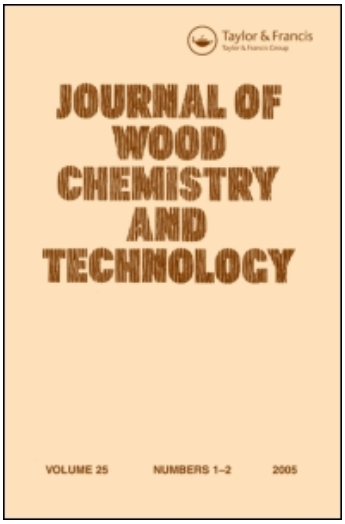

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ENZYMATIC HYDROLYSIS OF STEAM TREATED ASPEN WOOD: INFLUENCE OF PARTIAL HEMICELLULOSE AND LIGNIN REMOVAL PRIOR TO PRETREATMENT

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ABSTRACT

Aspen wood chips were treated with acidified chlorite to remove 90% of the lignin, or were extracted with concentrated alkali to remove 50% of the hemicellulose. Samples of the original chips, the resulting low-lignin aspenwood (LLA) and the low-hemicellulose aspenwood (LHA) were treated with saturated steam at 240°C for 20-180 s. Chemical analysis and enzymatic hydrolysis of the resulting water-washed substrates showed that, after 40 s of steaming, the rate and extent of enzymatic hydrolysis correlated better with removal of alkali-insoluble lignin than with removal of xylan. The original wood, LLA and LHA had similar crystallinity indices which increased in the water-insoluble fractions with time of steaming. Carbohydrate degradation products ("pseudo-lignin") formed in equal amounts on steaming original wood or LLA, indicating furfural-lignin condensation to be not involved. Development of accessibility in LHA resembled that in original aspenwood, despite the absence of acetic acid for autohydrolysis.

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INTRODUCTION

Some form of pretreatment is necessary to increase the rate and extent of the enzymatic hydrolysis of the carbohydrate components of lignocellulosic substrates (1-7). The main objective of most of the physical and chemical procedures used is to increase the accessibility of the cellulose to the cellulase enzyme complex.

Various factors have been reported to influence enzymatic hydrolysis. Grohmann et al. (8) related the improvement in enzyme digestibility of pretreated wood substrates to the removal of hemicellulose, which, according to Grethlein (9,10), results in an increase in both the accessible pore volume and the specific surface area. Stone et al. (11) showed that the median pore size is also strongly dependent on the degree of swelling. Fan et al. (12) found a linear relationship between crystallinity and rate of hydrolysis for different cellulose substrates and concluded that an effective pretreatment method should decrease the degree of crystallinity. However, other authors (13) have suggested that the correlation between the amorphous character and digestibility of cellulose appears not to be a causal one, and some researchers have indicated (10,14) that crystallinity is not of major importance. A similar contradiction can be observed when evaluating the relative influence of delignification on enzyme digestibili-

ty. Baker (15) and Stone et al. (11) reported the beneficial effect of lignin removal on enzymatic hydrolysis of wood pulps, whereas Wong et al. (16), Grethlein and Converse (10) and Brownell and Saddler (2) obtained little or no increase in digestibility after extraction of lignin from pretreated wood substrates.

Chum et al. (7) concluded from a study of the kinetics of enzymatic hydrolysis of organosolv pulps from aspen and black cottonwood, that the higher the residual xylan in these pulps, the smaller was the proportion of fast-hydrolyzable glucans and the lower was the ultimate glucan digestibility. Also, for samples containing ~13% xylan, the higher the residual lignin content, the smaller was the digestibility.

In the present work we have tried to obtain a better understanding of the relative importance of lignin and hemicellulose in the enzymatic hydrolysis of steam pretreated cellulosic substrates. By selectively removing the lignin or hemicellulose prior to steam treatment we hoped to relate the chemical composition, in particular the degree of delignification, to the rate of hydrolysis

EXPERIMENTAL

Wood and Wood Fractions

Commercial never-dried chips of aspen wood (A) (Populus tremuloides), of moisture content (MC) 37.2 % (wet-wood

basis), were used both directly in the steam pretreatment and as a source of wood fractions.

Low-lignin aspen wood (LLA). Commercial never-dried chips were reduced in size to 5-10 mm x 1-2 mm by passing them through a Wiley mill with a very coarse screen (openings of 1 cm in diameter). They were then substantially delignified by 3 treatments with sodium chlorite. Except for the larger scale of treatment and larger particle size, the treatment was essentiallly as described by Wise et al. (17). After extensive washing with distilled water and partial air-drying, the MC of the resulting LLA was 63.7 % (wet wood basis).

