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The Role of Ester Groups in Resistance of Plant Cell Wall Polysaccharides to Enzymatic Hydrolysis

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ABSTRACT

Xylan backbones in native plant cell walls are extensively acetylated. Previously, no direct investigations as to their role in cellulolytic enzyme resistance have been done, though indirect results point to their importance. An in vitro deesterification of aspen wood and wheat straw has been completed using hydroxylamine solutions. Yields of 90% acetyl ester removal for both materials have been accomplished, with little disruption of other fractions (i.e., lignin). Apparently, as the xylan becomes increasingly deacetylated, it becomes 5–7 times more digestible. This renders the cellulose fraction more accessible, and 2–3 times more digestible. This effect levels off near an acetyl removal of 75%, where other resistances become limiting.

Index Entries: Xylan; ester groups; cellulase; pretreatment.

INTRODUCTION

Xylans are major hemicelluloses in cell walls of hardwoods and grasses (*1–5*) that are, either as crops or residues, the prime candidates for biological conversion to fuels and chemicals. The xylan backbones in native cell walls are extensively acetylated, and experimental evidence indicates that xylans may be crosslinked to lignin by ester and ether bonds (*6,7*). The xylans in immature grasses are also esterified with phenolic acids (*8–18*). The phenolic acid esters, primarily ferulic acid, are thought to anchor lignin chains to xylans. There is also evidence that phenolic acid

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MATERIALS AND METHODS

Materials

Baled wheat straw was purchased at a local feed store. It was harvested locally in 1986 and visually inspected for absence of fungal decomposition. Debarked aspen logs 4–12 in. in diameter were obtained from a local lumber mill. A cellulase preparation (Celluclast 1.5L) and a β-glucosidase preparation (Novozym SP188) were kind gifts of NOVO Industries, Inc. These preparations in liquid form contain slight amounts of glucose that must be accounted for in the enzymatic digestibility analysis. Both preparations can be stored at 4°C with no detectable loss in activity. Aliquots of enzyme to be used in the assays were dissolved in deionized water, and stored at 4°C. The remaining chemicals were purchased from national laboratory supply houses (Aldrich Chemical Co., Sigma Chemical Co., and VWR Co.).

Size Reduction

The wheat straw and aspen logs were coarsely reduced by methods described previously (*40*). Once reduced to 0.125 in. mesh, both the wheat and aspen were then milled in a laboratory knife mill (Willey, model 4) equipped with a 2 mm rejection screen. These milled samples were used directly in the chemical pretreatment.

Pretreatment

Both aspen wood and wheat straw were washed exhaustively with deionized water and air dried at 45°C prior to any chemical pretreatment. All hydroxylamine pretreatment experiments were carried out in 500 mL vacuum flasks, since the washed and dried biomass must be impregnated completely with the deacetylating solution by evacuation (30 min at 20 in. Hg) to insure reproducibility and high reaction yield. Each flask contained 200 mL of slurry. The slurry consisted of 1–4% w/v hydroxylamine hydrochloride, 0.5% w/v ethylenediamine tetraacetic acid trisodium salt (EDTA), 0.5–2.5% w/v NaOH (to adjust the pH to between 8.5–9.0), 5% dried biomass, and a makeup of deionized water. The flasks were constantly stirred at room temperature for 10 d. The progress of the reaction was monitored by a spectrophotometric assay using a Milton-Roy Spectronic 601.

One mL samples collected for assay were first centrifuged to pelletize the solids in an Eppendorf desk top centrifuge. The supernatant was then drawn off, thus quenching the reaction within the liquid fraction of the sample. For convenience and consistency in assaying, the liquor could then be stored for several days.

The assay, based on the absorbance at 530 nm of the iron chloride ($FeCl_3$)–hydroxamic acid complex that forms between $FeCl_3$ and hydroxamic acids at low pH (pH < 1), detected the major product of the pre-

treatment (e.g., acetohydroxamic acid). Crystalline acetohydroxamic acid was used as the standard by which concentrations of the complex were calculated. The assay protocol consisted of adding a 2.0 mL aliquot of $FeCl_3$ stock (prepared by adding 25 mL 1 *N* HCl and 12.5 mL deionized water to 100 mL of a 1% w/v $FeCl_3$ solution in 1 *N* HCl) to a 1 cm path length cuvet. To this was added 50 μL of sample, standard, or water for a blank. The solution was well mixed and the absorbance at 530 nm was determined immediately.

esters interfere with enzymatic hydrolysis of polysaccharides in cell walls of grasses (*15,19–21*). The role of acetyl ester groups in the resistance of cell walls to enzymatic hydrolysis is less clear, since no direct investigations have been done, but indirect evidence (*15,22–26*) points to their protective role as well. Also, the chemical acetylation of carbohydrates in wood renders the wood resistant to enzymatic hydrolysis and microbial decay (*27–29*). Furthermore, the holocellulose prepared by mild chlorine dioxide oxidation of lignin in spruce and birch wood contains partially acetylated xylan (*30–33*). Neither cellulose nor xylan in this preparation are completely digestible by fungal cellulase and hemicellulase enzymes, including mixed cellulase/hemicellulase preparations produced by *Trichoderma reesei* Rut C-30 (*30–33*).

