are currently applied in textiles processes where mechanical action is always present such as in jets or rotating drum washers.[1] It is already recognized that cellulases with strong EG activity are preferred for achieving the aged look on denims and this effect is best obtained in machines that provide vigorous beating action.[1,6-9] It is also known that a careful balance between cellulase activity and mechanical action is required to achieve efficient fuzz-fiber and pill removal without excessive fabric strength loss.[1,2,8] These facts indicate that cellulase composition and mechanical action are key features during cellulase processing of cotton.

Cellulase Activities

The characterization of cellulase enzymes poses special problems related to the multicomponent nature of the enzymes and their complex synergistic action in various substrates. Crystalline or amorphous forms of celluloses as well their derivatives are all degraded by cellulases.[10] Therefore, the specific action of cellulases is related to the hydrolysis of the 1,4-ß-D glycosidic bonds and it is incorrect just to relate them to the hydrolysis of cellulose polymers.

The activity towards cellulose is measured as soluble-reducing sugars formed and is understood as the full activity of all cellulolytic components.[10,11] Amorphous substrates are also used because there are some cellulase components not active in crystalline cellulose.[10] The activity towards carboxymethylcellulose (CMC), or hydroxyethylcellulose, is measured as reducing sugars formed or by viscosimetric methods and is understood as endoglucanase activity.[10,11] The presence of side groups in CMC seems to have inhibitory action on most CBH enzymes produced by fungal systems.[10] The activity in cellobiase is measured towards cellobiose.[10,11]

There are specific recommendations by IUPAC in measuring cellulase activities based on the following substrates: filter paper, CMC (HEC) and cellobiose.[11] However, these recommendations have been ignored by most researchers who have adopted methods appropriate to their specific studies.[10]

Cellulase preparations used in cotton processing may be characterized using substrates like CMC, cellobiose and amorphous cellulose such as swollen Avicel (phosphoric acid swollen Avicel), or swollen cotton. The source of crystalline cellulose should, however, be fully scoured cotton. Since textile chemists are interested in which activities deliver certain finishing effects, the activity on scoured cotton fabrics should be measured at different levels of mechanical action (to simulate large scale treatments) and the properties of the treated fabrics should be carefully evaluated.

Trichoderma reesei Engineered Cellulases

The cellulolytic complex of *Trichoderma reesei* is one of the most extensively investigated fungal enzyme systems.[3-5] It is known to contain at least one cellobiase, CBH I, CBH II, EG I, and EG II. The genes of these hydrolases can be manipulated such that some

[a]International style differs from U.S. style by the use of commas for decimal points. Periods are used as delimiters. Note the use of commas with Tables I and IV and Fig. 1.

activities are deleted to produce new cellulase combinations.[4,5] Furthermore, it has been reported in recent literature that two more cellulase components known as EG III and EG V are always present in the crude mixtures from *Trichoderma reesei*.[12,13]

Characterization of the Enzyme Mixtures

Comparisons have been made between the activities (Table I) of crude cellulase mixture from *Trichoderma reesei* (TC) and mixtures in which the activities of EG I and EG II (C-EGs) or the activities of CBH I and CBH II (C-CBHs) had been deleted. These activities were determined as described previously.[8,9,14]

The measured activities illustrate the expected increments in classical endo and exo type activities for C-CBHs and for C-EGs, respectively. While TC was found to have lower measured activity than C-EGs towards cellobiose, CMC, and PASA, it caused consistently greater cotton weight loss than C-EGs. This apparent contradiction shows that care should be exercised in predicting cellulase activity on cotton from data obtained using other forms of cellulose or its derivatives. The result also points to the importance of synergy between the various components in the hydrolysis of cotton cellulose.

The deletion of CBH I and CBH II activity from the total crude mixture dramatically reduced the cotton weight loss thus confirming the importance of exo type activity in solubilization of the polymer. The deletion of EG I and EG II activity also caused some reduction in the cotton weight loss which is expected from the synergy between endo and exo type activity. The surprisingly high activity of C-EGs may possibly be due to the previously reported synergy between the two CBH components and/or to some endo activity in our C-EGs.[15] The latter may be accounted for the remaining EGs such as EG III and EG V.