Low-hemicellulose aspen wood (LHA). Commercial aspen wood chips, reduced in size as for LLA, were extracted under nitrogen with a 24 % potassium hydroxide solution essentially as described by Wise and Ratliff (18), except for the larger scale and much larger particle size. The solid residue of LHA was washed with distilled water until the washings were neutral. After partial air-drying, the MC of the resulting LHA was 59.2 % (moisture-containing basis).

Steam treatment

Samples (equivalent dry weight, 60 g) of never-dried aspen wood chips, LLA and LHA, were loaded separately in a thin-walled stainless-steel canister which was then lowered through an open ball valve into a 2-L stainless steel pressure vessel (2), which had been preheated to

240°C with saturated steam. The ball valve was then immediately closed and the steam inlet valve was opened. In 5 s, the steam temperature inside the vessel and the canister reached 240°C, and was maintained constant for treatment times from 20 to 180 s. The steam inlet valve was then closed within 2 s, and the pressure was bled down to atmospheric at a controlled rate, without explosion. The canister, with contents at 100°C, was then immediately lifted out through the opened ball valve and was cooled in a water bath.

The cooled, steam-treated chips were broken up in a blender and were washed twice with distilled water, both times by stirring for 1 hour as 5 % slurries, and recovering the washed solid by filtration. In some cases, samples of the water-washed products were also similarly washed twice with 0.4 % sodium hydroxide, again by stirring as 5 % slurries.

Analytical Methods

The apparent Klason lignin contents of the original aspen wood (A), the LLA and LHA, as well as of the water-insoluble fractions (WI) of the steam-heated (SH) products - SHA-WI, SHLLA-WI and SHLHA-WI, were determined by TAPPI standard method T 222 os-74 for "acid-insoluble" lignin.

HPLC was used: a) for characterizing the water-soluble (WS) fractions of the steam-heated products, b) for

determining cellulose (or other glucan) and xylan in the water-insoluble fractions (by analyzing the sugars in the Klason lignin filtrates), and c) for analyzing for the sugars in enzymatic hydroysates. For the water solubles, and for the Klason filtrates, an HPX-87H column (Bio-Rad Labs., CA, U.S.A.) with a guard column (H-Cation, Bio-Rad) was operated under conditions specified by Schwald and Saddler (19), and by Irick et al. (20). For the enzymatic hydrolysates, an HPX-87P column (Bio-Rad) with a guard column (Carbo P, Bio-Rad) was used with water as eluent. Before injection, samples were passed through a 0.45 μm filter. For all HPLC analyses a Varian HPLC system (Model 5000; Varian, N.J., U.S.A.) interfaced with a Varian CDS 401 data system was employed. A refractive index detector (RI-3; Varian) and a sample injector (Model 7126; Rheodyne, CA, U.S.A.) fitted with a 20 μL sample loop were used.

The crystallinity of the cellulose in the aspen wood, LLA and LHA before steam treatment, and in the water insoluble fractions after steam treatment, was measured by X-ray diffraction, using a Norelco diffractometer operated at 20 kV and 20 mA. Ground samples were pressed at 11 metric tons to form pellets. The crystallinity indices were calculated according to Segal et al. (21), using the intensities from the crystalline and amorphous fractions of the cellulose.

TABLE 1

Chemical Composition of Aspen Wood, "Low-lignin" Aspen Wood (LLA) and "Low-hemicellulose" Aspen Wood (LHA) before Steam Treatment

Material	Yield (% of orig. wood)	Analysis (% of original wood)		
		Lignin	Xylan	Cellulose
aspen wood	100	19.8	21.9	43.2
LLA	85.1[a]	2.1	22.5	44.9
LHA	78.2[b]	17.3	11.6	42.0

a) weight of solid residue obtained after delignification of wood with sodium chlorite
b) weight of solid residue obtained after KOH extraction of wood

Enzymatic Hydrolysis

All substrates were hydrolysed with an enzyme preparation from Trichoderma harzianum E58, at an enzyme concentration of 35 filter-paper units per gram of oven-dry substrate. The substrate concentration was 2 % w/v in a 0.05 M acetate buffer at pH 4.8. The flasks were shaken at 140 rpm in an incubator at 45°C.

