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0273-2289/02/101/0077/$13.75

Possibilities for Recycling Cellulases After Use in Cotton Processing

Part II: Separation of Cellulases from Reaction Products and Released Dyestuffs by Ultrafiltration

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Received June 2001; Revised October 2001; Accepted November 2001

Abstract

The adsorption and activity of a total cellulase (*Trichoderma reesei*) was measured and compared on undyed and dyed cotton fabrics. Recovery of enzymes from the reaction mixture and by desorption from the cotton substrate was evaluated. About 80% of the initial protein could be recovered. The removal of released products (soluble reducing sugars and dyes) from the treatment liquor and subsequent concentration of cellulase proteins was performed using an ultrafiltration membrane. Strong protein-dye interactions made it impossible to separate efficiently the dyes from the enzyme-containing treatment liquors. The use of surfactants did not enhance cellulase desorption from cotton fabric. Although anionic surfactants have a deactivating effect on cellulases, this effect seems to be reversible, since after ultrafiltration the cellulase activity was similar to that of enzymes desorbed with buffer only. *Humicola insolens* cellulases were shown to be much more sensitive to anionic surfactant than *T. reesei* cellulases. The use of cellulases that bind reversibly to cellulose is suggested for achieving more efficient cellulase recycling and for reducing backstaining by dye-cellulase complexes.

Index Entries: Cellulases; textile processing; dyes; surfactants; recovery; desorption; ultrafiltration; tailoring.

Introduction

When cellulases are used in textile processing they are not consumed or destroyed and are therefore potentially available for recycling. In Part I

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of this series *(1)*, deterioration in the activity of recycled cellulases was examined regarding: the inhibitory effect of reaction products such as glucose and cellobiose, the deactivation effects of heat and mechanical agitation, and the irreversible adsorption of specific cellulase components on cotton cellulose substrates.

In the present article, the separation of cellulases from reaction products and substrates is considered with a view to making more cost-effective use of the recycled enzymes.

Since cellulase finishing is very often carried out on dyed textiles, dye molecules are released into solution during processing, and these are likely to cause cross-staining of other substrates if the liquor is recycled *(2,3)*. Consequently, the present work includes cellulolytic hydrolyses of substrates dyed with dyes representing two important application classes for cellulosic substrates (vat and reactive). An attempt was made to separate released dye from the cellulases in recycled treatment liquors.

Various methods have been used to recover cellulases from treatment liquors or substrates in nontextile applications. Some investigators *(4–7)* have studied the possibilities for recycling cellulases by using these enzymes in repeated cycles. A decrease in cellulase activity with increasing numbers of recycling steps was always observed. The accumulation of hydrolysis products in the recycled liquors was found to be a problem because the end products cause cellulase inhibition *(1,6)*. It is therefore necessary to provide a method for separating cellulases from end products to improve the activity of the recycled enzymes. The use of ultrafiltration techniques, for the removal of sugars produced during cellulose hydrolysis and for recovery and concentration of cellulases, has been suggested by several groups of researchers *(8–10)*. Other techniques that have been proposed for the separation of cellulases from end products include carrying out the hydrolysis in a two-phase system *(11)* and selective adsorption of cellulases from the liquor by using various types of adsorbents *(12–14)*.

Other aspects of enzyme recycling such as improving the desorption of cellulases from the substrate have been investigated in nontextile applications *(15,16)*. Otter et al. *(15)* found that the best cellulase activity recovery was achieved by using alkali and surfactant to elute the adsorbed enzymes.

For the present work, an ultrafiltration system was chosen to concentrate cellulases and separate them from cotton fabric reaction products (soluble reducing sugars and dyes). Reaction medium buffer and anionic and nonionic surfactant solutions were used in an attempt to increase desorption of the enzymes from the cotton substrate.

Materials and Methods

Substrate

The substrate was commercially scoured and bleached 100% cotton poplin fabric having 60/32 ends/picks/cm and an area density of 100 g/m^2.

