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Possibilities for Recycling Cellulases After Use in Cotton Processing

Part I: Effects of End-Product Inhibition, Thermal and Mechanical Deactivation, and Cellulase Depletion by Adsorption

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Abstract

Preliminary recycling experiments with cellulase enzymes after cotton treatments at 50°C showed that activity remaining in the treatment liquors was reduced by about 80% after five recycling steps. The potential problems of end-product inhibition, thermal and mechanical deactivation, and the loss of some components of the cellulase complex by preferential and or irreversible adsorption to cotton substrates were studied. End-product inhibition studies showed that the build-up of cellobiose and glucose would be expected to cause no more than 40% activity loss after five textile treatment cycles. Thermal and mechanical treatments of cellulases suggested that the enzymes start to be deactivated at 60°C and agitation levels similar to those used in textile processing did not cause significant enzyme deactivation. Analysis of cellulase solutions, by fast protein liquid chromatography, before and after adsorption on cotton fabrics, suggested that the cellobiohydrolase II (Cel6A) content of the cellulase complex was reduced, relative to the other components, by preferential adsorption. This would lead to a marked reduction in activity after several treatment cycles and top-up with pure cellobiohydrolase II would be necessary unless this component is easily recoverable from the treated fabric.

Index Entries: Cellulases; textile processing; enzyme recycling; adsorption; depletion; inhibition; deactivation.

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Introduction

Cellulases are a group of enzymes capable of catalyzing the hydrolysis of cellulose. Natural cellulolytic systems are composed of three major classes, which act synergistically. Endoglucanases (EGs, EC 3.2.1.4) cause random hydrolytic chain scission at the most accessible points of the cellulose chain, creating new chain ends on the cellulose surface for exoglucanase attack. Exoglucanases or cellobiohydrolases (CBHs, EC 3.2.1.91) attack chain ends in a stepwise fashion, releasing cellobiose. Finally, β-glucosidase (EC 3.2.1.21) or cellobiase hydrolyses cellobiose to glucose. The cellulase system of the filamentous fungus *Trichoderma reesei* has been found to be one of the most effective for the hydrolysis of crystalline cellulosic materials *(1)* and has been used extensively in textile processing *(2)*. It produces two cellobiohydrolases (CBHI and CBHII) at least four endoglucanases (EGI, EGII, EGIII, and EGV) and one β-glucosidase *(3,4)*. A new system of nomenclature for cellulases has been suggested *(5)* in which CBHI becomes Cel7A, CBHII→Cel6A, EGI→Cel7B, EGII→Cel5A, EGIII→Cel12A, and EGV→Cel45A, but for greater clarity, when referring to the enzyme suppliers specifications and to earlier publications in the literature, the more familiar nomenclature has been retained in the present paper.

In recent years the use of cellulases in the textile finishing and household detergent industries has been increasing, and these industries now represent the largest markets for these enzymes *(6)*. Since individual cellulases are highly specific in the ways which they hydrolyse crystalline cellulose, they can be used to create a variety of finishing effects on cellulosic textiles, such as enhancement of fabric surface appearance by removing fuzz fibres and pills and delivering softness benefits. Furthermore, enzymatic treatments of textile materials often constitute a more ecological alternative to traditional and more polluting chemical processes.

More recently, much attention has been given to using genetic engineering techniques to provide cellulase compositions tailored to give better-controlled processes. For example, for depilling cotton fabrics, preparations of EGI and EGII have been developed *(7)*.

The textile industry is extremely competitive and cost conscious, and, since enzymes are often regarded as expensive reagents, there continues to be some resistance to their more widespread use. Enzymes, however, are not consumed in the reactions which they catalyze; therefore, they are potentially recyclable. Recycling can significantly reduce on-costs associated with enzymatic processing *(8)*, but surprisingly no attempts to recycle cellulases after use in textile processing have been found reported in the literature. A study of the possibilities in this area therefore seems to be novel and timely.

The recycling of cellulases has been studied in the context of other industrial applications *(9–12)* in order to reduce costs and make their use more economically attractive. Potential barriers to cellulase recycling such

Table 1
Some Properties of the Cellulolytic System Produced by *T. reesei (26,27)*

Enzyme	Percentage in the mixture	Isoelectric point (p*I*)
CBHI	60	3.5–4.2
CBHII	20	5.1–6.3
EGI	10	4.6
EGII	1	5.5
EGIII	Not determined	7.4
EGV	Not determined	2.8–3.0
β-glucosidase	Not determined	8.7

as end-product inhibition *(13–17)*, thermal and mechanical deactivation *(18–23)*, and depletion of specific cellulase components by adsorption with the subsequent loss of synergism *(24)* have been widely reported. In the present work the same problems were studied in the context of recycling after cotton processing. Changes in the composition of cellulase complexes, caused by differences in the reversibility of binding of individual components to the textile substrate, could be particularly serious in textile finishing where small but reproducible changes in the physical properties of the substrate surface are required *(25)*. Consequently, this aspect of recycling has been given careful attention. In further work (Part II of this series) the separation, by ultrafiltration, of cellulases from reaction products and dyes released by cotton fabrics has also been investigated.

