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CELLULASE HYDROLYSIS OF COTTON CELLULOSE: THE EFFECTS OF MECHANICAL ACTION, ENZYME CONCENTRATION AND DYED SUBSTRATES

A. CAVACO-PAULO§, L. ALMEIDA§*

§*Dep. Eng. Têxtil, Universidade do Minho, P-4800 Guimarães and*
**CITEVE, P-4760 V. N. Famalicão, PORTUGAL*

Experiments with dyed and undyed cotton, using different cellulase concentration and with or with out mechanical action showed a high effect of those factors. The process with mechanical action have a higher weight-loss. Dyed cotton with vat and sulfur dyes have almost the same weight-loss than undyed cotton, but on reactive dyed cotton the cellulase hydrolysis is shorter. It was also verified a decrease of hydrolysis extent with an increase of the reactive dye concentration on the cotton substrate. Some synergism due to the enzyme concentration was apparently observed on the changes of the length of the leaving sugars.

KEY WORDS: Cellulase Enzymatic Hydrolysis, Cellulase Concentration Synergism, Cotton Cellulose, Dyed Cotton Substrates, Mechanical Action

INTRODUCTION

The cellulase treatment is an ecological way of finishing cotton textiles. Applications like the reduction of pilling, softening and ageing are widely used in textile processing (Cavaco-Paulo and Almeida, 1991). The degree of enzymatic hydrolysis of cotton must be carefully controlled, in order to get the desired effect without a severe damage of the fabrics.

All cellulase producers recommend a process with mechanical action, like the treatments on jets or winches and some times the use of stones with the enzyme in washing-machines to produce the desired second-hand look on jeans (Cavaco-Paulo and Almeida, 1991).

Those applications are obtained after dyeing stages, and it is important to know to what extent a certain dye group will change the cellulase hydrolysis. We can consider the dyed samples as cellulose derivatives, because the dye used is of a reactive type which is covalently bonded to the glucose units of the cellulosic chain. Also, the dye is on the surface and first layers of cellulose fibre and it is on this area that the enzymatic hydrolysis possibly takes place (Sagar, 1985). The action of the cellulase complex on cellulose and cellulose derivatives seems to be different; on the cellulose derivatives the endo type enzyme from the all cellulase complex is the most active (Finch, 1985).

In a previous work (Almeida and Cavaco-Paulo, 1993) we have verified, to improve textile properties on cotton fabrics, a cellulase treatment without mechanical action, of less than one hour will be enough, with a concentration of 3% enzyme/substrate. In the present work, all the experiments were made in one hour and with a range of 0.1 - 2 % enzyme/substrate. Even testing different kinds of cotton, we try to cover all concentration range where the synergism between cellulase components is higher as it was observed before for Avicel (Woodward *et al.*, 1988).

In the present work, it is showed the effect of the mechanical action, enzyme concentration, and dyed substrates on enzymatic hydrolysis of cotton. The studies were made on cotton fabrics and on scoured cotton fibres. The cellulase preparations used are commercial and they are a mixture, at least, of endoglucanases (EC 3.2.1.4), cellobiohydrolases (EC 3.2.1.91) and cellobiases (EC 3.2.21). The parameters studied are weight loss, mean length of the leaving sugars, reducing sugars of the fibres, strength loss and thickness. Scanning electronic microscopy photographs of the fibers are also shown.

EXPERIMENTAL PART

Cellulases: Cellusoft L (used for pilling reduction and softening, maximum activity pH = 5); Denimax T (used on second hand effects, maximum activity pH = 7), both kindly supplied from Novo Nordisk, A/S.

Enzyme Activity: The activities were measured towards carboxymethylcellulose (CMC), phosphoric-acid-swollen Avicel (PASA) and cellobiose, adapted from as described previously (Evans *et al.*, 1992). We have also measured the activity towards scoured cotton fabric, measuring the weight loss, the reducing sugars liberated (as cuprous neocuproine complex) and its mean chain length.

Weight loss and Mean Chain Length of the Leaving Sugars: The weight loss were measured before and after the treatment relative to the dry samples 4 hour at 105°C; then measuring the reducing ends of the leaving sugars as cuprous neocuproine complex we obtained its mean chain length.

