Enzymatic Treatment of Lyocell—Clarification of Depilling Mechanisms

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ABSTRACT

We have characterized cellulose from the original structure of a lyocell fabric, from pills mechanically removed from the surface with a razor blade and from cellulose microfibrils removed from the surface and dispersed in the liquor during a cellulase depilling process. We find that cellulases first attack the cellulose from the microfibrils on the surface of the fabric because they are more externally exposed than the cellulose of the original fabric structure and have the same characteristics in terms of molecular weight, polydispersity, and crystallinity index as the base fabric. Cellulose from the microfibrils removed from the base fabric by the cellulase and recovered from the liquor shows significantly increased polydispersity, therefore indicating that the microfibrils have been widely attacked by the enzymes. Cellulases prove to be thorough surface finishing agents, since they change only the fiber surface, not the crystallinity.

Lyocell is the name of solvent-spun cellulosic fibers from wood pulp in an amine oxide solvent system. Lyocell fibers have high dry and wet tenacity and a luxurious drape [1, 7]. The major drawback of these fibers is fibrillation, especially after wet processing stages with mechanical agitation. This primary fibrillation induces microfibrils that are very heterogeneous in their size [1, 7]. Cellulase enzymes improve the appearance of lyocell fabrics and produce a further homogeneous, short fibrillation in a second process with high mechanical agitation. This fibrillation, also called secondary fibrillation, gives the "peach skin feeling" to lyocell garments [1, 7].

Cellulase enzymes are well known finishing agents successfully applied to remove fuzz fibers and pills not only from lyocell fabrics, but also from all cellulosic based fabrics [1]. Cellulases can be found in multicomponent enzyme systems, with endoglucanases (EG) that randomly hydrolyze cellulose chains, cellobiohydrolases (CBH) that hydrolyze cellobiose from the polymer ends, and cellobiases that hydrolyze cellobiose to glucose. All these enzyme components act in a synergistic fashion during the degradation of cellulose. However, the slow kinetics of enzymatic degradation of crystalline cellulose improves fabric and fiber properties without excessive damage [2].

Industrial processes to remove pills include strong mechanical action together with the action of cellulase enzymes [1, 2, 4]. Those processes produce considerable amounts of cellulosic microfibrils liberated to the liquor along with soluble sugars [2]. Previous studies indicated that EG activity can produce those cellulosic microfibrils in a process with high mechanical agitation without the presence of any CBH activity [2]. In this paper, we try to clarify the depilling mechanisms by characterizing the cellulose of the enzymatically released microfibrils (identified here as insoluble powder or IP), the mechanically removed microfibrils cut from the fabric with a razor blade (identified here as pills), and the main fabric structures.

Experimental

Twill lyocell (with the commercial name of Tencel[2]) fabric with 41/24 ends/picks per cm was kindly supplied by Courtaulds, U.K. The enzymes TC (total crude mixture that contains all the *Trichoderma* cellulases) and EG rich (crude mixture that does not contain any known CBH activity) were given by Röhm Enzyme Finland. Those enzymes were previously characterized [1, 2, 3, 5].

Treatments with no mechanical agitation (identified as 0MA) were done in an incubator with the fabrics in a bath of the cellulase [50 mg total protein/g fabric with an

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[2] Use of a company or product name is for information only and does not imply approval or recommendation by the U.S. Department of Agriculture to the exclusion of others.

acetate buffer at pH 4.8 (0.2 *M* acetate)] for 120 hours (5 days) at 50°C. The control treatment was done with the buffer only on unwashed fabric.

Fabrics were treated with high mechanical agitation as received (before washing, BW) and after primary fibrillation produced by washing at 40°C during 60 minutes in a jet with high mechanical agitation and without detergent (after washing, AW), dried in a tumble dryer. Fabrics were then treated in 50 ml of the treatment solution [cellulase bath = 50 mg protein/g fabric, with an acetate buffer at pH 4.8 (0.2 *M* acetate)] in Linitest equipment for 60 minutes at 50°C; mechanical agitation was produced by eight steel disks normally used in the test for fastness to dry cleaning. After treatment, the samples were washed in sodium carbonate solution (10%) and then with hot and cold water.

