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A Comparative Study of Different Cellulase Preparations in the Enzymatic Treatment of Cotton Fabrics

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Abstract

Twenty-nine cellulase preparations from different sources were compared in terms of their abrasive activities (the ability to remove Indigo from denim) and their ability to saccharify cellulose. No direct relationship could be found between these two abilities. The preparations were divided into three groups: (1) with a high yield of reducing sugars after 24 h hydrolysis of Avicel cellulose but low abrasive activity; (2) universal cellulases that could both effectively hydrolyze cellulose and remove Indigo from denim; and (3) cellulase samples with high abrasive activity but low saccharification ability.

Cellobiohydrolase (CBH) and xylanase were purified from different fungi by chromatofocusing on a Mono P column and subjected to limited proteolysis with papain yielding cellulose-binding and core (catalytic) domains. The adsorption ability and backstaining index of both CBH and xylanase core proteins were notably lower than the respective parameters for the initial nondigested enzymes indicating that protein adsorption on the surface of cotton fibers is a crucial factor causing Indigo backstaining during the enzymatic denim washing procedure.

Index Entries: Cellulase; limited proteolysis; adsorption; textile; denim fabrics; stone washing; Indigo; backstaining.

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Introduction

Several groups of enzymes, including amylases, cellulases, proteases, catalases, and peroxidases, are widely used in the textile industry *(1,2)*. Amylases are used for desizing, i.e., the removal of starch from textile materials. Cellulases find such applications as biopolishing of cotton fabrics and denim treatment. They can also induce special effects on cellulosic fabrics and garments. Proteases are used for modification of wool and silk. Catalases and peroxidases are applied for removal of excess hydrogen peroxide after bleaching of fabrics. Lipases, pectinases, and ligninases also have a potential for use in textile processing *(2)*.

In the last decade, cellulases have been widely applied for a denim garment treatment as an alternative to the stone washing process in order to achieve an aged look of denim fabrics *(3–5)*. In practice, they displaced pumice stones and chemical agents used in denim treatment processes previously. One of the problems arising during the enzymatic procedure is Indigo backstaining *(3)*. An ideal enzyme for the denim biowashing process would provide high abrasive activity (the ability to remove Indigo from denim) and low backstaining. At the same time, the enzyme must softly modify the surface of fabrics without losing the strength of cellulose fibers.

Recently, it has been shown that the high ability of cellulase enzyme protein to bind cotton cellulose is the major issue that causes backstaining *(6,7)*.

The aim of this work was a comparative study of different cellulase preparations from the point of view of their action on denim fabric in the process of Indigo removal. Hydrolytic activities of the preparations (in the reaction of cellulose saccharification) were also compared, since the ability of cellulase to conduct a deep conversion of cellulose to sugars may serve as a measure of its aggressiveness toward cellulose fibers. Additional evidence that protein adsorption on the surface of cotton fibers is a crucial factor causing Indigo backstaining is provided as a result of studying adsorption and backstaining properties of two purified enzyme components subjected to limited proteolysis.

Materials and Methods

Enzymes and Activity Assays

Twenty-nine commercial and laboratory cellulase preparations produced by different fungi (from genera *Trichoderma, Penicillium, Chaetomium, Humicola*, and others) were used. Specific activities of the preparations are given in Table 1. Filter paper activity (FPA) was determined by the IUPAC standard assay *(8)*. Endoglucanase activity was determined by the viscometric method *(9)* at pH 4.5 and 40°C using carboxymethylcellulose (CMC, medium viscosity, Sigma) as a substrate. CMCase activity was measured by assaying reducing sugars released after 5 min of enzyme reaction with CMC (5 g/L) at pH 5.0 and 50°C *(9)*. Avicelase activity was determined by

Table 1
Specific Activities (U/mL or U/g) of Cellulase Preparations Used in the Study