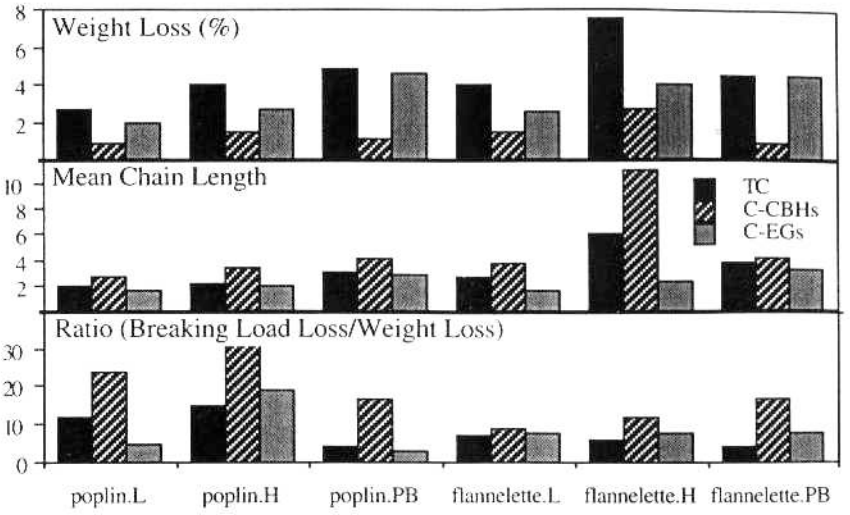

Fig. 2. Weight loss, mean chain length of the soluble sugars and breaking load loss/weight loss ratio for the various treatments. Treatment conditions: L—65 rpm of Linitest pots at 50C, 1g of fiber to 10 mL of liquor, enzyme dilution of 1/50 and pH = 4,8 (acetate buffer), 30 min; H—similar to L but with 15 steel discs used in various fastness tests; PB—pad-batch simulation, beaker, enzyme dilution of 1/50 and pH = 4,8 (acetate buffer), three days at 20C.

After short hydrolysis times (one hour in the Linitest pots), the relative activity of C-CBHs is higher and the relative activity of C-EGs is lower when compared with longer hydrolysis times (six days in a beaker). This seems to be due to the rotation of the reactor where the hydrolysis occurs. A more detailed analysis of the role of mechanical action in cellulase hydrolysis is discussed next.

The increased slope of the plot of cotton fluidity versus cotton reducing power indicates increasing randomness of cellulolytic attack.[14] Thus C-CBHs causes the most random hydrolysis and C-EGs the most localized attack, with TC having an intermediate effect (Fig. 1). The randomness of cellulolytic action of different EGs is commonly measured this way using CMC as a soluble substrate.[16]

It is interesting to note that the relative randomness or localization of cellulase action on cotton or hydrolysis extension did not change the measured crystallinity of the fiber (Table II). Similar results has previously been observed for several other crude cellulases.[14,18,19] Thus the present work tends to confirm the view that the action of cellulase is not confined initially to noncrystalline regions, irrespective of the cellulase components present. It has also been reported that the hydrolysis of cellulose by cellulase concentrates of *Trichoderma viride* is first order with respect to substrate.[20] This suggests uniform reactivity of cellulose (crystalline and amorphous) and therefore implies that no change in crystallinity should result from cellulase hydrolysis.

