Treatment of cotton fabrics with purified *Trichoderma reesei* cellulases

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Two different types of cotton fabrics, i.e. cotton twill and cotton poplin, were treated with purified *Trichoderma reesei* cellulases. Methods used to evaluate the enzyme effects included weight loss, tear strength, bending hysteresis, wrinkle recovery and pilling tendencies. When the different fabric properties at the same weight loss level were compared, it was apparent that cellobiohydrolase I (CBH I) and endoglucanases had different effects on cotton fabrics. Endoglucanases caused more strength loss than CBH I but had also positive effects on the bending behaviour and pilling properties. The two endoglucanases tested differed in their action. At low hydrolysis levels practically no strength loss was obtained with these endoglucanases whereas at higher dosages EG II caused significantly higher strength losses as compared to EG I. A positive result in the pilling was, however, also obtained with these low enzyme levels, indicating practically no strength loss.

INTRODUCTION

The possibilities for treating cellulosic materials such as cotton, viscose or lyocell with cellulase enzymes have grown recently, especially with increasing consumer and industrial concern for environmental issues. The best known applications of cellulases are in denim garment washing processes, as an alternative to stone washing and in the modification of cotton fabrics to improve the surface properties [1,2]. In stone washing processes cellulases are replacing pumice stones and resulting in less damage to the machinery, environment and garments. Cellulases are also increasingly used in domestic fabric washing products [3].

A variety of bacteria (e.g. *Clostridium, Cellulomonas)* and fungi (e.g. *Humicola, Trichoderma, Penicillium*) produce cellulases. Enzymes from different origins have been identified and purified; their physicochemical, hydrolytic and molecular properties have been extensively characterised. One of the most important industrially used strains for cellulase production is *Trichoderma reesei*. The cellulolytic system of *T reesei* is composed of two cellobiohydrolases (CBH I and CBH II), at least four endoglucanases (EG I, EG II, EG III and EG V), and at least one β-glucosidase [4]. Cellulases act synergistically in the hydrolysis of crystalline cellulose. Endoglucanases randomly attack the amorphous regions in cellulosic substrates, resulting in a rapid decrease in cellulose chain length, whereas cellobiohydrolases can also act on crystalline regions of cellulose, releasing cellobiose from the end of cellulose chains [5]. Most of the endoglucanases and cellobiohydrolases are composed of a cellulose-binding domain (CBD) and a core protein, which contains the active site. These two domains are linked together via a linker region [6,7].

All processes in which cellulases are applied to treat textile goods include the cooperative action of mechanical friction during enzymatic hydrolysis. This friction plays a role in opening up the substrate and allowing the enzyme to cleave to the cellulose chains [8]. The strong mechanical friction provided by the rotation of a washing machine, in spite of partial or total substitution of pumice stones by the enzymes, is also essential for a good contact and removal of indigo from denim garments [9,10].

The mechanism of cellulase action has been investigated by using different mixtures of cellulases [9–11]. Commercial enzymes for textile treatments are usually mixtures of various cellulases. Due to the different mode of action of endoglucanases and cellobiohydrolases, the effect of cellulase treatment may be greatly influenced by the composition of the enzyme mixture. In this work cotton fabrics were treated with purified *T reesei* cellulases CBH I, EG I, and EG II [13,14], and the effects of the individual cellulases were evaluated.

MATERIALS AND METHODS

Fabrics

A 100% cotton twill woven fabric was used, commercially scoured and bleached (properties: fabric count 30 ends/cm, 21 picks/cm, 0.57 mm thickness, 250 g/m^2 weight). In addition a plain woven 100% cotton poplin was used

g/m^2 weight).

Enzymatic treatments

Cellulases CBH I, EG I and EG II were purified from culture filtrate of *T reesei* as described previously [13,14]. Enzymatic treatments were carried out in stainless steel pots (500 ml) in a Linitest machine for 60 min at 50 °C, using about 10 g fabric in 0.05 mol/l citrate buffer at pH 5.0, liquor ratio of 10:1. The dosage of purified cellulases (EG I, EG II, CBH I) were 0.2–5.0 mg of protein per gram of fabric.

Mechanical agitation was achieved by adding ten stainless steel discs (supplied with the Linitest machine) to each Linitest pot. After the treatments, the enzymes were inactivated by immersing the pots in boiling water for 5 min, and the fabrics were washed subsequently with hot and cold water. Reference treatments were also carried out but without enzyme in the buffer solution.