Materials and Methods

Substrates

The textile substrate used was scoured and bleached 100% cotton poplin fabric having 60/32 ends/picks cm^{-1} and area density of 100 g m^{-2}.

Carboxymethylcellulose (CMC, sodium salt, degree of substitution less than 0.4) from BDH Chemicals, Ltd. (Poole, England) and filter paper No 1 (FP, Whatman, Maidstone, England) were used as substrates for the measurement of endoglucanase and total cellulase activities, respectively.

Enzymes

The enzyme mixtures used in this study were *Trichoderma reesei* cellulases supplied by Röhm Enzyme Finland Oy (Rajamäki, Finland). Three non-commercial preparations were used: Total Crude (TC) with all the cellulolytic components present, and two genetically engineered preparations; endoglucanase enriched (EG-rich) with CBHI and CBHII activities deleted and cellobiohydrolase enriched (CBH-rich) with EGI and EGII activities deleted. Some details of the TC preparation are represented in Table 1. The main cellulase components claimed to be present in the

EG-rich composition were EGI (50%) and EGII (50%) and in the CBH-rich composition the main components claimed to be present were CBHI (75%) and CBHII (25%). These composition details were provided by the supplier.

Cellulase Inhibition by End-Products

Cellulase (TC) solutions (3.8 mg protein/g fabric) were incubated (50°C at pH 5.0 in 0.1 *M* acetate buffer for 1 h) with cotton fabrics in presence of increasing concentrations of cellobiose and glucose. After the enzymatic treatments, the residual enzymes on the fabric were inactivated by immersing the treated fabrics in sodium carbonate (5%) and further rinsing in boiling and cold distilled water. The fabrics were dried and weighed for weight loss determination (see *Analytical Methods*). Cellulase activity was measured in terms of fabric weight loss and was expressed in terms of percentage relative to the activity obtained in the absence of sugars. Samples of the reaction mixtures, before and after the enzymatic treatments, were taken to measure total protein in solution and calculation of protein adsorption by difference.

Thermal and Mechanical Deactivation of Cellulases and Conformational Changes in Protein Structure Caused by Heating

Cellulase (TC) solutions (≈ 0.8 g protein/L in acetate buffer 0.1 *M* pH 5.0, without substrate) were subjected to different thermal and agitation treatments given in the legend of the Fig. 3. After these treatments, CMC and FP activities of the treated enzymes were measured.

In addition, the conformational changes in protein structure caused by heating these solutions were followed by fluorescence emission spectroscopy. The fluorescence emissions of the cellulase solutions (TC in 0.1 *M* acetate buffer) were determined using a FLUOROG-2 fluorescence spectrometer (F 2127, Spex Industries, Edison, NJ), equipped with a magnetic stirrer and water-jacketed sample cell holder. The temperature was varied by using an external circulating bath. The excitation monochromator was set at 285 nm (excitation wavelength of tryptophan) and the fluorescence emission was followed from 300 to 450 nm. Maximum emission wavelengths were recorded for each sample over the temperature range 20–80°C.

Adsorption and Hydrolysis Experiments on Cotton Fabrics

The adsorption and hydrolysis treatments were performed in a Rotawash machine for 1 h at 50°C and 40 rpm. Samples of cotton fabric (7 g) were placed in the stainless-steel pots (500 mL) with 100 mL of a cellulase solution (TC, EG-rich, or CBH-rich) in sodium acetate buffer (0.1 *M*, pH 5.0) to give a protein concentration of 9 mg/g of cotton. Liquor samples were taken to measure the total protein present in solution and for protein analysis by fast protein liquid chromatography (FPLC). The treatment liquors (hydrolysates with the fabric removed) were centrifuged (room tempera-

ture, 10 min, 2875*g*) to remove any traces of insoluble material (cotton debris). The supernatant was analyzed for total protein and total soluble reducing sugars. Fabric weight loss was also determined as described in *Analytical Methods*. The protein adsorbed by the fiber was determined as the difference between initial protein concentration and the concentration of the protein remaining in the supernatant. The supernatants were then ultrafiltered using a Prep/Scale™ TFF cartridge containing a 0.09 m^2 polysulfone ultrafiltration membrane with a molecular weight cut off (MWCO) of 10 kDa (Millipore Corporation, Bedford, MA) to remove the soluble reducing sugars. The ultrafiltration membrane was washed with acetate buffer to recover protein that remained inside the ultrafilter (avoiding loss of protein in the membrane) and this washing liquor was combined with previous concentrate. FPLC analysis of this liquor was performed to determine the change in composition of the cellulase complex remaining in solution after cotton treatment.

