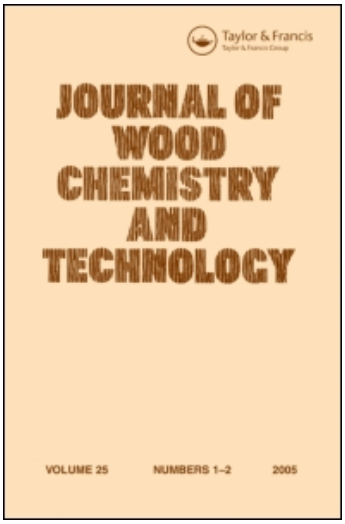

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Removing Hemicellulose from Pulps by Specific Enzymic Hydrolysis

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REMOVING HEMICELLULOSE FROM PULPS BY SPECIFIC ENZYMIC HYDROLYSIS

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ABSTRACT

The hemicellulose content (solubility in 18% NaOH) of a delignified mechanical aspen pulp was lowered from 23.4% to 18.2% by one-hour hydrolysis with xylanase isolated from the fungus Schizophyllum commune by fractional precipitation. After 24 h hydrolysis, the hemicellulose content was reduced further to 12.9%. The predominant hydrolysis products, xylose and xylobiose, confirmed the specificity of hydrolysis. A crude mixture of cellulolytic and hemicellulolytic enzymes from the same microorganism gave glucose as the major hydrolysis product, and a pulp with higher relative hemicellulose content. Xylan-specific hydrolysis of a low-yield sulfite pulp gave only a small decrease in pentosan content.

INTRODUCTION

The removal of hemicellulose from wood fibre is a necessary step in the manufacture of dissolving pulp and, at present, is usually accomplished by an ultra-hot alkaline extraction stage within a bleaching sequence[1]. An alternative method to remove hemicelluloses would be by treating pulps with enzymes which react only with the hemicellulose portion in pulp. Enzymes are usually very specific catalysts; those that hydrolyse xylan, for example, will not generally attack cellulose or mannan. The specificity of

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cellulases and various hemicellulases has been used previously to identify morphological features in wood[2,3] and to determine xylan distribution in holocellulose fibres[4]. However, highly purified enzymes were used in these studies and, as such, these would be expensive to produce for any industrial application. We now report a simple fractional precipitation technique for crude separation of cellulase and xylanase enzymes produced from the fungus Schizophyllum commune.

The objective of the present work was to determine whether the fungal enzymes could produce a hemicellulose-free pulp which might then be suitable for the manufacture of rayon. In the first stage of the investigation an attempt was made to remove as much hemicellulose as possible from a delignified mechanical pulp, which could then be further extracted with alkali. Success with this led to further tests with a conventionally bleached chemical pulp.

MATERIALS AND METHODS

Pulps

The mechanical pulp was aspen thermomechanical pulp (TMP) (about 90% yield; 2.1 MJ/kg) delignified by chlorite bleaching[5] as follows:

Sodium chlorite (80 g) was dissolved in one litre of water. Glacial acetic acid (15 ml) was added and the solution was further diluted to 1.1 litre. Never-dried pulp (30 g, oven-dry basis) was mixed with this solution at 40° C for 40 h. The pulp was drained and washed with water. The yield was 84% based on unbleached pulp, and the Klason lignin content was less than 2%.

The chemical pulp was a screened mixed hardwood sulfite pulp (unscreened yield about 44%). The pulp was either chlorinated and washed (partially bleached) or subjected to a conventional CED bleach sequence. The brightness of this bleached pulp was 89.4% ISO; the kappa number measured after CE was 2.9.