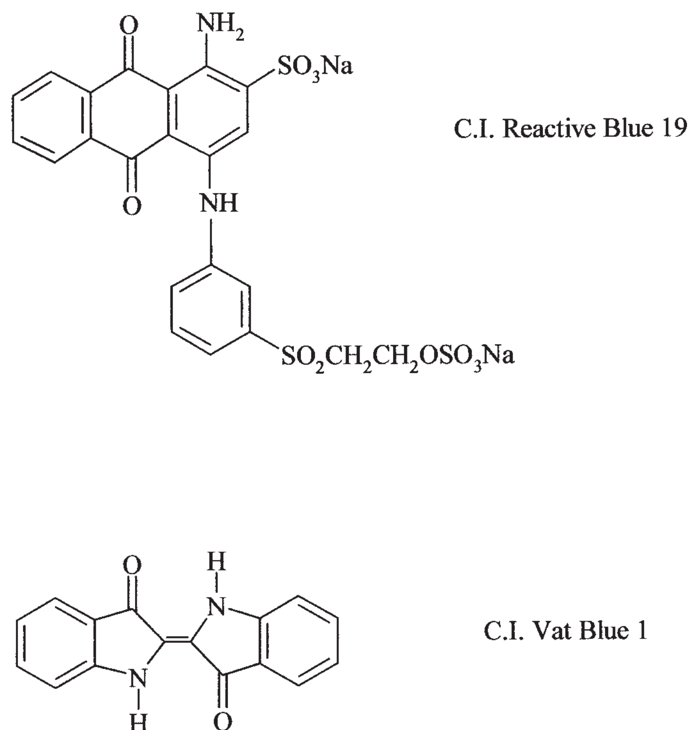

Fig. 1. Chemical structures of Remazol Brilliant Blue R (C.I. Reactive Blue 19) and indigo (C.I. Vat Blue 1).

The cotton fabrics were used without any further treatment, or they were dyed with Remazol Brilliant Blue R (C.I. Reactive Blue 19) or indigo (C.I. Vat Blue 1) (see chemical structures in Fig. 1) following the procedures recommended by the suppliers.

Carboxymethylcellulose ([CMC], sodium salt, degree of substitution <0.4) from BDH (Poole, England) and filter paper no. 1 (FP, Whatman, Maidstone, England) were used as substrates for measuring endoglucanase and total cellulase activities, respectively.

Enzymes

The cellulases used were total crude commercial preparations produced from *Trichoderma reesei* (Ecostone L 883042) and *Humicola insolens* (Denimax L). These preparations were supplied by Röhm Enzyme Finland Oy (Rajamäki, Finland) and by Novo Nordisk (Bagsvaerd, Denmark), respectively.

Surfactants

The surfactants were Lutensit A-LBN 50 (sodium salt of C_{10-13}–alkylbenzesulfonic acid) and Lutensol A 7 N (an alkylpolyethylene glycol made from a saturated, 100% linear C_{12-14} fatty alcohol and having an aver-

age ethoxyl chain length of 7). Both surfactants were from BASF (Ludwigshafen, Germany).

Enzymatic Treatments and Preparation of Treatment Liquors for Ultrafiltration

Dyed or undyed fabric samples (25 g) were placed in pots of a Rotawash machine with 250 mL of the cellulase (total crude from *T. reesei*) solution in acetate buffer (0.1 *M*, pH 5.0). An enzyme load of about 100 mg of protein/g of fabric was used with sodium azide (0.02%) added as an antimicrobial agent. The incubation was performed at 50°C, using an agitation rate of 20 rpm for 8 h. To increase the level of mechanical agitation, 10 stainless steel discs were added to the reaction mixture. Control treatments were made with the fabrics in buffer solution under the same incubation conditions. Two replicates were carried out for each fabric treatment.

After the enzymatic treatments, the solutions were filtered through a 2.7-μm glass microfiber filter GF/C (Whatman). The solids retained on the filters and the treated fabrics were washed with acetate buffer (1 L) to elute adsorbed enzymes. Samples of the filtrate and washings were collected for analysis of protein, soluble reducing sugars, and dye in the case of fabric dyed with Remazol Brilliant Blue R. The filtrate and washings were mixed, and buffer was added to make a volume of 2 L for the ultrafiltration experiments.

For studies of dye protein interactions at different pHs, the same procedures were followed but 5 g of fabric dyed with Remazol Brilliant Blue R or indigo was used in 50 mL of cellulase solution. In this case, agitation was provided by two stainless steel discs. After filtration, washing was with 100 mL of buffer, and the final volume was made up to 250 mL with buffer.

Ultrafiltration Experiments

The solutions (2 L) obtained from the procedure described in the previous section were ultrafiltered using a Prep/Scale™ TFF cartridge (Millipore, Bedford, MA), containing a 0.09-m^2 polysulfone ultrafiltration membrane with a mol wt cut-off of 10 kDa. Before ultrafiltration of the hydrolysate solutions, the ultrafilter was prewashed with acetate buffer. The ultrafiltration was performed at 1–1.2 bar until the desired reduction in volume of the retentate was achieved. Samples of the feed, retentate, and permeate solutions were analyzed for protein, sugars, and dye content. After ultrafiltration, the cartridge was washed with buffer to recover protein present in the solution that remained inside the ultrafilter and in the lines. Ultrafiltration of a sugar solution (containing glucose and cellobiose dissolved in acetate buffer) was also done to study the removal of sugars in the absence of proteins.