Analysis of Cellulase Components by Fast Chromatofocusing (FPLC)

Analysis of the cellulase components was carried out using a chromatofocusing system. Column (Mono P HR 5/20, Amersham Pharmacia Biotech, Uppsala, Sweden) equilibration was achieved by running with the start buffer [25 m*M* Bis-Tris adjusted to pH 6.9 with hydrochloric acid (2 *M*) supplemented with 10% of betaine monohydrate] until the column effluent was at the same pH as the start buffer. Cellulase samples diluted in start buffer were filtered through a small syringe filter (Millex-GV13, diameter 13 mm, sterile, low protein binding, polyvinylidene difluoride filter membrane, pore size 0.22 µm, Millipore, Bedford, MA) before being applied to the column. The sample was injected by first running 5 mL of eluent to avoid protein exposure to extremes of pH. In some cases, 0.5–4 mL centrifugal ultrafiltration units (Ultrafree-4, Millipore Corporation, Bedford, MA) with 5000 MWCO, polyethersulfone membranes, were used to concentrate the samples before injection. The elution was then carried out at 1 mL/min with 37 mL of 1:10 diluted Polybuffer 74 (Amersham Pharmacia Biotech, Uppsala, Sweden) adjusted to pH 4.0 with hydrochloric acid (2 *M*) supplemented with 10% of betaine monohydrate, followed by a further 43 mL of 1:10 diluted Polybuffer 74 adjusted to pH 3.0 with hydrochloric acid (2 *M*) also supplemented with 10% of betaine monohydrate. All eluents and cleaning solutions were continuously vacuum degassed before entering the column. The protein detection was monitored using a UV detector (Welchrom K-2500, Knauer, Berlin, Germany) set to 280 nm. The pH of the column effluent was also monitored and recorded to check the formation of the pH gradient and to observe the elution pH of protein fractions.

Determination of Cellulase Activity

Cellulase activity toward cotton fabrics was measured in terms of fabric weight loss and production of soluble reducing sugars after enzy-

matic treatments. These parameters were determined as described in the analytical methods.

Activities toward CMC and FP were measured by incubating the cellulase samples with the substrates at 50°C and pH 5.0 during 30 and 60 min, respectively. The soluble reducing sugars produced during the reaction were determined as described below.

Analytical Methods

Total protein in solution was measured by the Bradford assay *(28)* with BSA as the protein standard. Soluble reducing sugars were determined using the neocuproine method described by Cavaco-Paulo et al. *(29)* and glucose was used as standard sugar. Fabric weight loss was determined by difference in the weights of fabric samples before and after enzymatic treatments and always after conditioning for 24 h at 20°C and 65% relative humidity. In each experiment a control fabric, subjected to the same conditions but without cellulase, was included.

Results and Discussion

After enzymatic treatments of cotton with cellulases, the enzymes are distributed between the liquor and the substrate. Thus cellulases may be recoverable from either phase.

Preliminary experiments were carried out in which the treatment liquor from cotton fabric hydrolyses (carried out as described above) was recycled for five consecutive treatments without any liquor volume adjustment, addition of fresh enzyme, or removal of reaction products. The relative amounts of recyclable enzyme present in the liquor and adsorbed to the substrate were determined. The cellulases remaining in the treatment liquor after five treatment cycles were still active, but the adsorbable fraction decreased and its activity toward cotton fabric (measured as weight loss) was reduced by about 80% over the five cycles (Table 2). It is suggested that this apparent activity loss could be due to a combination of end-product inhibition, thermal and/or mechanical deactivation, and to loss of some components of the cellulase complex by preferential and irreversible adsorption to cotton. Each of these factors was studied separately as described in the following sections.