The xylans in native cell walls are insoluble in all known unreactive solvents, because of their covalent crosslinking to lignin (*1–5*). This prevents their isolation in an unmodified state. The procedures commonly used for xylan isolation are harsh and usually involve oxidative degradation of lignin, extraction of oxidized lignin fragments, and final extraction of hemiculluloses from delignified residues (holocelluloses) with strong (4–15%) sodium or potassium hydroxide solutions (*1–5*). The hemicelluloses are then usually recovered through precipitation by neutralization and addition of ethanol. The treatment with strong solutions of alkaline metal hydroxides saponifies most or all ester groups and frees ester crosslinks. Therefore, these xylan preparations are extensively modified. When tested as substrates for xylanase activities, the results are misleadingly high (*30–33*). As shown in the Results and Discussion section, the xylans in untreated hardwoods and mature grasses (e.g., wheat straw) are as undigestible as cellulose. Only intensive mechanical or chemical pretreatments make hemicelluloses susceptible to enzymatic attack. Chemical pretreatments, which are often designed to depolymerize and remove lignin, modify hemicelluloses at the same time (*34–37*). It is then difficult to assign proper roles to hemicelluloses and lignin in resistance of untreated plant cell walls to enzymatic hydrolysis.

The acetyl or other ester groups can be removed from xylan chains by selective chemical or enzymatic treatments. Esterases have recently been identified in mixed cellulase–hemicellulase preparations produced by some fungi including *T. reesei*. The enzymes have been shown to remove acetyl groups from xylans, but do not depolymerize the xylans (*30–33*). Chemical reactions that could be effective for selective cleavage of acetyl

and other ester groups must proceed near neutral pH values, at low temperatures and preferably in an aqueous phase. We have chosen amidation with hydroxylamine for current investigations because it can fulfill all the criteria stated above and has been proposed as a method for quantitative determination of acetyl groups in woods and pulps (*38*). Hydroxylamine is a weak base (pKa=8), but it is highly nucleophilic and readily transforms esters of organic acids to hydroxamic acids (*38,39*). The hydroxamic acids can then be easily detected and quantitatively determined by spectrophotometric analysis of a purple complex, which forms in acidic iron chloride solutions (*39*).

Sample Storage

Solid residues were frozen for digestibility studies, air dried at 45°C for chemical analysis, or oven dried at 105°C for recovery analysis. Volumes of combined filtrate and washes were measured and stored at room temperature. The presence of hydroxylamine and EDTA inhibits microbial growth.

Analysis of Solid Residues

Dry weight was determined by standard methods (i.e., oven drying at 105°C to constant weight) (*41,42*), as were Klason and acid soluble lignin. Ash analyses were preformed by gravimetric analysis according to A.O.A.C. methods (*43*). Nitrogen content was determined by high temperature pyrolysis utilizing anhydrous acetohydroxamic acid as the standard (Huffman Labs, Golden, CO).

Anhydrosugars in the solids and reaction liquor were determined by a procedure slightly modified from the procedure developed at the US Forest Products Laboratory (*41*). Rationale and details of the modification have been discussed previously (*44*).

Acetate content of the solid residues was measured by conversion of the acetate esters to free acetic acid by hydrolyzing the biomass in 2% w/v sulfuric acid at moderate temperatures (121°C for 2 h). Included as a control, several saponifications (1 *N* NaOH at 121°C for 2 h) followed by acidification, were performed (*15*). The free acetic acid content of the subsequent hydrolyzates was analyzed by gas chromatography using a Hewlett-Packard model 5840A gas chromatograph equipped with a flame ionization detector and a Carbopack C/ 0.3% CW 20M/ 0.1% H_3PO_4 packed column (6 ft x 2 mm, Supelco). Column, injection port, and detector temperatures were maintained at 175, 190, and 190°C, respectively. Nitrogen served as carrier gas. The column was calibrated with C-2-C-5 volatile fatty acids standards (0.1%, Supelco).

Phenolic acids present in the biomass were determined by saponification (1 *N* NaOH for 24 h at room temperature), followed by acidification (pH<2) and butanol extraction (*8-18*). These extracts were evaporated

and redissolved in eluant appropriate to the HPLC system used, as discussed below.

Analysis of Pretreatment Liquids

Phenolic acids that reacted to form hydroxamic acids in the supernatant after pretreatment were quantified by methods described above, with the exceptions that no saponification step was needed, and the butanol extract was hydrolyzed by boiling in 1% HCl for 1 h. Lignin-like compounds in the liquor were determined spectrophotometrically by absorption at 280 nm using a Beckman model 260 spectrophotometer and extinction coefficients found in literature (*48*).