Total weight loss was measured after conditioning the fabric samples at 20°C and 65% relative humidity for 24 hours before and after treatments. Insoluble weight loss was measured using a strong oxidant to digest insoluble material produced during hydrolysis. This method consisted of digesting organic matter with an oxidant (dichromate) in an acidic medium at a high temperature. Specifically, a sample of liquor was taken and centrifuged. The supernatant was removed and the residue was washed with two portions of distilled water to remove all soluble sugars, then dried at 103 ± 2°C overnight. Two ml of 1.8% of potassium dichromate in sulfuric acid (50%) reagent was added, mixed, and heated in a boiling water bath for 30 minutes. After dilution with 50 ml of distilled water, the absorbance of the solutions was read in a UV-VIS spectrophotometer against a water blank at 441 nm. Glucose was used as a standard.

Air permeability of the fabrics was measured according to ASTM Method D 737-96 "Standard Test Method for Air Permeability of Textile Fabrics."

Molecular weight distributions and the crystallinity index were measured on cellulose from the main fabric structures, from the pills cut with a razor blade from the washed fabric, and from the microfibrillar mass (insoluble powder, identified here as IP) obtained in the Linitest treatments after sedimentation and centrifugation of the baths remaining after treatments.

Molecular weight (MW) parameters of MW_w (weight-average MW), MW_n (number-average MW), and polydispersity ($M_w/M_n - P_d$) were determined by gel permeation chromatography as described before [8], with the modification of shorter heating times. The samples were heated in dimethylacetamide at 150°C for 1 hour, cooled to 100°C for addition of lithium chloride, and maintained at that temperature for 1 hour, cooled to 100°C for addition of lithium chloride, and maintained at that temperature for 1 hour, followed by heating for 1 hour at 50°C. The longer heating times required for cotton cellulose [8] were not required for lyocell cellulose. The mobile phase was 0.5% lithium chloride in dimethylacetamide; columns were Polymer Laboratories Mixed B; calibration with polystyrene standards was by means of universal calibration.

The crystallinity index of the treated fabrics and the powders was obtained by the formula $CrI = ((I_{12} + I_{21.5})/2 - I_{14})/(I_{12} + I_{21.5})/2$, where I_x is the intensity measured at x degrees adapted from Cidambareswaran *et al.* [6]. X-ray diagrams were obtained with a Philips analytical PW1710 diffractometer and an x-ray tube using Ni filtered CuKα radiation. The angular limits were 10° and 30°.

Results and Discussion

The results are shown in Tables I, II, and III, and the discussions of the results focus on the effects of the level of mechanical agitation and previous primary fibrillation.

Treatment with No Mechanical Agitation

The total crude mixture seems to be able to fully hydrolyze the lyocell substrate, while the mixture from which CBH activity is deleted seems not to solubilize the fabric more than 0.6%. We have verified a slight increase

Table I. Insoluble and total weight loss (%).

Treatment	TC	EG rich
Total weight loss, long treatment, no agitation	12.0 ± 0.2	0.6 ± 0.2
Insoluble weight loss, long treatment, no agitation	0[a]	0[a]
Total weight loss, before washing	3.0 ± 0.2	1.9 ± 0.2
Insoluble weight loss, before washing	0.14 ± 0.01	0.15 ± 0.01
Total weight loss, after washing	3.9 ± 0.2	3.8 ± 0.2
Insoluble weight loss, after washing	0.48 ± 0.01	1.27 ± 0.01

[a] The treatment liquors were clear with no turbidity.