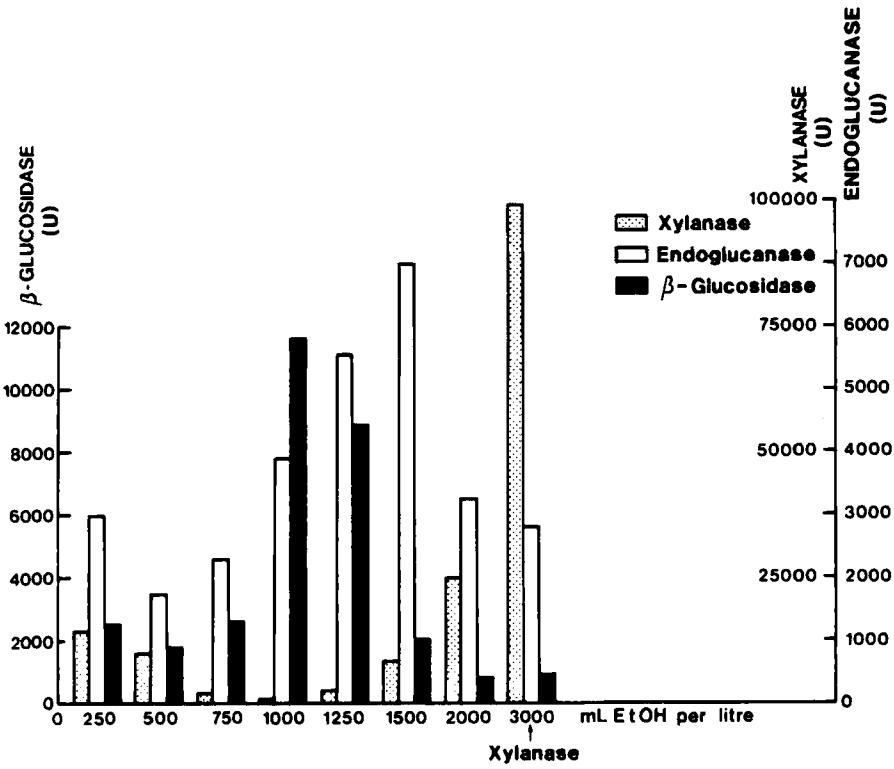

FIGURE 1. Step-wise additions of ethanol to the crude Schizophyllum commune culture filtrate at 4°C gave precipitates which were removed by centrifugation after each addition. The bars represent total enzyme activities found in the individual precipitate fractions. The fraction labelled xylanase was isolated.

Enzyme treatments

Enzymes were produced by culturing Schizophyllum commune for nine days in the presence of cellulose, as described previously[6]. The culture solution was clarified by centrifugation (7000 x g at 5°C for 10 min) and either stored frozen or fractionally precipitated with ethanol; Figure 1 shows the fractionation profile and composition of the xylanase-rich material.

Xylanase and endoglucanase activities were determined by incubating suitably diluted enzyme solutions (1 ml) with either a

1% solution of larch xylan (Sigma Chemical Co.) or carboxymethylcellulose (type 7LT; Hercules Inc.), as described previously[7]. Enzyme units (U) were defined as the release of one µmol of reducing sugar (as xylose or glucose equivalents) per min. β-Glucosidase activity was assayed by hydrolysis of p-nitrophenyl-β-D-glucoside (Sigma Chemical Co.) at pH 5.0 as described previously[7], and units were expressed as µmol of nitrophenol produced per minute.

Pulps were treated at 3% consistency in 0.2M sodium acetate buffer, pH 5.0 at 30°C. Pulp slurry (200 ml) was reacted with enzyme (or just buffer for blanks) in Erlenmeyer flasks (500 ml) shaken at 250 rpm (New Brunswick Scientific Co. Gyratory Shaker). Pulps were thoroughly washed with pH 5.0 buffer (mechanical pulp) or 1% NaOH (chemical pulp) followed by water, and handsheets were made on a 200 mesh screen. Experiments with mechanical pulps were performed once except yield and 18% NaOH solubility determinations which were duplicated for two experiments to establish the error limits shown in Table 1. All experimental points for chemical pulps are plotted (Figs. 3 to 5).

TABLE 1

Properties of Delignified Aspen TMP Before and After 24 h Treatment With Enzymes

Treatment	Time, h	Xylanase conc., U/ml	Yield*, % (±2)	18% NaOH solubility**, % (±0.5)	Pentosans**, %	Viscosity, 0.5% cuene, mPa.s
Buffer control	1	0	99	23.4	20.4	16.8
Xylanase fraction	1	300	87	22.3	16.9	10.4
	24	300	74	20.2	12.7	9.2
Xylanase fraction	1	1600	71	18.2	13.4	8.7
	24	1600	65	12.9	9.1	6.4
Crude total enzyme	1	300	67	22.0	17.7	8.4
	24	300	18	28.0	n.d.	n.d.

* Delignified pulp = 100% n.d. = not determined
** Pulp remaining after the treatment = 100%

Soluble sugar analysis

Sugars in the reaction mixture were determined by high performance liquid chromatography (HPLC; Waters Associates, Model 204 system). Samples up to 100 µl were filtered (8 µm filter, Millipore) and injected onto a HPX-87 ion exchange column (Bio-Rad Ltd.) maintained at 85°C. The sugars were detected with a differential refractometer (Waters Associates, Model R401), identified by comparison of retention times with standards, and quantified by an internal standard (myo-inositol) peak height method, as described previously[8]. Sugar standards were Fisher reagent grade, except xylobiose which was tentatively identified by comparison with chromatograms of xylan hydrolysates.