Cellulase Inhibition by End-Products

The cellulase inhibition effects of glucose and cellobiose are represented in Figs. 1 and 2, and it is clear that both sugars caused reduced adsorption onto the cotton substrate and inhibited the enzymatic activity. Cellobiose shows a stronger inhibition effect than glucose. With 5 g/L of cellobiose present in the initial hydrolysis reaction, adsorption was reduced by about 29% and activity toward cotton was reduced by about 25% (Fig. 1). With 20 g/L of cellobiose, both adsorption and cellulase activity were reduced by about 45%. The effect of glucose was less severe, and even in the

Table 2
Percentage of Protein Adsorbed
(Relative to the Protein Present at the Beginning of Each Step),
Relative Activity in Terms of Fabric Weight Loss,
and Concentration of Reducing Sugars in the Supernatants
After One Enzymatic Treatment and After Four Recycling Steps

Step	Protein adsorbed (%)	Relative activity (%)	[Reducing sugars] (g/L)
1	35	100	1.64
5	4	19	8.11

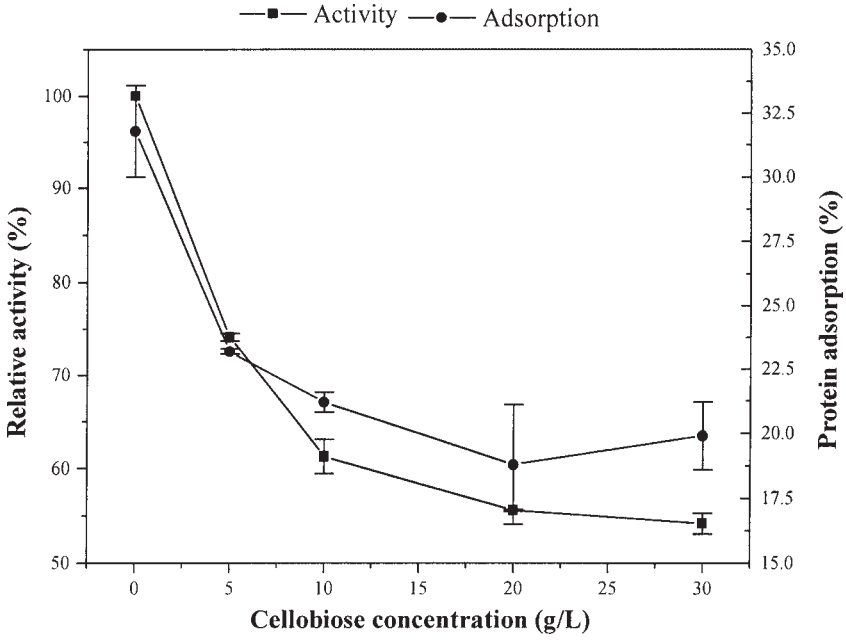

Fig. 1. Effect of cellobiose concentration on cellulase (TC) activity and adsorption (50°C, pH 5.0, 3.8 mg protein/g fabric). The values in the figure represent the mean of two in independent experiments and error bars the standard deviation.

presence of 30 g/L of glucose the total cellulase still retained 78% of its initial activity although adsorption was reduced by 29%. The effect of the products of cellulose hydrolysis on cellulase activity has been analyzed by other workers *(14,15)* and they found comparable results. The inhibitory effect caused by the reaction products (soluble oligosaccharides, cellobiose, and glucose) can be explained by the fact that these sugars can bind to EGs and CBHs and then inhibit their adsorption to the fabric. Such effects have been noted by Lee and Fan *(15)*.

Since cellobiose is the stronger inhibitor, it is important to control its concentration in recycled reaction mixtures. β-Glucosidase is the cellulase