Reducing Ends on the Cotton Fibers: The reducing ends of the cotton fibres are quantified by the produced cuprous neocuproine complex in alkaline media at 95°C.

Thickness: Were at 2,5 gf measured on KES-FB 3 - Compression Tester, Kato Tech. Co, LTO.

Strength Loss: Were measured in relation to the fabric untreated in a Instron Machine.

Fibre Parameters: Mean Length (by scanning the fibre bundle pneumatically), Short Fiber Content (% on a weight basis of fibres which have less than 12.7 mm of length), Strength and its Elongation (relative to a break tensile force) were obtained by measurement on a HVI 4000 (High Volume Instrument for testing cotton fibres; Motion Control). All samples were conditioned one day, to 20°C and 65% of relative moisture before the measurements.

Scanning Electronic Microscopy Photographs (Leica Cambridge Stereoscan 360) were taken after 2 minutes of gold metalization (Bio-Rad SC 502).

Mechanical Action

Samples: All samples used were 100% cotton scoured fibres and 100% cotton fabrics after industrial scouring and bleaching.

Treatments: The fabrics were treated with 0.1, 0.5, 1 and 2 g/l of cellulase using the ratio: 1g of fiber to 10 ml of bath; buffer 4.8 (acetate, 0.5 M) for Cellusoft L, buffer 7.0 (phosphate, 0.5M) for Denimax T, temperature of 50°C. The treatments were stopped (1 h) by addition of a solution of Sodium Carbonate 10 %. The fabrics were washed after treatment with hot and cold water. The machine used was Linitest machine with 65 rpm of the 250 ml steel reactors. The mechanical action was provided when required with 12 steel disks used on fastness to dry cleaning.

Dye Concentration

Samples: All samples used were 100% cotton carded fabrics after industrial scouring and bleaching.
Dyeing: The dye used were Levafix Yellow E-3RL (C. I. Reactive Orange 30) from Bayer, with a dichloroquinoxaline reactive group. The initial concentration of the dye bath were 0,4, 1 and 4 % in relation to fabric weight. We used the "All-In" method described by the technical information of Levafix E Dyes from Bayer.
Treatments: The fabrics were treated with 1% of cellulase (enzyme/fibre), 1g of fiber to 10 ml of bath, buffer 4.8 (acetate, 0.5 M) for Cellusoft L, buffer 7.0 (phosphate, 0.5M) for Denimax T, temperature of 50°C. The treatments were stopped (1 h) by addition of a solution of Sodium Carbonate 10 %. The fabrics were washed after treatment with hot and cold water. The machine used was Linitest machine with 65 rpm of the 250 ml steel reactors. The mechanical action was provided when required with 12 steel disks used on fastness to dry cleaning.

Dye Effect

Samples: All samples used were 100% cotton fabrics after industrial scouring and bleaching.
Dyeing: The reactive dyes used were Procion Yellow H-EXL and Procion Blue MX 2GN from ICI, Marine Cibacrone FG from Ciba, Ramazol Yellow 4GL from Hoescht and Levafix Yellow E-3RL from Bayer. The vat dye was Indranthren Red FBB from BASF and the sulfur dye was Hydrosol Brilliant Red BLL from Hoescht. The dyeing methods used are described for which dye on its technical information. The initial concentration of the dye bath was 4 % to the fabric weight.
Treatments: The fabrics were treated with 1% of Cellusoft L (enzyme/fibre), 1g of fiber to 10 ml of bath, buffer 4.8 (acetate, 0.5 M), temperature of 50°C. The treatments were stopped (1 h) by addition of a solution of Sodium Carbonate 10 %. The fabrics were washed after treatment with hot and cold water. The machine used was Linitest machine with 65 rpm of the 250 ml steel reactors.

