TEXTILE DEPILLING: USE OF ENZYMES AND CELLULOSE BINDING DOMAINS

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Abstract. Textile fabrics biopolishing is one of the most important industrial application of cellulases. These are widely used to remove fibrils and fuzz fibres from cotton fabrics, or to produce the "stone-washed" look of denim garments. The depilling effect and the achievement of desirable touch properties are among the applications sought by the users. This process, although effective, is associated to a significant tensile strength loss. The biopolishing mechanism is still the subject of controversy. Interfacial properties are not considered in the removing of the pills. It is believed that the hydrolytic activity of cellulases is the only responsible process for the biopolishing. In this work, we aim at introducing a new perspective in the understanding of the biopolishing mechanism, specifically we consider the contribution of interfacial properties. Cellulose Binding Domains (CBD) with a much lower hydrolytic activity than cellulases were produced in laboratory by ultrafiltration after digestion with a protease. Some were purified by ion-exchange chromatography to reduce even more catalytic activity. Cotton fabrics were treated with different cellulases and the CBD. Soluble sugars, tensile strength loss and pilling degree were measured to evaluate the effect of enzymes and CBD, to understand the tensile strength loss and to conclude if interfacial properties are important in the biopolishing process.

Keywords: Depilling; Cellulose binding domains; tensile strength loss; interfacial properties

1. Introduction

Cellulases are increasingly being applied to textile finishing. Cellulases are widely used to remove fibrils and fuzz fibres from cotton fabrics, or to produce the "stone-washed" look of denim garments. However, a major problem with cellulases is the subsequent loss in tensile strength. Lenting and colleagues (Lenting and Warmoeskerken, 2001a) provided guidelines to prevent tensile strength loss. According to these authors, the strength loss is caused by the degradation of crystalline cellulose.

Cellulases are produced by a wide variety of bacteria (e.g. *Clostridium*, *Cellulomonas*) and fungi (e.g *Humicola*, *Trichoderma*, *Penicillum*). Among these, the more important commercially is the one from *Trichoderma reesei* (Heikinheimo et al, 1998). The cellulolytic system of cellulases is composed of cellobiohydrolases (CBHs) and endoglucanases (EGs). CBHs act on the crystalline regions of cellulose releasing cellobiose from the end of cellulose chains, meanwhile EGs attack amorphous regions (Chanzy and Henrissat, 1985). Most cellulases share a common structural organisation, with a catalytic domain (or core domain) connected to a cellulose-binding domain (CBD) via a linker peptide sensitive to proteolysis. The fungal CBDs are usually located either at the C- or N- terminus of the enzyme, and are about 30-40 aminoacids long (Lenting and Warmoeskerken, 2001b).

It has been proposed that CBD increases the effective enzyme concentration on the surface of the solid substrate, promoting the release of a single cellulose chain from the crystallite, by breaking hydrogen bonds (Tomme et al, 1995; Linder and Teeri, 1997; Sridodusk et al, 1998). It has also been reported that CBDs have the ability to perform a nonhydrolytic disruption of cellulose fibres, releasing small particles (Din et al, 1991). Banka and colleagues (1998) demonstrated that a fibril-forming protein from *Trichoderma reesei* caused nonhydrolytic disruption of cotton fibers. Small fibrils were released, without detectable production of reducing

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sugars. Ida Lee and colleagues (2000) obtained images, by atomic force microscopy, of holes left in cotton fibres treated with hexachloropalladate-inactivated CBH I. The holes are believed to be caused by the penetration of the binding domain. In the same study, a cellulase from *Thermotoga maritime* without CBD was used, and no effect on the cotton fibers was detected. Recently, Irina Kataeva and colleagues (2002) detected modifications on the surface of the fibres caused by action of CBDs and fibrinectin from *C. thermocellum*.

Lemos and colleagues (2002) developed a simple method to obtain CBDs from fungal cellulases after digestion by a protease. Using this process, it is possible to obtain gram amounts of CBDs, although with some contaminating enzymatic activity. In this work, we aim at analysing whether fabrics treated with CBDs - with very low hydrolytic activity - present the same finishing quality of fabrics treated with cellulases.

2. Materials and Methods

2.1. Enzymes

Cotton fabrics were treated with Cellulose-Binding-Domain (CBDs), obtained from a cellulase preparation (Celluclast, Novozymes) and with commercial enzyme (Cellusoft APL, Novozymes, industrially used for depilling).