Preparation	FPA	Endoglucanase	CMCase	Avicelase
1	17	333	1037	13
2	18	321	1100	15
3	18	167	520	6
4	30	742	884	11
5	23	388	814	9
6	18	396	895	8
7	28	440	1141	11
8	22	681	2812	7
9	28	340	530	8
10	49	560	1100	19
11	170	760	1050	178
12	500	3875	7000	276
13	126	927	4815	329
14	129	1569	4968	182
15	161	1900	4137	192
16	131	1262	3333	244
17	52	1772	21480	52
18	68	94	904	84
19	98	532	6288	115
20	27	153	790	38
21	22	847	1351	3
22	11	43	259	15
23	11	243	134	14
24	12	115	841	3
25	155	1250	4500	184
26	111	1782	4893	232
27	3	44	79	1
28	27	1552	1835	8
29	16	272	1240	8

analyzing reducing sugars released after 5 min of enzyme reaction with 5 g/L Avicel cellulose (Serva, Germany) at pH 5.0 and 40°C (*9*). Reducing sugars were analyzed by the Somogyi–Nelson method *(10)*. All activities were expressed in International Units, where 1 U of activity corresponded to the quantity of enzyme hydrolyzing 1 μm of glucoside bonds per 1 min.

Saccharification of Cellulose

The concentration of reducing sugars after 24 h hydrolysis of Avicel cellulose was chosen as a criterion of saccharification ability of cellulase preparations. Conditions of the hydrolysis were the following: substrate concentration 50 g/L, cellulase activity 1 filter paper unit (FPU)/mL, 50°C, pH 4.5–5.0. Hydrolysis of Avicel was carried out under magnetic stirring in 10-mL test tubes placed in a thermostatic water bath.

Treatment of Denim with Cellulase

Enzymatic treatment of denim fabric was conducted as follows. Denim swatch (10 × 10 cm), desized with α-amylase, was placed in a special reactor containing 30 mL of enzyme solution in 0.1 *M* Na-acetate buffer (pH 5.0). Processing was carried out at 50°C under intensive agitation for 1 h. As a control, another piece of denim fabric was processed under the same conditions in the reactor containing the acetate buffer without an enzyme. Samples were rinsed with tap water for 1 min and dried overnight at room temperature, and then a colorimetric analysis of the samples was carried out. The analysis included scanning the surface of samples at 300 dpi resolution on a MFS-12000SP scanner ("Mustek") followed by computer processing using Adobe Photoshop 4.0 software. Efficiency of Indigo removal was assessed as a difference in the color intensity between control and enzyme-treated samples using a blue channel of the software. For each enzyme preparation, an assay experiment was conducted in duplicate.

Adsorption and Backstaining Assays

Adsorption and backstaining properties of xylanase and cellobiohydrolase (CBH) from cellulase preparations nos. 2 and 14, respectively, were studied as described in a preceding paper *(7)*. Enzyme purification procedure using chromatofocusing on a Mono P (Pharmacia, Sweden) column is also described in that paper.

Limited Proteolysis of Enzymes with Papain

Limited proteolysis of the CBH and xylanase was carried out incubating the enzymes with activated papain (Sigma) *(11)*. The enzyme to papain ratio was 30:1 (w/w). Enzyme sample (300 μL, 2 mg/mL) was incubated with 10 μL of papain (2 mg/mL, 30 BAEE U/mL) at pH 6.5 for 6 h.

Core proteins of CBH and xylanase were separated by gel-filtration on a Superose 12 HR 10/30 column (Pharmacia) using a Pharmacia FPLC System. Column was equilibrated and elution was carried out with 0.1 *M* acetate buffer (pH 5.0). Protein concentration at the column outlet was monitored photometrically at 280 nm using an ultraviolet detector.

Results and Discussion

Saccharification Ability and Abrasive Activity of Different Cellulase Samples

In a comparative study of different cellulase preparations, we equalized conditions in the reaction system by the filter paper activity. The activity was 1 FPU/mL in the Avicel saccharification experiments. During the enzymatic treatment of denim fabric, 0.05 FPU/mL were used.

