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Desorption of cellulases from cotton powder

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Abstract

Cotton fabrics were treated with three different *Trichoderma reesei* cellulase preparations (total crude – TC, endoglucanase enriched – EG-rich, cellobiohydrolase enriched – CBH-rich) using mechanical agitation to produce cotton powder. Desorption of cellulase enzymes from the cotton powder was then performed by washing with buffer. After 3 washings most of the protein was desorbed from the cotton powder and the amount of sugars released in the latter washings was negligible. TC and CBH-rich preparations produced a finer cellulose powder than EGs. The desorption process caused a decrease in degree of polymerisation (DP) specially for the cotton treated with EGs and a marked increase in polydispersity (P_d) for all preparations.

Introduction

In recent years there has been an increased interest in the use of cellulases in textile processing for the modification of cellulosic fibres. This has provided the creation of a variety of finishing effects (depilling and ageing) and constitutes a more ecological alternative to traditional and more polluting chemical processes. The treatment of cotton with cellulases in textile processes is normally performed with high level of mechanical agitation (Cavaco-Paulo 1998). Depending on the cellulase preparation (total cellulase, endo-enriched, exo-enriched or monocomponent), its concentration, degree of agitation and treatment time, insoluble particles of cellulose are released during the enzymatic hydrolysis being enhanced by mechanical agitation.

The adsorption of cellulases on insoluble cellulosic substrates is a prerequisite step for subsequent hydrolysis and this subject has been studied by several authors (Kim *et al.* 1997, van Wyk 1997). Since the adsorption/desorption process is a surface phenomenon, it is important to characterize the structural properties of the cellulosic substrates. The insoluble cellulose powder released during the enzymatic treatments presents a high specific surface area for enzyme adsorption (Duff *et al.* 1995, Lee *et al.* 1982, Morgado *et al.* 2000). This means that there is a potential source of enzymes adsorbed to these particles which can be recovered by desorption and recycled and this can contribute to reduce costs associated with enzymatic processing.

In this work, cotton fabrics were treated for long periods of time with different cellulase preparations and using high mechanical agitation to produce a maximum of insoluble cellulose powder. The desorption of cellulases from the cotton powder was carried out by successive washings to investigate what amount of enzymes could be recovered with the perspective of recycling these enzymes. The cellulose material was characterized in terms of degree of polymerisation (DP) and particle size to analyse the changes occurred in the cellulose material and the modes of action of the different enzymes.

Materials and methods

Enzymes and substrate

Three cellulase preparations from *Trichoderma reesei* were used. A complete cellulase system (Total Crude – TC) and two genetically engineered preparations: endoglucanase enriched (EG-rich, cellobiohydrolase activities deleted) and cellobiohydrolase enriched (CBH-rich, main endoglucanase activities deleted). These commercial liquid preparations were supplied by Rhöm Enzyme Oy (Rajamäki, Finland).

Hundred % scoured and bleached cotton poplin fabrics (60/32 ends/picks per cm and area density of 100 g/m^2) were used as the cellulosic substrate for the enzymatic treatments.

Enzymatic treatments

Cotton fabrics (5 g) were treated with the three cellulolytic preparations in 0.1 M acetate buffer pH 5 (50 ml) with approximately 100 mg protein g^{-1} fabric enzyme dosage at 1:10 fabric to liquor ratio, for 24 h at 50 °C. Stainless steel discs were added to the reaction mixture to promote high level of mechanical agitation. After the enzymatic reaction, samples were centrifuged (2875 *g*). The supernatant was analysed for protein and soluble sugars determination (see Analytical methods) and the cotton powder with adsorbed enzymes was used to carry out the desorption experiments. Samples from the reaction mixture were also collected for particle size analysis. Two replicates were carried out for each enzymatic treatment.

Desorption experiments

The desorption of enzymes from the cotton powder was made by successive washings of the cellulosic material with buffer (50 ml) at 25 °C, 125 rpm in a shaker bath for 30 min at 25 °C to avoid extensive hydrolysis during the desorption process and enhance preferential desorption. After each washing, the samples were centrifuged and the supernatant collected for determination of protein desorption and sugar production (see Analytical methods). The cotton powder was then used in a subsequent washing. The process was repeated until no protein was found in the supernatant. After this process, the cotton powder was washed with distilled water to remove acetate buffer used during washing and dried in a oven (30 °C) and analysed for determination of DP. Two replicates were carried out for each desorption experiment.

Analytical methods

Total protein in solution was measured by the Bradford (1976) method with BSA as standard. Soluble reducing sugars were determined using the neocuproine method as described by Cavaco-Paulo *et al.* (1996) with glucose as standard. Each determination was done in duplicate.

The degree of polymerisation (DP_n) of the cotton powder was determined by gel permeation chromatography (GPC) as described before (Ramos *et al.* 1999). It was also calculated the polydispersity value which measures the distribution amplitude of DP. The size of the cotton particles were measured using a Mastersizer Analyser (Malvern Instruments Ltd., Malvern, UK).