Determination of Enzymatic Digestibility

Biomass samples from hydroxylamine pretreatments and controls were assayed for in vitro digestibility using a NOVO Celluclast 1.5L/Novozym SP188 cellulase/β-glucosidase preparation. In order to minimize variable equilibrium effects encountered by codigestion with an added β-glucosidase (Novozym SP188), the enzyme hydrolysis products were assayed directly by HPLC for cellobiose, glucose, and xylose. Where appropriate, the glucose concentration in cellobiose was added to the free glucose to generate the total glucose yield.

Enzyme preparations were made by mixing Celluclast 1.5L and Novozym SP188 11/1 v/v. The resulting solution had a cellulase activity 85 international FPU/mL, and a xylanase activity of 55 IU (1 IU being 1 μmole xylose equivalents released/mL-min). The cellulase activity was determined, at 50°C in 0.05 *M* citrate buffer pH 4.8, by the usual method (*51*). The xylanase activity was determined under the same conditions, using a 1% w/v oatspelt xylan in water solution as substrate.

Enzyme digestions were performed with 10 mg/mL anhydroglucose as the basis for substrate concentration. Tetracycline (40 μg/mL) and cycloheximide (30 μg/mL) were added to the mixture to combat microbial infections; the antibiotics do not inhibit enzyme activity at the concentrations used (*45*). The resulting mixtures were then incubated at 50°C with gentle agitation. Enzyme loadings of 75 international FPU/g substrate were used in all experiments. Samples for analysis were removed at regular intervals and subjected to heat denaturation (70°C for 20 min) to inactivate and precipitate the enzymes. After high-speed pelleting, the supernatants were assayed immediately by HPLC. The samples were hydrolyzed for periods up to 150 h to insure that the release of soluble sugars from pretreated biomass became negligible. As external control, SigmaCell 50, was digested cocurrently with the biomass samples to assess the variation between enzyme batches and thus the validity of the biomass digestion. Digestion is calculated as percentage of the total sugars (corrected for hydration) released from the anhydrosugars in the biomass sample at the end of the pretreatment.

NMR Spectroscopy

The ^{13}C NMR spectra were obtained on a Nicolet NT-150 spectrometer at a carbon frequency of 37.735 MHz. This instrument was equipped with a custom cross-polarization magic-angle spinning (CP/MAS) unit and probe. The decoupling field was 13 G (55 KHz). The spinning system is a modified version of Wind's (*46*), with a sample volume of 0.3 cm^3. The samples were spun at 3800 rps. The CP contact time was 1 ms, and the repetition time was 1 s. 2 K data points were collected with a spectrum width of 20 KHz and an acquisition time of 52 ms. The number of scans ranged from 6 to 180 k. Chemical shifts are relative to external tetramethylsilane, with hexamethylbenzene as a secondary standard (methyl signal at 17.35 ppm).

Liquid Chromatographic Analysis

Digestion and anhydrosugar analysis were obtained by high pressure Ion-Moderated Partition (IMP) chromatography using a Bio-Rad HPX-87P column controlled at 60°C. The monosaccharide profile was obtained with a Hewlett-Packard model 1037A refractive index detector. A Beckman 110A HPLC pump was used to deliver the eluant (water) at a flowrate of 0.3 mL/min.

The phenolic acids analysis required the use of a Bio-Rad Aminex HPX-87H column controlled at 60°C, and a Waters model 450 UV detector equipped with 254 nm filters. 10% (v/v) acetonitrile in 0.01 *N* sulfuric acid was used as eluant for phenolic acids analysis, in order to decrease the column's adsorption of the phenolic compounds and thus decrease retention times.

A high pressure size exclusion chromatography (HPSEC) system was designed to allow estimation of the apparent molecular weights of the butanol-soluble fraction obtained after hydroxylamine treatment. Since lignins and other phenolic organic compounds may be found in this fraction, an HPSEC system was designed to function in 1 *N* NaOH eluant. This system consisted of a Beckman model 110B HPLC pump, a Pharmacia Superose 6 and Superose 12 HR 10/30 column pair, and a Waters model 440 ultraviolet detector. Flow was maintained at 1.0 mL/min. The system was calibrated with polystyrene sulfonate standards (Polymer Labs, UK), tannic acid (Aldrich), vanillic acid (Sigma Chem.), and acrylic acid standards (American Polymer Standards Corp.). Molecular weights of unknowns were estimated using conventional procedures for HPSEC analysis (*47*).

