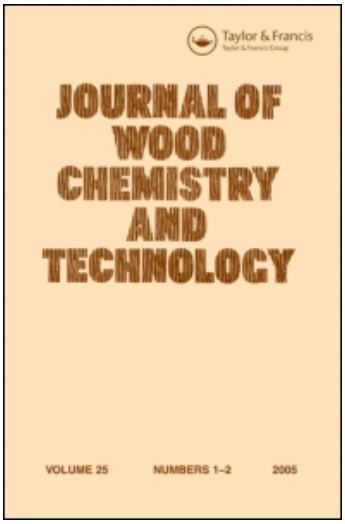

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A CP/MAS ^{13}C NMR Study of the Effect of Steam Explosion Processes on Wood Composition and Structure

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A CP/MAS ^{13}C NMR STUDY OF THE EFFECT OF STEAM EXPLOSION PROCESSES ON WOOD COMPOSITION AND STRUCTURE

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ABSTRACT

Two wood species - the softwood Pinus radiata and the hardwood Eucalyptus regnans have been examined by CP/MAS ^{13}C NMR spectroscopy before and after treatment by two steam explosion processes, namely, the Canadian Iotech Process and the Australian Siropulper process.[1,2] While the main effects of treatment appeared to be, in general, similar for both processes and both species of wood, e.g. an apparent increase in cellulose crystallinity, hydrolysis of the hemicellulose component, de-etherification and depolymerization of the lignin component, some differences were also detectable, particularly in samples of extracted exploded wood. These included differences in cellulose crystallinity, composition and residual lignin structure. In some cases, signal area ratios could be used both to confirm and to provide a comparative measure of these effects and differences. Signal ratios were also the basis for development of a useful, non-destructive method of examining lignin content, which may have an advantage over the Klason method for comparing exploded wood and extracted exploded wood samples, in that it does not degrade carbohydrate. Some implications of the results for bioconversion processes are mentioned briefly. CP/MAS NMR spectroscopy is thought to be a valuable and promising technique for examining and comparing the effects of pretreatment on the structure and composition of biomass substrates, as it provides estimates, from a

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single experiment, of cellulose crystallinity and lignin content, as well as qualitative comparisons, e.g. of lignin structure.

INTRODUCTION

Steam explosion processes, such as the Iotech and Siropulper processes,[1-5] are currently of considerable interest as a promising pretreatment in the conversion of biomass to high value products such as liquid and gaseous fuels, protein, chemicals and phenolics for use in adhesive resins manufacture.[6]

The treatments result in substantial breakdown of the structure of the lignocellulosic material involving hydrolysis of the hemicellulosic component, depolymerization of the lignin component and defibration and therefore, an increase, which can be very large, in the accessibility of the carbohydrate components to digestion by enzymes or microorganisms and the possibility of conversion to valuable products. The hydrolysed carbohydrate and depolymerized lignin are accessible to solvent extraction and the latter is a low molecular weight and potentially valuable new type of lignin.[1-6]

To date, steam explosion treatment has been less successful in making softwood biomass accessible to bioconversion, than it has been with hardwood and possible reasons for this need investigating.[1,5]

Solid state (CP/MAS) ^{13}C NMR spectroscopy is a rapidly developing new technique which appears to be ideal for studying the changes in structure and composition that take place in biomass on steam explosion treatment. Some work using this technique to examine wood and exploded woods has recently been reported.[7-9]

In this paper, the technique has been used to study two wood species - the softwood Pinus radiata and the hardwood Eucalyptus regnans, treated by two explosion processes, namely, the Canadian Iotech Process (IP) and the Australian Siropulper Process (SP).[1-4] Additional substrates with bioconversion potential, prepared by removal of lower molecular weight material from the exploded woods have also been examined using the technique. Pinus radiata wood pretreated with SO_2, to improve breakdown, prior to treatment by the Siropulper Process (SP(SO_2)), was included in the study.[5]

RESULTS AND DISCUSSION

1 Steam Explosion Treatment of Pinus Radiata Wood

The following were examined: untreated wood (W), exploded wood (EW) and extracted exploded wood (EEW), prepared by removing the bulk of the lower molecular weight or solubilized material by a single stage extraction with aqueous acetone. The extraction removed about 90% of the solubilized lignin and carbohydrate from the Siropulper treated wood, but less of the soluble carbohydrate from wood treated by the Iotech process.