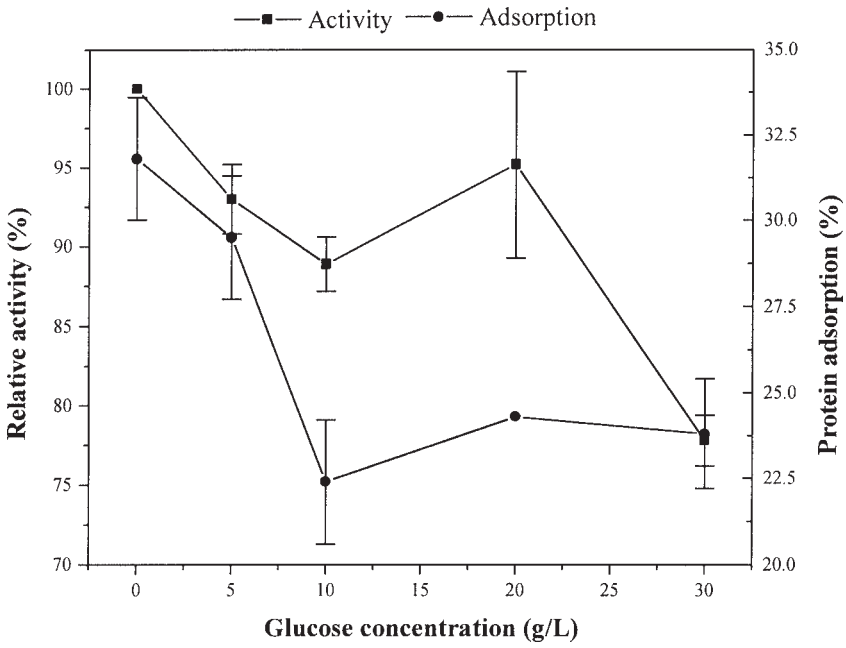

Fig. 2. Effect of glucose concentration on cellulase (TC) activity and adsorption (50°C, pH 5.0, 3.8 mg protein/g fabric). The values in the figure represent the mean of two independent experiments and error bars the standard deviation.

component responsible for cellobiose degradation, but it is known that cellulases from *T. reesei* are relatively deficient in β-glucosidase activity *(24,30)*. Measurement of the β-glucosidase activity present in the TC mixture gave 0.76 U/mg total protein (at 50°C and pH 5.0 using cellobiose as substrate). This value is very low when compared with activities of isolated β-glucosidases from other sources. For example Dekker *(30)* and Calsavara et al. *(16)* found activities of 251 U/mL (50°C, pH 5.0) and 17.8 U/mg protein (65°C, pH 4.5), respectively, for β-glucosidase from *Aspergillus niger*. It is therefore suggested that when cellulases from *T. reesei* are recycled, they should be supplemented with exogenous cellobiase activity to increase the degradation of cellobiose produced during the enzymatic treatments thereby reducing its inhibitory effect. Alternatively, the separation of products, by ultrafiltration or selective adsorption could be considered (see Part II of this series).

Typical cellulase treatments on cotton fabrics produce an amount of soluble reducing sugars equivalent to 1–2 g/L of glucose. In the present work, five treatment cycles produced a concentration of reducing sugars (0.044 *M*) equivalent to about 8 g/L of glucose (Table 2) but even if all the reducing sugars were cellobiose, the inhibitory effect (0.044 *M* equivalent to about 15.2 g/L of cellobiose) should not have reduced activity by more than about 40%, and other factors were therefore likely to be involved in the 80% activity loss that was recorded.

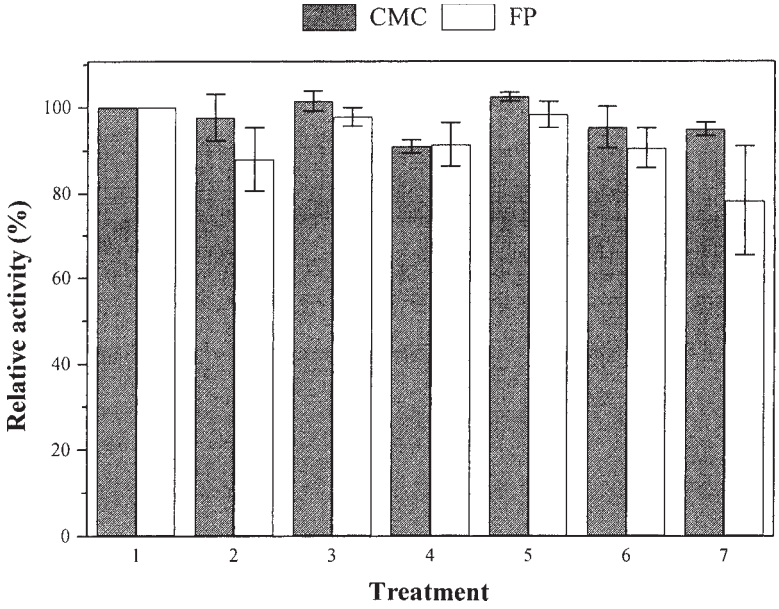

Fig. 3. Effect of thermal and mechanical treatments on CMC and FP activities of a total cellulase (TC). **1**, untreated; **2**, rotawash, 22°C, 40 rpm, 1 h; **3**, rotawash, 22°C, 5 discs, 40 rpm, 1 h; **4**, rotawash, 50°C, 1 h; **5**, rotawash, 50°C, 40 rpm, 1 h; **6**, rotawash, 50°C, 5 discs, 40 rpm, 1 h; **7**, rotawash, 60°C, 40 rpm, 1 h. The values in the figure represent the mean of two independent experiments and error bars the standard deviation.