RESULTS AND DISCUSSION

This study reflects an attempt to identify the role of ester groups, inherent in the cell walls of aspen wood and wheat straw, in the resistance of polysaccharides to enzymatic hydrolysis. Since the major source of

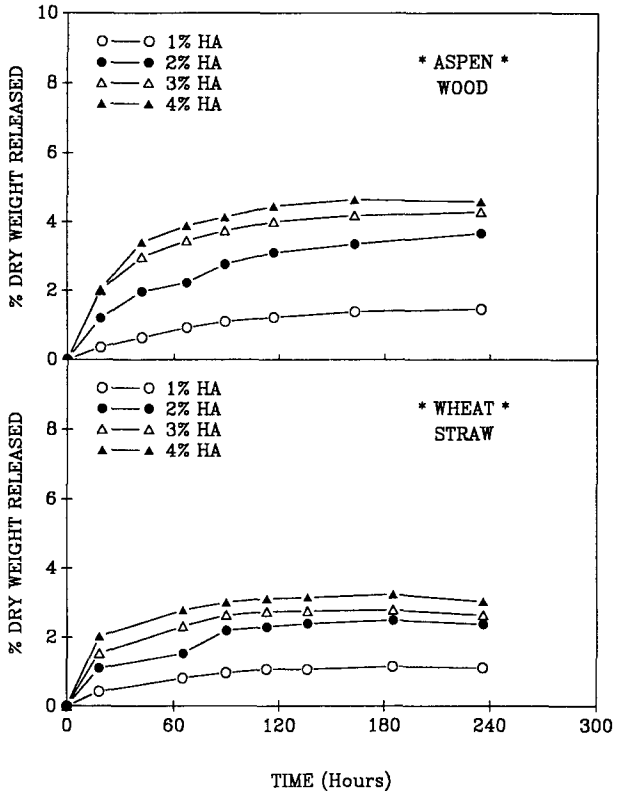

Fig. 1. Percent of total dry weight released as hydroxamic acids during pretreatment.

ester groups were found to be the acetyl esters present in the hemicellulose xylan fraction, the results have been based on the relative deacetylation rather than the relative deesterification.

Pretreatment

Figure 1 shows the formation of hydroxamic acid-Fe^{3+} complexes during hydroxylamine pretreatment for aspen wood and wheat straw. In the figure, the abscissa is the percent of total dry weight before treatment, released as hydroxamic acids and calculated as acetohydroxamic acid. Since the effects of inadequate impregnation of reagent were quite pronounced (data now shown), it was determined that a minimum of 30 min at 20 in. Hg vacuum were required for sufficient wetting and hydroxylamine penetration. The reaction of 1% hydroxylamine solutions in both cases still seem to be somewhat limited by the reagent concentration. The reactions in the 3–4% hydroxylamine range, however, seem to be limited by accessibility of acetyl groups. The removal of acetyl groups converge

at a value of approximately 90% of the total acetate present (found by mild acid hydrolysis and reported in Table 1). This could be a result of incomplete impregnation or some steric inaccessibility. In either case, this incomplete deacetylation could not be overcome with longer evacuations. Obviously, other hydroxamic acids that may be present would shift the true percentage released because of the molecular weight difference. These other hydroxamic acids could be the products of organic acids esterified in the biomass (e.g., aromatic acids). However, these groups were determined not to be present in appreciable amounts, and the curves based on acetohydroxamic acid were quite accurate.

Characterization of Pretreated Residues

Table 1 includes the important characteristics, based on the original, post-water wash, pre-pretreatment dry wt of the hydroxylamine treated aspen wood and wheat straw. Both 2% and 4% hydroxylamine treated samples are included with the water-washed "raw" biomass in Table 1.

The results for aspen wood indicate that even though the biomass was water washed exhaustively, 2% of the total dry wt of the aspen was still water soluble. This amount must be accounted for when determining losses during pretreatment, by including it to the summation of the characteristics for each concentration. The results also show that approximately 5–10% of the ash was removed for both solution concentrations. The 2% and 4% hydroxylamine solutions also removed 75% and 90% of the acetate present, respectively. There was no appreciable nitrogen incorporation through hydroxylamine side reactions. No cellulose or hemicellulose was lost since all these recoveries are approximately 100%. Apparently, some Klason lignin was converted to acid soluble lignin during the reaction since more than 110% of the acid soluble lignin was recovered after pretreatment. Therefore the total lignin, the summation of both Klason and acid soluble lignin, is a better measure of lignin content. The aspen wood consistently lost 10% of the total lignin in all cases. All major components were accounted for in the liquid and solid portions of the pretreated biomass and component losses (which must be obtained by the summation of the component values in each of the columns) agree well with dry wt recoveries reported in Table 1.

The wheat straw results were quite similar, but with 7% water solubles remaining and nearly 20% losses for ash. Again, approximately 75% and 90% acetate removal for the 2% and 4% hydroxylamine treatments, respectively, there was no nitrogen incorporation, and some Klason lignin was converted to acid soluble lignin. It should be noted that the total lignin lost in this case was consistent, but somewhat larger (e.g., $\approx 20\%$). The recoveries of cellulose and hemicellulose were slightly greater than 100%, indicating minor analytical imprecisions. Again, with this variance in mind, and the 7% water extractables, the major components were accounted for and losses agree well with dry wt recoveries for the wheat straw in Table 1.