Apparent changes in structure and composition were detected by comparing spectra and further examined using signal area ratios to explore the potential of this technique for measuring and comparing these changes. Signal assignments were made using literature data and some data obtained in this laboratory.[7,8,10,11] Signal intensities were integrated by the cut and weigh method. Spectra are shown in Fig. 1.

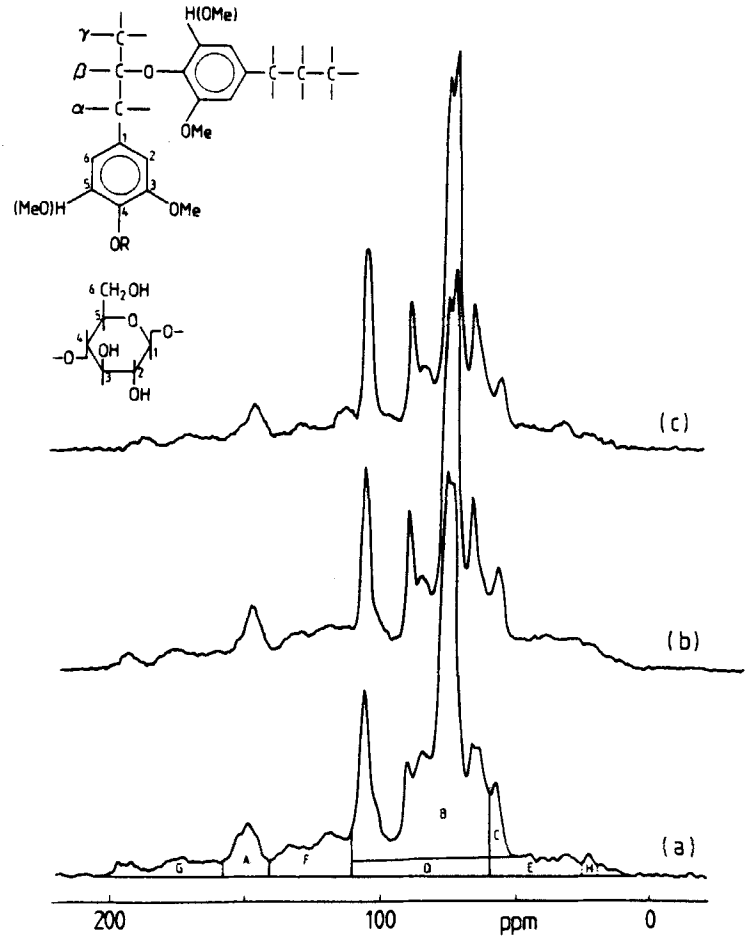

FIGURE 1. CP/MAS ^{13}C NMR spectra of steam exploded P. radiata wood. (a) untreated wood, (b) (IP.EW), (c) (IP.EEW), cont'd.

(a) Measurement of Lignin Content and the Effect of Steam Explosion on Lignin/Carbohydrate Composition

The composition of (W), and (IP), (SP) and (SP(SO_2)) (EW) and (EEW) is shown in Table 1 in terms