To study the influence of pH on the separation of dyes from the reaction mixture, ultrafiltration was carried out on a smaller scale. Portions (20 mL)

of feed solution were separated for pH adjustment in a range from 3.0 to 9.0, by making additions of sodium hydroxide or acetic acid as necessary. These experiments were made using a 4-mL centrifugal ultrafiltration unit with polysulfone membranes having a 5000 kDa mol wt cutoff (Ultrafree-4; Millipore). The ultracentrifugation was carried out at room temperature in a centrifuge tube EBA 8 (Hettich, Tuttlingen, Germany) (2875*g*) with 4 mL of feed solution for 1 h. This procedure was repeated three times, and the retentate and permeate were taken for analysis of protein, sugars, and dye. The ultrafilters were washed with 4 mL of acetate buffer.

Enzymatic Treatments in Presence and Absence of Surfactants

To compare the effects of the anionic and nonionic surfactants on cellulase activity, hydrolysis experiments were performed in the presence and absence of these compounds. The same enzyme concentrations (3.6 mg of protein/g of fabric) and incubation conditions (Rotawash machine, 50°C, 20 rpm, 1 h) were used in buffer (acetate buffer, 0.1 *M*, pH 5.0) alone and buffer plus anionic or nonionic surfactant (both used at 1 g/L). In the case of *H. insolens* cellulase (neutral cellulase), the activity was determined in phosphate buffer solution (0.1 *M*, pH 7.0). Activities were measured in terms of the concentration of soluble reducing sugars produced.

Desorption of Cellulases from Cotton Fabrics Using Buffer and Surfactants

Before use in these experiments, the cotton fabrics were washed in 2 g/L of sodium carbonate solution and rinsed in hot and cold distilled water to remove any residual surfactant from previous scouring and bleaching processes.

To compare the effectiveness of surfactants in buffer and buffer alone, for the desorption of cellulases from fabric, pieces of cotton fabric (6.9 g) were incubated in a cellulase solution (1.0 mL of total crude from *T. reesei* in 100 mL of acetate buffer, 0.1 *M*, pH 5.0) in a Rotawash machine (50°C, 20 rpm, 1 h). Protein concentration in solution was determined before and after the treatment. The fabric was removed and washed in 100 mL of buffer or surfactant solution (Rotawash, 25°C, 20 rpm, 30 min). The fabric was removed and the solution containing desorbed enzymes was then incubated (Rotawash machine, 50°C, 20 rpm, 1 h) with a new cotton fabric (6.9 g). The cellulase activity was measured in terms of liberated soluble reducing sugars.

Determination of CMC and Filter Paper Activities

The endoglucanase activity (CMC activity) of cellulase samples was measured towards CMC (0.5%) at 50°C for 30 min. Activity on filter paper (total cellulase activity) was measured at 50°C for 1 h. The soluble reducing sugars released during the reaction were determined by the method described next.

Table 1
Protein (total crude) Adsorption (on fabric plus fine cotton particles) and Sugar Production After Enzymatic Hydrolysis Using Undyed and Dyed Cotton Fabrics as Substrates
(Rotawash machine, 50°C, pH 5.0, 100 mg protein/g fabric, 20 rpm, 10 discs, 8 h)

Fabric	Protein adsorption (%)[a]	Sugar production (g)[a]
Undyed	37.1 (1.1)	0.709 (0.093)
Dyed with Remazol Brilliant Blue R	39.6 (6.8)	0.451 (0.201)
Dyed with indigo	38.2 (6.6)	1.215

[a]Values in parentheses represent the SD of two independent experiments.

Analytical Methods

Total protein in solution was measured by the Bradford *(17)* assay with bovine serum albumin as the protein standard. Soluble reducing sugars were determined using the neocuproine method described by Cavaco-Paulo et al. *(18)*. The dye content of the solutions was measured spectrophotometrically by reading the absorbance of the samples at the wavelength of maximum absorption of the dye. Two determinations were made for each sample.

Results and Discussion

Removal of Sugars and Dyes from Processing Liquors Using an Ultrafiltration System

The results given in Table 1 show the levels of cellulase adsorption and the reducing sugar production for the undyed and dyed cotton fabrics. There were no significant differences in the adsorption percentage for dyed and undyed fabrics, although cellulase adsorption was less reproducible on the dyed fabrics. With respect to the liberation of reducing sugars, it seems that the presence of a reactive dye inhibits the hydrolysis rate, because covalent binding of the bulky dye molecules at the more accessible points of the cellulose molecules makes their accommodation in the reactive sites of cellulase more difficult. This is in accordance with the findings of other investigators *(19–21)*. The result of sugar production for the indigo-dyed fabric is anomalous, but it could be owing to interference of indigo on the determination of reducing sugars.