Results and discussion

The enzymatic hydrolysis of cellulosic substrates is a result of a concerted action of different types of cellulases. 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.

In this work, cotton fabrics were treated with three cellulase preparations at a high agitation level. After the enzymatic treatments the percentage of protein adsorption was determined and how much of that protein could be desorbed by washing with buffer. The results of these experiments are shown in Figure 1.

When a high level of mechanical agitation and high enzyme concentration are used there is a production of great amounts of cotton powder which has a high surface area. This leads to higher levels of adsorption (46, 39 and 34% for TC, EG-rich and CBH-rich preparation, respectively) than when it is used, in mild conditions (lower enzyme dosages, shorter treatment times and low level of mechanical agitation). TC shows greater level of adsorption and this leads to a greater extent of hydrolysis shown by higher production of soluble reducing sugars (Figure 1). This greater solubilization of the cotton fabric caused by the action of TC is also shown by the lower size of the cotton particles produced (Table 1). With CBH-rich mixture, however, there is also a low value for particle diameter

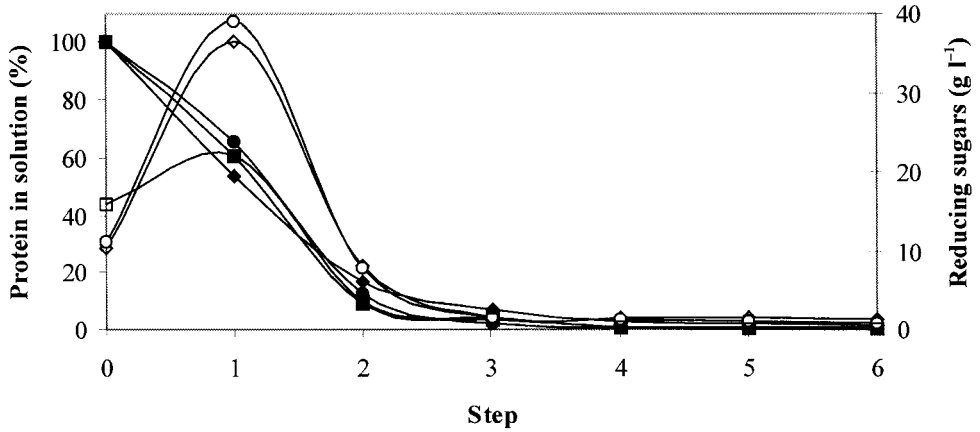

Fig. 1. Protein and sugar content of the supernatants before (step 0) and after (step 1) the enzymatic treatments of cotton fabrics with the three cellulases preparations and during the desorption process (steps 2, 3, 4, 5 and 6). Protein: ♦, TC; ■, EG-rich; ●, CBH-rich (protein content of the supernatants is given in percentage, corresponding 100% to the amount of protein present initially; TC: 7.7 g l^{-1}, EG-rich: 7.3 g l^{-1}, CBH-rich: 11.2 g l^{-1}). Reducing sugars: ◊, TC; □ EG-rich; ◯, CBH-rich.

Table 1. Characterization of the cotton powder in terms of degree of polymerisation (DP), polydispersity and particle size after cotton fabric has been subjected to enzymatic treatment using different cellulase preparations (≈100 mg protein g^{-1} fabric, 1:10 fabric to liquor ratio, pH 5, 50 °C, 24 h, high level of mechanical agitation).

Sample	DP_n[b]	Pd[c]	Particle size[d] (μm)
Original fabric	507	7	–
Control fabric[a]	429	4.9	–
Cotton powder after hydrolysis with TC	348	4.7	21.8
Cotton powder after hydrolysis with EG-rich	303	5.3	26.8
Cotton powder after hydrolysis with CBH-rich	388	4.4	21.3
Cotton powder after hydrolysis with TC and 5 washings	250	6.7	–
Cotton powder after hydrolysis with EG-rich and 5 washings	183	6.9	–
Cotton powder after hydrolysis with CBH-rich and 5 washings	370	5.7	–

[a]Fabric treated with buffer and discs.
[b]Number average degree of polymerization.
[c]Polydispersity ($P_d = DP_w/DP_n$ where DP_n is weight average degree of polymerisation).
[d]Average particle diameter.

but with this preparation the percentage of adsorption is lower. Since the mode of action of EGs is more in the way of cutting the cellulose chain at most accessible points, it is expected that bigger fragments of cellulose would be released to the solution. This is confirmed by the results given in Table 1 which show that the cotton particles obtained from the EG-rich treatment present a bigger size.