RESULTS AND DISCUSSION

TABLE 1 - Activities - 1 Unit is the enzyme amount who realize 1 μmol of reducing sugars as glucose by minute

Enzyme	Denimax T	Cellusoft L
Cellobiose	1,7 U/g	1,5 U/g
CMC	115 U/g	159 U/g
PASA	144 U/g	361 U/g
Cotton	10 U/g	51 U/g
Weight Loss-Cotton	0,91 %	1,33 %
Mean Chain Length-Cotton	2,4	1,7

By looking at the weight-loss graphics of figure 1, we observe a general higher hydrolysis extent for the fibres than for the fabrics. The process is also higher with mechanical action. But, the weight-loss becomes smaller when the dye concentration of the substrates increases.

Those observations are applicable for both enzymes used, but the cellulases are different as it was verified before (Cavaco-Paulo and Almeida, 1993). Cellusoft L is a total cellulase and it is more active than Denimax T (the ageing enzyme), having the last cellulase a relatively higher endo action (tab. 1). The photos of SEM (fig. 3-a) confirm that the first one produces a deeper damage while the last just produces a superficial damage on the fibres. Also, looking at the (fig. 1) weight loss, the effects are greater for the total cellulase than for the ageing one.

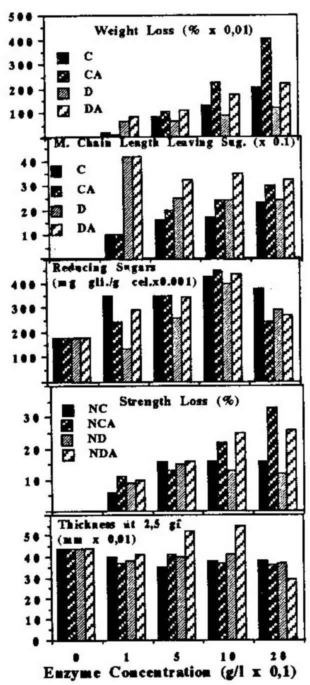

Figure 1-a) - Graphics of the parameters studied for cotton fabric with enzyme concentration. (C - treated with Cellusoft L, D - treated with Denimax T, A - mechanical agitation)

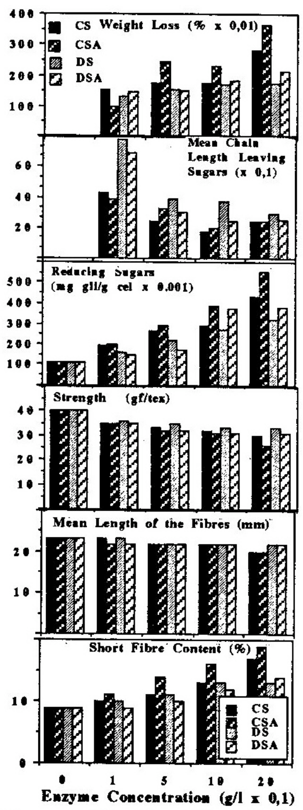

Figure 1-b) - Graphics of the parameters studied for scoured cotton fibres with enzyme concentration. (S - scoured fibres, C - treated with Cellusoft L, D - treated with Denimax T, A - mechanical agitation)

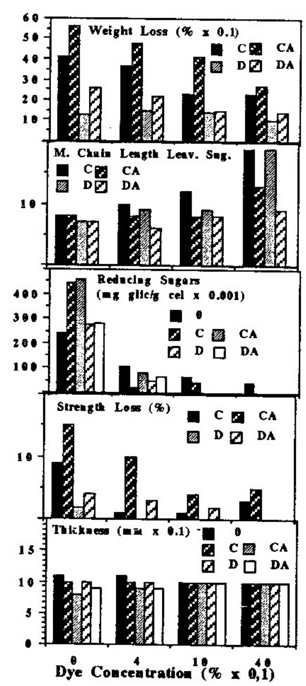

Figure 1-c) - Graphics of the parameters studied with the dye concentration on the cotton fabric. (O - original fabric, C - treated with Cellusoft L, D - treated with Denimax T, A - mechanical agitation)