Pulp analyses

The following standard tests (Canadian Pulp and Paper Association Standard number bracketed) were performed:

Viscosity	(0.5% cuene; G24)
Pentosans	(G12)
Kappa number	(G18)
Klason lignin	(G 9)

RESULTS AND DISCUSSION

Mechanical Pulp

The enzyme treatments were performed on hardwood pulps, since their predominant hemicellulose, xylan[9], is hydrolysed by xylanase from Schizophyllum commune. Chlorite delignification of aspen TMP gave a pulp almost free of lignin (less than 2% Klason), but with a high hemicellulose content as shown by the sodium hydroxide solubility and pentosan content (see control in Table 1).

The fractional precipitation of crude enzyme solution from Schizophyllum commune with ethanol (Figure 1) gave a xylanase fraction which contained relatively little cellulase activity.

This is shown by the endoglucanase and β-glucosidase activity distributions; the total enzyme contained the sum of all the fractional activities which would allow synergistic hydrolysis of cellulose fibres[6]. The xylanase fraction contained low endoglucanase and, especially, β-glucosidase activity and the synergistic action of the latter two was thus lost, resulting in very limited ability to hydrolyze cellulose to glucose.

The objective of the enzyme treatment was to obtain a pulp in high yield with low hemicellulose content (solubility in 18% NaOH and pentosans) and high viscosity. Table 1 shows the pulp properties obtained. Hydrolysis of the pulp with non-specific crude total enzyme (last two lines) gave no significant relative decrease in hemicellulose, and poor yields. On the other hand treatment with xylanase-rich fractions at two different concentrations gave better yields of pulp and the hemicellulose content was lower than the control. However, all treatments resulted in lower viscosity pulps, indicating cellulose hydrolysis. The higher specificity observed with xylanase is confirmed by HPLC analyses of the sugars produced from the pulps during hydrolysis (Figure 2). The pentose sugars xylobiose and xylose predominate in both xylanase treatments, while glucose is the major sugar from the total enzyme hydrolysis (Table 2).

TABLE 2

Sugars Produced By 1 h Treatment of Delignified Aspen TMP With Various Enzymes

Treatment	Xylanase Conc. U/ml	Endoglucanase Conc. U/ml	Xylobiose Wt. %*	Xylose Wt. %*	Glucose Wt. %*	Ratio Pentose/Hexose	Total Sugars Wt. %*
Xylanase fraction	300	9	2.6	0.9	0.5	7.0	4.0
Xylanase fraction	1600	48	2.9	3.2	1.1	5.5	7.2
Crude total enzyme	300	23	1.7	8.0	14.7	0.66	24.4

* Untreated pulp = 100%

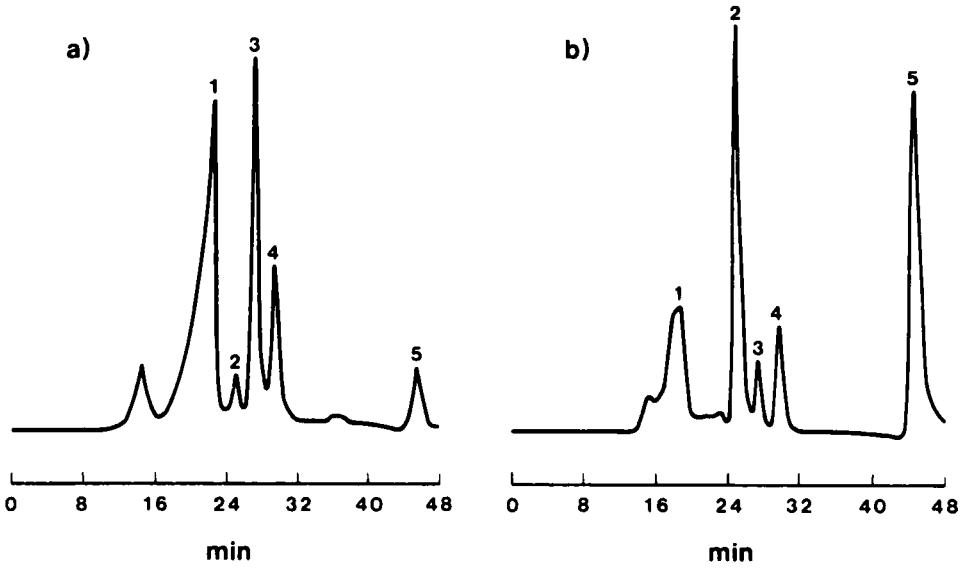

FIGURE 2. HPLC of sugars extracted from aspen TMP by (a) total enzyme and (b) xylanase fraction (300 U/mL) from S. commune. The numbered peaks represent (1) oligosaccharides and enzyme, (2) xylobiose, (3) glucose, (4) xylose, and (5) myo-inositol (internal standard).