Table 1
Characterization of Hydroxylamine Pretreated Aspen Wood and Wheat Straw[a]

	Aspen Wood			Wheat Straw		
	Raw[b]	2% HA[c]	4% HA[c]	Raw[b]	2% HA[c]	4% HA[c]
Water solubles	2±0.5	NA	NA	7±1	NA	NA
Ash	0.59±0.03	0.53±0.08	0.56±0.10	4.4±0.3	3.5±0.1	3.6±0.1
Acetate content	4.6±0.2	1.1±0.2	0.5±0.1	2.2±0.2	0.5±0.1	0.2±0.1
Nitrogen content	0.12±0.02	0.20±0.02	0.25±0.02	0.28±0.02	0.29±0.02	0.34±0.02
ANHYDROSUGARS						
Glucan	46±2	48±2	48±3	41±3	43±2	44±2
Xylan	19±1	19±1	19±1	20±1	20±2	20±3
All others	3±1	3±1	3±1	3±1	4±1	4±1
LIGNIN:						
Klason	22.0±0.7	19.0±0.2	19.2±0.5	19.0±0.9	15.4±0.6	14.1±0.9
Acid soluble	+2.6±0.1	+3.1±0.3	+3.0±0.3	+1.6±0.1	+1.7±0.1	+1.8±0.1
Total lignin	24.6±0.7	22.1±0.4	22.2±0.6	20.6±0.9	17.1±0.6	15.9±1.0
Dry weight recovery (w/w%)	NA	95±1	94±1	NA	94±1	91±1

[a] All values are reported in percent total dry weight *before* pretreatment.
[b] Post-water wash, pre-hydroxylamine pretreated biomass.
[c] 2% or 4% (w/v %) hydroxylamine pretreated biomass.

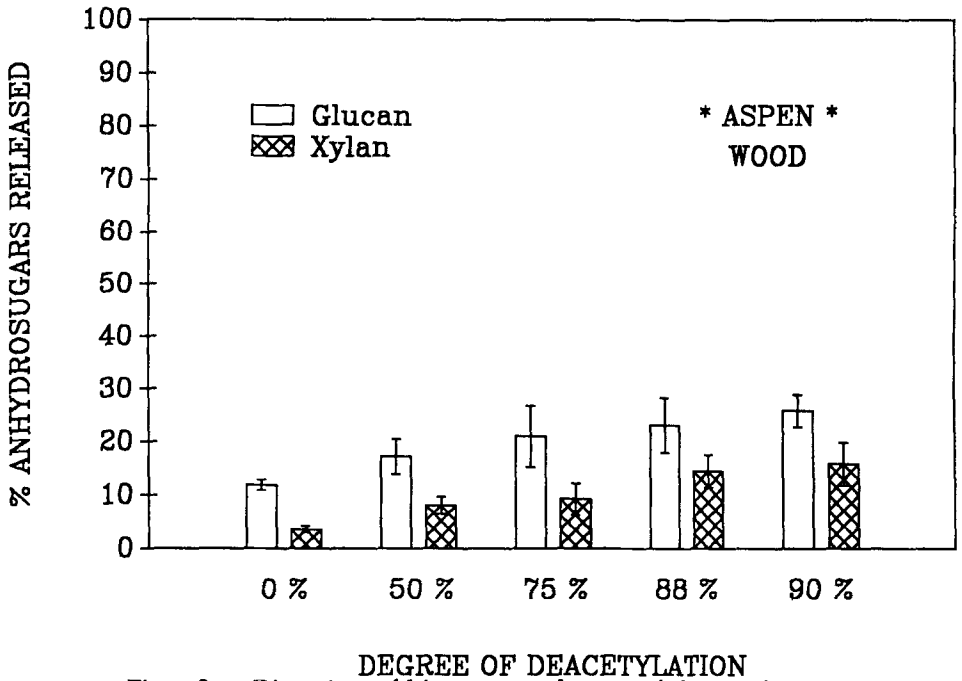

Fig. 2. Digestion of biomass vs degree of deacetylation.

Enzyme Digestibility

Digestibility studies were performed on the hydroxylamine pretreated wheat straw and aspen wood samples as described in the Materials and Methods section. The enzyme to cellulose ratio, cellulose concentration, and temperature were selected to ensure the most complete digestion possible of the α-cellulose control (Sigmacell 50) (e.g., 90–95%) in ca. 48 h. These conditions approximate those reported in the literature, but a clear distinction should be made between the digestibility studies reported here and the kinetic results that can be obtained at the same time. Our primary concern is the determination of the ultimate digestibility of all the samples. Therefore, no effort to optimize the enzyme loading was made. Excessive amounts of cellulase enzyme were added to ensure that the results reflect the resistance of the substrate and not enzyme loading as a limiting factor.

In order to follow the specific monosaccharide production rates, samples were taken at regular intervals up to 150 h, as described above. Results were corrected for hydration, and the percent of total available glucan and xylan released from each sample was calculated. Typically, with the enzyme loadings employed, the release of each monosaccharide was complete in 100 h, however, the samples were left incubating as long as 150 h as a control.