2.2. CBD Production

Celluclast (1.5L) was diluted in a total volume of 5 L with distilled water, washed in a Pellicon 2 Ultrafiltration Module (Millipore) equipped with a 30-kDa nominal weight cut-off membrane for about 12h with sodium acetate buffer (25 mM, pH 5), to remove low molecular weight compounds.

Proteolysis was done with papain (papaya latex, Sigma) in a concentration of 1g papain/3000 g protein, for 3 hours at room temperature. After digestion, the mixture was passed through an Ultrafiltration Module (Millipore) equipped with a 10-kDa nominal weight cut-off membrane. CBDs were collected in the permeate.

Some CBDs were further purified by ion-exchange chromatography, using a XK 50/60 column packed with DEAE Sepharose^{TM} Fast Flow and a FLPC system from Pharmacia.

2.3. Depilling Treatment of Cotton Fabrics

The tests were performed in a Mathis, Drum Dyeing and Washing Unit, Type TWA 1.5/5 kg machine and in a smaller machine, a Mathis Beaken Labomat, Type BFA. The fabric was a RIB 1×1 100% Cotton. Two pre-treatments were carried out. In the first, called boiling, the fabric was treated with 0.5 g/l of Kieralon ED 835, 1 g/l of Lufibrol DK and 2 g/l of caustic soda, for 30 minutes at 100ºC. In the other one, called ½ white, the fabric was treated with 0.5 g/l of Kieralon ED 835, 0.5 g/l of Tinoclarite 4525, 2 g/l of caustic soda 50% and 2g/l of H_2O_2, for 25 minutes at 95ºC. Then Baylase EFR was added to neutralize the H_2O_2 for 10 minutes.

The depilling treatments were made in 30 l of water with 2 kg of fabric, in the larger Mathis machine. The agitation rate was 25 rpm for 45 minutes at 55ºC and pH 5-5.5 for the Cellusoft APL. The assays with CBDs were performed for 90 minutes, at the same temperature. In the smaller machine, 200 g of fabric were treated in 3 l of water with an agitation rate of 55 rpm and the temperature set at 55ºC. The reaction was terminated adding NaOH to the mixture, to a final concentration of 0.5 g/l, to remove the protein adsorbed to the fabric. Different concentrations of enzyme and CBDs were used.

2.4. Analysis

Hidrolytic activities of cellulases and CBDs preparations, expressed as Filter Paper Units (FPU), were determined as the amount of sugars released in one hour at 50ºC, using filter paper (Whatman nº1) as insoluble substrate. Soluble sugars were measured using the dinitrosalicylic acid method (DNS), using glucose as standard (Miller, 1959).

Supernatants Analysis. The soluble sugars released during depilling were measured using the dinitrosalicylic acid method (DNS), using glucose as standard (Miller, 1959). The adsorption of protein was calculated as the difference between the concentrations before and after treatment.

Fabric Analysis. Fabrics were characterized for the pilling degree, weight and strength loss.

Weight loss was determined as the difference of the fabric's weight before and after treatment.

The fabric resistance to tension was measured using the Truburst, Bursting Strength Tester from James H. Heal & Co Ltd. Five tests were performed, and the difference between the reference and treated fabrics was calculated.

Pilling tendency was measured using the Nu-Martindale 404 Abrasion & Pilling Tester, from James H. Heal & Co Ltd. After the tests, samples were rated from 0 to 5 according to the degree of pilling (K3 value), by the experienced personnel from Vale de Tábuas.

Fabrics samples were observed by electron microscopy (Leica, Cambridge S360). The samples were coated with gold particles in a Fisons Instruments Polaron SC502 Sputter Coater.

3. Results and Discussion

3.1. CBD Production

The CBD production was carried out using a modification of the method described previously (Lemos et al, 2002): the cellulase was treated with papain, and the binding domains were separated by ultrafiltration. In these experiments a UF Pellicon system from Millipore was used, allowing a gram scale production of peptides. This preparation was found to have residual catalytic activity (figure 1), higher than the obtained in the lower scale systems used in previous work (Lemos et al, 2002). A further purification of the binding domains was carried out by anion exchange chromatography. The FPU activity of the obtained CBDs, and also of several other commercial enzymes, is shown in Figure 1.