Figure 1 shows the yield of reducing sugars after 24 h hydrolysis of Avicel cellulose and abrasive activities (the ability to remove Indigo from

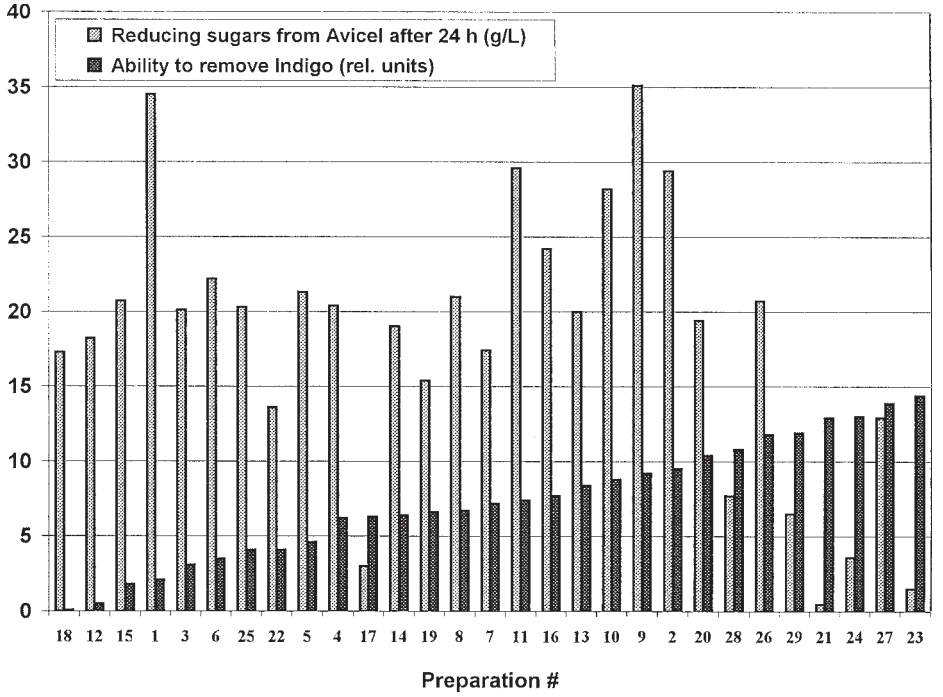

Fig. 1. Comparison of abilities to hydrolyze cellulose (left bars) and abrasive activities (right bars) for different cellulase preparations. The numbering of cellulase samples corresponds to Table 1. The samples are sorted in the order of increasing ability to remove Indigo from denim.

denim) for cellulase preparations under study. It can be seen from Fig. 1 that the preparations differed dramatically in their ability to saccharify cellulose and their abrasive activity. It should be noted that, as it would be expected, we observed a satisfactory correlation between the Avicelase activity (Table 1) and the yield of sugars after 24 h hydrolysis of Avicel. Taking into account all the enzyme preparations under study, the correlation coefficient was found to be 0.51. For a selected pool of enzyme samples, including preparations from one fungal genus (i.e., for the series of *Penicillium* preparations produced under different fermentation conditions and differing by component composition), the correlation coefficient reached 0.96. However, we could not find any direct correlation between the ability of preparations to remove Indigo from denim fabric and any specific activity given in Table 1. So, a question about a key component of the cellulase multienzyme system responsible for the dye removal is still open.

