The Role of *Trichoderma reesei* Cellulases in Cotton Finishing

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ABSTRACT

Knitted and woven cotton fabrics were treated with a monocomponent *Trichoderma reesei* endoglucanase II (EGII) and with experimental *Trichoderma* cellulase mixtures containing different cellulase profiles. The cellulase action was evaluated by measuring weight loss, pilling, and bursting strength. Of the experimental mixtures, Cellulase F, being an EGII-enriched preparation, was the best mixture to obtain the highest pilling reduction with the lowest strength and weight losses on cotton knitted fabric. The monocomponent EGII was, however, even more efficient with respect to good depilling with minimal negative effect on the knitted fabric. The results obtained with the different cellulases were found to depend on the fabric type. In the case of ring-spun woven fabric, purified EGII was the best enzyme in depilling with the lowest weight loss. Cellulase B (containing CBHI and EGII) reduced the pilling tendency, but the weight loss was higher as compared to that obtained with the monocomponent EGII. In the case of open end-spun woven fabric, Cellulase B and Cellulase E (EGII enriched, CBHI negative) resulted in improved depilling, but at the same time they caused relatively high weight loss.

Key Terms

Cellulase
Cotton
Finishing
Pilling
Trichoderma reesei

The biofinishing of cotton textiles using cellulolytic enzymes provides an environmentally friendly route to achieve certain desirable effects. Biofinishing with cellulases has been applied also on a variety of other cellulosic fabrics.[1] In the biofinishing process, the fiber ends protruding from the fabric surface are weakened and subsequently separated from the material with the aid of mechanical action. As a result, the surfaces are cleaned by reducing fuzz. Removal of the fibers also prevents pilling and subsequently reduces permanently the tendency to form pills during wearing and washing. Biofinishing enzymes are suitable both for the wet processes of the textile industry and for finishing textiles, either as continuous lengths or as single items in washing machines.

Trichoderma reesei is the most commonly used fungus for cellulase production in the textile industry. The cellulolytic system of *T. reesei* is composed of two cellobiohydrolases (CBHI, CBHII) and at least five endoglucanases (EGI, EGII, EGIII, EGIV, and EGV).[2,3] The cellulases act synergistically in the hydrolysis of crystalline cellulose. Endoglucanases randomly attack the amorphous regions in cellulosic subtrates, resulting in chain scission and a consequent reduction in cellulose molecular weight. Cellobiohydrolases act on the ends of the cellulose chains releasing cellobiose.[4,5]

Mainly crude cellulase preparations have been used in biofinishing processes. Due to the unoptimized cellulase composition in these preparations, the enzymatic treatment has, in some cases, resulted in significant strength losses. By treating cotton interlock fabric with purified *T. reesei* cellulases, Heikinheimo and Buchert showed that EGII-based combinations always resulted in good pilling properties.[6] Furthermore, with an EGII:CBHI ratio of 25:75, practically no decrease in strength was observed despite the high improvement in the pilling resistance. Furthermore, single purified CBHs did not affect pilling properties of the interlock fabric, whereas EGI and EGII treatments clearly improved pilling values.[6] However, in order to obtain similar improvement in pilling resistance, relatively more EGI was required as compared to EGII.[6]

The aim of this work was to define the optimum cellulase compositions for biofinishing of different types of cotton fabrics in order to achieve the cellulase finishing effects with minimum negative impacts. The effects of experimental cellulase mixtures containing desired cellulase profiles of *T. reesei* cellulases were studied on cotton fabrics.

EXPERIMENTAL

Materials

Enzymes

The experimental cellulase mixtures A-F with different cellulase profiles were produced by genetically modified *T. reesei* strains. The strains were constructed using

TABLE I.

Enzyme Profiles in Different Experimental *T. reesei* Cellulase Samples

Code	Cellulase Overproduced	Gene Deleted	Cellulases Present
A		*egl2, cbh2*	CBHI, EGI
B		*egl1, cbh2*	CBHI, EGII
C		*egl1, cbh1*	CBHII, EGI
D		*egl1, cbh1*	CBHII, EGII
E	EGII	*cbh1*	EGI, EGII, CBHII
F	EGII		EGI, EGII, CBHI, CBHII

TABLE II.