Figures 2 and 3 show the digestibility of biomass vs degree of deacetylation for the xylan from aspen wood and wheat straw, respectively. The extent of digestion of both cellulose and xylan increase with increased deacetylation. Although the change in digestion appears more dramatic

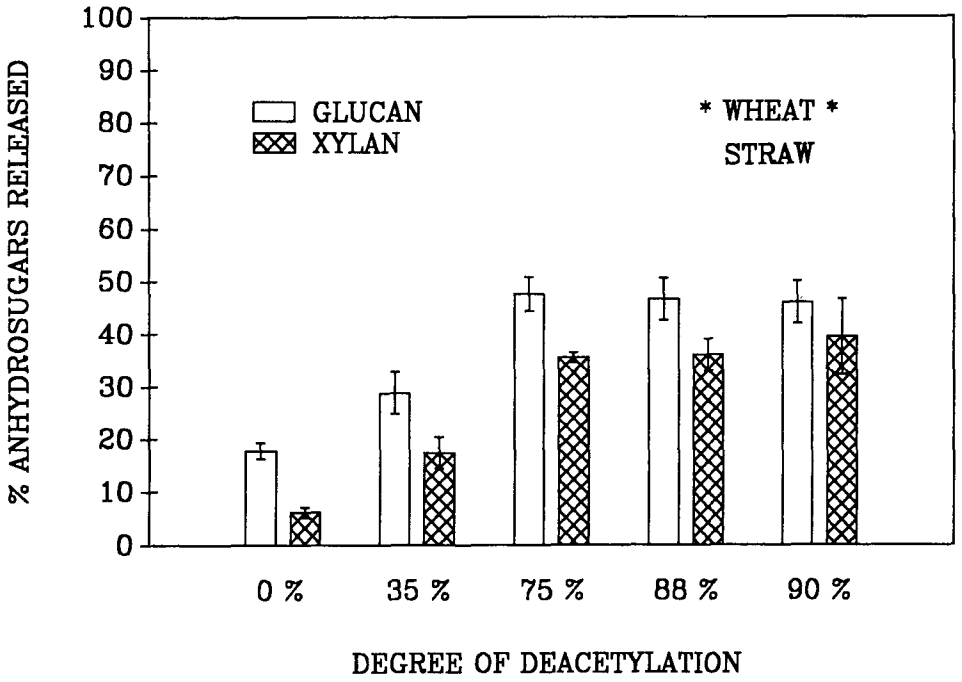

Fig. 3. Digestion of biomass vs degree of deacetylation.

for wheat straw, (a threefold increase in cellulose and a sevenfold increase in xylan), the results for aspen wood are quite similar (a 2.5-fold increase for cellulose and a fivefold increase for xylan). It would seem that as the protective hemicellulose (xylan) becomes more deacetylated, it becomes more digestible. This, in turn, may make the cellulose fraction more accessible and therefore more digestible.

NMR Spectroscopy

The ^{13}C CP/MAS difference spectra for aspen wood and wheat straw (raw versus 4% hydroxylamine treated) appear in Figs. 4 and 5, respectively. Peak assignments are well documented (*49,50*), and are shown in the figure legends. Upon inspection it is apparent that the only major changes in the spectra are the differences at the methyl peak of acetyl esters (j) and at the ester carbonyl (a), with some minor changes under the C_2, C_3, and C_5 carbohydrate peaks (f). These results are in excellent agreement with the gravimetric analysis mentioned above. It is also important to note that the areas under the peaks at (a) and (j) are comparable in magnitude. This would indicate that these two species are present in similar amounts, since the areas are directly integrable. Specifically, this indicates that the major fraction of ester groups within both aspen wood and wheat straw are acetyl esters. Also, it must be noted that the difference at (f) is a small portion of two relatively large peaks, whereas the changes in the ester-carbonyl and acetyl-methyl peaks are large portions of two relatively small peaks. It is also important to note that there was no change in the aromatic lignin peaks (b) and (c), indicating that