Table II. Air permeability.[a]

Fabric sample	Air permeability, $ft^3/min/ft^2$
Control (buffer, BW)	57.2 ± 1.4
Washed control	26.4 ± 0.6
TC 0MA	68.6 ± 6.5
EG 0MA	54.2 ± 0.9
TC BW	55.9 ± 4.2
EG BW	32.5 ± 0.3
TC AW	55.5 ± 2.7
EG AW	64.2 ± 2.9

[a] See Table III for sample codes.

TABLE III. MW_n, MW_w, polydispersity, and crystallinity index.[a]

Sample	$MW_n \times 10^3$	$MW_w \times 10^3$	Polydispersity	Crystallinity index
Control (buffer, BW)	63.0 ± 0.7	123.2 ± 1.6	1.954 ± 0.009	0.19
Washed control	67.3 ± 5.5	129.3 ± 7.1	1.930 ± 0.160	0.19
TC 0MA	68.8 ± 3.9	127.2 ± 1.5	1.853 ± 0.087	0.19
EG 0MA	66.0 ± 1.4	124.7 ± 2.1	1.889 ± 0.056	0.19
TC BW	65.0 ± 2.8	127.0 ± 1.8	1.956 ± 0.062	0.19
EG BW	61.1 ± 3.9	123.0 ± 0.2	2.021 ± 0.127	0.19
TC AW	60.5 ± 4.6	126.3 ± 2.2	2.101 ± 0.160	0.19
EG AW	60.4 ± 3.0	127.0 ± 1.6	2.108 ± 0.099	0.19
Pills (raised from washed control)	60.7 ± 1.5	120.7 ± 1.1	1.989 ± 0.032	0.18
TC BW-IP	53.6 ± 6.5	151.1 ± 24.4	2.841 ± 0.491	0.19
EG BW-IP	47.5 ± 4.9	138.7 ± 10.9	2.935 ± 0.173	0.20
TC AW-IP	51.5 ± 7.3	149.4 ± 8.4	2.935 ± 0.249	0.17
EG AW-IP	36.4 ± 2.3	98.2 ± 5.6	2.704 ± 0.121	0.17

[a] TC = total cellulase crude, EG = EG enriched mixture, BW = before washing, AW = after washing, 0MA = zero level of mechanical agitation (in a shaker bath), IP = insoluble cellulose powder collected in liquor baths.

in MW and a small decrease in polydispersity for the long treatment with both cellulases. The crystallinity index is unchanged, independent of a weight loss of 12% or 0.6%.

These results confirm the stepwise fashion in which cellulose chains are removed [2]. The EGs, after cleaving all accessible glycosidic bonds at the fiber surface, stop their action. We verified this for cotton at 0 level mechanical agitation by the limited weight loss produced. The presence of CBH activity to start hydrolysis on the cleaved bonds or the extremities of the chain is essential to full hydrolysis of cellulose at 0 level mechanical agitation. In this manner, cellulose chains may be split off chain by chain, with subsequent hydrolysis into soluble sugars, resulting in no free microfibrils remaining in the fabric. With this sort of hydrolysis, it is understandable that the degree of polymerization is not affected significantly and the slight increase is due to the removal of some broken ends already present in the control sample, sharpening the distribution curve (or lowering the polydispersity) of the molecular weight. For the low levels of hydrolysis, the crystallinity of the samples is unchanged, since the cellulase enzyme changes the surface of the fibers and not the way cellulose chains are packed within the fibers. We further speculate that a 3D globular protein with a size between 40 and 60 kDa will not penetrate the high density (tightly packed) cellulose structure unless the structure is opened by mechanical means as described below.

TREATMENT WITH HIGH MECHANICAL AGITATION

The EG-enriched crude mixture produces a relatively higher weight loss in a process with higher levels of mechanical agitation, and the insoluble weight loss is always higher for the EG-enriched crude mixture. There are no significant changes in MW parameters, only a slight increase in polydispersity of the treated samples. This has also been verified before for cotton cellulase treated with a total cellulase mixture [8]. However, the insoluble powders collected in solution show a significant decrease in MW_n and a significant increase in polydispersity. Air permeability, used here as a measure of fibrillation, decreases significantly for the washed control, demonstrating the well-known washing-induced fibrillation, and decreases significantly with the cellulase treatments only in the case of treatment of unwashed lyocell with the EG-enriched crude mixture. The crystallinity index does not show any significant changes for the fabrics and insoluble powders.