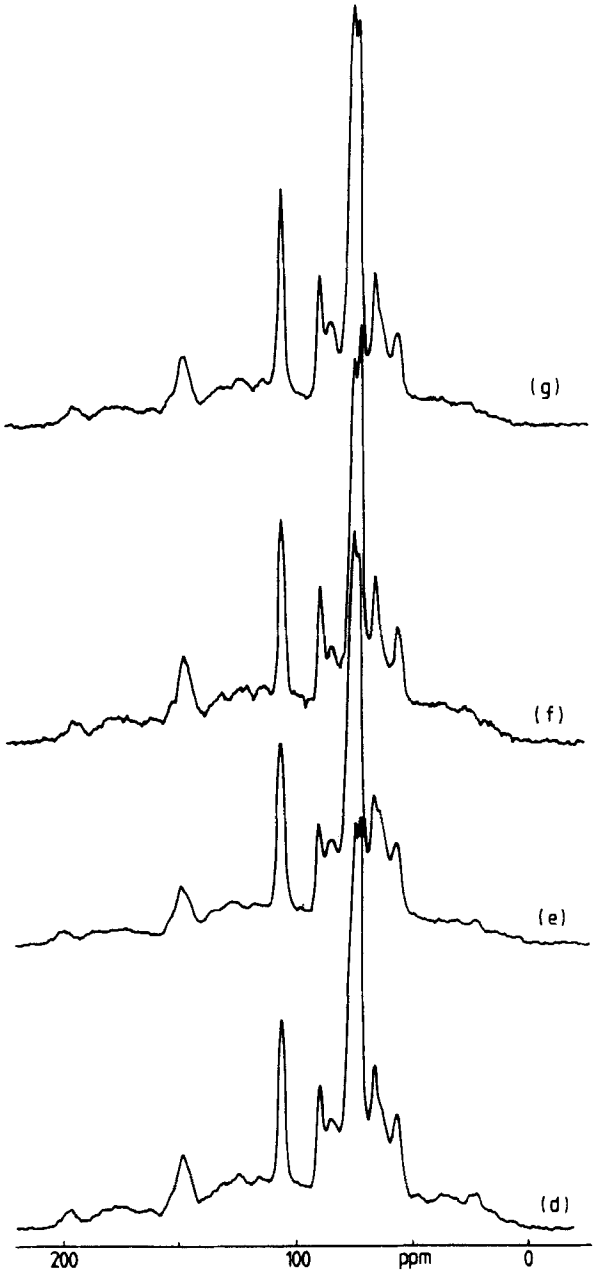

FIGURE 1 CONT'D. (d) (SP.EW), (e) (SP.EEW), (f) (SP(SO_2).EW), (g) (SP(SO_2)EEW).

TABLE 1

Composition and ^{13}C NMR Signal Area Ratios for Pinus Radiata Samples (and Pure Cellulose)

Sample	C/L	wt.%L	C_4^{ci}/C_4^{as}	C_1/C_4^{ci}	C_1/C_4^{ci+as}
W	2.2	34	0.62	2.5	1.0
IP.EW	1.6	42	1.3	1.6	0.9
IP.EEW	2.2	34	1.4	1.5	0.9
SP.EW	1.6	41	1.1	1.6	0.8
SP.EEW	2.1	35	0.9	2.0	0.9
$SP(SO_2)$.EW	1.2	49	1.4	1.6	0.9
$SP(SO_2)$.EEW	1.5	43	1.1	1.8	0.9
Cellulose*	-		1.3	1.8	1.0

C/L = carbohydrate/lignin monomer units
C_1, C_4^{ci}, C_4^{as} = signals of cellulose, ci = crystalline (or interior) as = amorphous (or amorphous + surface) IP = Iotech process, SP = Siropulper process, $SP(SO_2)$ = Siropulper process with SO_2 pretreatment. EW = exploded wood, EEW = extracted exploded wood.
* = Whatman No. 1 filter paper

of the ratio of carbohydrate to lignin monomer units (C/L) and also the weight percentage of lignin. Details of the method of measurement and calculation are given in the experimental section. A simplifying assumption allows ratios of carbohydrate to lignin carbon atoms to be converted readily to (a) ratios of monomer units and (b) weight %'s of lignin and hence comparison with other methods, e.g. the Klason, of examining lignin content.

Although the Klason value for untreated wood,[12] 27.4% is substantially lower than the NMR value, the difference is not unreasonable for such completely different methods as, for example, the problem of overlap of signals and spinning side bands and simplifying assumptions may inflate the NMR value, while soluble lignin and the use of extractives-free wood may lower the Klason value. Even if the NMR value is inflated by systematic errors, this does not detract from the usefulness of the method for comparing lignin contents within a set of samples.