Table I. Enzyme Activity of *Trichoderma reesei* Engineered Cellulases

Substrate/Enzyme[a]	TC	C-CBHs	C-EGs
Cellobiose[b] (U/g)	2,2	4,4	4,7
CMC[c](U/g)	94	159	120
Phosphoric Acid Swollen Avicel[c] (U/g)	194	93	275
Cotton[d] (%)	63	3	52
Weight Loss[e] (%)	1,40	0,47	1,05

[a]Enzyme samples kindly supplied by Primalco, Ltd. [b]One unit yields 2 µmol of glucose per minute in the cellobiase hydrolysis. [c]One unit yields 1 µmol of reducing sugars as glucose per minute. [d]Six days, beaker, 50C, 1g of fiber to 20 mL of liquor, enzyme dilution of 1/60, pH = 4,8 (acetate buffer). [e]One hour, 65 rpm of Linitest pots, 50C, 1g of fiber to 10 mL of liquor, enzyme concentration of 1mg/mL, pH = 4,8 (acetate buffer).

Table II. Crystallinity Index of the Treated Cotton Samples[17]

Enzyme	Untreated	TC	C-CBHs	C-EGs
Time (days)	6	6	6	6
Crystalline Index (%)	83	83	83	81

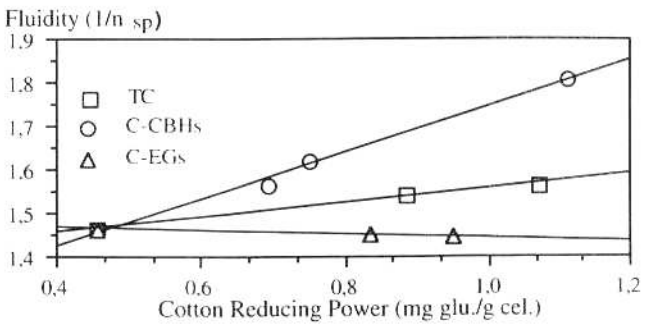

Fig. 1. Relation between cotton reducing power and fluidity.[14] Treatment conditions indicated in Table I-d during 0-13 days of hydrolysis.

The Role of Mechanical Action During Hydrolysis

The relative activities of the three cellulases are different for the different processes and fabrics (Fig. 2). Increasing the mechanical action will increase the activities of all enzymes, particularly of the C-CBHs (EG richer) cellulase. The mean length of the leaving sugars is always higher for C-CBHs, confirming their endo action. Mechanical action also increases the length of the leaving sugars, and cotton powder deposits appear at the bottom of the reactors pots after processes with a high level of mechanical agitation. The fabric strength loss is essentially due to the endo action as shown in Fig. 2.

Previous adsorption studies indicate that increasing the mechanical action will decrease enzyme adsorption, thus increasing the number of free sites where enzyme can attack.This is verified in particular for the crude rich in EG activity.[21]

The pad-batch simulation (PB) process gave similar weight loss (Fig. 2), length of the leaving sugars, and fabric strength loss ratio for both fabrics types. But for the other processes with higher mechanical action, the flannelette has a higher weight loss, longer leaving sugars, and relatively low loss of fabric strength. This seems to indicate that the cooperation between cellulase and mechanical action on flannelette results mainly in the degradation of pills, while for the poplin this cooperative action results mainly in the degradation of the underlying structure (Table III).

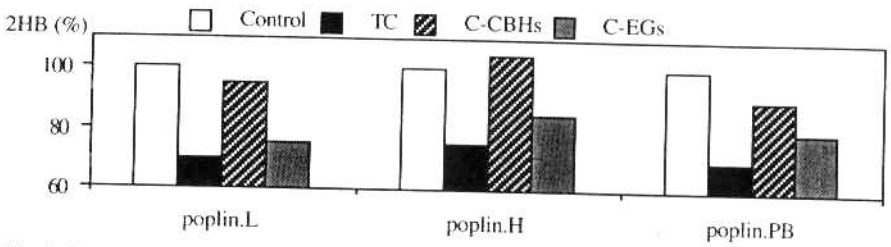

Fig. 3. Bending hysteresis (KES-F2) of the cellulase-treated fabrics. See treatment conditions in Fig. 2.