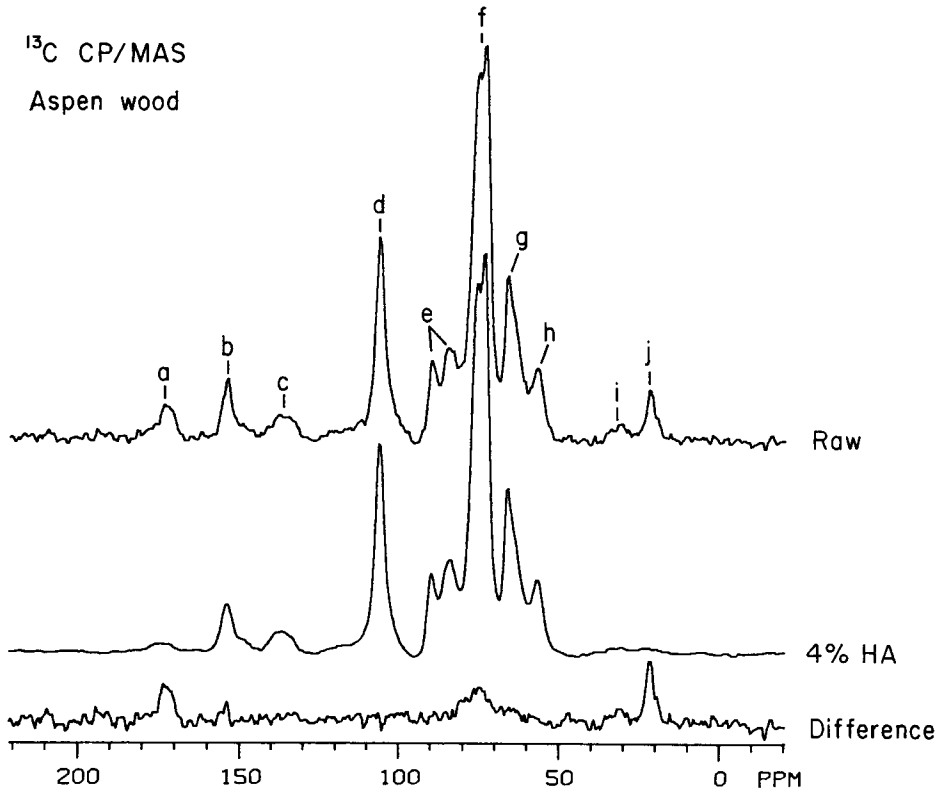

Fig. 4. ^{13}C CP/MAS NMR Peak Assignments: a. Carbonyl, b. Oxygen-substituted aromatic, c. Aromatic, carbohydrate (d. C-1, e. C-4, f. C-2, C-3, C-5, g. C-6,) h. Methoxy, i. Aliphatic, j. Acetyl methyl.

there was no significant removal of true polyphenolic lignin. The minor losses of Klason lignin after hydroxylamine pretreatment thus appear to be caused by the removal of other biomass constituents (e.g, waxes or steroids), which do not dissolve during the two stage sulfuric acid hydrolysis of the Klason lignin analysis.

Lignin Analysis

In order to better understand the minor lignin removal during hydroxylamine treatment, experiments were designed to identify the amount, type, and molecular weights of the fraction removed. First, in order to determine the type and amount of aromatic acids present in the starting materials, samples were saponified and analyzed by the HPLC methods described in the Materials and Methods section. Aromatic acids were released from both aspen wood and wheat straw, but the results show they represent less than 1% by weight of either aspen wood or wheat straw. Results show that aspen wood contained 0.15% para-hydroxy-

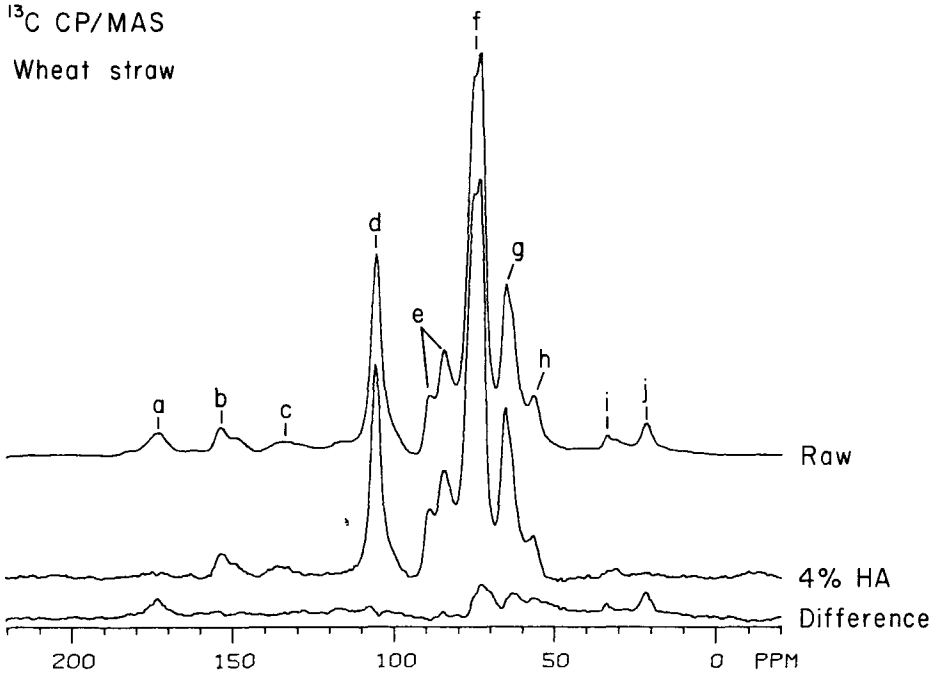

Fig. 5. ^{13}C CP/MAS NMR Peak Assignments: a. Carbonyl, b. Oxygen-substituted aromatic, c. Aromatic, carbohydrate (d. C-1, e. C-4, f. C-2, C-3, C-5, g. C-6,) h. Methoxy, i. Aliphatic, j. Acetyl methyl.

benzoic acid with traces of protocatechuic acid and caffeic acid, whereas wheat straw contained 0.11% coumaric acid with traces of vanillic acid and ferulic acid. Moreover, the HPLC analysis of the pretreatment liquors described above indicate that less than 20% of the total aromatic acids present were released as aromatic-hydroxamic acids. Therefore, these aromatic acids cannot account for the amount of Klason lignin lost.