For each process, (IP) and (SP), the ratio of carbohydrate to lignin monomer units (C/L) appears to fall substantially and by a similar amount on explosion treatment of the wood, but to rise again to near the original value in the extracted exploded wood. In the case of the SO_2 pretreated wood (SP(SO_2)), the fall appears to be much greater and the rise in the (EEW) to be somewhat smaller in magnitude than that for the above processes. The fall would be in accordance with some of the carbohydrate, particularly hemicellulosic carbohydrate, being degraded by the treatment,[3,13] and the rise in (EEW) samples with removal of lignin. Non-volatile degradation products of carbohydrate give broad bands of signals in both the aromatic and aliphatic spectral regions. For example, CP/MAS spectra of thermally degraded cellulose show a broad band of signals in the 100-180 ppm region.[14] The measured lignin content may therefore include a contribution from those carbohydrate degradation products, such as furan polymers, which give signals in the spectral region used for integration of signal intensities. However, as shown

below, the Klason method for lignin content not only includes degraded carbohydrate, but may also degrade and include some of the low molecular weight carbohydrate, particularly xylose, released by the steam explosion processes.[3,15] The NMR method therefore appears to be a useful, non-destructive way of examining wood composition and to have the advantage of not degrading carbohydrate, when applied to (EW) and (EEW) composition. However, the possibility of some distortion of the measured lignin content, due to contributions from carbohydrate degradation products and possibly modified lignin side-chains should be kept in mind. e.g. The lignin extracted from ($SP(SO_2)$.EW) gives a prominent signal at 145.3 ppm, which may contain a considerable contribution from C_β in enol ethers.[15]

(b) The Effect of Steam Explosion on the Cellulose Component of the Wood

The carbohydrate region of the spectra (Fig. 1) shows that the explosion treatments bring about major changes in two pairs of signals assigned to (a) crystalline (or interior, as proposed recently for native cellulosic materials)[16,17] cellulose and (b) amorphous (or amorphous + surface)[16,17] cellulose, i.e. in the C_6^{ci} and C_6^{as} signals, which occur at 65.3 and 62.6 ppm respectively and the C_4^{ci} and C_4^{as} signals which occur at 89.2 and 83.9 ppm respectively.[7,16,17] Smaller changes in the doublet for $C_{2,3,5}$, which occurs at 72.5 and 75.1 ppm respectively, are also observed. Signals for amorphous (or amorphous + surface) cellulose contribute to the downfield component of this doublet.[7] The C_6 signals, which appear as a doublet in the spectrum of untreated

wood, become almost a singlet in the spectra of the exploded woods due to a massive reduction in the intensity of the C_6^{as} signal, while the C_4 signals show a very marked increase in the intensity of the C_4^{ci} signal and a decrease in that of the C_4^{as} signal. The change in the $C_{2,3,5}$ doublet is an apparent increase in the ratio of the intensities of the upfield and downfield components from <1 to >1. However changes in the structure of hemicellulosic carbohydrate, which gives signals of greatest intensity in the region between the two components of the doublet could also have affected these signals.[7] Cellulose signals in general overlap the broader signals of the hemicellulosic component.[7] However distortions appear to be minor (see below) and our interest is in comparing crystallinities rather than in absolute values.

The apparently increased crystallinity of the cellulose component in the exploded woods does not appear to be significantly affected by removal of extractable material in the case of wood treated by the Iotech process. In contrast, Siropulper treated wood appears to undergo a decrease in crystallinity on removal of extractable material, which is shown by a decrease in the relative intensities of the $C_{4,6}^{ci}$ and $C_{4,6}^{as}$ signals and a reversal in the relative intensities of the components of the $C_{2,3,5}$ doublet from >1 to <1.

The signal area ratios C_4^{ci}/C_4^{as} (Table 1) were determined to examine and provide a measure of the magnitude of the crystallinity changes. They support a substantial, real and fairly similar increase for all the (EW)'s and Iotech processed (EEW) and smaller

increases, relative to the (EW)'s, for the Siropulper processed (EEW)'s. The validity of using these ratios as a measure of changes in cellulose crystallinity is supported by the C_1/C_4^{ci} ratios and also by the C_1/C_4^{ci+as} ratios, which are reasonably close to the theoretical value. (Table 1). Distortions due to signal overlap with signals of the other wood components (such as C_4 of hemicellulosic material) and products of the explosion treatments therefore appear to be minor.

This increase in crystallinity on steam explosion treatment could be due to removal of, and/or an increased degree of order in, the 'as' component. The latter has recently been suggested or implied for other species of wood.[9,18] For example, the increase may be due in part to cleavage of interunit linkages in amorphous regions and to destruction of bonding interactions of surface cellulose with matrix components. The apparently process-related crystallinity difference observed on removal of solubilized material, which does not appear to be a spectral artifact, may in part be due to differences in the physical characteristics of the (EW)'s and different changes in structural features of the cellulosic fibre (such as pore size), occurring on extraction of soluble material and subsequent drying of the fibre.

