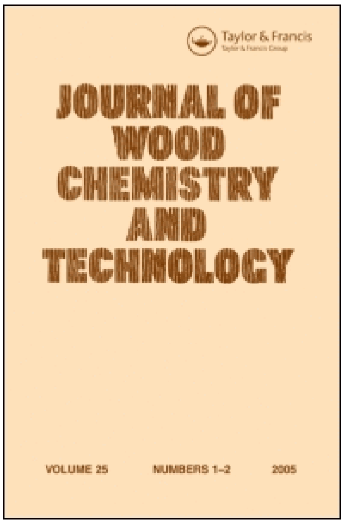

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A. S. Schmidt[a]; S. Mallon[a]; A. B. Thomsen[a]; S. Hvilsted[b]; J. M. Lawther[c]
[a] Plant Research Department, Risø National Laboratory, Roskilde, Denmark [b] Danish Polymer Centre, Department of Chemical Engineering, The Technical University of Denmark, Lyngby, Denmark [c] Plant Fiber Laboratory, Royal Veterinary and Agricultural University of Denmark, Taastrup, Denmark

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COMPARISON OF THE CHEMICAL PROPERTIES OF WHEAT STRAW AND BEECH FIBERS FOLLOWING ALKALINE WET OXIDATION AND LACCASE TREATMENTS

**A. S. Schmidt,[1],* S. Mallon,[1] A. B. Thomsen,[1]
S. Hvilsted,[2] and J. M. Lawther[3],†**

[1]Plant Research Department, Risø National
Laboratory, PO Box 49, DK-4000 Roskilde, Denmark
[2]Danish Polymer Centre, Department of Chemical
Engineering, The Technical University of Denmark,
DK-2800 Lyngby, Denmark
[3]Plant Fiber Laboratory, Royal Veterinary and
Agricultural University of Denmark, Agrovej 10,
DK-2630 Taastrup, Denmark

ABSTRACT

Wheat straw (*Triticum aestivum*) and beech (*Fagus sylvatica*), were used to evaluate the effects of two pre-treatment processes (alkaline wet oxidation and enzyme treatment with laccase) on lignocellulosic materials for applications in particleboards and fiberboards. Wheat straw and beech fibers

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*Corresponding author. Novo Nordisk A/S, Protein Chemistry, Hagedornsvej 1, DK-2820 Gentofte, Denmark. E-mail: asks@novonordisk.com

†Current address: BioVelop AB, Karbingatan 10, S-25467 Helsingborg, Sweden.

reacted differently in the two processes. The chemical composition changed little following enzyme treatment. After alkaline wet oxidation, fibers enriched in cellulose were obtained. With both materials, almost all hemicellulose (80%) together with a large portion of the lignin were solubilised by alkaline wet oxidation, but essentially all cellulose remained in the solid fraction. Following enzyme treatment most material remained as a solid. For wheat straw, reaction with acetic anhydride indicated that both treatments resulted in more hydroxyl groups being accessible for reaction. The enzyme treatment gave a more reactive surface than alkaline wet oxidation for wheat straw, whereas the opposite was observed for beech. Fourier transform infrared (FT-IR) spectroscopy showed an almost complete loss of the ester carbonyl stretching signal and the corresponding C-C-O stretching in wet-oxidized materials. This proved that alkaline wet oxidation breaks ester bonds in wheat straw and beech. On the other hand, FT-IR could not detect any tangible effects of the laccase treatment.

Key Words: Wet oxidation; Laccase treatment; Thermomechanical pulping; Wheat straw; Beech; Chemical composition; Reactivity; Fourier transform infrared spectroscopy

INTRODUCTION

Lignocellulosic residues from agricultural and forestry crops, e.g., straw and wood chips, are potential raw materials for industrial production of several value-added products such as fuel ethanol, xylitol, oligosaccharides, and biofibers.[1–4] Renewable raw materials are particularly important because of their massive abundance world-wide, and hence, are promising feedstocks for "green" products. Due to the low density and good stiffness and strength of cellulosic fibers, they may be able to replace traditional fibers such as glass, carbon and other inorganic fibers in composite applications. However, straw and the stalks and by-products of other cereal crops possess a dense coating on their surface of wax, silica and protein, which serves to protect the epidermis against moisture loss. This layer also inhibits bonding with resins and glues, secondary bonding (hydrogen bonding) and auto-adhesion, and thus is a problem in the production of particleboards and fiberboards from straw.