Application of acidified-butanol extracts from the 4% hydroxylamine reaction liquor to alkaline HPSEC result in a profile showing three distinct maxima superimposed on a broad distribution envelope (*see* Fig. 6). This chromatogram indicates the absence of material greater than approximately 2,000 mol wt and the presence of two distinct molecular species. Based on comparison with the predetermined weight-average mol wts for the polymeric acid standards used to calibrate the column (data not shown), these species elute at volumes corresponding to apparent mol wts of approximately 700 and 200. These UV absorbing species account for 75% and 80% of the lignin removed from aspen wood and wheat straw, respectively, using an extinction coefficient of 18 L/g-cm at 280 nm (*48*). This information would tend to support the assertion that the small percentages of Klason lignin removed were nonpolyphenolic Klason lignin fractions, since the low molecular weight aromatic species appearing in the HPSEC are not of mol wts consistent with polymeric lignin fractions.

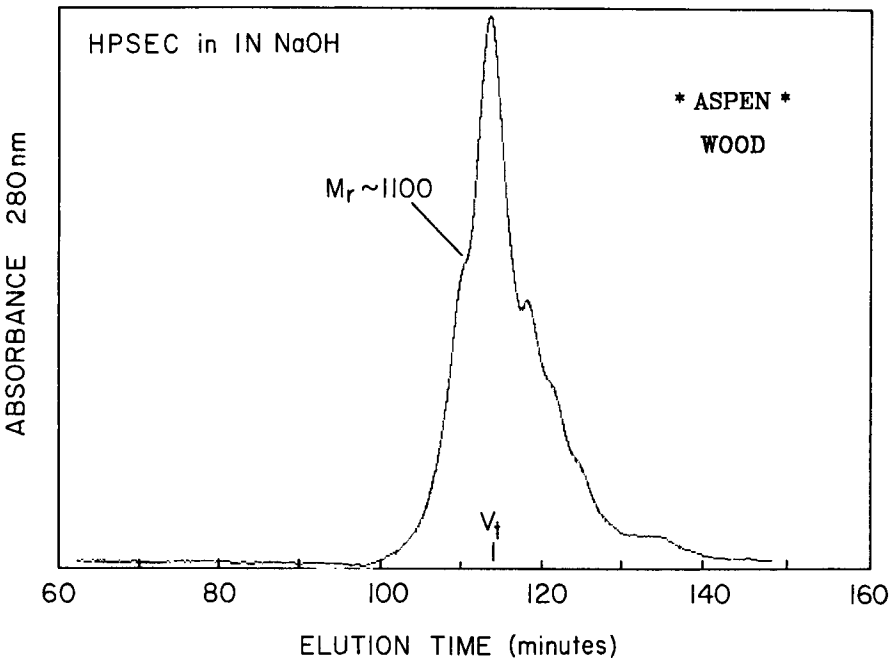

Fig. 6. Lignin Analysis: HPSEC in 1N NaOH.

One obvious question concerns the effect that the removal of "lignin" in each of the samples might have on the enzymatic digestion. Previous workers have shown that removal of lignin apparently enhances digestibility, however, their work does not account for the deacetylation that must have occurred under the conditions employed (*35,37*). Moreover, the losses of Klason lignin are comparable at all hydroxylamine concentrations, whereas the enzymatic digestibilities are not. The enzymatic digestibilities do correlate, however, with acetyl group removal. The observation that the digestion of both pretreated samples leveled off at a value much less than 100% supports the idea of the important role of lignin shielding. However, the discovery that increased digestion occurs with increased deacetylation, whereas lignin removal remains small and constant, tends to indicate that the lignin shield is not the only major factor in the resistance of carbohydrates of plant cell walls to enzymatic attack, and that acetylation of xylans plays an important role as well.

As a final note, the highly selective hydroxylamine pretreatment investigated in this work would be impractical for scaleup since hydroxylamine is an expensive, toxic, and mutagenic chemical. However, the observation that the removal of acetyl groups dramatically increases the enzymatic digestibility of carbohydrates can be exploited for design of milder pretreatments than those that have been developed thus far.

CONCLUSIONS

Ester groups play an important role in the mechanism of plant cell wall resistance to enzyme hydrolysis in both aspen wood and wheat straw. The aqueous hydroxylamine solutions, under specific reaction conditions, deacetylate plant materials to values up to 90% while affecting other mechanistically important fractions very little. The results reported here indicate that as the xylan fraction becomes deacetylated, it becomes 5–7 times more digestible. This, in turn, makes the cellulose fraction more accessible and 2–3 times more digestible. This effect apparently levels off near an acetyl removal of 75%, where other mechanisms come into play (e.g., lignin shielding).

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