The differences in weight-loss between both enzymes become smaller when we have a cellulose with higher concentration of dye (fig. 1-c). This factor increases the length of the leaving sugars (fig. 1-c) after the treatment and decreases the loss of strength (fig. 1-c). For the fabric and the fibres, generally, the main chain length is higher for the ageing cellulase than for the total one, which agrees with the results of table 1, but for the reactive dyed cotton the reverse happens. Also, the weight loss for vat and sulfur dyed fabrics is almost the same than for the undyed fabric, after cellulase hydrolysis; but for the different reactive dyed fabrics the weight loss is smaller, about 60-70 % of undyed (fig. 2). Those observations support the idea that on cellulose derivatives the endo enzyme is the most active. In recent work (Ueda *et al.*, 1993), it was showed a similar result of figure 2, but also a inhibitory action for direct dyes suggesting that the molecular size and ionic groups of the dye could also influence cellulase hydrolysis.

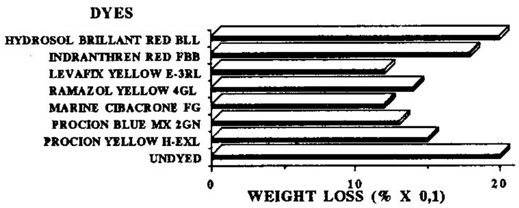

Figure 2 - Weight-Loss for the dyed fabrics

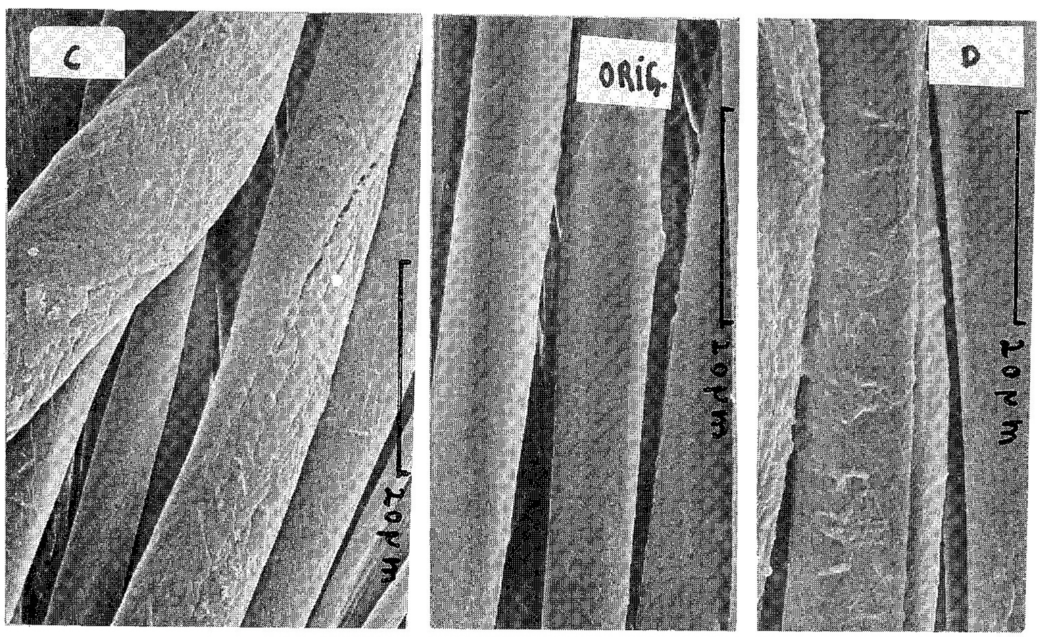

Figure 3 a) - Photographs of Scanning Electronic Microscopy for samples treated with out mechanical agitation (ORIG.- Untreated cotton; C - treated with Cellusoft L, 2g/l; D - treated with Denimax T, 2g/l)

As expected, the length of the leaving sugars decreases with enzyme concentration, for both enzymes for the fibres (fig. 1-b). But for the cotton fabric (fig. 1-a), the increase of enzyme concentration makes the action of both enzymes different: the mean chain length of the leaving sugars obtained with the ageing cellulase decreases with enzyme concentration and the reverse happens for the total cellulase; this seems to indicate a higher synergism between the endo and exo-glucanases at lower enzyme concentration. A similar synergism was observed before on Avicel

(Woodward *et al.*, 1988). Cellulose in a fabric is less accessible than on a bundle of fibres, being this a possible explanation for the different behavior of fibres and fabric.