Wet oxidation pre-treatment (water, oxygen, and elevated temperature) in alkaline conditions has proven to be an efficient method for fractionating agricultural crops for conversion purposes.[1,5] This process removes most of the non cell-wall material (such as wax, pectin and proteins) and solubilises most of the hemicelluloses, which then can be enzymatically converted to ethanol.[1] In this study, the properties of the fiber residue was examined for utilization in fiber based or fiber/plastic composite products. The reactivity of the fibers towards acetic anhydride correlates directly with the number of accessible hydroxyl groups on the fiber and the reactivity towards coupling agents and binders such as maleic anhydride modified polypropylene (MAPP), polymethylene polyphenyl isocyanate (PMPPIC), silanes, titanates etc.[6] Concentrations of hydroxyl groups on fiber surfaces also give information about their hydrophilicity/hydrophobicity, and hence, their affinity towards hydrophobic/hydrophilic adhesives and matrices.

Enzyme treatment using laccase (developed in collaboration with Novozymes A/S) is also examined as it has been shown to increase the auto-adhesion of wood fibers showing improved properties of binder-less boards presumably by forming e.g., phenoxy radicals in the lignin component.[7,8] As the substrate for the enzyme is lignin, the lignocellulose must first be thermomechanically defibrated (Figure 1). Laccase treatment attacks lignin resulting in lower molecular weight fragments, increasing the thermoplasticity of lignin and loosening its rigid structure allowing improved flow during board consolidation and increased interfacial adhesion.[8]

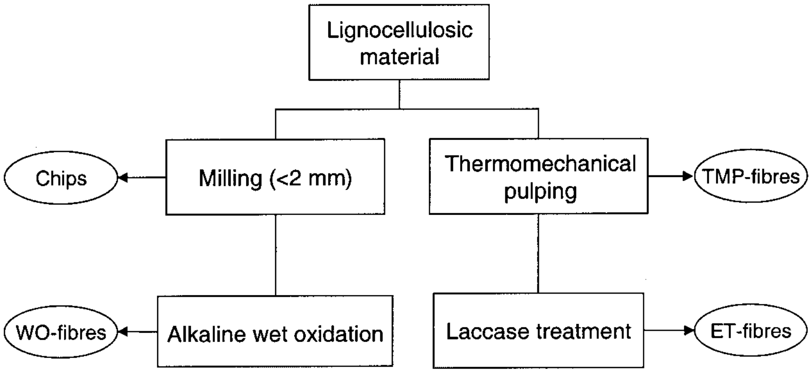

Figure 1. Flow chart of wheat straw and beech treatments.

In this study, two materials, wheat straw and beech, were used to evaluate the potential of the two pre-treatment processes for generating fibers with improved properties for use in fiber-based products (Figure 1). The fibers were evaluated by analysis of their chemical composition, by their reaction with acetic anhydride and by non-invasive Fourier Transform Infrared (FT-IR) spectroscopy.

RESULTS AND DISCUSSION

Chemical Composition

The chemical composition of the raw materials was very different (Table 1). Wheat straw had considerably higher hemicellulose and non-cell wall material (NCWM) content, while beech had considerably higher cellulose and lignin contents. The TMP treatment of both materials only slightly changed the fiber composition by reducing the hemicellulose

Table 1. Chemical Composition (% w/w dry matter) of Wheat Straw and Beech Before and After Treatment by Alkaline Wet Oxidation or Laccase Enzyme and the Percentage of Each Component Removed During Treatment Based on Mass Balances.