These results indicate that EG activity cooperates with mechanical agitation, producing microfibrillar material that can be released into the bath or stay attached in the fibers. Evidence for that had been shown previously [4] and also here by the extreme increase or decrease of air permeability on the EG-treated fabrics depending on the washing step prior to primary fibrillation. Apparently the MW distribution on the main structure of the fabric remains unchanged for the lower levels of weight loss produced. Higher levels of weigh loss probably will not decrease the MW, but will destroy the fabric structure, producing a microfibrillar mass, as we verified previously [2]. Since adsorption of the enzymes onto the insoluble cellulose powders is about six to seven times higher than onto the cellulose of the main fabric structure [2], those powder particles are hydrolyzed faster, as shown by the considerably increased polydispersity of those samples. Despite that, the crystallinity index of those powders is not significantly changed. It seems that cellulases and mechanical agitation will not change the crystallinity of cellulose, since the changes are just at the fiber surface and not within their structure.

EFFECTS OF WASHING-INDUCED PRIMARY FIBRILLATION

The washing-induced primary fibrillation increases the amount of insoluble weight loss, especially for the EG-enriched crude mixture. This seems to indicate that EG activity is best at producing good depilling effects. However, this will be the case only in fabrics that are already highly pilled. Fabrics with no pilling at all (like the control fabric) can be fibrillated in a short treatment time (see decreased air permeability of fabric treated with an EG-enriched mixture before washing). We verified fibrillation with EG-rich activities previously for cotton fabrics [4].

Pills produced by primary fibrillation and then cut from the washed fabric with the razor blade do not show significant differences in the crystallinity index, MW, or polydispersity when compared with the cellulose of the main fabric structure of the washed fabric or the control (buffer only, unwashed) fabric. Previously we suggested that mechanical agitation will drive the action of cellulase enzymes toward the pills instead of toward the cellulose of the main fabric structure. This indeed seems to be the case, since previous fibrillation yields higher amounts of insoluble weight loss. The reason enzymes are driven toward the pills is not because the pills are more accessible structurally (*i.e.,* they have the same crystallinity index as the cellulose of the main fabric structure), but because they are more exposed at the fabric surface than the main fabric structure.

The results also suggest that cellulosic material is only effectively degraded (understood as an effective reduction of the size of cellulose chain) after removal from the main fabric structure. This indicates (by a mechanism still unclear to us) that mechanical agitation will remove the more exposed pills after a slight enzymatic weakening (hydrolysis) that is not really detectable as a reduction of MW or increase of polydispersity.

Conclusions

Both the removal of microfibrils and their formation can be induced by cellulase enzymes, in particular by EG activities. We have shown this in our work and also in previous papers [2, 4]. The fact that the same enzyme activities can produce those apparently opposite phenomena might be the cause of some confusion in understanding the mechanisms of cellulase action in the context of a process with high mechanical agitation.

The results of our work clearly show that for short treatment times, EG activities will attack what is more exposed, *i.e.,* the pills or raised microfibrils at the fabric surface if they exist. It seems that only after weakening with a joint enzymatic action and mechanical agitation are those pills removed. The removed microfibrillar material has a wider distribution of MW (higher polydispersity) than the original fabric structure. However the original fabric structure has a polydispersity similar to the control fabric, suggesting that effective degradation of cellulose material will only happen after the cellulose is out of the fabric structure. If the fabric doesn't have any pills or raised microfibrils [2, 4], they will be formed (in short treatment times) and removed afterward (if the treatment is long enough).

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