The C_4^{ci}/C_4^{as} ratios correspond to crystallinities of only 38% for the untreated wood and from 51-59% for all the treated samples except (SP.EEW) 46%. A value of 56% was also obtained for Whatman No. 1 filter paper (see Table 1). All of these values are considerably below the 70% range reported for native celluloses,[19] which is interesting in view of the

recent proposal for native cellulosic materials, that surface cellulose makes a substantial contribution to the signals attributed to amorphous cellulose.[16,17,20] This results in estimates of crystallinity based on relative signal intensities being low, compared with those given by other measures.[16,17,20] For wood samples, some distortion due to overlap of signals is possible, but it should not affect the usefulness of these measurements for comparing substrates.

The magnitude of the crystallinity change on steam explosion treatment suggests that slow steam penetration of the wood, which was thought to contribute to the treatment being less satisfactory with softwoods,[1] may not be a significant problem.

The crystallinity of cellulosic substrates is reported to be one of the factors affecting accessibility to bioconversion processes and the rate of enzymatic hydrolysis, while others include the lignin matrix and the surface area and porosity of the cellulose fibre.[21] Studies have shown that amorphous cellulose reacts at a faster rate than crystalline resulting in an increase in the X-ray measured crystallinity index.[22] The apparent increase in crystallinity on steam explosion treatment could therefore be detrimental to bioconversion. However, despite the increase, the crystallinity of all the samples appears to compare favourably with that of filter paper, which is a readily digestible substrate.[23]

The NMR method of examining crystallinity should be quite useful in studies comparing the enzymatic digestibility of cellulosic substrates, but the implications for hydrolysis rates of the possible inclusion of surface cellulose in the signal for amorphous cellulose need to be explored.

(c) The Effect of Steam Explosion on the Hemicellulose Component of the Wood

Although most of the hemicellulose signals are obscured by those of cellulose, the signal for C_1 at 102.0 ppm, which shows as a distinct shoulder on cellulose C_1 in the spectrum of (W), is at least markedly reduced in the spectra of the treated woods, Fig.1. The small signal at 21.0 ppm, which is assigned to the methyl carbon of hemicellulose acetyl groups appears to be similarly affected, with a possible exception in the case of (SP.EW). These results indicate that the treatments hydrolyse and deacetylate a substantial amount of the hemicellulosic component and are consistent with the substantial monosaccharide content (primarily mannose, xylose and glucose) of the water soluble extracted material.[15]

They may also again indicate that steam penetration of the wood is reasonably efficient.

A measure of the acetyl group content in the untreated wood is given by the ratio of acetyl methyl groups to carbohydrate monomer units, i.e. by 6H/B. (See Fig. 1 and Experimental). The value obtained, 0.1, corresponding to an acetyl group content of 2% is reasonably close to the range reported for softwoods (1.1-1.7%) and the value of 1.3% reported for P. radiata wood,[12,24] although the error in this determination could be large (see Experimental).

(d) The Effect of Steam Explosion on the Lignin Component of the Wood

Although the propanoid side-chain signals are obscured by the signals of the carbohydrate components and the visible signals are broad with low intensities, the aromatic signals in the 141-158 ppm region can

yield useful information within a reasonably short data accumulation time. Preliminary experiments on lignin model compounds have shown that chemical shifts for CP/MAS spectra rarely differ by more than 2 ppm from chemical shifts for solution spectra. The signals from methoxy-substituted C_3, (at 149 ppm) and the etherified plus free hydroxy-substituted C_4 carbon atoms, which occur downfield and upfield of C_3 respectively, were therefore used, not only to determine lignin/carbohydrate composition, as described in (a) above, but also to examine structural changes in the lignin. Most important of these was de-etherification at the C_4 carbon atom, which gives a measure of the extent to which interunit linkages have been cleaved, especially the major β-0-4, and hence information on depolymerization.