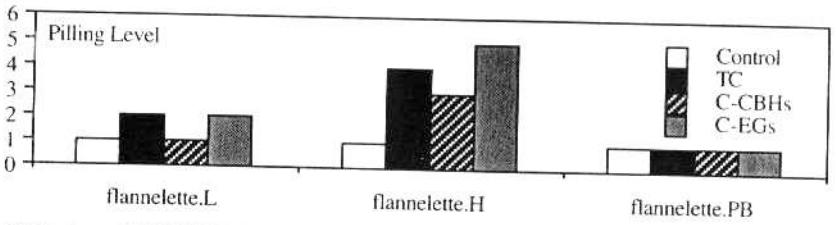

Fig. 4. Pilling Level (ASTM 3512—Level 5 = low degree of pills, Level 1 = high degree of pills) of the cellulase-treated fabrics. See treatment conditions in Fig. 2.

Properties of the Treated Fabrics

Previous studies on cellulase-treated fabrics indicate that bending hysteresis and pilling level are the most representative parameters for poplin and flannelette type fabrics, respectively.[22]

The reduction of bending hysteresis was verified for the cellulase with all components (Fig. 3). The PB process giving higher weight loss, also gave a greater reduction in bending hysteresis. The process with higher mechanical friction increased bending hysteresis for the treatment with C-CBHs cellulase. These changes in bending hysteresis are related to the formation and removal of microfibrils at the fiber surface with consequent variations in interfiber friction. An upcoming section includes a discussion of these changes as well as scanning electron micrograph photos.

Mechanical action is essential to efficient removal of pills from the flannelette surface (Fig. 4). These effects are best obtained with C-EGs which give good microfibril removal and low strength loss.

Cellulase Treatment of Denim Fabrics

The color loss in denim fabrics is best obtained with the total crude, but it seems by comparison of C-EGs and C-CBHs effects that the endo action is the most important in removing the indigo dye from fabric surface. The ratio between the percentages of dye removed and dye backstained is higher for the crude richer in CBHs action (Fig. 5). These results indicate that both pH and enzyme composition influence backstaining. CBH action produced higher soluble sugars in solution and these sugars could reduce indigo increasing the redeposition of dye.[7] Backstaining is also dependent on the reducing ends formed in the fabric during hydrolysis. The reduction of indigo is moderate at pH 5-7 (indigo is best reduced at alkaline pH), suggesting that physical adsorption could also be important in dye redeposition. Thus, indigo redeposition during cellulase treatment is a complex process which is dependent on several mechanisms occurring at the same time.

Commercial Cellulases

The enzyme activity of two commercial cellulases—Cellusoft L, used to obtain depilling effects, and Denimax T, used to obtain the aged look of denims—is shown in Table IV. The activity measured as weight loss on flannel-

Table III. Fabric Specifications

Fabric	Ends/Picks per cm	Thickness at 2.5 gf/cm² (mm)	Weight per Area (g/m²)	Pilling Level (ASTM 3512)	Previous Treatments
Poplin, 100% Cotton	60/32	0.5	100	5	scoured and bleached
Flannelette, 100% Cotton	21/15	1.0	160	1	scoured, bleached and raised

Table IV. Enzyme Activity of Two Commercial Cellulases

Substrate\Enzyme[a]	Cellusoft L[e]	Denimax T[e]
Cellobiose[b] (U/g)	0,75	0,85
CMC[c] (U/g)	159	115
Phosphoric Acid Swollen Avicel[c] (U/g)	361	144
Cotton Weight Loss[d] (%)	1,33	0,91
Flannelette Weight Loss (%) (L)	1,9	1,0
Flannelette Weight Loss (%) (H)	2,7	2,0

[a]Enzyme samples kindly supplied by Novo Nordisk A/S. The names of commercial products are given for information only and their mention does not constitute a recommendation. [b]One unit yields 2 µmol of glucose per minute in the cellobiase hydrolysis. [c]One unit yields 1 µmol of reducing sugars as glucose per minute. [d]One hour, 65 rpm of Linitest pots, 50C, 1g of fiber to 10 mL of liquor, enzyme concentration of 1mg/mL. [e]Activities measured at pH = 4,8 (acetate) for Cellusoft L and pH = 7,0 (phosphate) for Denimax T. (L) is identical to d. (H) is identical to d, but with 12 steels discs in the Linitest Pots.