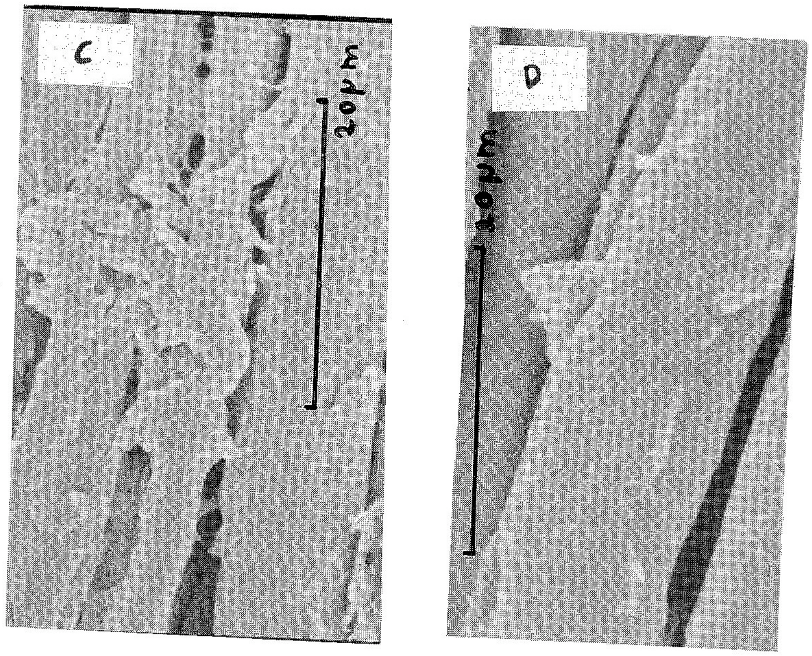

Figure 3 b)- Photographs of Scanning Electronic Microscopy for samples treated with mechanical agitation (C - treated with Cellusoft L, 2g/l ; D - treated with Denimax T, 2g/l)

The weight loss for the ageing enzyme is almost independent of dye concentration of the substrates, but when we have mechanical agitation the weight loss is double for the undyed fabric and decreases to the same value to the highest dyed fabric (fig. 1-c). The mean chain length of the leaving sugars is always smaller for the process with mechanical action for the dyed substrates and fibres using ageing cellulase (fig. 1-b-c). Those facts seems to indicate that mechanical action could help on the cellulose degradation by cellulase less rich in exo type enzyme. But using a total cellulase on fibres and both enzymes on the fabric (fig. 1-a-b), the main chain length is higher with mechanical action, because the friction provided by the steel disks is higher on the cellulose on a fabric than a cellulose on the bundle of fibres.

The reducing sugars of the treated samples increases with enzyme concentration for both enzymes and for both samples (fig. 1-a-b), but for the less accessible cellulose a decrease is verified at higher concentration of enzyme, perhaps due to the removal of the broken chain by the excess of enzyme.

The reducing ends of the fibres (fig. 1-c) after enzymatic treatment increases on the original fabric, but for dyed fabrics the reducing sugars decreases and on the fabric with higher concentration of dye the reducing ends group falls to zero. One possible explanation to this fact is that the reactive dye is linked to the hydroxyl group of carbon 1 of the reducing end and hence looses its reducing capacity. The cellulase action seems to remove almost all the free cellulose, hydrolyzing less the dyed cellulose.

The strength loss increases with enzyme concentration (fig. 1-a-b), but almost falls to zero for the treated higher dyed substrates (fig. 1-c). Mechanical action makes a higher strength loss, like observed on weight loss.
The thickness tends to decrease with enzyme concentration (fig. 1-a), but for the ageing enzyme with mechanical action we observe an increase of thickness at lower/middle concentrations. This is consistent with the microfibrils produced by mechanical action during degradation on fibre surface, showed on SEM photos (fig. 3-b). The increase of thickness at lower enzyme concentration doesn't happen for the total cellulase because this enzyme makes a deeper degradation and the microfibrils are removed making thickness smaller.
Thickness (fig. 1-c) will decrease with the enzymatic treatment, due to the removal of pilling, on undyed and less dyed fabrics, but on more dyed fabrics the thickness does not change.
The mean length of the fibres decreases with enzyme concentration, being more or less independent of mechanical action. The short fibre content or percentage of fibres having less then 12,7 mm increase with the process. The mechanical action just increase the short fibres content for the total cellulase, supporting what was observed to fig. 3-a-b that total cellulase produces a deep damage and the ageing cellulase just a superficial damage on cotton fibres.