If the treatment of the pulp with enzymes resulted solely in partial conversion into sugars, then the sum of soluble sugars should approximately equal the pulp yield loss. Inspection of Tables 1 and 2 shows that the soluble sugars are always lower than expected. This apparent discrepancy may be due to a number of factors, including unquantified oligosaccharides in the hydrolysate, and fibre fragmentation during hydrolysis followed by loss of fibre fines and less-soluble oligosaccharides during handsheet formation.

Chemical Pulp

The chemical pulp was subjected to similar enzyme treatments as was the mechanical pulp, but since less hemicellulose was pre-

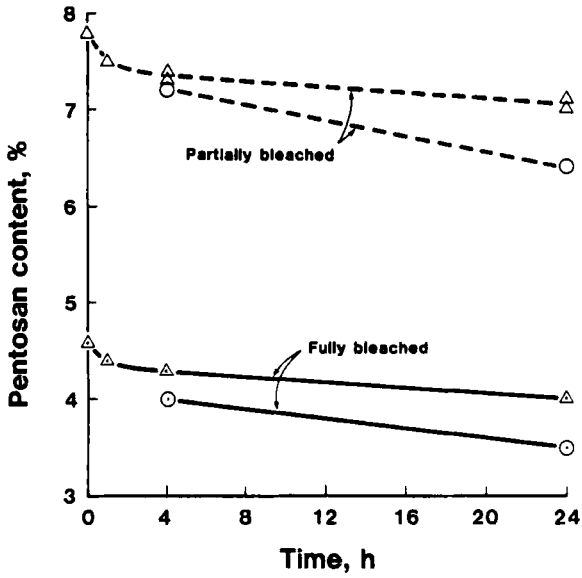

FIGURE 3. Pentosan content of partially-bleached (----) and fully-bleached (——) chemical pulps following treatment with total enzyme (Δ) or xylanase fraction (0).

sent, more dilute enzyme solutions were used. Each enzyme solution contained 13 units/mL of xylanase; the total enzyme solution also contained 0.6 units/mL of endoglucanase. Two kinds of chemical pulps were compared to investigate the effect of residual lignin on hemicellulose accessibility; one was partially bleached (chlorinated and washed with water - no alkaline extraction) while the other was fully bleached in a CED sequence. Figures 3, 4 and 5 show the effect of enzyme treatment of these pulps on pentosan content, yield and viscosity, respectively.

Two observations follow from these figures. First, the xylanase treatment is more specific for hemicellulose removal than the total enzyme treatment. This is shown by the lower pentosan content (Figure 3) and higher yield (Figure 4) of pulps treated ith xylanase. Second, the pentosans in the fully-bleached pulps

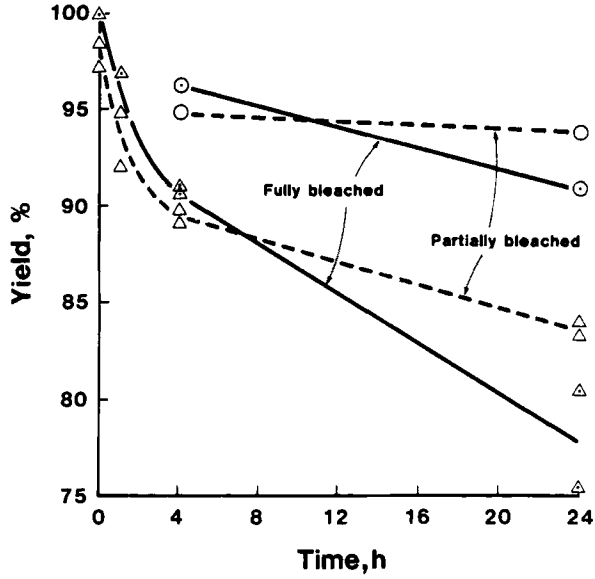

FIGURE 4. Yield of partially-bleached (----) and fully-bleached (——) chemical pulps following treatment with total enzyme (Δ) or xylanase fraction (0).