As expected, the obtained CBDs preparations have a much lower activity than Cellusoft, a cellulase industrially used for depilling. The chromatography step further reduces the residual activity. Although still bearing some hydrolytic activity, this CBD sample is used in this work to test the effect (regarding depilling) of possible interfacial modifications associated to peptide adsorption

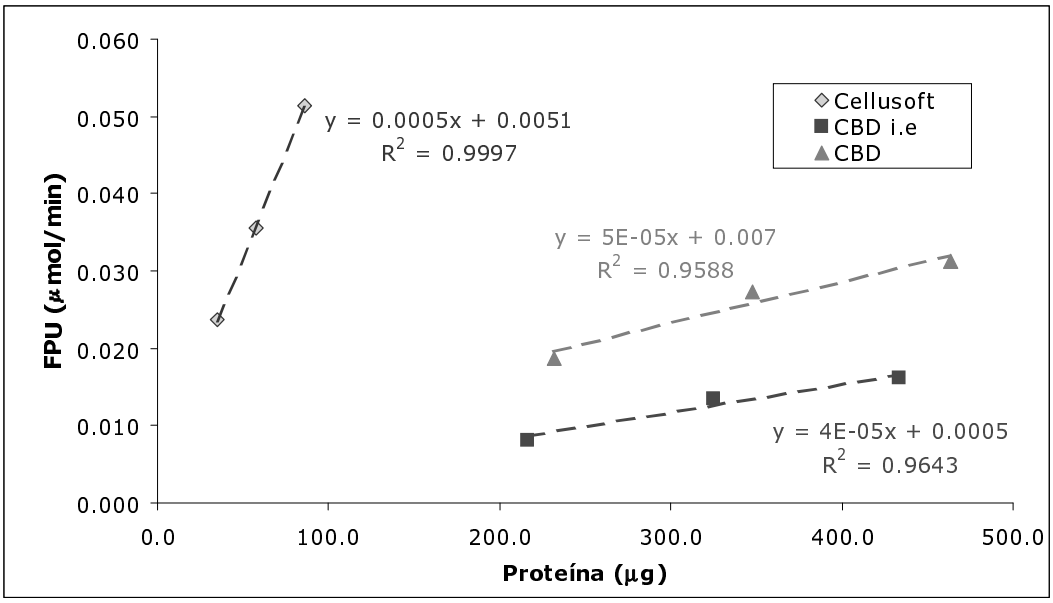

Figure 1. Enzymatic activity of Cellusoft and CBD on filter paper

- CBD ie: CBD after ion exchange chromatography

3.2. Depilling Experiments

Figure 2 shows images obtained by electron microscopy of the treated and non-treated fabrics. As may be observed in the figures with magnification 30x, both the enzymatic and CBD treatments mainly remove the loosen fibres in the surface of the fabric. The fibres removed in the depilling process are apparently 1-2 mm long (Figure 2), being loosely bound to the woven fabric. Smaller fibres or fuzz are also probably removed.