After fabric treatments, the enzymes are distributed between the hydrolysate, the particles of cotton debris, and the cotton fabric itself. In principle, both the "free" and adsorbed cellulases can be recovered and recycled for further treatments. The proteins that remained in solution were filtered from the debris and enzymes that remained adsorbed to the fabric and to the cotton debris were partially recovered by simple desorption by washing with fresh acetate buffer solution. Table 2 represents the

Table 2
Protein Recovery After Hydrolysis
Using Undyed and Dyed Fabric as Substrates

Fabric	Sample	Protein recovery (%)[b]
Undyed	Filtrate	62.9 (1.1)
	Washings[a]	18.1 (2.6)
Dyed with Remazol Brilliant Blue R	Filtrate	60.4 (6.8)
	Washings[a]	15.8 (1.2)
Dyed with indigo	Filtrate	61.9 (4.4)
	Washings[a]	17.3 (0.4)

[a]Washing was with fresh acetate buffer solution.
[b]Values in parentheses represent the SD of two independent experiments.

percentage of recovery that can be achieved from the filtrate and from the washing process after hydrolysis of undyed and dyed cotton fabrics. About 60% of the cellulase protein remained in the filtered hydrolysate, but a significant percentage (15–18%) was also recovered by washing the fabric and cotton debris with buffer solution. Thus, with these processes it was possible to achieve a total cellulase protein recovery of 75–80%. The presence of dyes on the substrate made no significant difference in the percentage of protein recovery.

The 20–25% loss of protein must be the result of incomplete desorption of the cellulases from the fabric and cotton debris. To obtain higher levels of desorption, greater volumes of buffer and or different conditions should be used for washing. If more buffer solution is used, more dilute enzyme solutions are obtained, but these can be concentrated by using ultrafiltration.

After the recovery procedure, ultrafiltration was carried out to concentrate the proteins and remove the sugars and dyes. The results (Table 3) show that when only soluble sugars were present in the acetate buffer feed solution, without cellulases or dyes, it was possible to achieve about 77% sugar removal, when the permeate represented about 80% of the initial feed volume. The presence of cellulase proteins in the feed solution caused a reduction in sugar removal. It seems that interaction between cellulases and their degradation products prevents the latter from passing through the filtration membrane. Possibly, some sugars remain "adsorbed" or "attached" to the enzymes, causing them to remain together in the retentate. With respect to the protein in the retentate, about 90–95% was found to be present in this solution. Washing the ultrafilter with acetate buffer and then analyzing the resultant retentate showed that there was some protein retained on the filter, which usually constituted 5–10% of the initial amount.

When reactive dye was also present in the hydrolysate solution, only about 50% of the dye was removed by ultrafiltration. This is attributed to strong protein-dye interactions, which may also involve protein-sugar

Table 3
Percentage Volume, Sugar, and Dye Content
of Permeate Solutions Relative to Their Initial Feed Solutions

Sample	Volume (%)	Sugar (%)	Dye (%)
Sugar solution in acetate buffer	61.0	56.8	—
(glucose + cellobiose)	82.4	76.7	—
Hydrolysate of undyed fabric	81.0	58.9	—
(soluble sugars + proteins)	80.4	58.5	—
Hydrolysate of fabric dyed	79.5	60.4	54.1
with Remazol Brilliant Blue R	78.8	52.5	43.0
(soluble sugars + soluble dye + proteins)			
Hydrolysate of fabric dyed with indigo	77.9	60.4	None
(soluble sugars + dye + proteins)			

interactions, because reactive dyes bind covalently to cellulose, and after cellulolytic hydrolysis, reactive dyes that appear in solution remain bound to glucose, cellobiose, or cellulose oligomers *(22)*.

When indigo was present in the hydrolysate, none of this dye passed through the filtration membrane. This is not surprising because, as a vat dye, indigo is insoluble in water. For dyeing, indigo is reduced to its acid leuco form (yellow), which penetrates the fibers. Fixation of the dye is then achieved by oxidation to its blue insoluble form. After the enzymatic hydrolysis of indigo-dyed cotton fabrics, some of the indigo trapped in the fibers is released into solution in its insoluble form. There is, however, some solubilization of the dye, owing to interaction between the dye and the enzymes *(23)*. Since indigo cannot be separated from cellulases (by ultrafiltration) after enzymatic stone washing, and it is known that the indigo-cellulase complex causes backstaining of undyed fabrics *(3,24)*, it seems unlikely that cellulases used in this application can be recycled unless some other means is found for separating indigo from cellulases.