	Treatment	DM (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	NCWM (%)	Ash (%)
Straw	Chips	–	38.6	34.1	7.9	19.2	0.3
	WO	–	71.5	13.7	5.5	8.9	0.4
	Removed	46.3	0.5	78.4	62.1	75.0	26.4
Straw	TMP	–	41.0	31.8	9.5	17.5	0.2
	ET	–	43.6	32.3	9.7	14.1	0.4
	Removed	6.5	0.6	5.1	4.4	24.8	1.8
Beech	Chips	–	46.2	28.6	14.1	10.8	0.2
	WO	–	66.0	9.0	11.7	13.1	0.2
	Removed	40.8	15.5	81.4	51.0	28.2	54.9
Beech	TMP	–	45.7	22.7	16.3	15.0	0.4
	ET	–	47.9	22.7	16.7	12.2	0.4
	Removed	5.0	0.4	4.8	2.7	22.8	2.4

WO: 60 g biomass/L; 185°C; 12 bar O_2; 6.5 g Na_2CO_3; 15 min.
TMP: 1) steaming, 140°C, 4 min; 2) refining, 180°C, 8 atm.
ET: 50 g fibers/l; Laccase: 3 LACU/g dry fiber; 40°C; pH 4.5; 1 h.
Abbreviations are explained in Figure 1.

content. In wheat straw, this was accompanied by a reduction in the NCWM content. Enzyme treatment of the TMP-fibers had only minor effects on fiber composition. However, the composition changed considerably during wet oxidation giving a fiber fraction with much lower hemicellulose content, and for straw, much lower NCWM and lignin contents. Hence, the fiber fraction for both materials was highly enriched in cellulose by wet oxidation.[2,5] The lower content of hydrophilic components, such as hemicellulose, might improve the compatibility of the fibers in thermoplastic composite applications.

With alkaline wet oxidation, most of the original hemicellulose (80%) was removed, based on simple mass balances, as a result of both the alkaline solubility of hemicellulose and its oxidative degradation.[5] A large portion of the lignin was also removed by oxidation, 50% from the beech and 60% from wheat straw. For wheat straw, 75% of the NCWM was removed by alkaline wet oxidation compared to only 28% for beech. All of the cellulose in wheat straw remained in the fiber fraction, but about 15% were solubilised from beech during wet oxidation. This is probably due to the loss of alkali soluble low molecular weight portions of cellulose. The difference between beech and wheat straw was presumably due to better penetration of the more hydrophilic beech fibers. This can be seen by the fact that wheat straw fibers tend to float in water, whereas beech fibers sink.

With enzyme treatment, most of the material remained as a solid. For both materials, mainly NCWM had been solubilised during the treatment. Additionally, some hemicelluloses (5%) were solubilised, presumably due to the loss of water-soluble hemicelluloses such as galactans and some xylans.[9] All cellulose remained in the solid fraction.

Fiber Reactivity

The reaction of hydroxyl groups on the fibers with acetic anhydride before and after the treatments give information on how the surface was altered during the treatment. The reaction depends heavily on the distribution of reactive functional groups and their accessibility. Stated in the literature lignin is the most reactive of the cell wall components,[10] however, this is somewhat misleading. While the phenyl hydroxyl groups of lignin are more reactive than hydroxyl groups on polysaccharides, the larger number of reactive sites on hemicellulose and the greater abundance of hemicellulose mean that hemicellulose displays a greater level of reaction than lignin. Cellulose is relatively unreactive mainly due to steric factors and its high degree of crystallinity.