Analyses

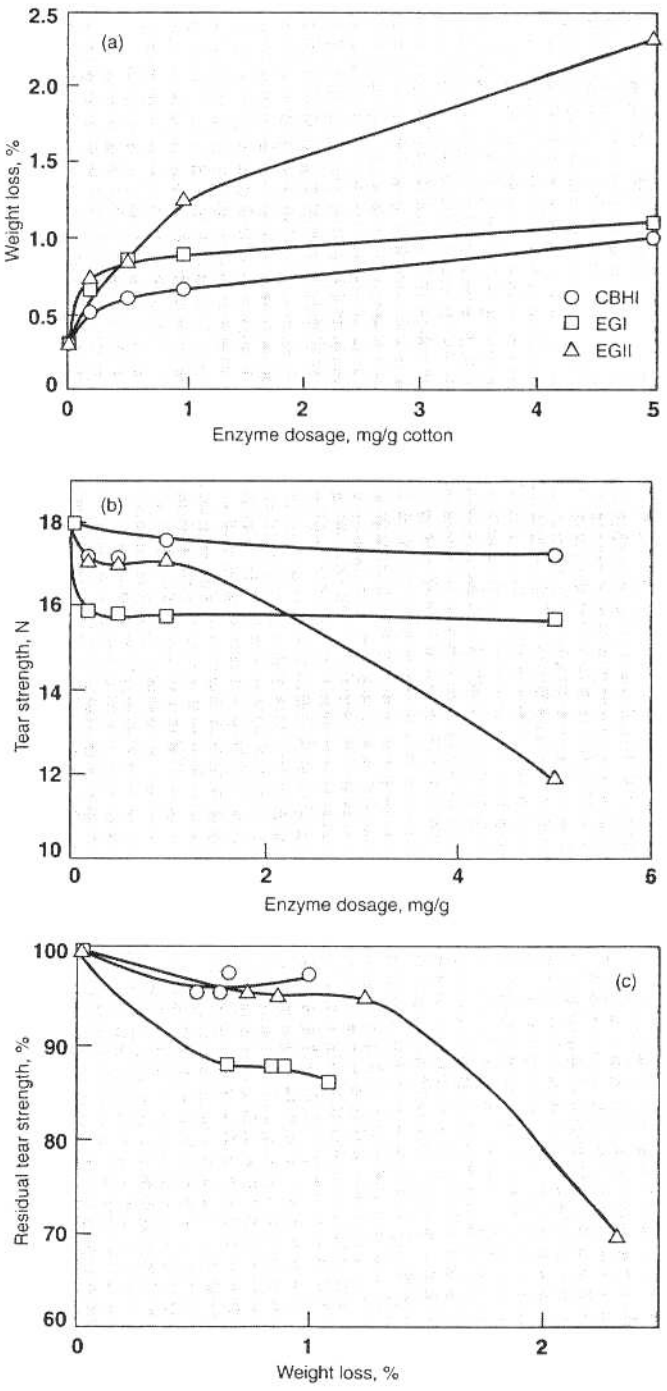
Fabric weight loss was determined as the difference in the weights of fabric sample before and after treatment, and after conditioning for 24 h at 20 °C and 65% RH. The tear strength of treated fabrics was measured on an Alwetron TCT 10 machine, with the 100 N cell (SFS 3981). Tear strength was measured in the warp and weft directions in poplin samples and only in the warp direction in twill samples. The pilling tendency of the enzyme-treated fabrics was tested using the modified Martindale abrasion testing method, with 500 cycles of abrasion. The modification apparatus for the Martindale abrasion testing method, described in ISO 12947-1, was from the maker of the Martindale machine. The test specimen was abraded against the face of the wool fabric specified in ISO 12947-1. The test results were evaluated by a panel on a scale 1 to 5, where 1 was the highest pill and fuzz level of the treated fabrics. Wrinkle recovery of the fabrics was tested by the Monsanto method (ASTM D 1295) along the warp direction. Fabric bending hysteresis was measured using a KES-FB2 instrument from Kato Tech Co. Ltd (at 20 °C and 65% RH).

RESULTS AND DISCUSSION

Treatment of cotton twill fabric with purified cellulases

Cotton fabric was treated with monocomponent *T reesei* CBH I, EG I and EG II with dosages of 0–5 mg/g for 1 h. After the treatments the extent of solubilisation of cotton cellulose was measured as weight loss. With an enzyme dosage of 5 mg/g, the EG II caused 2.3% weight loss, whereas the weight losses caused by both EG I and CBH I was significantly lower: 1.1 and 1.0% respectively (Figure 1a). By increasing the EG I and CBH I dosage from 1 to 5 mg/g only a slight effect on weight loss was obtained, whereas the weight loss obtained with EG II increased from 1.3 to 2.3%. In the reference treatment a weight loss of 0.3% was obtained, attributable to the mechanical action.