Effects of Different Cellulase Treatments on Weight Loss

	Weight Loss %					
	Knitted		Ring-Spun		Open End-Spun	
Enzyme	1 mg/g	0.5 mg/g	1 mg/g	0.5 mg/g	1 mg/g	0.5 mg/g
EGII	1.0 (+/-0)	0.7(+/-0)	1.7	1.5	1.9	1.5
A	0.7(+/-0.2)	0.8(+/-0)	2.2	1.7	2.5	2.1
B	0.6(+/-0.2)	1.0(+/-0)	2.2	2.1	2.4	2.1
C	1.0(+/-0.2)	0.9(+/-0.1)	-	-	-	-
D	1.1(+/-0.3)	1.4(+/-0.3)	1.9	1.6	2.0	2.2
E	1.4(+/-0.3)	1.2(+/-0.3)	1.9	1.8	2.5	2.2
F	1.2(+/-0.1)	0.7(+/-0.1)	2.6	2.1	2.7	2.4
Reference	0.2(+/-0.1)	0.2(+/-0.1)	1.2	1.2	1.4	1.4

standard genetic engineering techniques as described previously.[7,8] The cellulase profiles in each mixture are presented in Table I. *T. reesei* cellulase EGII was purified as described previously and used as comparison in the treatments.[9]

Fabrics

The fabrics used for the enzyme treatments were 100% cotton knitted interlock (203 g/m^2), 100% open-end spun woven cotton twill (223 g/m^2), and 100% ring-spun woven cotton twill (237 g/m^2). The fabrics were commercially scoured and peroxide bleached, rinsed, and dried on a stenter. No optical brightener or softener was applied.

Methods

Enzymatic Treatments

For the enzymatic treatment of knitted fabric, about 10 grams of samples were placed in the stainless steel pots (500 mL) of a Linitest machine with liquid ratio 1:10. Treatment time was one hour at pH 5 and 50C. Enzyme dosages were 0.5 and 1 mg of protein per gram of cotton substrate. Treatments of ring-spun and open end-spun fabrics were also carried out in a Linitest for one hour at pH 5 and 50C with dosages of 0.5 and 1 mg of protein per gram of fabric.

After the treatments, the enzymes were inactivated by immersing the pots in boiling water for five minutes, and the fabrics were subsequently washed with hot and cold water. Four parallel tests were done with knitted fabric and one with woven fabrics. Reference treatments were carried out correspondingly but without enzyme in the buffer solution.

Analyses

Weight loss was determined as the difference in fabric weight before and after the enzyme treatment. The fabric weight was measured after drying for four hours at 105C and cooling overnight in a desiccator. Strength properties of knitted fabrics were measured using Psi-Burst tester according to a standard ISO 2960 with 10 parallel measurements. The pilling tendency of the knitted fabrics was measured by a tumble pilling tester (ASTM D 3512-82) with two parallel tests. The fabrics were visually evaluated by a panel giving the highest grade 5 to the best specimen (no pills). Pilling properties of woven fabrics were tested using the Martindale method. Tested fabrics were rubbed against the standard woolen fabric 2000 times. After the rubbings the fabrics were visually evaluated by a panel.

RESULTS

Knitted Fabric

Cotton knitted fabric was treated with experimental cellulase mixtures A-F containing different cellulase profiles (Table I). The purified EGII was used as comparison in the treatments. All enzymes were dosed based on their protein content and the dosages used were 0.5 and 1 mg/g. The effects of enzyme treatments were evaluated according to weight loss, pilling values, and bursting strength. Of the cellulase preparations tested, the monocomponent EGII and Cellulases E and F were found to improve the depilling significantly and with the smallest enzyme dosage used the highest pilling improvement; i.e., a pilling value of 5 could be reached (Fig. 1). Both Cellulases E and F are EGII-enriched cellulase preparations (Table I). The difference between Cellulase E and F is that the gene coding for CBHI has been deleted from the strain used for production of Cellulase E, and therefore CBHI is not present in that preparation. When higher enzyme dosages were used; i.e., the enzyme dosage was 1 mg/g, the Cellulases C and D also resulted in a

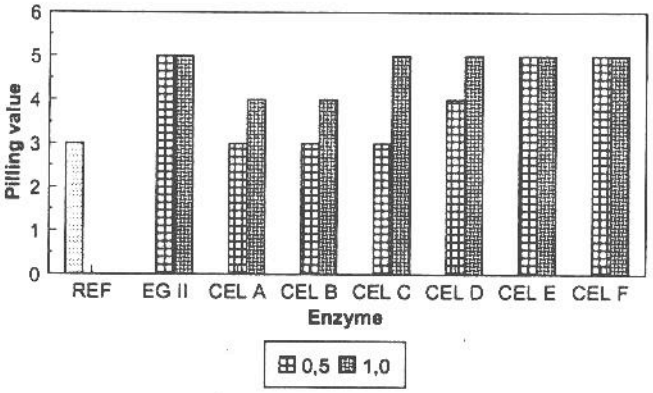

Fig. 1. Effect of different experimental cellulase mixtures and monocomponent EGII on pilling properties of knitted cotton fabric. The enzyme dosages (0.5 or 1.0) are expressed as mg protein/g of fabric.