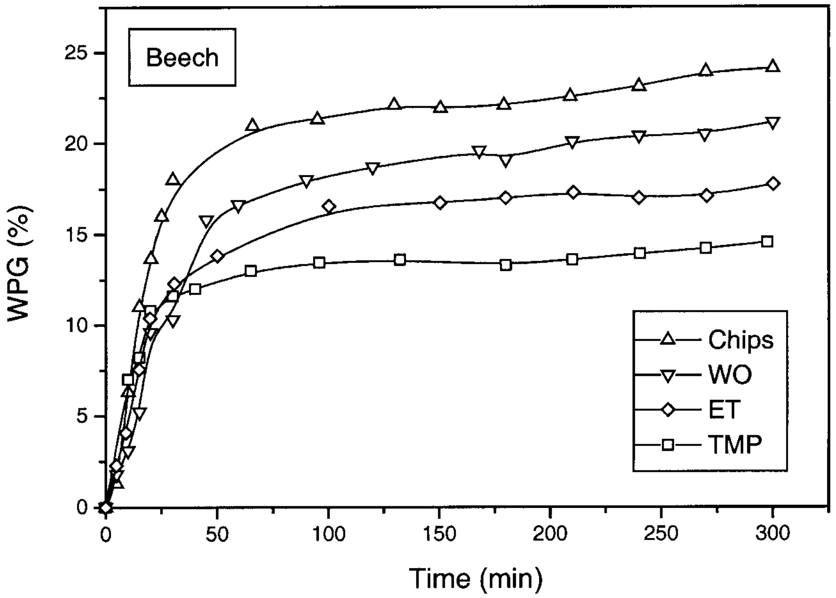

Figure 2. Weight percent gain (WPG (%)) of beech fibers by acetylation as a function of reaction time. Abbreviations are explained in Figure 1 and conditions in Table 1.

The reaction profiles show weight percent gains (WPG) due to acetylation both before and after alkaline wet oxidation and enzyme treatment for beech and wheat straw (Figures 2 and 3, respectively). The shape of the reaction curves indicated that they were the sum of reaction with surface and reaction with bulk hydroxyl groups.[11] At first the reaction takes place at or near the fibre surface, but as the reaction proceeds, the bulk reactions such as diffusion of reagent into the fibre matrix assume increasing importance. However, due to swelling it is difficult to separate the reactivity of the different groups from the fiber accessibility.

The initial rates (Table 2) were calculated from experimental data in Figures 2 and 3 by linear regression though zero using WPGs until the reaction began to level off. The correlation coefficients ranged between 0.91 and 0.99. All initial reaction rates were of the same order of magnitude, because the reactivity of the present hydroxyl groups did not change in the treatments. However for wheat straw, all treatments slightly increased the initial rate compared to that of chips. This suggested an exposure of more reactive hydroxyl groups on or near the surface

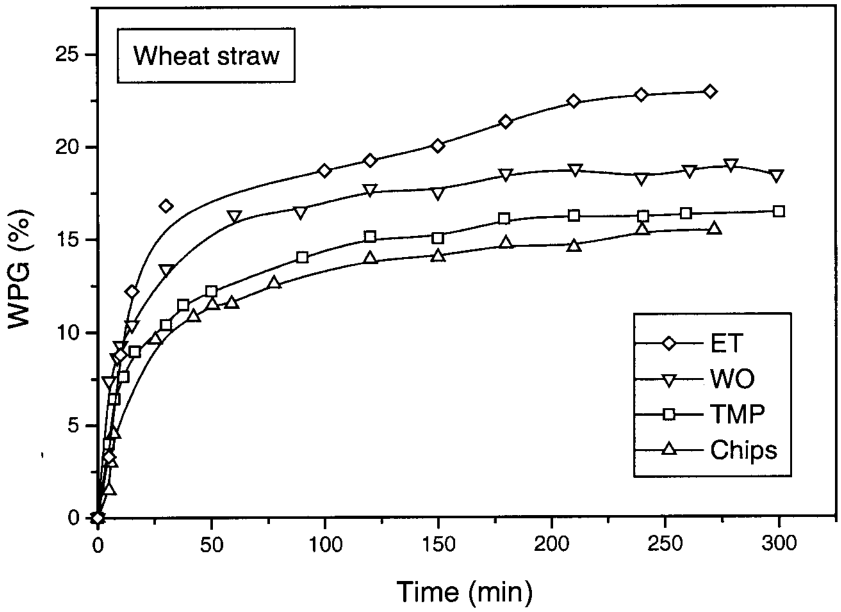

Figure 3. Weight percent gain (WPG (%)) of wheat straw fibers by acetylation as a function of reaction time. Abbreviations are explained in Figure 1 and conditions in Table 1.