RESULTS AND DISCUSSION

The yields and chemical compositions of the "low-lignin" aspen wood (LLA) and "low-hemicellulose" aspen wood (LHA), are listed in TABLE 1, together with the composition of the original aspen wood from which they were prepared. In order to avoid the difficulty of steam treating wood meal, and to avoid the introduction of a

TABLE 2

Mass Balance, and Chemical Composition of Water-insoluble Fraction of Steam-heated Aspen Wood (SHA-WI), after Steam Treatment at 240°C (without Explosion)

Treatment time (s)	Recovery[a] (% orig.wood)			Analysis of SHA-WI (% of orig.wood)		
	Total SHA	WS	SHA-WI	Lignin[b]	Xylan	Cellulose
20	99.7	23.9	75.8	17.4	9.2	43.5
40	97.8	23.5	74.3	19.0	6.5	44.7
80	93.2	23.3	69.9	20.2	2.1	43.7
120	88.6	19.0	69.6	21.4	1.0	40.8
180	82.5	11.0	71.6	24.1	-	40.2

a) Recovery of SHA (steam heated aspen wood) consisting of a WS (water-soluble fraction) and a SHA-WI (water-insoluble fraction)
b) Apparent Klason lignin, i.e. Klason lignin plus pseudolignin

new variable (particle size) in the steam pretreatment, commercial aspen chips which had been broken up as described above, were used in the preparation of LLA and LHA. Lignin and xylan contents were reduced by about 90 % and 50 %, respectively (TABLE 1), which resulted in products of approximately equal accessibility.

Steam treatment of the aspenwood, LLA and LHA substrates at 240 °C for 20-180 s gave yields of water-soluble and water-insoluble fractions listed respectively in TABLES 2, 3 and 4, together with the analysis of the water-insoluble fractions. Alkali extraction of the SHA-WI removed the alkali-soluble fraction of the lignin, and

TABLE 3

Mass Balance, and Chemical Composition of Water-insoluble Fraction of Steam-heated "Low-lignin" Aspen Wood (SHLLA-WI), after Steam Treatment at 240°C (without Explosion)

Treatment time (s)	Recovery[a] (% orig. LLA)			Analysis of SHLLA-WI (% of orig. LLA)		
	Total SHLLA	WS	SHLLA-WI	Lignin[b]	Xylan	Cellulose
0	-	-	-	2.5	26.4	52.8
20	95.6	36.5	59.1	1.6	5.0	49.7
40	93.9	34.3	59.6	2.8	3.4	49.0
80	87.9	30.5	57.4	4.0	0.5	49.1
120	82.5	24.7	57.8	5.3	-	49.3
180	76.8	18.8	58.0	6.8	-	47.7

a) Recovery of SHLLA (steam treated "low-lignin" aspenwood) consisting of a WS (water-soluble fraction) and a SHLLA-WI (water-insoluble fraction)
b) Apparent Klason lignin, i.e. Klason lignin plus pseudolignin

TABLE 4

Mass Balance, and Chemical Composition of Water-insoluble Fraction of Steam-heated "Low-hemicellulose" Aspen Wood (SHLHA-WI), after Steam Treatment at 240°C (without Explosion)

Treatment time (s)	Recovery[a] (% orig. LHA)			Analysis of SHLHA-WI (% of orig. LHA)		
	Total SHLHA	WS	SHLHA-WI	Lignin[b]	Xylan	Cellulose
0	-	-	-	22.2	14.9	53.7
20	97.4	14.9	82.5	20.6	4.6	52.8
40	95.2	15.4	79.8	20.5	4.1	51.9
80	93.5	14.5	79.0	21.4	1.6	52.9
120	91.1	14.0	77.1	21.3	-	48.7
180	89.2	12.7	76.5	22.2	-	46.4

a) Recovery of SHLHA (steam heated "low-hemicellulose" aspen wood) consisting of a WS (water-soluble fraction) and a SHLHA-WI (water-insoluble fraction)
b) Apparent Klason lignin, i.e. Klason lignin plus pseudolignin

TABLE 5

Recovery and Chemical Composition of Water-and-alkali-insoluble Fraction of Steam-heated Aspen Wood (SHA-WIA), after Steam Treatment at 240°C (without Explosion)

Treatment time (s)	Recovery of SHA-WIA (% of original wood)	Analysis of SHA-WI (% of orig.wood)		
		Lignin[a]	Xylan	Cellulose
20	60.8	9.4	5.7	40.5
40	55.6	7.0	3.8	43.4
80	50.5	4.2	0.9	42.6
120	46.2	1.6	0.5	41.6
180	45.6	1.0	<0.5	40.8

a) Apparent Klason lignin, i.e. Klason lignin plus pseudolignin

left the alkali-washed residues (SHA-WIA) with the compositions listed in TABLE 5.