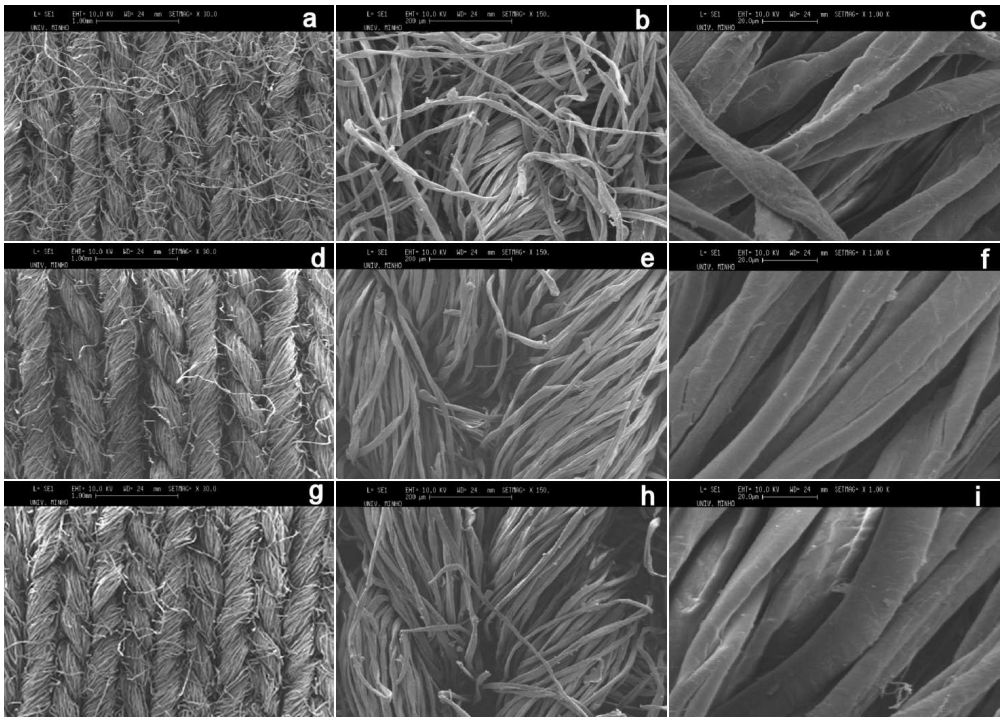

Figure 2. Non-treated fabric (a - 30×; b – 150×; c - 1000×), enzyme treated fabric (d - 30×; e – 150×; f - 1000×) and CBD treated fabric (g - 30×; h – 150×; i - 1000×) observed by electronic microscopy.

The released soluble sugars, tensile strength loss, and the depilling effect were measured. The results obtained (both with the larger and the smaller machine) are shown in Table 1. The sugars solubilised by enzymes and CBDs, correspond, as described in the Materials and Methods section, to incubation periods of 45 and 90 minutes. The larger machine has a different and stronger agitation than the smaller one. This difference on the agitation affects the finishing of the fabric. In fact, as can be seen in table 1, for the tests in the Mathis Labomat (small machine), the obtained pilling degree was worse, for comparable enzyme concentrations. As compared to the hydrolytic activities detected in the filter paper assays, it appears that the hydrolysis carried out by CBDs is enhanced in the presence of mechanical action of the Mathis machines.

Table 1. Effect of CBDs and Cellusoft on the treatment of cotton fabrics *

		Soluble sugars (g/l)	Insoluble weight loss (%)	Total weight loss (%)	Strength loss (%)	Pilling degree (K3)
CBD	Raw fabric	-	-	-	-	2
	3 mg/g	0.20	0.80	1.0	2	4
	4 mg/g	0.45	2.55	3.0	13	5
	3.5 mg/g	0.28	2.22	2.5	9	4.5
	25ºC, 3.8 mg/g	0.17	2.83	3.0	15	3.5
	½ white 4 mg/g	0.34	1.66	2.0	12	4
	CBDs purified (i.e.)	0.00	2.0	2.0	15	2.5-3
	Labomat, 3.3 mg/g	0.23	n.d.	n.d.	17	3.5-4
	Labomat, 5.8 mg/g	0.21	n.d.	n.d.	17	4-4.5
Cellusoft	0.75 mg/g	0.37	n.d.	n.d.	n.d.	3
	1.1 mg/g	0.40	n.d.	n.d.	n.d.	3.5
	1.5 mg/g	0.55	1.45	2.0	9	5
	1.5 mg/g	0.88	5.02	5.9	15	5
	1.2 mg/g	0.70	3.90	4.6	18	4
	1.12 mg/g	0.68	3.42	4.1	15	4
	½ white 1.5 mg/g	0.90	3.00	3.9	12	4
	Labomat, 2.6 mg/g	0.49	n.d.	n.d.	14	4-4.5
	Labomat, 3.9 mg/g	0.54	n.d.	n.d.	21	4-4.5

* Except when otherwise stated, all assays where conducted at 50ºC, in the larger MATHIS machine, with the RIB fabric. The protein concentration used in each assay (either CBD or enzyme) is shown. n.d. – not determined; i.e.-ion exchange.