Table 2. Total Weight Percent Gain (WPG (%)) (After 200 min Reaction) and the Initial Reaction Rates for the Acetylation Reaction of Different Wheat Straw and Beech Fiber Types. Abbreviations Are Explained in Figure 1 and Conditions in Table 1

Material	Fiber Type	WPG (%) (After 200 min)	Initial Reaction Rate (min^{-1})
Beech	Chips	22.4	0.64
	WO	19.8	0.36
	TMP	13.5	0.47
	ET	17.2	0.45
Wheat straw	Chips	14.6	0.40
	WO	18.7	0.85
	TMP	16.2	0.64
	ET	22.0	0.63

compared to untreated chips. Alkaline wet oxidation treatment gave the highest initial rate but after short acetylation times the rate decreased as the reaction began to level off. For beech, all treatments decreased the initial rate compared to that of chips probably by removing highly reactive hydroxyl groups from or near the surface of the beech. Alkaline wet oxidation treatment gave the lowest rate. For both materials, TMP- and enzyme-treated fibers had the same initial rate, but enzyme treatment did increase the degree of acetylation of TMP-fibers (Figures 2 and 3).

The small differences observed for the initial reaction rates (Table 2) were a bit disappointing. Thus, the acetylation technique might not be optimal for investigating surface reactivity of fibers as described above. On the other hand, the main change obtained by the treatments was reflected in the number of hydroxyl groups being available for reaction which is where the reaction profiles level off. These differences give the most information on fiber properties. When the reaction level off, further bulk reaction can occur as new groups continue to be made available for reaction, hence, the profiles (Figures 2 and 3) do not reach a constant level of maximum weight gain.[12]

For beech (Figure 2), chips were found to reach significantly higher weight gains than treated fibers. As the chips had been ground to pass a 2-mm sieve their dimensions were comparable to TMP-fibers. The low WPG of TMP-fibers was surprising, as TMP-fibers normally are known to be reactive.[13] Wood chips are normally heated by high-pressure steam to around 140°C, which is above the softening temperature of lignin. When the chips are defibrated in a refiner at high temperature, the wood structure is broken apart at the lignin-rich middle lamella yielding fibers with a coating of lignin. In the case of fibers produced for medium density fiberboard, as used in this study, even higher temperatures were employed (up to 180°C) (the Asplund process).[13] Such pulp gives weak paper due to reduced hydrogen bonding. High processing temperatures have also been shown to reduce lignin content in beech[14] and to bring about condensation reactions within lignin reducing its hydrophilicity.[15]

After alkaline wet oxidation, the overall reactivity of the beech chips decreased. Wet oxidation removed almost all the hemicellulose, a large amount of the lignin and a small amount of the cellulose, leaving more of the less reactive cellulose behind, hence, making a decreased number of reactive sites available for reaction. Even though wet-oxidized fibers had the lowest initial acetylation rate, the WPG increased over a longer period of time reaching a higher WPG level than both TMP-fibers and enzyme treated fibers.

Wheat straw (Figure 3) was an altogether different system, as the reactivity of the chips was limited by the presence of a hard protective surface layer of wax, silica and protein which likely prevented penetration of the acetic anhydride. Both alkaline wet oxidation and enzyme treatment increased reactivity indicating that the treatments have opened the material in such a way that more hydroxyl groups became available for reaction. Alkaline wet oxidation solubilised most of the protective layer in the form of NCWM giving increased reactivity, despite removing reactive lignin and hemicellulose.

Wheat straw TMP-fibers were found to be less reactive (Figure 3) like those of beech (Figure 2). The TMP process will break up the protective outer layer of the wheat straw but the resultant layer of condensed lignin limits reactivity. However, after enzyme treatment the reactivity was greatly increased to a level similar to that of untreated beech chips. The treatment apparently disrupted the condensed lignin surface, greatly increasing the number of sites available for reaction.