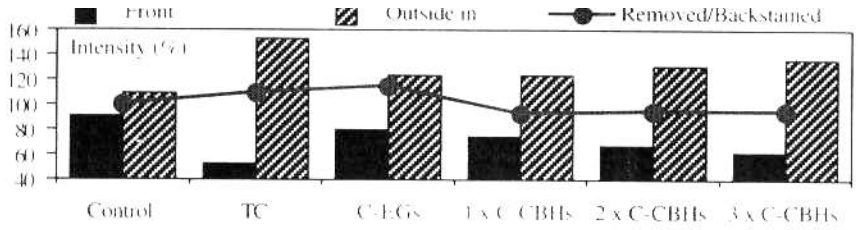

Fig. 5. Intensities (ACS CS 5 Chroma Sensor) measured at 640 nm for denim fabrics treated with *T. reesei* crude mixtures. Treatment conditions: 1g of fiber to 40 mL of liquor, enzyme dilution of 1/100 and pH = 4,8 (acetate buffer), 45 min, 65 rpm of Linitest pots at 50C, mechanical action provided with 15 steel discs used in various fastness tests.

ettes treated under conditions of low and high mechanical action is also shown because we intend to show the relationship between the activities of these commercial products and the parameters of the treated flannelettes.

Cellusoft L is the most active enzyme but Denimax T has a relative higher endo activity. The relative weight loss produced by the high mechanical action treatment also confirms the relative endo character of Denimax T (Table IV).

The mechanical properties of the

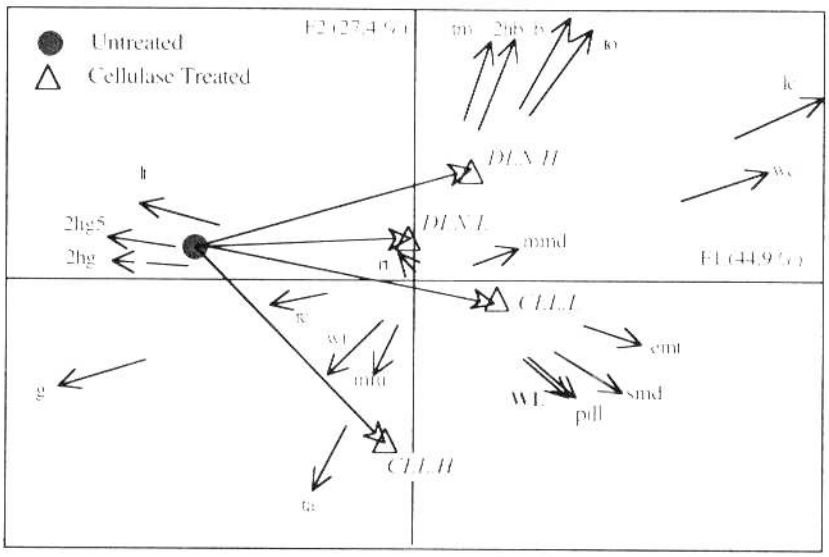

Fig. 6. Vectors map between extracted factors 1 and 2. (All parameters are represented by their small letter according to KES nomenclature; TA—thermal absorptivity, pill—pilling level (ASTM 3512), WL—weight loss, *CEL*—Cellusoft L, *DEN*—Denimax T. See treatment conditions in Table III-H, L).[23]