FIGURE 1a) shows that enzymatic hydrolyzability of the water-insoluble fraction of the steam-treated aspenwood (without explosion), increased with the length of steam treatment, as has previously been observed after analogous steam-explosion treatments (2,3). Relatively high glucose yields (85 % of theoretical) were obtained after an 80-s steam treatment and water extraction, which removed about 90 % of the xylan present in the original aspen wood (TABLE 2) without noticeable decomposition of the cellulose. Longer treatments (120 and 180 s), however, did cause some hydrolysis and solubilization of the cellulose, thereby reducing the potential amount of

glucose that could be obtained after enzymatic hydrolysis. In the later stages of the longer treatments, much of the xylan already solubilized in the earlier stages was further degraded to an insoluble material which has been previously termed pseudolignin. This xylan decomposition was reflected both in the increased "apparent Klason lignin" content of the SHA-WI and in the low recovery of xylan in the water-soluble fraction.

The longer treatments (120 and 180 s) significantly increased the enzymatic hydrolyzability of the cellulose remaining in the water-insoluble fraction, despite the fact that only a slight further reduction in the already low xylan content was possible. Xylan hydrolysis and removal were apparently not the only factors involved in increasing the accessibility of the cellulose.

It has been suggested that the hydrolysis of lignin, which restricts the swelling of the cell walls (11), plays an important role in the development of cellulose accessibility. The hydrolytic breakdown of the lignin, although not evident in the data shown in TABLE 2, is reflected in TABLE 5 after alkali extraction, in the decreasing lignin content remaining with increasing steaming time. Removal of the free alkali-soluble lignin did not significantly alter the yields of glucose obtained after enzymatic hydrolysis, although it did appear to reduce the rate of hydrolysis (FIGURE 1b). It

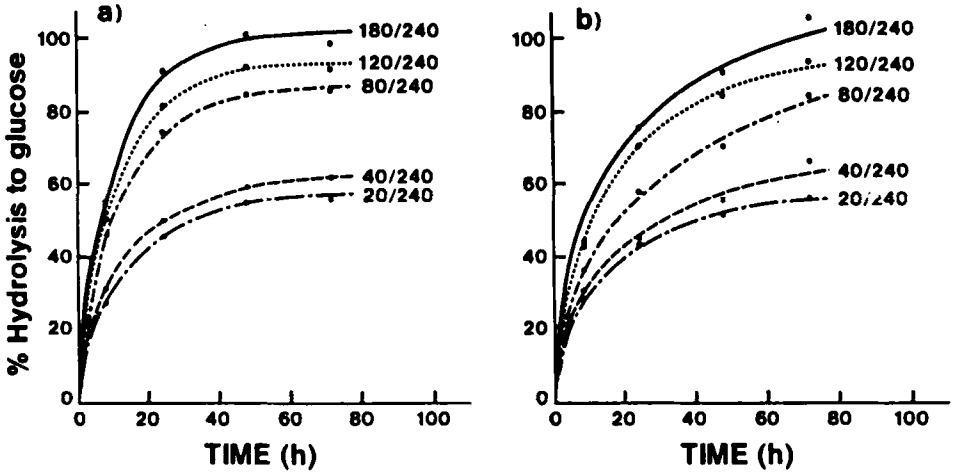

FIGURE 1: Course of reaction for the enzymatic hydrolysis of a) SHA-WI, and b) SHA-WIA; glucose yield from cellulose, % of theory;

240 - steam temperature in °C
20-180 - treatment time in s

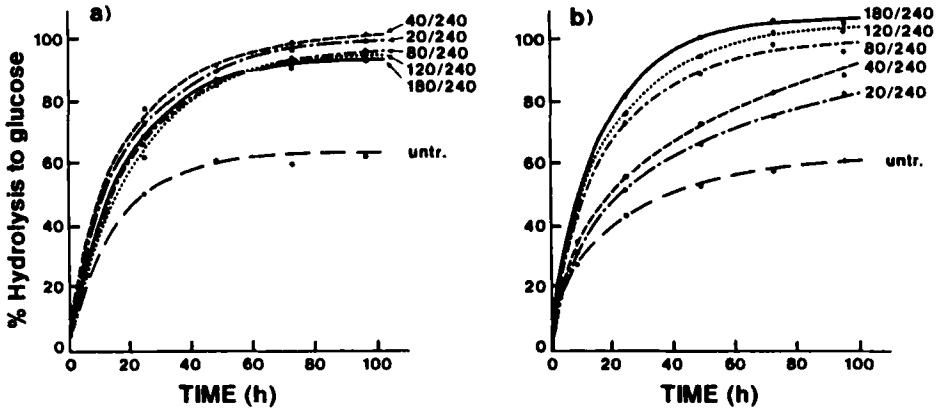

FIGURE 2: Course of reaction for the enzymatic hydrolysis of a) SHLLA-WI and untreated "low-lignin" aspen wood, and b) SHLHA-WI and untreated "low-hemicellulose" aspen wood; glucose yield from cellulose, % of theory;

steaming conditions same as in Figure 1

is noteworthy that the increase in cellulose accessibility, after 80 s of steaming, was accompanied by a significant further reduction in the content of residual alkali-insoluble lignin. The already low xylan content was not reduced significantly.