Analyzing data presented in Fig. 1, one could divide the enzyme preparations into three major groups. Preparations pertaining to the first group possessed a high-saccharification ability (they provided a high yield of reducing sugars after 24 h hydrolysis of cellulose), but they had a relatively low-abrasive activity (sample numbers 1, 3, 5, 6, 12, 15, 18, 22, and 25). Enzyme samples from the second group represented universal prepara-

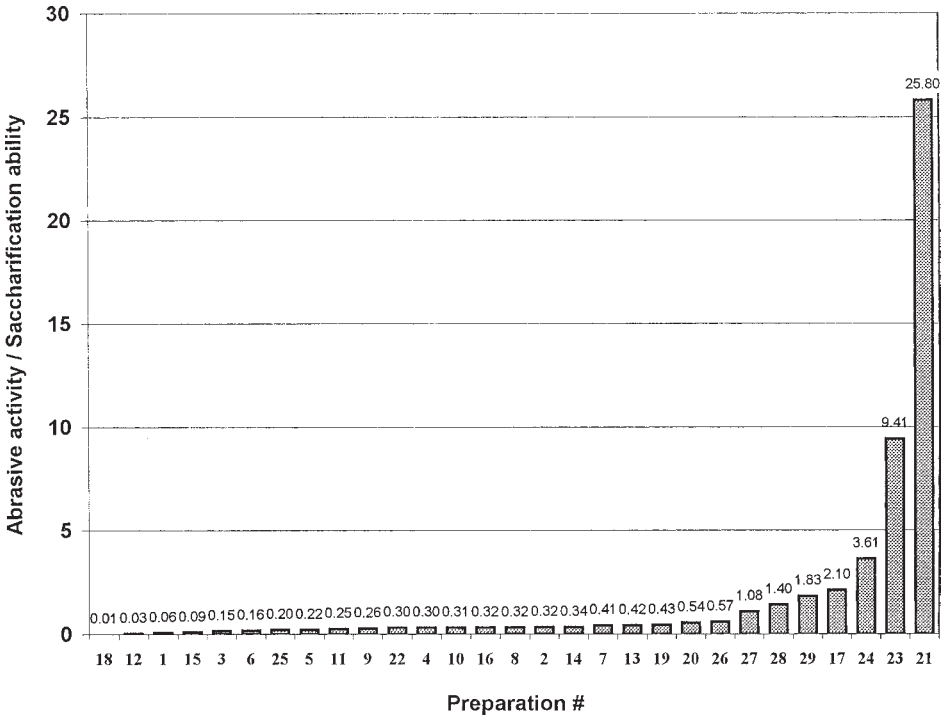

Fig. 2. Comparison of cellulase preparations from the point of view of their "softness" and "aggressiveness." Numeration of cellulase samples is the same as in Fig. 1 and Table 1. The samples are sorted in the order of increase in cellulase softness.

tions, able to both effectively hydrolyze cellulose and remove Indigo from denim (numbers 2, 4, 7, 8, 9, 10, 11, 13, 14, 16, 19, 20, and 26). The third group included cellulase samples possessing a high abrasive activity but exhibiting low saccharification ability (numbers 17, 21, 23, 24, 27, 28, and 29).

If we introduce an arbitrary parameter representing a ratio of the abrasive activity to the yield of reducing sugars from cellulose (*see* data in Fig. 1), it can be used as a criterion of "softness" or "aggressiveness" of cellulase toward cellulose. Such ratios for the cellulase samples under study are shown in Fig. 2, where the preparations are sorted in the order of increase in cellulase softness. From the analysis of data shown in Figs. 1 and 2, one may conclude that the first group of enzymes (according to the classification described in the preceding paragraph) represented the most aggressive cellulases, for which the Indigo removal efficiency/saccharification ability ratio varied within the range from 0 to 0.25. For the second group of cellulases (universal enzymes), the ratio varied from 0.25 to 1. The most soft cellulases were in the third group, for which the ratio had a value higher than 1.

It is obvious that the last group, including the most soft cellulases, is the most appropriate for textile processing, where the enzyme can gently modify the surface of fabrics without losing the strength of cellulose fibers.