Effect of pH on Removal of Reactive Dye

Proteins are amphoteric molecules, which can be positively or negatively charged, depending on solution pH. Since many dyes are also amphoteric, it seems probable that the major interactions between proteins and dyes will be electrostatic in nature. To study this type of interaction, ultrafiltration experiments were performed on the hydrolysates of fabrics dyed with Remazol Brilliant Blue R and indigo at different pHs. In these experiments, the percentages of liquor volume, sugar, and dye content of the permeate solution relative to the initial feed solution were determined.

Figure 2 shows that for Remazol Brilliant Blue R there was a peak at pH 5.0, where maximum ultrafiltration efficiency occurred (higher permeate volume and higher separation of sugar and dye). There were also two minima (pH 3.0 and 5.3) of lower-efficiency separation. At pH 3.0, the

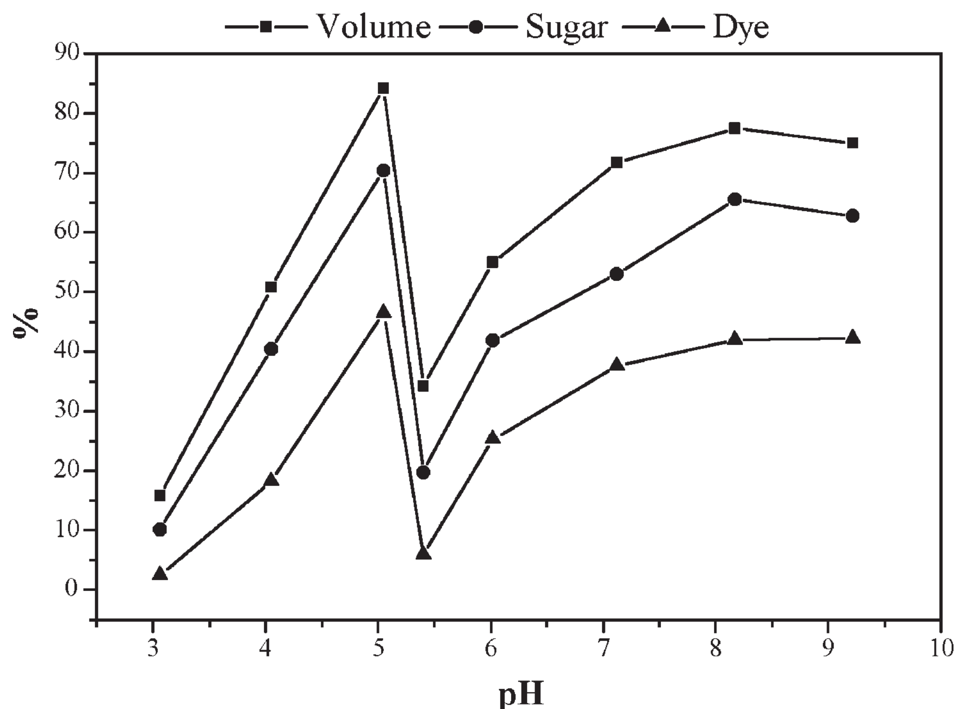

Fig. 2. Percentages of volume, sugar, and dye content of permeate, relative to initial feed solution, at different pHs for hydrolysate of fabric dyed with Remazol Brilliant Blue R.

enzymes became denatured and deactivated; the solutions presented a turbid aspect; and after sedimentation, the bottom of the tube showed a deeper blue than the top, owing to the precipitation of protein together with the dye. Since there was precipitation of the protein in the feed solution, it was more difficult to ultrafilter the solution, hence the low volume of permeate. The pH value of 5.3 corresponds to the hydrolysate obtained after hydrolysis without any adjustment in pH, and the lower ionic strength of this solution may account for stronger dye-protein interactions. After this second minimum, the filtration efficiency increased with increasing pH. At pH 9.0, the dye content of the permeate was approximately the same as that achieved at pH 5.0.

The results in Fig. 2 also show that there was, as expected, a direct correlation between the percentage of permeate volume and its sugars and dye content. This means that if the permeation rate can be increased, better separation in terms of sugars and dye can also be achieved.