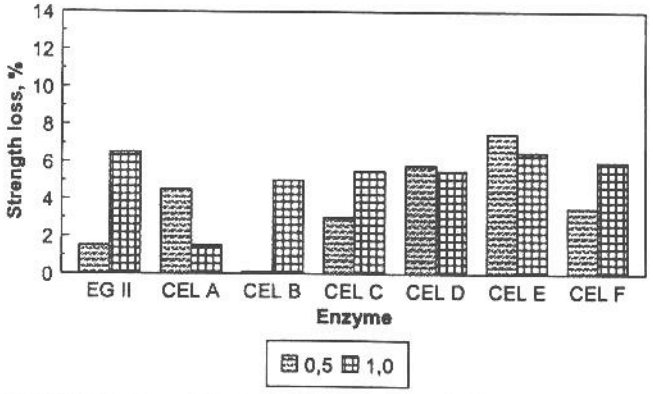

Fig. 2. Comparison of the strength loss percent obtained by treatment of knitted cotton fabric with different experimental cellulase mixtures and single purified EGII with dosages of 0.5 mg/g and 1.0 mg/g.

pilling value of 5. These two preparations contained cellulases CBHII+EGI and CBHII+EGII, respectively (Table I). Under the conditions tested, the pilling value remained on the level of 4 with Cellulases A (CBHI+EGI) and B (CBHI+EGII).

When the weight losses of the cellulase treated fabrics were determined, it was found that the best pilling value with the lowest weight losses were obtained with EGII and Cellulase F (Table II, Fig. 1). The strength loss caused by monocomponent EGII alone was the lowest (1.5%), whereas Cellulase F caused slightly higher strength loss, (Fig. 2). Clear differences between the experimental enzyme mixtures were observed. For example, to obtain the pilling value of 5 with Cellulase C and D, a higher enzyme dosage was needed as compared to Cellulase F, and also the strength loss obtained was slightly higher (Fig. 2). With Cellulase E, the pilling value of 5 could be reached with the smallest concentration, but the strength and weight losses were higher than those obtained with Cellulase F, C, or D at the same pilling level. Thus, a single EGII would be the most efficient cellulase in depilling with minimal negative effects on the fabric (Table III). Of the experimental mixtures, Cellulase F was the best preparation to obtain the highest pilling value with lowest strength and weight losses.

Woven Fabric Treatment

Results obtained with the experimental cellulase mixtures and purified EGII for knitted fabric were compared further to woven fabrics treated with the same preparations. Pilling properties of woven fabrics were tested using the Martindale method. Tested fabrics were rubbed against the standard woolen fabric 2000 times. After the rubbings the fabrics were visually evaluated by a panel. In the case of ring-spun fabric, EGII and Cellulase B reduced the pilling tendency (positive effect on pilling), whereas in the case of open end-spun fabric, only B and E treatments resulted in improved depilling (Table IV).

When ring-spun fabric was treated with dosage of 0.5 mg/g, the Cellulases A, D, E, and monocomponent EGII caused the lowest weight losses (Table II). In the case of open end-spun fabric, EGII caused the lowest weight loss, and no great differences among the other enzyme preparations were observed. Thus, the fabric type affected the weight loss obtained. As comparison with the same enzyme concentration the lowest weight losses for knitted fabric were obtained with Cellulases F and EGII (Table II). In general the weight losses obtained with knitted fabrics were lower as compared to those obtained with woven fabrics. For ring-spun fabric the monocomponent EGII alone was the best enzyme to achieve the best pilling value with the lowest weight loss. For open end-spun fabrics Cellulase E, being an EGII-enriched preparation, was the best preparation to reduce pilling, but at the same time it caused relatively high weight loss.

TABLE III.

Enzyme Dosages Needed to Obtain a Pilling Value of 5 for Knitted Fabric

Enzyme	Dosage (mg/g)	Strength Loss (%)	Weight Loss (%)
EGII	0.5	1.5	0.7
F	0.5	3.5	0.7
C	1	5.5	1.0
D	1	5.5	1.1
E	0.5	7.5	1.2

TABLE IV.

Effect of Different Enzymes on Pilling of Woven Fabrics[a]

Enzyme	Ring-Spun Woven 0.5mg/g	Open End-Spun Woven 0.5mg/g
EGII	++	0
A	0	0
B	+	+
D	0	0
E	0	++
F	0	0
Reference	0	0

[a] + Symbol indicates positive effect, and 0 no effect on treated fabric

CONCLUSION

With experimental cellulases, it was found that high pilling removal was dependent on the fabric type. In all cases, EGII-based cellulase products gave the most positive depilling result. Similar results have been reported by Heikinheimo and Buchert using purified *Trichoderma* cellulases on cotton interlock fabric.[8] According to the results the strength reduction caused by the cellulase treatment can be minimized by having only EGII present in the cellulase mixture.

Acknowledgements

This research has been financed by the Commission of the European Union ("Biofinishing of cotton with cellulases" BRPR-CT-95-0009).

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