TABLE 3 shows that steam treatment of LLA resulted in a faster solubilization of xylan than did steaming of whole aspenwood. After only 20 s, 80 % of the xylan was removed during subsequent water washing, and on enzymatic hydrolysis (FIGURE 2a) the glucose yield rose from 58 % to 93 % of theoretical (i.e. close to the highest yield of 95 % obtained after 40 s). On further steaming, the glucose yield declined slowly as the apparent Klason lignin content increased.

This increase in apparent lignin, which resulted from xylan degradation, was roughly equal to that obtained with original aspenwood, despite the fact that only one-tenth as much true lignin was present during the steaming of LLA. It is unlikely, therefore, that the relatively large increases resulted from recondensation of degradation products (i.e. furfural or hydroxymethylfurfural) on the lignin, as was recently suggested by Tekely and Vignon (22) to explain changes in the ^{13}C-NMR spectra of Populus tremula wood during steaming. Further evidence, that at least part of the pseudolignin was not chemically attached to the true Klason lignin, was ob-

tained by exhaustive extraction of the apparent Klason lignin from SHA-WI, with boiling water. The total weight of methoxyl in the residue remained unchanged, although the sample lost weight.

FIGURE 2 shows that removal of 50 % of the xylan from aspenwood (in the preparation of LHA), or 90 % of the lignin (in the preparation of LLA), resulted in similar enzymatic hydrolysis curves before steam treatment. TABLES 3 and 4 show that, after the steam treatments, corresponding water-washed products from LHA and LLA had identical xylan contents, within 1 % (original wood basis), but had substantial differences in lignin contents. During steaming, the LHA with its much higher lignin content developed accessibility much more slowly than did the LLA. The hydrolysis curves from the steamed and washed LHA resembled more closely those from steamed and washed aspenwood, except after the shorter steaming times where hydrolysis of the LHA product was faster, in accordance with its lower xylan content.

A second difference was that during the steam treating of the LHA no acetyl groups were present, as a consequence of the alkali extraction. Formation of acetic acid from the hydrolysis of acetyl groups was therefore not possible. We were unable to detect any acetic acid in the water-soluble fractions even after 180 s of steaming. It was shown, by HPLC, to be present to up to

TABLE 6

Crystallinity Index Values of Different Untreated and Steam Pretreated Wood Preparations

Treatment time (s)	Crystallinity index in %		
	SHA-WI	SHLLA-WI	SHLHA-WI
0	58.6	57.9	58.8
20	74.8	78.0	75.1
40	-	80.7	75.6
80	78.6	82.8	76.4
120	-	84.5	77.0
180	80.2	83.8	77.1

4 % of the original wood in similar fractions from LLA. The rapid solubilization of xylan in the LHA therefore occurred without this acid.

TABLE 6 shows that the crystallinity index of aspen wood was essentially unchanged during removal of lignin in the preparation of LLA, and during removal of xylan in the preparation of LHA. After steam treatment for only 20 s at 240°C the crystallinity indices of the SHA-WI, SHLLA-WI and the SHLHA-WI all increased by 15-20 %. Prolonged steaming for up to 180 s resulted in only slight additional increases.

CONCLUSIONS

The accessibility of cellulosic materials to cellulase enzymes was found to be similar whether the materials were steam-treated with explosion or without. Prepara-

tion of LHA samples from aspen wood resulted in complete removal of acetyl groups. Steam treatment of LHA nevertheless enhanced the accessibility of the cellulose fraction even though no acetic acid was present as a catalyst. No correlation was evident between saccharification yields and the crystallinity indices of SHA-WI, SHLLA-WI and SHLHA-WI.

When the hemicellulose content had been reduced to below 1 %, the alkali-insoluble lignin content was still more than four times as great. The further substantial increase in conversion of cellulose to glucose, obtained after longer steam treatment, can be related to the removal of this lignin. Additional evidence was obtained after selective removal of lignin prior to steam treatment, which resulted in a material that was highly accessible to enzymatic hydrolysis, even after very short steam treatment times.

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