Although the signals are observed as a poorly resolved broad band, the explosion treatments appear in all cases to result in a reduction in the intensity of the signals downfield of C_3 in this band and changes in the intensity of signals upfield of C_3, i.e., in a decrease in signals comprised primarily of C_4 in etherified guaiacyl units and changes in those which include C_4 in non-etherified units. These changes are also shown by the spectra of the (EEW)'s. (These samples contain about two-thirds of the lignin present in the original wood.) Interpretation of these apparent changes as being due to lignin de-etherification was assisted and supported by the structures of the extracted lignins, which have undergone extensive cleavage of the β-0-4 linkages and release of non-etherified C_4 hydroxy-groups.[15,25] The similarity of the spectra for each (EW) and its

corresponding (EEW) suggests that the residual lignin in the (EEW) has also undergone substantial β-O-4 cleavage. This, together with its insolubility suggests that it could be a fairly condensed lignin formed by repolymerization of lower molecular weight fragments, rather than an 'unreacted' lignin in regions not penetrated by the steam treatment. Some incorporation of carbohydrate degradation products and reaction with carbohydrate during repolymerization would not be unlikely.[13,26]

Further work is aimed at verifying and further exploring these structural changes, as lignin content, structure and distribution are factors reported to affect accessibility of cellulosic substrates to enzymatic hydrolysis.[27]

The high 'lignin' content of the exploded woods, which was not greatly reduced by the extraction procedure and the possibly condensed structure of the residual lignin suggested that accessibility of the cellulosic carbohydrate to enzymatic hydrolysis could be rather poor in all samples, including the SO_2 pretreated, unless the lignin and particularly the residual lignin was aggregated by the steam explosion treatments. This has been suggested by Iotech Corporation to occur in hardwoods treated by this process. However the enzymatic digestibility of (SP(SO_2).EW) is reported to be much improved compared with that of (SP.EW),[5] which suggests that lignin structure and distribution are more important than lignin content. The removal of lower molecular weight soluble lignin from the treated wood could however improve the substrate, by removing material toxic to microorganisms.

2 Steam Explosion Treatment of Eucalyptus Regnans Wood

Samples of untreated wood, (IP) and (SP) exploded wood and extracted exploded wood from each process were examined as described above for P. radiata. Spectra are shown in Fig. 2.

(a) Lignin/Carbohydrate Composition

The composition of (W), (IP.EW), (SP.EW), (IP.EEW) and (SP.EEW) is shown in Table 2. Lignin contents determined by the Klason method are also shown. Details of differences in the method of calculation for this type of wood are given in the experimental section.

The NMR value for the lignin content of untreated wood is again substantially higher than the value given by the Klason method. The C/L ratio falls substantially in wood treated by the Siropulper process but appears to be unchanged in wood treated by the Iotech process.
However, another batch of (IP.EW) had a C/L ratio of 1.8 and therefore showed a similar fall to that for (SP.EW), so no significance can be attached to the apparent process difference in C/L. In both cases, the changes in lignin content are very much smaller than those given by the Klason method. This would be consistent with the latter method degrading a substantial amount of the low molecular weight carbohydrate rich in xylose, which is present as a result of hemicellulose hydrolysis and the high xylan content of hardwoods, as xylose has been found to be particularly susceptible to degradation.[15]

The C/L ratio rises again in both of the (EEW)'s and in contrast to the softwood, to values considerably