Thermal and Mechanical Deactivation of Cellulases and Conformational Changes in Protein Structure Caused by Heating

The results given in Fig. 3 show that agitation levels typical of textile processing do not cause significant mechanical deactivation. One-hour treatments at 50°C did not cause significant deactivation but treatments at 60°C did cause partial deactivation, which was more apparent for FP activity. This suggests that CBHs are less thermally stable than EGs.

In previous work *(31)* it was found that cellulase (TC) solutions lost 20% of their activity after 5 h treatment at 50°C (equivalent to five treatment cycles). It may therefore be expected that some thermal deactivation of cellulases will occur on recycling at 50°C and addition of stabilizers may be advisable. It should be noted, however, that in the present work the thermal treatments were made in the absence of the substrate and it is known that in some cases substrates may act as enzyme stabilizers *(32)*, but this was not, however, observed specifically for cellulase enzymes.

Proteins contain aromatic amino acids such as tryptophan, tyrosine, and phenylalanine, which exhibit fluorescence. Fluorescence is sensitive to the environment of the fluorescer and both the wavelength of maximum emission (λ_{max}) and the fluorescence intensity may be affected. Usually a

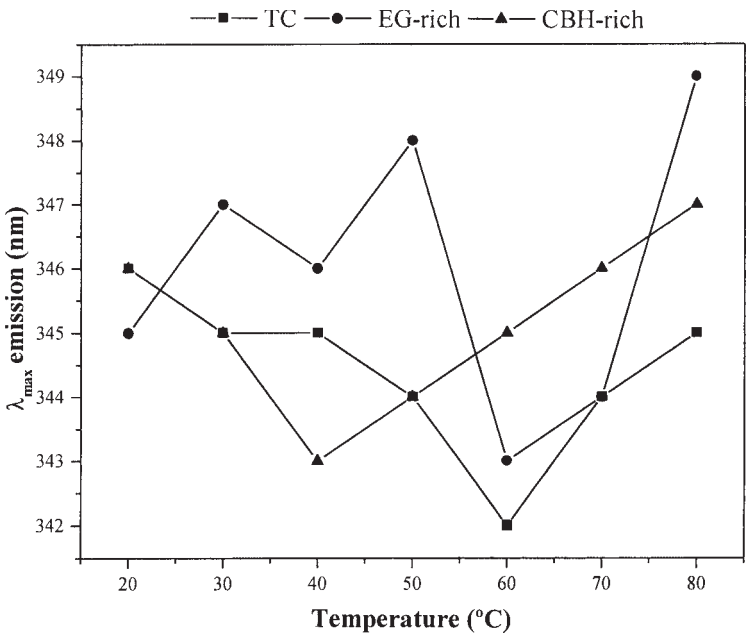

Fig. 4. Temperature dependence of the wavelength of maximum fluorescence emission for the three cellulase preparations in 0.1 *M* acetate buffer (pH 5.0).

shift in λ_{max} indicates a change in polarity of the environment of the fluorescer. In general, the fluorescence intensity increases as the polarity of the environment decreases. Fluorescence spectroscopy has been used widely in studies of protein denaturation, since it reflects the properties of the immediate environment of protein chromophores and, consequently, provides information about protein structure *(33)*. The effect of temperature on cellulase protein conformation was monitored using this technique. It can be seen from Fig. 4 that the three cellulase crudes showed different behaviors over the temperature range studied. CBH-rich showed a shift in λ_{max} to shorter wavelength between 20 and 40°C and then a progressive shift to longer wavelength between 40 and 80°C. EG-rich, in contrast, showed an initial shift in λ_{max} to longer wavelength between 20 and 50°C followed by a very sharp shift to shorter wavelength at 60°C and then a marked shift to longer wavelength between 60 and 80°C. TC being a mixture of these EGs and CBHs showed intermediate behavior with a minimum in λ_{max} at 60°C reflecting the EG behavior more than that of the CBHs. When considered together with the effects of temperature shown in Fig. 3, it is tempting to suggest that the reversals in direction of the shift in λ_{max} represent the onset of conformation changes associated with deactivation. These would imply that the EG-rich crude was more thermally stable by 10–20°C than the CBH-rich crude, and this would be consistent with the findings of Baker et al. *(23)* who found that the EGII of *T. reesei* is more thermally stable (by about 10°C) than the other main components. It should be noted that a clearer picture

Table 3
Characterization of Cellulase Preparations
in Terms of Protein Adsorption and Activity
(Weight Loss and Production of Reducing Sugars) on Cotton Fabrics[a]

Cellulase preparation	Protein adsorption (%)	Weight loss (%)	[Reducing sugars] (g/L)
TC	25.8 (3.1)	4.45 (0.58)	1.60
EG-rich	11.9 (0.6)	1.38 (0.33)	1.35 (0.03)
CBH-rich	18.7 (0.8)	3.02 (0.26)	1.69 (0.06)

[a]Rotawash machine, 50°C, 40 rpm, 1 h, pH 5.0, 9 mg protein/g fabric, 1:14 liquor ratio. Values in brackets represent the standard deviation of two independent experiments.

of the effects of temperature on fluorescence would be obtained for pure enzyme preparations which could be obtained using the FPLC system if a preparative FPLC column was used.