In Figure 1 it can be seen that most of the protein remains in the supernatant after the cotton hydrolysis (step 1 in Figure 1). About 54% for TC, 61% for EG-rich and 66% for CBH-rich. In the following washing (step 2), and for the TC preparation, 37% of the protein adsorbed was recovered, which corresponds to 17% of the protein present initially before the treatment (1.3 g protein l^{-1}). In the case of EG-rich, 23% of the adsorbed enzyme was recovered in the first washing, representing 17% of the total protein used (0.7 g protein l^{-1}). For the CBH-rich preparation, 37% and 13%, respectively (1.4 g protein l^{-1}). In the following washings it is possible to recover more enzymes but in a decreasing way. At the end of the fifth washing (step 6) almost no protein could be detected in the supernatant. In the total of the five washings it is possible to recover 57, 35 and 47% of the protein adsorbed for the three cellulase preparations. Combining all the supernatants it is possible to recover 80, 75 and 82% of the total protein used initially.

This washing process leads to diluted enzyme solutions, which can be concentrated for example using an ultrafiltration membrane with an appropriate molecular weight cut-off. This technique also allows the separation of reaction products from the supernatants, which are known to cause cellulase inhibition. After this separation, cellulases remain active and therefore the enzymes can be reused in subsequent cotton treatments.

The concentration of reducing sugars in the supernatant was followed to investigate if there was considerable production of sugars during the desorption process (Figure 1). Some reducing sugars were found in the initial solution (cellulases + buffer) because cellulases are glycoproteins and have therefore sugars on their composition. Sugars were always detected in the supernatant but in decreasing concentrations. The producing of sugars can be due to the liberations of soluble sugars attached to the cellulose powder during washing. Some hydrolysis during the desorption can also be expected. Indeed, the DP decreases after the washing of the cotton powder with buffer. The increase in polydispersity (Table 1) after the washings also reveals some action of the enzymes towards the cellulose particles.

Analysing the results shown in Table 1, it can be seen that the EG-rich preparation has a major effect in the DP_n (reduces the DP) than the other two preparations, despite the particle size being higher. This is in agreement with other findings that the EGs cause more decrease of DP (Morgado *et al.* 2000, Ramos *et al.* 1999). The DP analysis results show that TC and CBHs act more in terms of cellulose solubilization (higher sugars yield and lower particle size) and EGs are more effective on decrease the cellulose chain length, producing cellulose particles with lower size. With CBH-rich preparation, however, the DP was not significantly reduced after washing but there was an increase in the polydispersity value, revealing some endoglucanase activity. Despite in this preparation the main endoglucanase activities were deleted, there are some contaminations of minor EGs which can be responsible for this increase in polydispersity.

Conclusions

The insoluble cellulosic material produced during enzymatic hydrolysis of cotton presents a high surface specific area for cellulase adsorption (shown by the determination of particle size and by the higher levels of protein adsorption). This insoluble material can be separated from the liquor treatment by simple sedimentation, filtration or centrifugation and the desorption of enzymes from this powder can be carried out by simple washing with buffer. In this way it is possible recover a significant amount of cellulases which can be recycled in posterior treatments.

TC and CBH-rich preparations produce a cellulose powder more fine than EGs. The desorption process caused a decrease of DP specially for the cotton treated with EGs but an increase in polydispersity for all preparations.

References

Cavaco-Paulo A (1998) Mechanism of cellulase action in textile process. *Carbohydr. Polym.* **4**: 423–428.

Cavaco-Paulo A, Almeida L, Bishop D (1996) Effects of agitation and endoglucanase pre-treatment on the hydrolysis of cotton fabrics by a total cellulase. *Textile Res. J.* **66**: 287–294.

Duff SB, Moritz JW, Casavant TE (1995) Effect of surfactant and particle size reduction on hydrolysis of deinking sludge and nonrecyclable newsprint. *Biotechnol. Bioeng.* **45**: 239–244.

Kim DW, Jang YH, Jeong YK (1997) Adsorption behaviors of two cellobiohydrolases and their core proteins from *Trichoderma reesei* on Avicel PH 101. *Biotechnol. Lett.* **19**: 893–897.

Lee SB, Shin HS, Ryu DDY, Mandels M (1982) Adsorption of cellulase on cellulose: effect of physicochemical properties of cellulose on adsorption and rate of hydrolysis. *Biotechnol. Bioeng.* **24**: 2137–2153.

Morgado J, Cavaco-Paulo A, Rousselle M-A (2000) Enzymatic treatment of lyocell – clarification of depilling mechanisms. *Textile Res. J.* **70**: 696–699.

Ramos LP, Zandoná Filho A, Deschamps FC, Saddler JN (1999) The effect of *Trichoderma* cellulases on the fine structure of a bleached sofwood kraft pulp. *Enzyme Microbiol. Technol.* **24**: 371–380.

van Wyk JPH (1997) Cellulase adsorption-desorption and cellulase saccharification during enzymatic hydrolysis of cellulose materials. *Biotechnol. Lett.* **19**: 775–778.