The results show that the depilling obtained with Cellusoft is dependent on the enzyme concentration. By lowering the protein dosage from 1.5 to 1.1 and 0.75 mg/g, a less effective depilling is observed (K3 values of 5, 3.5 and 3, respectively). Interestingly, it was possible to obtain a similar depilling effect using the CBDs preparation. Although with a much lower hydrolytic specific activity, these peptides effectively remove fibrils from the fabric´s surface. The results clearly show that it is possible to obtain the highest depilling effect with a much lower sugar release than with Cellusoft. Lowering the sugars release is convenient, since there is lower weight loss and effluents treatment costs are reduced. For instance, a sugar release up to a concentration of 0.7g/L corresponds to a depilling class 4 using Cellusoft, the same classification being obtained using CBDs corresponding to a sugar release of only 0.20-0.28g/L. Roughly, it appears that, with Cellusoft (45 minutes

treatment, with protein concentration of 1-1,5 mg/g), a sugar solubilization 2 fold superior to the one produced with CBDs (90 minutes treatment, with protein concentration of 3-4 mg/g) is necessary for a similar depilling to be obtained. The CBD preparation obtained in our laboratory is more effective than Cellusoft in promoting a depilling effect, in that the removal of pill is achieved with lower sugar release. The positive results obtained with CBDs raise intriguing questions about the depilling mechanism: why do peptides with a low specific activity perform well compared with commercial cellulases? In our view, two explanations may be advanced to answer this question: 1) the residual activity present in the CBDs preparation matches the needs for the removal of pills, with no unnecessary release of soluble material; 2) the adsorbed binding modules, while having no catalytic activity, somehow affect the surface properties, leading to the desired effect. In order to evaluate whether the removal of the trace hydrolytic activity would affect the depilling ability of CBDs, two further assays were carried out. The treatment of the fabric was conducted at room temperature (25ºC), and the CBDs purified by anionic chromatography were used. In both cases (table 1), the catalytic activity is much lower or negligible, and the depilling effect was substantially reduced. It is thus apparent that the hydrolytic activity associated to the CBDs preparation is necessary. However, in our view, this does not rule out the second hypothesis above suggested.

A significant loss of tensile strength arises while using enzymes. This is considered the major limitation for a more widespread use of this technology. The development of a process involving lower risk in this regard seems highly desirable. We analysed the effect of successive CBD treatments, to see whether the reduced sugar release would lead to a better control of the fabric´s tensile strength. As can be seen from data on Table 2, successive treatments were performed in order to observe the tensile strength loss with Cellusoft and CBDs. Tests were performed on jersey+lycra and on 100% cotton fabrics.

Table 2. Effect of successive treatment of fabrics using Cellusoft and CBD

Fabric		Control	Cellusoft	CBD
Jersey+Lycra	***1rst treatment***			
	Pressure (kPa)	460	378	380
	Strength loss	-	**21%**	**18.7 %**
	2nd treatment			
	Pressure (kPa)	460	379	375
	Strength loss	-	**21.1 %**	**24.4 %**
	3d treatment			
	Pressure (kPa)	480	352	357
	Strength loss	-	**26.8 %**	**25.5 %**
100% RIB Cotton	***1rst treatment***			
	Pressure (kPa)	776	622	651
	Strength loss	-	**19.9%**	**16.2 %**
	2nd treatment			
	Pressure (kPa)	776	661	648
	Strength loss	-	**14.4 %**	**16.6 %**
	3d treatment			
	Pressure (kPa)	762	559	584
	Strength loss	-	**26.7%**	**23.3 %**

There is no significant difference between the use of Cellusoft and CBDs. In fact, for all these successive treatments the tensile strength loss was very similar, regardless of using CBDs or Cellusoft.

The following hypothesis can be suggested to explain this matter: 1) the removal of the loosen fibers on the surface of the fabric may be responsible for the loss of the tensile strength; 2) the adsorption of the proteins may modify the cellulose structure, by breaking hydrogen bonds (Tomme et al, 1995; Linder and Teeri, 1997; Sridodusk et al, 1998) or nonhydrolytic disruption (Din et al, 1991); 3) Finally, it may be suggested that a very low hydrolysis rate may suffice to affect the mechanical properties of cotton fabrics.

4. Conclusions

The following conclusions may be drawn from these results: 1) it is not possible to simply relate the extent of hydrolysis with the depilling effect and tensile strength loss; 2) *The CBDs preparation is, among the formulations tested in this work, the more effective one*; however, the depilling effect cannot be attributed, at least integrally, to an effect associated with the binding modules alone. Indeed, using CBDs at room temperature (lowering the residual catalytic activity), or using CBDs purified by anionic chromatography, resulted in a poorer depilling effect. Thus, the adsorption of the binding domains alone is not sufficient for the removal of the fuzz, small fibres, or loosely bound fibres. 3) Based on the results obtained with the successive treatments, it appears that total elimination of the tensile strength loss cannot be avoided, even substantially reducing the release of sugars.

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