Analysis of Cellulase Components by FPLC Before and After Adsorption in Hydrolysis Experiments on Cotton Fabrics

In Table 3, results of cellulase adsorption and activity on cotton fabrics are given for the different cellulase compositions. With respect to the adsorption, it seems that CBHs adsorbed more (18.7%) on cotton than EGs (11.9%) and, when both EGs and CBHs were present, the percentage adsorption (from the same total concentration) increased to 25.8%, probably reflecting different site specificities for EG and CBH adsorption as well as their relative proportions in TC. The weight loss results (Table 3) were consistent with known EG (endo) and CBH (exo) type activities and with the expected synergy between them in the TC preparation. The relative concentrations of soluble reducing sugars (expressed as g/L glucose) produced by the three cellulase preparations cannot simply be related to weight loss. They may be expected to reflect different distributions of glucose, cellobiose, and soluble oligomers as well the different specific activities of the components of the three preparations.

The analysis of cellulase compositions before and after enzymatic treatments of cotton fabrics was made by using chromatofocusing. In this technique, the elution of the proteins is in order of decreasing isoelectric points. The identification of the cellulase components on the chromatogram (Fig. 5) was made considering their p*I*s, their percentage in the preparation (Table 1), and the activities on CMC and FP of the fractions collected. This identification is represented in the pH–chromatographic profile shown in Fig. 6. CBHI shows slightly different eluting pHs in the TC and CBH-rich preparations but this is likely to be due only to errors associated with pH measurement (pH meter). Analysis of the chromatographic profile of the EG-rich preparation suggests that CBHII is also present. Since the CBH activities were deleted in this cellulase composition, the peak may be due to another EG (EGIII has a pI of 7.4, Table 1) or to a contaminant protein.

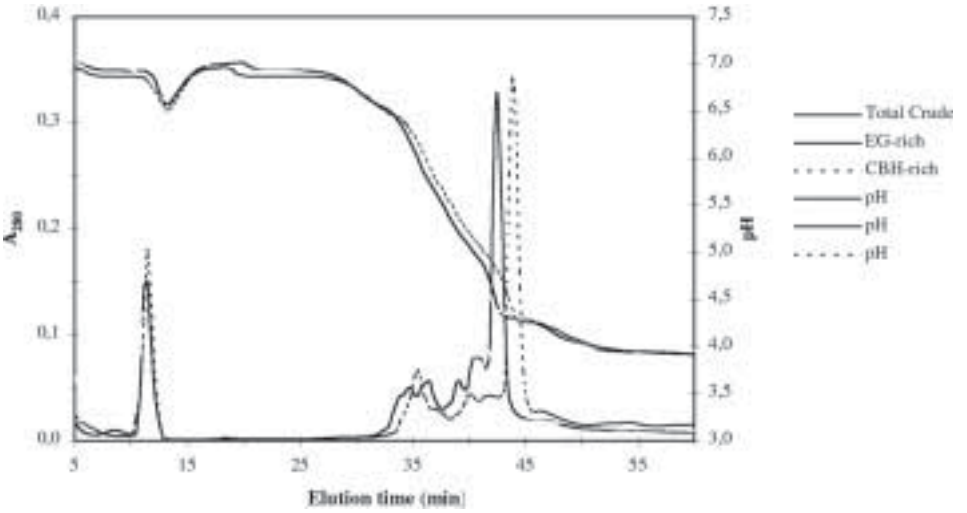

Fig. 5. Simultaneous comparison of the chromatograms of the three cellulase preparations with their elution pH (see chromatographic conditions in *Materials and Methods*).

The cellulase samples were analyzed before and after the enzymatic treatments of cotton fabrics, and the peak area for each component was calculated. Adsorption of cellulase components was expressed as the percentage difference between their peak area in the original cellulase preparation and those in the supernatant after adsorption.