FT-IR Spectroscopy

Despite the complexity of IR-spectra of wood and plant cell walls giving difficulties in the assignment of the IR signals, they have proven to give useful information on changes in composition.[16] The FT-IR transmission spectra of wheat straw and beech are shown in Figures 4 and 5, respectively. The intense O-H band at 3600–3100 cm^{-1} has components arising from all three cell wall constituents, but the broadness of this signal due to hydrogen bonding, meant that little information could be extracted from this band. C-H stretching bands can be seen at 2940 and 2905 cm^{-1}, which correspond to CH and CH_2 groups, respectively. The carbonyl signal at 1745 cm^{-1} (C=O stretching of alkyl esters)[17] is due almost entirely to lignin, with a minor contribution from the polysaccharides. The fact that it occurred well over 1700 cm^{-1} means that the carbonyl group was not conjugated to the aromatic ring of lignin but corresponded to ester linkages between cinnamic acids and lignin/polysaccharides.[18,19] The band was more dominating for beech (Figure 5) than for wheat straw (Figure 4) due to the higher lignin content in beech. Following alkaline wet oxidation, this carbonyl signal was eliminated almost completely, due to breakage of ester bonds and solubilisation of lignin during the process. The 1637 cm^{-1} band is also a carbonyl stretching band due to para-substituted ketones or aryl aldehydes,[18,20] and was eliminated by alkaline wet oxidation.

The two small characteristic bands at 1600 and 1510 cm^{-1}, corresponding to aromatic ring vibrations (aryl-H vibration) in lignin,[17,18]

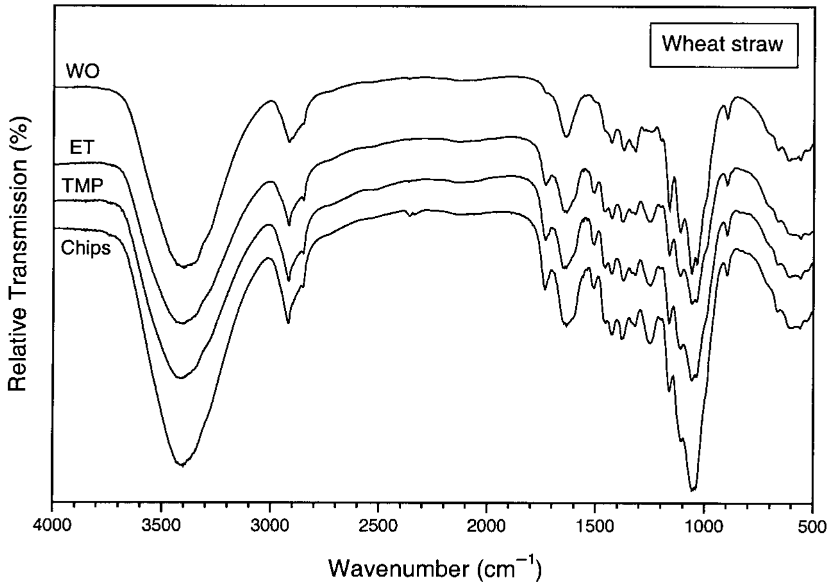

Figure 4. FT-IR transmission spectra of wheat straw fibers. Abbreviations are explained in Figure 1 and conditions in Table 1.