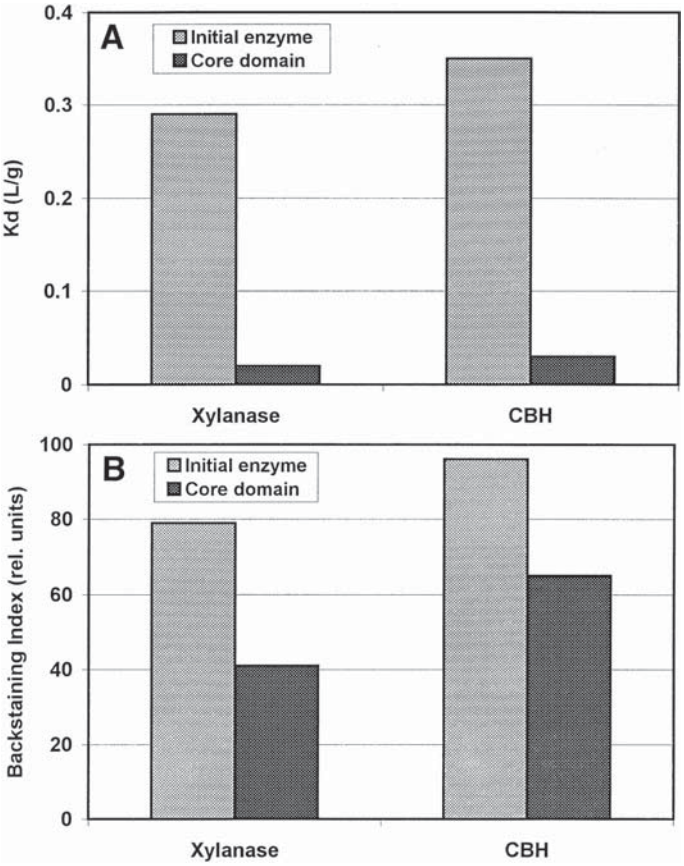

Fig. 3. Adsorption distribution coefficients **(A)** and backstaining indexes **(B)** for the initial nondigested xylanase from the enzyme preparation no. 2, CBH from preparation no. 14, and their core domains obtained by limited proteolysis.

Indigo Backstaining in the Presence of Purified CBH and Xylanase

In a previous paper, we found direct correlations between the ability of protein to bind to cellulose and Indigo backstaining *(7)*. The relationships have been found for both crude cellulase samples and purified enzyme components. In the present paper, an additional study focusing on enzyme adsorption and backstaining was carried out for two purified enzymes from different sources having a high ability to bind to cellulose.

Xylanase and cellobiohydrolase (CBH) from cellulase preparations nos. 2 and 14, respectively, were purified by chromatofocusing on a Mono P column *(7)*. Both enzymes were homogeneous as judged by sodium dodecyl sulfate (SDS)-electrophoresis and isoelectrofocusing in polyacrylamide gels. Xylanase had a molecular weight of 65 kDa and p*I* 4.5. CBH had the same molecular weight as xylanase (65 kDa), and its p*I* was 3.9. After the adsorption on 5% Avicel cellulose at 50°C and pH 5.0 for 15 min, 82 and 69% of the enzymes (by protein assay) were bound to cellulose.

The purified xylanase and CBH were subjected to limited proteolysis with papain. As a result of the proteolysis, the core proteins with molecular weights of 51 and 56 kDa, respectively, were formed according to the SDS-polyacrylamide gel electrophoresis (PAGE) analysis. Also, proteins with molecular weights of approx 14 and approx 10 kDa, respectively, representing cellulose-binding domains of xylanase and CBH, were detected. The core proteins were separated by gel-filtration on a Superose 12 column.

Figure 3A and B show the adsorption distribution coefficients and backstaining indexes for the initial purified enzymes and their core proteins. It can be seen from Fig. 3 that both the adsorption ability and backstaining significantly decreased after removal of cellulose-binding domains of the enzymes by limited proteolysis.

These results provide an additional proof to the previously published data *(6,7)* that the enzyme adsorption on cellulose fibers is a crucial parameter causing Indigo backstaining.

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