In the indigo-enzyme separation experiments (Fig. 3), with the exception of pH 3.0, the ultrafiltration efficiency remained approximately the same throughout the pH range studied. With this dye, better ultrafiltration efficiency (80–95% for permeate volume and 70–80% for sugar remotion) was obtained than with the reactive dye, but there was no indigo in the permeate at any pH. These results indicate that the interactions between

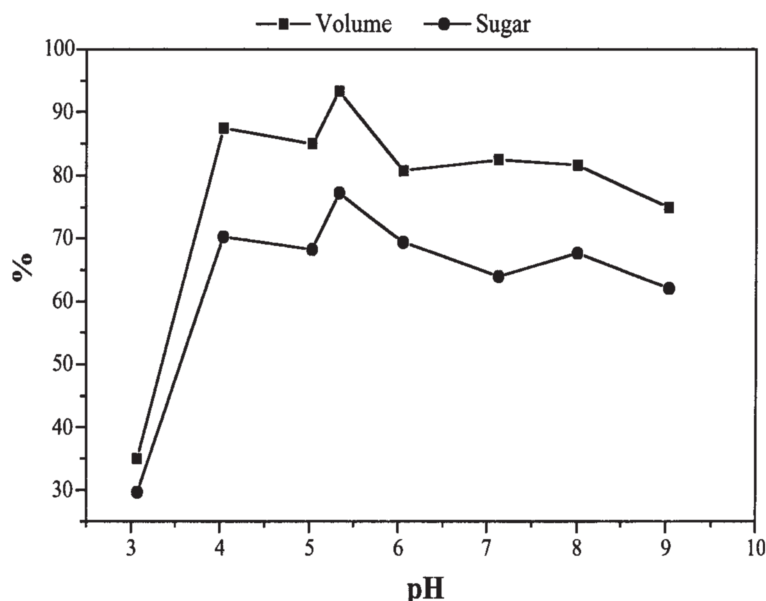

Fig. 3. Percentages of volume and sugar content of permeate solution, relative to initial solution, at different pHs for hydrolysate of fabric dyed with indigo.

indigo and the cellulase enzymes are hydrophobic (between hydrophobic residues of the cellulase molecule and the aromatic rings of the indigo dye; see Fig. 1). This is in agreement with the findings of Gusakov et al. *(24)*, whose experiments with indigo interaction with immobilized amino acids indicate that the amino acids bind indigo via hydrophobic interactions and hydrogen bonding.

It is known that the cellulose-binding domains (CBDs) of cellulases present exposed aromatic residues on their surface (tyrosines on family I CBD and tryptophan on family II), which seem to mediate the binding interaction with cellulose *(25)*. It has also been reported that some CBD families bind irreversibly to cellulose *(26)*. It seems likely that the aromatic residues of the cellulose-bound CBD can also interact with indigo molecules and that backstaining is likely to be the result of this interaction. When cellulases that adsorb reversibly to cellulose are used, this type of interaction will not constitute a problem, because such cellulase enzymes together with bound dye are easily desorbed from the cellulose surface. Since in some cases it is the CBD that is responsible for irreversible adsorption and the CBD is not essential for cotton hydrolysis *(26)*, the problems of backstaining and cellulase loss by adsorption might also be reduced by using cellulases without CBDs.

Enzymatic Treatments in Presence and Absence of Surfactants

Before assessing the desorption of cellulases by surfactants, we decided to investigate their effects on cellulase activity. The activities of an

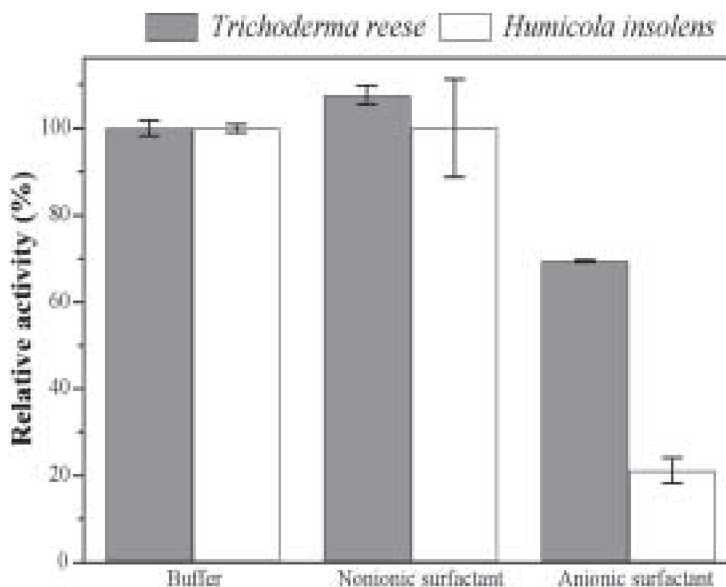

Fig. 4. Effect of surfactants (1 g/L) on cellulase activity during enzymatic hydrolysis of cotton fabrics (Rotawash machine, 3.6 mg protein/g fabric, 50°C, 20 rpm, 1 h). Values represent the mean of two independent experiments and error bars the SD.

acid cellulase (*T. reesei*) and a neutral cellulase (*H. insolens*) were measured in the presence of buffer alone and buffer plus nonionic or anionic surfactants.