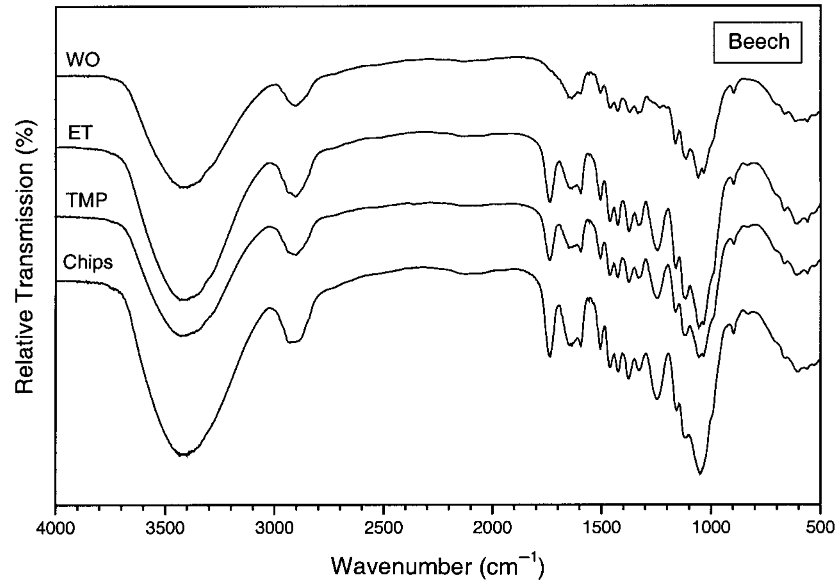

Figure 5. FT-IR transmission spectra of beech fibers. Abbreviations are explained in Figure 1 and conditions in Table 1.

could be seen clearly for all samples (Figures 4 and 5). Their intensity was diminished following alkaline wet oxidation, in particular for wheat straw. Besides aromatic ring vibrations, the 1600 cm^{-1} band also contained components due to polysaccharide-derived vibrations, suggesting high polysaccharide content in wheat straw.[18] The ester-linked feroyl and *p*-coumaroyl groups also contributed to the 1510 cm^{-1} absorbance.[19] Another characteristic feature was the band at 1245 cm^{-1}, C-O-H deformation, C-O stretching of phenolics and asymmetric C-C-O stretching of esters,[17] which was almost completely lost by the alkaline wet oxidation process. In other words, almost all the hemicellulose ester groups initially present in the material disappeared.

Given the complex structure of the cell wall, it was not surprising to observe a considerable overlap of absorption bands. In general, the fingerprint region of the spectra (1500–800 cm^{-1}) was very similar for TMP- and enzyme-treated fibers indicating that FT-IR was not able to detect any tangible effect of the enzyme treatment. The minor differences observed were probably due to solubilisation during the enzyme treatment.

CONCLUSIONS

Wheat straw and beech were used to evaluate the effects of the alkaline wet oxidation process and enzyme treatment with laccase on the chemical properties of lignocellulosic materials. As expected, wheat straw and beech fibers reacted very differently in the two treatments. The chemical composition changed little following enzyme treatment, whereas considerable changes were observed for alkaline wet oxidation yielding fibers with increased cellulose contents. For both materials, most of the hemicellulose (80%) together with a large portion of the lignin were solubilised during alkaline wet oxidation, but essentially all cellulose remained in the solid fraction (99.5%). Wet oxidation of wheat straw also solubilised much of the non-cell wall material including wax and pectin (75%).

Alkaline wet oxidation reduced the reactivity of beech towards acetic anhydride by removing much of the hemicellulose and lignin. High temperature thermomechanical pulping (TMP) yielded unreactive fibers. Subsequent enzyme treatment activated the fiber surface but only to a level lower than untreated beech. For beech, both alkaline wet oxidation and combined TMP- and enzyme treatment yielded fibers, which were less reactive towards acetylation in comparison to beech chips. For wheat straw, both alkaline wet oxidation and combined TMP- and enzyme treatment removed the outer protective layer of wax, silica and protein, resulting in increased reactivity towards acetic anhydride. Enzyme-treated TMP-fibers had reactivity similar to that of untreated beech.

FT-IR spectroscopy showed an almost complete loss of the ester carbonyl stretching signal and the corresponding C-C-O stretching in wet-oxidized materials. This proved that alkaline wet oxidation breaks ester bonds in wheat straw and beech. However, FT-IR could not detect any tangible effect of laccase treatment.

EXPERIMENTAL

Lignocellulosic Materials

Wheat straw (*Triticum aestivum*) was kindly provided by the Biocomposites Center, Bangor, UK and beech (*Fagus sylvatica*) by Junckers Industries, Køge, Denmark.