strength properties of the twill fabric, even at the highest enzyme dosage of 5 mg/g (Figure 1b). Similar results have also been reported when other types of cellulosic materials have been treated with cellobiohydrolases [13]. When the fabric was treated with EG I and EG II at dosages of less than 1 mg/g only a slight effect on tear strength was obtained (Figure 1b). However, by increasing the EG II dosage to 5 mg/g, significant reduction in strength was obtained. EG I, on the other hand, did not cause any



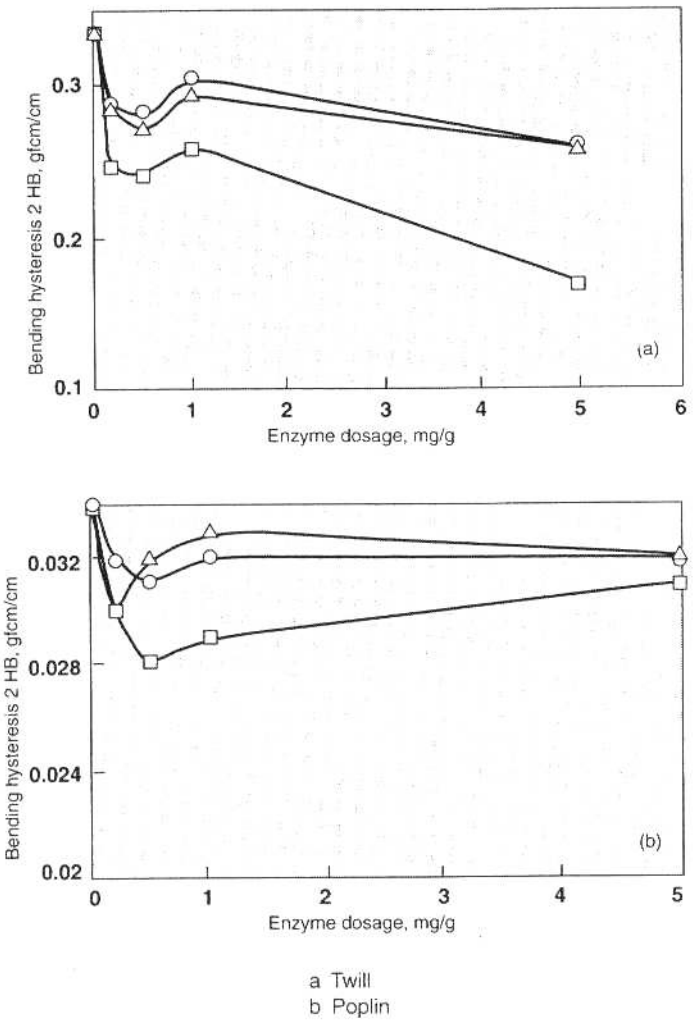
a Weight loss
b Tear strength
c Residual tear strength in relation to weight loss

Figure 1 Effect of purified cellulases CBH I, EG I and EG II on properties of woven cotton twill

further strength loss at increased enzyme dosages. By plotting strength decrease against weight loss, it was observed that at low weight loss levels the effect of EG I on strength loss was more pronounced (Figure 1c). When cellulose pulps have been treated with EG II, a negative impact on fibre viscosity has been observed at very low enzyme dosages [13,14]. This difference might be attributed to the more inaccessible cellulose matrix in cotton fabrics, compared with those of cellulose pulps.

All enzymes had a positive effect on drapability when used at low dosages, as measured by bending hysteresis (2HB) in both warp and weft directions. However, by increasing the dosages of CBH I and EG II, no clear further effect was obtained (Figure 2a), which could be due to the increased friction between the fibres. In this case, a combination of enzyme action and a high level of mechanical agitation may have raised a stubble of fibrillar material on the fibre surface, as already reported by Cavaco-Paulo *et al.* [10]. In contrast to the action of CBH I and EG II, increasing the EG I dosage brought about an improvement in drapability (Figure 2a).