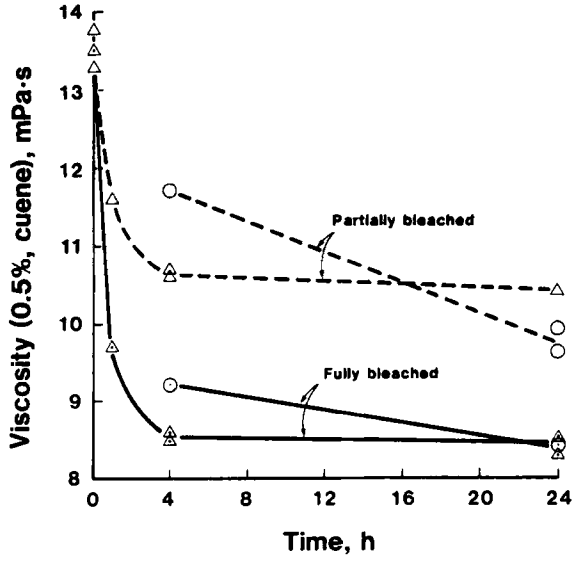

FIGURE 5. Viscosity of partially-bleached (----) and fully-bleached (——) chemical pulps following treatment with total enzyme (Δ) or xylanase fraction (0).

are no more accessible to enzymes than in the partially-bleached pulp, since the rate of pentosan removal is approximately equal for both pulps (Figure 3). This is somewhat surprising as one would expect increased accessibility to enzymes after lignin removal, and indeed the cellulose fibres are more easily hydrolysed, as shown by the drop in viscosity (Figure 5). Since full bleaching results in removal of both lignin and hemicellulose (see Figure 3, zero-time values), it may be that the more accessible hemicellulose was removed during the bleaching.

An alternative explanation for the incomplete hydrolysis of hemicellulose, even in delignified fibres, could be that the hemicellulose has been chemically modified during pulping and the resulting polysaccharide is not recognized by the enzyme. Some evidence exists for polysaccharide modification during sulfite cooking[10]. Also xylan is lost at a faster rate during sulfite cooking than glucomannan. Thus, the remaining xylan may also be interspersed with other hemicelluloses even though these were of relatively low abundance in the original wood.

CONCLUSIONS

The hemicellulose content of a holocellulose derived from mechanical pulp was lowered to a minimum of 12.9% by hydrolysis with a xylan-specific enzyme mixture. By comparison, a crude mixture of cellulolytic and hemicellulolytic enzymes gave a lower yield of pulp with higher hemicellulose content.

Treatment of low-yield chemical pulp (low pentosan content) with xylanase did not result in significant further removal of pentosan. A target value for dissolving pulp (2% pentosan) could not be attained, since the pentosans were either shielded from or not recognized by the enzyme. Viscosities of treated pulps were also unacceptable for dissolving pulp. Thus, the use of enzymes for removal of hemicellulose from pulps appears to be most effi-

cient if the hemicellulose content is high, such as in high-yield pulps. Enzymic treatments are less efficient with low-yield pulps.

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REFERENCES

1. F.R. Charles, Pulp Paper Mag. Can. 64 (6), 73 (1963).

2. M. Sinner, N. Parameswaran, N. Yamazaki, W. Liese and H.H. Dietrichs, Applied Polymer Symposium #28, 993 (1976).

3. M. Sinner, N. Parameswaran and H.H. Dietrichs, Adv. in Chem. Series, 181, 303 (1979).

4. J. Boutelje, K.E. Eriksson and G.H. Hollmark, Svensk Papperstidn. 74, 32 (1971).

5. W. Klauditz, Holzforschung, 11, 110 (1957).

6. M. Desrochers, L. Jurasek and M.G. Paice, App. Env. Microbiol. 41, 222 (1981).

7. M.G. Paice, L. Jurasek, M.R. Carpenter and L.B. Smillie, App. Env. Microbiol. 36, 802 (1978).

8. M.G. Paice, L. Jurasek and M. Desrochers, Tappi, 65 (7), 103 (1982).

9. B.L. Browning, (Ed.), The Chemistry of Wood, p. 70 (1975), Krieger Publishing.

10. J.K. Hamilton, E.V. Partlow and N.S. Thompson, Tappi, 41 (12) 803 (1958).