The analysis of the chromatographic profiles of the CBH-rich preparation before and after adsorption (data not presented) showed some depletion (about 40%) of the peak corresponding to CBHII. For the EG-rich preparation there appeared to be some depletion of EGII in the mixture after adsorption on cotton fabrics. These behaviors were also observed for the TC mixture, but since the relative amounts of the EGs in TC were much less than in the engineered preparations, the depletion of EGII from TC was not so noticeable. The depletion of CBHII may mean that it is adsorbed preferentially, or that it is adsorbed more irreversibly to the substrate than CBHI. The latter would be in agreement with the results reported by Linder and Teeri *(34)* and by Carrard and Linder *(35)*, who showed that the adsorption of the isolated CBD (cellulose binding domain) of CBHI was completely reversible, whereas the CBD of CBHII could not be desorbed from the substrate by buffer dilution. It is therefore concluded that significant deactivation of recycled TC occurs because of CBHII depletion through irreversible adsorption, but in further work the composition of components desorbed from the cotton substrate should be analyzed to confirm or deny that the strongly adsorbed fraction is in fact rich in CBHII.

To avoid the problem of TC deactivation through irreversible adsorption of specific components, it is suggested that cellulase compositions for specific fabric finishing effects might be designed to contain only compo-

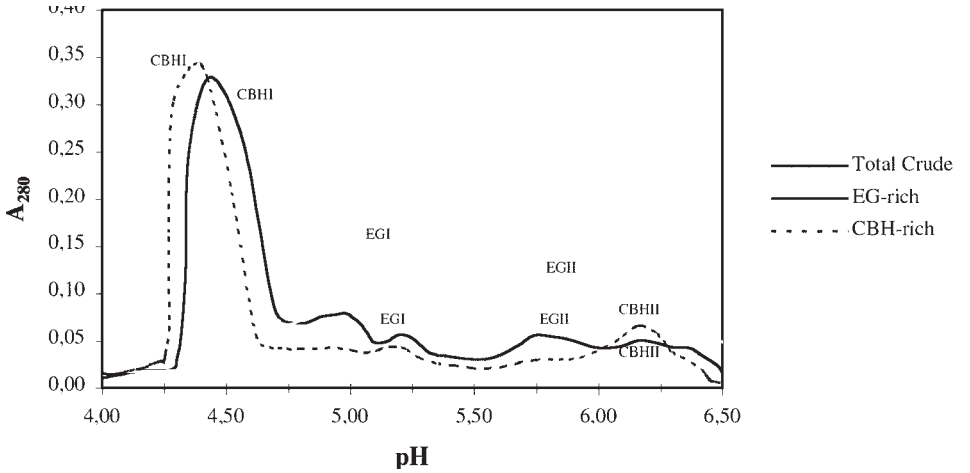

Fig. 6. pH profile of the three cellulase preparations and identification of the main components in the mixtures.

nents that are adsorbed reversibly. In some cases the isolated catalytic domains of cellulases might be used since it is believed to be the CBD that is responsible for the irreversible adsorption of some cellulase components. Although CBHI requires its CBD for efficient adsorption and hydrolysis of insoluble cellulose, its adsorption is completely reversible. On the other hand, EGII adsorbs to insoluble cellulose with or without its CBD *(36)*. It has also been demonstrated, with purified endoglucanases and with their isolated catalytic domains, that the presence of CBDs is not essential for activity in cotton in textile processes where high levels of mechanical agitation are applied *(37)*. In this work it was also shown that the adsorption of the core domains was completely reversible to cotton fabrics.

Conclusions

There was a 40% loss of cellulase activity toward a cotton substrate in the presence of 15 g/L of cellobiose, which is still greater than the possible cellobiose concentration after five typical fabric treatments.

Measurement of thermal and mechanical deactivation showed that cellulases start to be deactivated at about 60°C and that EGs are more thermostable than CBHs. The agitation levels used (typical of textile processing) did not cause significant cellulase deactivation effects.

The study of adsorption of individual cellulase components by FPLC showed that there was depletion of CBHII in the supernatant after cotton treatment. This may indicate that CBHII is irreversibly adsorbed to cotton. EGII also showed a similar behavior, but this could only be detected in the EG-rich preparation. These results suggest that there is a need to supplement the recycled liquor with selected pure cellulase components in order to maintain a constant composition and thereby to deliver, reproducibly, the desired effects to treated fabrics.

It can be concluded that cellulase activity losses during recycling result from end-product inhibition, thermal deactivation, and preferential and/or irreversible adsorption of CBHII. The loss of CBHII from the total cellulase complex appears to be the most serious of these effects with regard to maintaining reproducible fabric treatments. The design of cellulase preparations for textile processing that contain only components that are adsorbed reversibly is therefore suggested. These preparations would be more suitable for recycling and this could lead to significant cost savings.

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