The enzymatic treatments caused no significant changes in wrinkle recovery values of the cotton twill



a Twill
b Poplin

Figure 2 Effect of purified cellulases CBH I, EG I and EG II on bending hysteresis of cotton (mean values in both warp and weft directions)

Table 1 Effect of *Trichoderma reesei* CBH I, EG I and EG II treatments on degree of wrinkle recovery degree (Monsanto method) of 100% cotton twill and poplin

Enzyme dosage (mg/g cotton)	Twill			Poplin		
	CBH I	EG I	EG II	CBH I	EG I	EG II
0	53	53	53	78	78	78
0.2	51	59	55	86	81	86
0.5	57	59	58	73	88	81
1.0	60	59	61	67	72	82
5.0	56	62	60	72	83	81

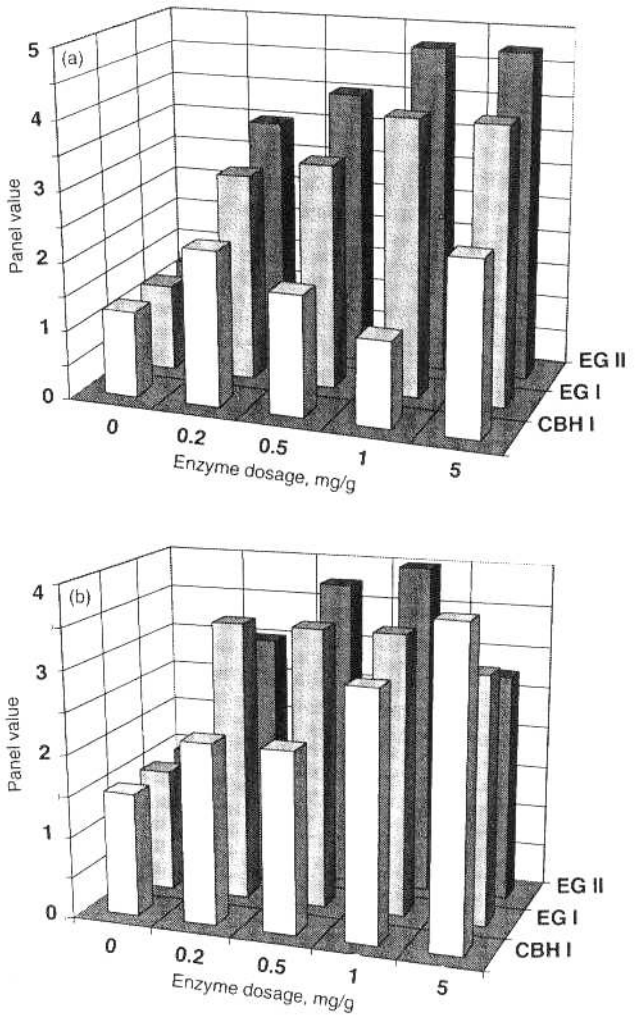


Figure 3 Effect of purified cellulases CBH I, EG I and EG II on pilling abrasion resistance of cotton; for key see Figure 2

fabric after CBH I treatment, even at the high enzyme dosages used, according to the Monsanto measurements. EG I and EG II treatments increased the wrinkle recovery slightly from 53 to 62 and 60 respectively (Table 1).

The pilling tendency of the enzymatically treated twill fabrics was tested by the Martindale abrasion testing machine. Treatment of the twill fabric with EG I and EG II