CONCLUSIONS

Mechanical action during cellulase hydrolysis is a important factor: it increases the process,being higher on a fabric than on a bundle of fibres and it seems to help the cellulose degradation with a mixtures less rich in exo type enzyme. Mechanical action also produces microfibrils and it can produce pilling at controlled conditions with a enzyme richer in endo type cellulase.
Not only the physical structure of the fabric is important on cellulase hydrolysis (Almeida and Cavaco-Paulo, 1993), but also cotton cellulose in a fabric is less accessible to enzyme attack than on a bundle of fibres. On the fabric at lower concentrations of enzyme apparently the synergism between the cellulase components is higher.
Enzymatic hydrolysis is smaller on reactive dyed fabric than on vat, sulfur dyed and undyed fabrics, and the results show on the reactive dyed fabric the endo type enzyme is the most active.

ACKNOWLEDGMENTS

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REFERENCES

Almeida, L. and Cavaco-Paulo, A. (1993) Softening of cotton by enzymatic hydrolysis. *Melliand Textilber.*, **74**, E184-E185/404-407

Cavaco-Paulo, A. and Almeida, L. (1991) A hidrólise enzimática do algodão - Nova Técnica de Amaciamento. *Nova Têxtil*, **22**, 52-57

Cavaco-Paulo, A. and Almeida, L. (1993) The scouring effect on cellulase enzymatic hydrolysis of raw cotton. In *Proccedings of a FEMS Symposium: Lignin Biodegradation and*

Transformation, edited by J. C. Duarte, M. C. Ferreira, P. Ander, pp 101-102, Forbitec, 18-21 April, Lisboa, Portugal

Evans, E., Wales, D., Bratt, R. and Sagar, B. (1992), Investigation of an endoglucanase essencial for action of the cellulase system of *Trichoderma ressei* on crystaline cellulose. *J. Gen. Microbiol.*, **138**, 1639-1646

Finch, P. (1985) Enzymatic degradation of cellulose. In *Cellulose Chemistry and its Applications*, edited. by T. P. Nevel, S. H. Zeronian, pp. 312-343, John Wiley & Sons

Sagar, B. F. (1985) Mechanism of cellulase action. In *Cellulose and its Derivatives: Chemistry, Biochemistry and Applications*, edited by J. F. Kennedy, G. O. Phillips, D. J. Wedlock & P. A. Williams, pp. 199-207, Ellis Harwood

Ueda, M., Koo H. L., Yoshimura, Y. and Wakida, T. (1993), Cellulase treatment of cellulosic fiber. Poster in the 16th. IFATCC, Maastricht, Holand, 7-9 June

Woodward, J., Lima, M. and Lee, N. (1988), The role of cellulase concentration in determining the degree of synergism in the hydrolysis of microcrystalline cellulose. *Biochem. J.*, **255**, 895-899

Corrections: page 355 - Table 1, by error of the Publishers. It will be a note in a near volume.

TABLE 1 - Enzyme Activity

Enzyme	Denimax T	Cellusoft L
Cellobiose a)	0.85 U/g	0.75 U/g
CMC b)	115 U/g	159 U/g
PASA b)	144 U/g	361 U/g
Cotton b)	10 U/g	51 U/g
Weight Loss-Cotton	0.91 %	1.33 %
Mean Chain Length-Cotton	2.4	1.7

a) - 1 Unit is the enzyme amount that produces in the cellobiose hydrolysis 2 μmol of glucose by minute
b) - 1 Unit is the enzyme amount that produces 1 μmol of reducing sugars as glucose by minute

The names of commercial products are provided for specific information, only. Their mention does not constitute a recommendation.