The results, illustrated in Fig. 4, show that the nonionic surfactant increased the activity of total crude from *T. reesei* on cotton fabric by about 7%, but that the effect on *H. insolens* cellulase was less consistent. The anionic surfactant, which is said to cause partial denaturation of cellulases *(27)*, caused some deactivation of both cellulases. The *T. reesei* cellulase retained about 70% of its activity whereas *H. insolens* cellulase retained only about 20% of its original activity. It was found that with nonionic surfactants, the enzymatic hydrolysis of cellulose was always improved *(28–30)*. It seems that nonionic surfactants act as enzyme stabilizers, preventing inactivation at the liquid-solid interface during hydrolysis and facilitating enzyme desorption from the substrate. Park et al. *(30)* found that the amount of free cellulase enzyme in the reaction mixture during cellulose hydrolysis was greater when surfactant was used than in the case of no surfactant. Another explanation for the increased hydrolysis rate of insoluble cellulose in the presence of nonionic surfactants is that the surfactant can adsorb to cellulose, thus lowering surface tension, improving wetting, and thereby making the cellulose more accessible to the enzymes *(28)*.

It has been shown, however, that cationic and anionic surfactants denature enzymes even at low concentrations *(31)*. The interactions between proteins and ionic amphiphiles have been widely studied. The results of

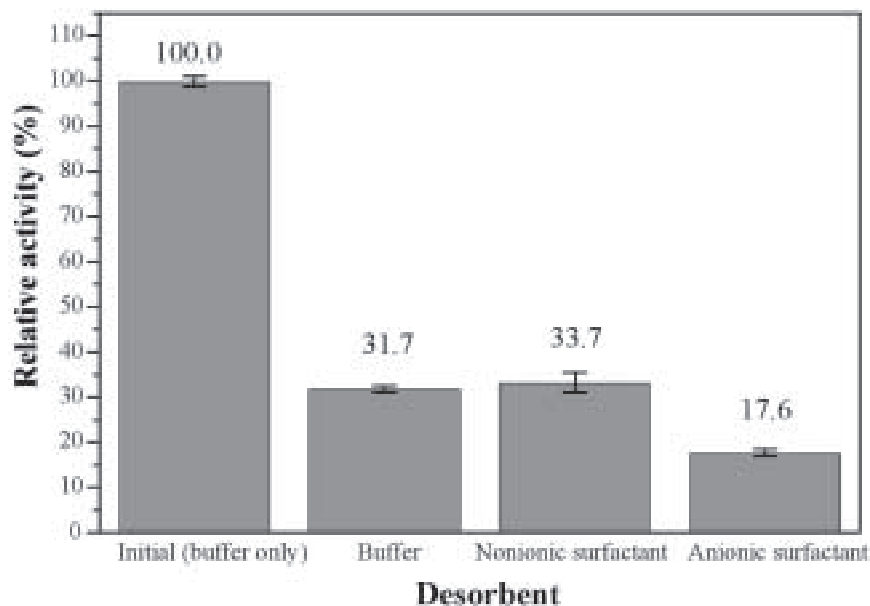

Fig. 5. Comparison of desorbed activities (as liberated reducing sugars) using different desorbents. Values represent the mean of two independent experiments and error bars the SD.

such investigations indicate that electrostatic and or hydrophobic interactions occur between protein and surfactant molecules. In this case, the hydrophobic residues of the protein are exposed to allow the association with the surfactant, and this usually results in conformational change of the native protein leading to its denaturation *(32)*.

Desorption of Cellulases from Cotton Fabrics Using Buffer and Surfactants

After a single cellulase treatment, the enzymes adsorbed to the cotton fabrics were eluted using different desorbents. The recovered enzymes were used in a subsequent cotton treatment. The resulted activity (recovered activity) was measured and compared with the initial activity. The results of these experiments are illustrated in Fig. 5, which shows that when buffer was used as a desorbent, the resulting solution contained 31.7% of the original activity toward cotton fabric. Analysis of the liquor after the first hydrolysis showed that 30–35% of the enzyme protein was adsorbed, but it must be remembered that the wet fabric also carried free enzyme over into the desorption bath. It is therefore not clear what percentage desorption of protein recovery is achieved in these experiments, and there is a need for more detailed study in this area. Analysis of the desorption liquid by fast protein liquid chromatography could be conducted in order to confirm or deny the irreversible binding of specific fractions of the cellulase