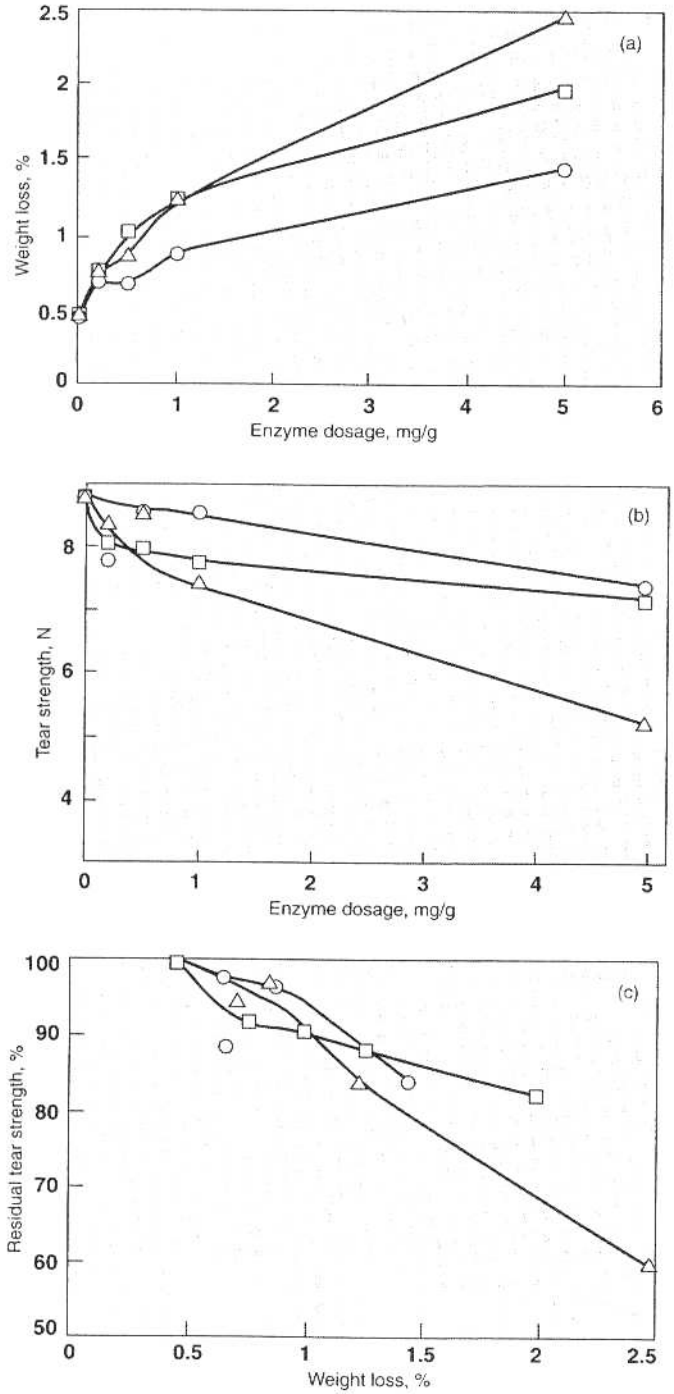


Figure 4 Effect of purified cellulases CBH I, EG I and EG II on properties of woven cotton twill; for key see Figure 1

resulted in significantly improved pilling abrasion resistance. Of the endoglucanases, EG II had the more pronounced effect and the panel value increased from 1.2 to 4.8 at the 5 mg/g dosage level (Figure 3a). The positive effect was also obtained in treatments with relatively low enzyme dosages, in which practically no strength decrease was observed. Thus, by optimising the endoglucanase dosage, the positive effect on pilling tendency could be obtained without any significant reduction in fabric strength properties.

Treatment of cotton poplin with purified cellulases

In order to obtain more information on the role of fabric type on the effects of enzyme treatment, cotton poplin fabric was treated with the same enzymes and dosages. This 100% cotton poplin was 2.5 times lighter than 100% cotton twill fabric. In contrast to the twill fabric, EG I and CBH I treatments resulted in significantly higher weight losses, whereas no difference was observed in the case of EG II (cf. Figures 1a and 4a). The mechanical action provided in the Linitest caused a higher weight loss on poplin than on twill fabric.

The effect of the EG I, EG II and CBH I treatments on tear strength was relatively weak at low enzyme levels (Figure 4a). Also in this case the negative effect of EG II was observed when high dosages were used (Figure 4a). When strength loss was plotted against weight loss, it was observed that at a weight loss of 1% and about 10% strength loss were obtained with all the enzymes tested (Figure 4b).

The effects of enzyme treatments on drapability of poplin, as measured by bending hysteresis, were less pronounced than the effects on twill fabric (Figures 2a and 2b). Treatment of the fabric with EG I increased drapability, while the EG II and CBH I treatments had practically no effect (Figure 2b).

No clear correlation between the enzyme dosage and wrinkle recovery was observed in the poplin treatments (Table 1). This may be due to the structure and light weight of the fabric.

The propensity towards pilling of poplin fabric was less pronounced, as compared with that of twill fabric, owing to the differences in structure between the two fabric types. In general poplin fabrics have more interlacing points than twill fabrics. In the evaluation, the panel value of the control was set to unity in order to facilitate comparison of the enzymes. As a result the panel values were not comparable with those obtained on twill (Figure 3a). All the cellulase treatments resulted in improved pilling abrasion values of the poplin fabric (Figure 3b). However, in this case the endoglucanases were also more effective at low enzyme dosages.

CONCLUSIONS

The impact of monocomponent *Trichoderma reesei* cellulases on the properties of two different cotton fabrics was tested. By comparing the different fabric properties at the same weight loss level, it was apparent that CBH I and EGs have different effects on twill and poplin fabrics. EGs caused more strength loss than CBH I but had a more positive effect on bending properties and pilling. However, at low hydrolysis levels practically no strength loss was obtained with the endoglucanases whereas at higher dosages EG II caused significantly higher strength losses as compared to EG I. Nevertheless, a positive pilling result was observed with these low enzyme levels, with virtually no concomitant strength loss. According to the results obtained, the technical properties derived from the enzyme treatment of cotton fabrics depend not only on

the type and concentration of enzyme used, but also on the type of fabric.

Understanding the impact of each individual cellulase enzyme on the fabric properties of different types of fabrics enables the production of novel optimised enzyme mixtures for the textile industry.

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