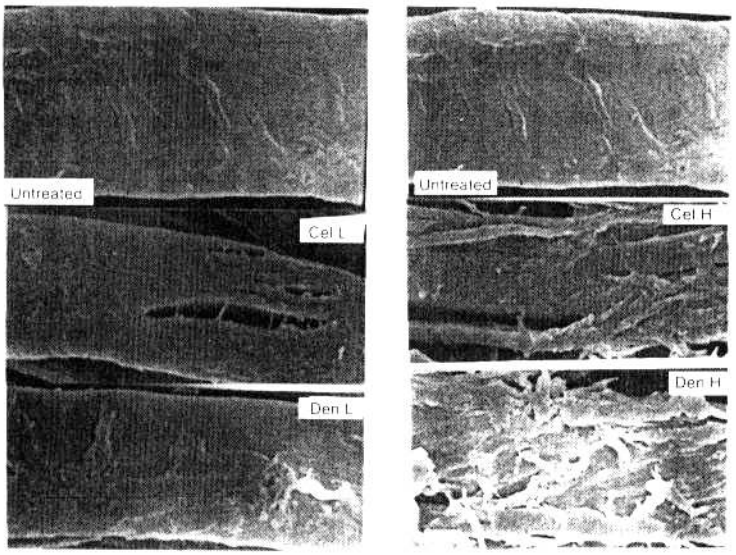

Fig. 7. Scanning electron micrographs of cotton fabrics (see Fig. 6 for abbreviations).

treated flannelettes were measured using the Kawabata Evaluation System for Fabrics.[23] The pilling level and the fabric thermal absorptivity (TA) were also measured because of the nature of flannelette fabrics (Table III).[24] To analyze all data produced on all treated fabrics, Multivariate Analysis Mapping Methodology was used. This technique which was recently suggested by Bishop and Cox, allows the treatment and further analysis of large databases with minimal loss of the original data.[25] The statistical treatment was developed using SPSS for Windows.[25,26] The relative positions of the vector parameters indicate how these parameters are interrelated.[26] For the studied fabrics, four groups were distinguished where the parameters are positively intercorrelated (Fig. 6).

- Group 1: EMT, SMD, WL and pilling level; the last two being highly correlated
- Group 2: 2HG, 2HG5 and LT
- Group 3: Tm, To, 2HB and B
- Group 4: LC and WC

Further analysis indicates a negative intercorrelation between

- Group 1 and 2
- G and Group 4
- TA and Group 2

All cellulase treatments led to a general increase of parameters of groups 1 and 4 and to a decrease of group 2. These changes are expected, as the cellulase treatment led to a weight loss of the flannelettes due to depilling and the claimed improvement of softness due to a decrease of shear properties.

Cellusoft L yielded a higher weight loss and depilling effect than Denimax T. Increasing the mechanical action emphasized these effects. Cellusoft L increased TA and decreased thickness and bending properties, while for Denimax T the reverse occurred. It seems that the action of Cellusoft L decreased bending and shear properties by cleaning microfibrils from the fiber surfaces. A higher mechanical action breaks the free surface fibers, thus cleaning the fabric surface and decreasing thickness. Denimax T with a more superficial action can create some microfibrils at fiber surfaces (Fig. 7), leading to an increase of interfiber friction and increasing bending properties and thickness.[7] Thus Cellusoft L with its cleaning action makes fabrics more permeable to heat, giving a cool feeling (TA changes), and the reverse occurs for Denimax T.

The fiber surface variations (Fig. 7) are consistent with the variations of the mechanical properties of the treated fabrics as well as with the activity nature of both enzymes. It is reported that cellulase-treated cotton fabrics will

increase their water retention due to microfibril formation.[19] The photos in Fig. 7 suggest that a microfibril formation is best obtained by a treatment with an EG-rich cellulase and higher mechanical action. Similar changes for other endoglucanases have been reported.[27]

Conclusion

The characterization of enzyme mixtures is essential to the understanding and control of the finishing effects obtained by cellulase enzymes. This characterization should be done in conditions that simulate the real conditions of processing. The fabric structure and the presence of pilling at the fabric surface are important factors that determine cellulase activity particularly at high levels of mechanical action.

Cellulase treatments change all properties that can be related to pill and microfibril remotion or formation and in accordance with the specific activities of the enzymes used.

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Author's Address

Artur Cavaco-Paulo, University of Minho, Department of Textile Engineering, 4800 Guimarães, Portugal; telephone 351-53-511670; fax 351-53-514400.