Defibration

The thermomechanical defibration of wheat straw and beech was carried out by maintaining chips at 140°C in a steam-saturated chamber for 4 min. These chips were then fed to twin disc refiners operating at 180°C and 8 atm. Beech was refined at Junckers Industries, Køge, Denmark, and straw at the Biocomposites Center, Bangor, UK.[7]

Alkaline Wet Oxidation Treatment

Alkaline wet oxidation was carried out in a specially designed loop-reactor.[2] The finely chipped lignocellulosic materials (60 g/l) were mixed with 6.5 g/l Na_2CO_3 and water before adding oxygen to a pressure of 12 bar, and heating the suspension to 185°C for 15 min.[5] After cooling, the suspension was filtered to separate the solid cellulose-rich fraction from the liquid.

Enzyme Treatment

Fungal laccase SP504 (EC 1.10.3.2) was kindly supplied by Novozymes A/S, Denmark. Laccase catalyses the oxidation of a wide range of phenolic substances according to the scheme:

$$4\,\text{Phe-OH} + O_2 \Rightarrow 4\,\text{Phe-O}^{\bullet} + 2\,H_2O$$

Defibrated lignocellulosic material (TMP-fibers) (10.0 g) was suspended in water (200 ml). Laccase was added at a dosage of 3 LACU/g dry fiber. The reaction was stirred for 1 h at 40°C and pH 4.5 (adjusted using H_2SO_4 and NaOH). The pH-value of wood fibers normally ranges between 4 and 5. After cooling for 15 min, fibers were filtered and washed with water.[8] The fibers were dried at 40°C overnight. Enzyme treated fibers were stored below −18°C until analysis. 1 LACU was defined as the amount of enzyme needed to oxidize 1 μmole syringaldehyde per minute under standardized conditions (30°C, pH 5.5).

Chemical Composition

The raw materials and the treated solid fractions were analyzed for their hemicellulose, cellulose, lignin, and the non-cell wall material (NCWM) contents. This was done using the method of Goering and van Soest.[21] This method was established for the analysis of low lignin content non-wood materials and employs neutral detergent, acidic detergent and permanganate solution in order to remove NCWM, hemicelluloses and lignin, respectively, in a sequential process. Detergent analysis methods were used because of the desire to use only one analysis method throughout this study, and the unreliability of wood analysis methods when applied to wheat straw. The impact of errors due to the use of detergent analysis methods for wood was minimal due to this being a comparative study.

The sample material was boiled in a neutral detergent solution for 1 h, after which the suspension was filtered, washed, dried and weighed. The solid fraction was defined as the neutral detergent fiber (NDF). The sample material was then boiled for 1 h in an acid detergent solution (0.5 M sulfuric acid). The solid fraction after filtration and drying was defined as the acid detergent fiber (ADF). The lignin content was determined by treating the ADF with potassium permanganate and acetate-buffer for 90 min. The solid residue was then incinerated. The content of NCWM was calculated to be the solid removed by the NDF analysis, the hemicellulose content was the solid removed by the ADF analysis, the lignin content the solid removed by the permanganate step, and the cellulose content the solid removed by the incineration step. All samples were analyzed in duplicate and results were given as a dry matter percentage.

Acetylation

All samples were Soxhlet extracted in a toluene/acetone/ethanol mixture (4 : 1 : 1 by volume) for a period of 5 h followed by drying at 105°C overnight. The dried fibers (*ca.* 300 mg) were acetylated by adding 50 ml acetic anhydride in dry pyridine (volume ratio of 4 : 6). Reactions were carried out in a preheated oil bath (80°C). Reactions were stopped by addition of acetone (*ca.* 25 ml) followed by filtration. Fibers were washed with acetone (3 × 100 ml), then washed in refluxing acetone for 1 h. Modified fibers were dried at 105°C overnight. Weight gain was calculated as a percentage based on the oven-dried unmodified material.

FT-IR Spectroscopy

Approximately 3 mg portions of straw or wood were mixed with 300 mg of KBr and homogenized by strong agitation in a Dismembrator for 5 min. The resulting finely divided mixture was pressed into transparent samples suitable for analysis and the IR-spectra were recorded in the Perkin-Elmer 1760X FT-IR spectrometer using KBr as a reference.

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