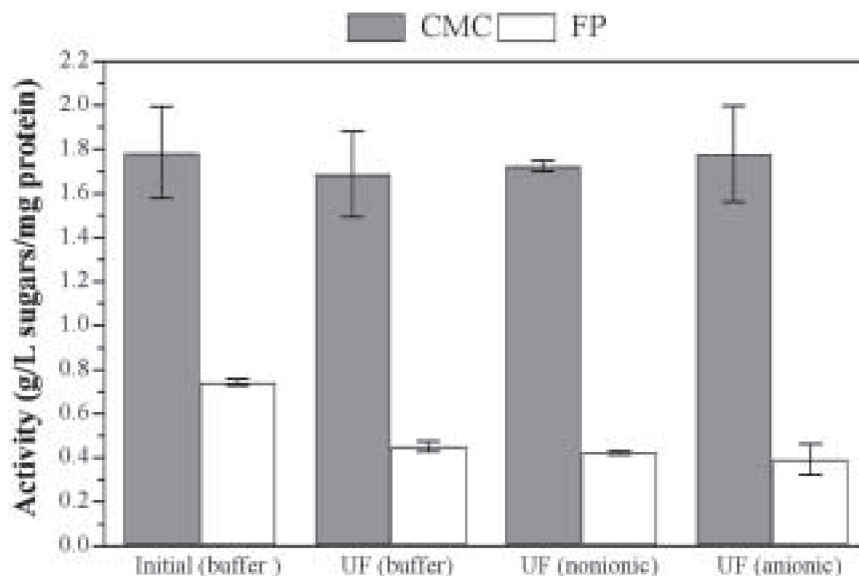

Fig. 6. Comparison of CMC and filter paper (FP) activities recovered by ultrafiltration (UF) using buffer alone and buffer plus nonionic or anionic surfactant (1 g/L) as desorbents. The recovery was carried out after enzymatic treatment of undyed fabrics. Values represent the mean of two independent experiments and error bars the SD.

complex that were found to be more depleted in the supernatant after the enzymatic treatment *(1)*.

There was no evidence that buffer plus surfactants desorbed more protein than buffer alone. Unfortunately, the protein concentrations in the presence of surfactants could not be measured by the Bradford (or Lowry) method because of the interference of surfactants with the formation and color of the dye-protein complex. The bicinchoninic acid method for protein determination is more tolerant of a variety of detergents (anionic, nonionic, and zwitterionic), but this assay is more sensitive to interference from reducing sugars.

Desorption of Cellulases Using Anionic and Nonionic Surfactants and Their Recovery by Ultrafiltration

We attempted to recover and combine the enzymes in the liquor after hydrolysis and the enzymes desorbed from the cotton fabrics and debris using buffer or surfactants and to concentrate and separate them from reaction products using an ultrafiltration system. After this procedure, the residual cellulase activity was measured and compared with that of "fresh" cellulase enzymes.

Figure 6 shows it was possible to recover most of the CMC activity present in the original cellulase sample (stock solution). The presence of surfactants did not enhance the desorbed activity. The filter paper activity

was, however, partially lost, which suggests that the cellobiohydrolases are more strongly adsorbed to the substrate or are more sensitive to deactivation. It is surprising that the desorption with anionic surfactant did not cause further deactivation (see Fig. 6), and it appears that ultrafiltration may separate anionic surfactants from cellulase proteins.

Conclusion

A total of about 80% of the cellulase protein could be recovered after use in cotton processing. About 62% was recovered from the processing liquor, and about 18% could be desorbed from the substrate using buffer solution. The 20% loss of protein is believed to be the result of incomplete desorption of some components of the cellulase complex that are bound irreversibly to the substrate. Cost calculations should therefore be made, for specific applications, to determine whether it could be more cost-effective to use more buffer to desorb the enzymes, or to use other potential desorbents in order to obtain greater levels of desorption with less buffer volume, and thus less need for subsequent protein concentration.

Surfactants did not seem to enhance cellulase desorption (measured in terms of desorbed activity) from the substrate. Although anionic surfactants have a deactivating effect on cellulases, this effect seems to be reversible since after ultrafiltration the cellulase activity was similar to that obtained for the desorption of enzymes with buffer. A total cellulase from *H. insolens* was shown to be much more sensitive to anionic surfactant than a total crude from *T. reesei*.

Although ultrafiltration was used successfully to reduce the concentration of sugars in treatment liquors, the presence of dyes, especially vat dyes (indigo), in these liquors proved to be a serious problem for recycling because ultrafiltration could not remove dye adsorbed on the cellulase protein. Unfortunately, this means that recycling of cellulases in enzymatic stone washing (the most important cellulase-finishing process) may not be possible, unless an alternative approach to the backstaining problem can be found. It may be that cellulase compositions containing only reversibly adsorbed components would be beneficial in this context.

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