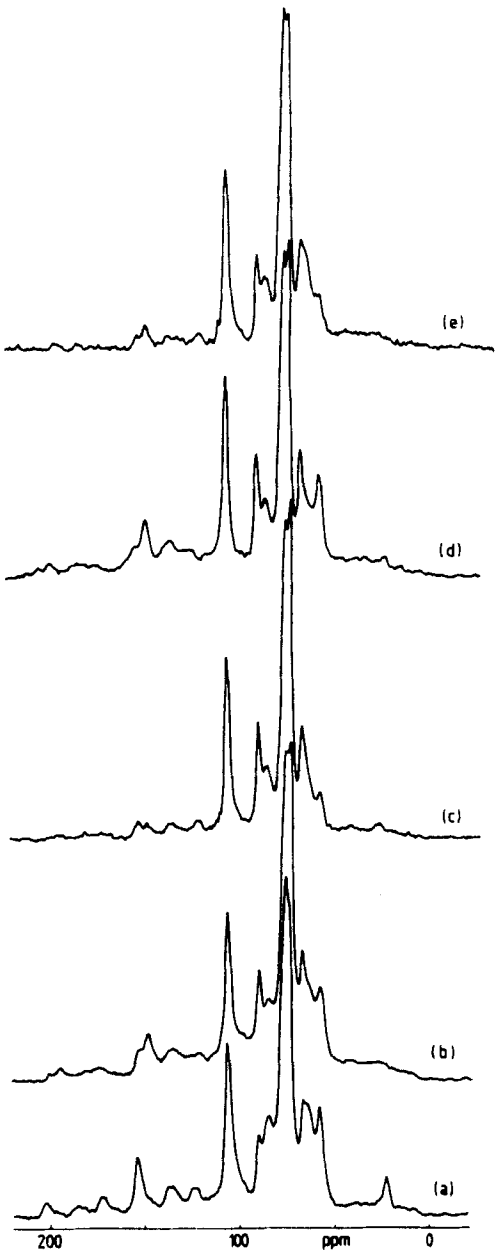

FIGURE 2. CP/MAS ^{13}C NMR spectra of steam exploded E. regnans wood. (a) untreated wood, (b) (IP.EW), (c) (IP.EEW), (d) (SP.EW), (e) (SP.EEW).

TABLE 2

Composition and ^{13}C NMR Signal Ratios for *Eucalyptus Regnans* Samples

Sample	C/L	wt.%L	% Klason L	C_4^{ci}/C_4^{as}	C_1/C_4^{ci}	C_1/C_4^{ci+as}
W	2.4	35	25	0.56	3.2	1.1
IP.EW	2.4	35	43	1.1	1.8	0.9
IP.EEW	5.4	19	19	1.2	1.7	0.9
SP.EW	1.7	43	47	1.2	1.9	1.0
SP.EEW	4.2	23	18	1.0	2.1	1.0

higher than that for the untreated wood, with (IP.EEW) apparently being somewhat richer in carbohydrate. The lignin contents relative to untreated wood are now in slightly better agreement with those determined by the Klason method, possibly due to removal of material degraded by this method. The more favourable C/L ratios in the (EEW)'s and the more extractable lignins support better accessibility to bioconversion and less inhibition with these hardwood substrates.

(b) The Cellulose Component

As observed with the softwood, both explosion processes brought about major changes in the intensities of the signals for (a) crystalline (or interior) and (b) amorphous (or amorphous + surface) cellulose (Fig. 2), consistent with an increase in cellulose crystallinity. Once again, removal of solubilized material appeared to have little effect on crystallinity in the case of Iotech processed wood, but to result in a decrease in crystallinity in Siropulper treated wood.

Measurement of the C_4^{ci}/C_4^{as} ratios confirmed that the crystallinity changes reflected in the intensities of the C_4,C_6 and $C_{2,3,5}$ signals were similar to those observed with the softwood for each type of explosion process. (See Table 2). The validity of using these ratios is again supported by the C_1/C_4^{ci} and C_1/C_4^{ci+as} ratios. Distortion of these ratios due to overlap of cellulose C_1 with lignin syringyl $C_{2,6}$ also appears to be insignificant.

These results appear to indicate that the efficiency of steam penetration into the wood and the effect of steam explosion treatment on cellulose crystallinity is similar for both types of explosion process and for both species of wood. Therefore, cellulose crystallinity does not appear to be a major factor in the poor accessibility of steam explosion treated (without SO_2 pretreatment), softwood fibre to enzymatic hydrolysis.

The process-related difference in crystallinity between the (EEW)'s is also similar for both species of wood, which may be further support for the suggestion given in the case of P. radiata in (b) above, of differences in changes in fibre structure brought about by the extraction procedure. There is growing evidence that changes in the digestibility of treated substrates may take place on drying or storage.[27]

(c) The Hemicellulose Component

The much higher content of acetyl groups in hardwoods is clearly shown by the spectrum of Eucalyptus regnans wood (Fig. 2), in which the signals for the methyl group at 21.2 ppm and the carbonyl group at 173.0 ppm are quite prominent. The ratio of

acetyl groups to carbohydrate monomer units is 0.3, corresponding to an acetyl group content of 5%, which is reasonably close to the range reported for hardwoods of 2.9-4.8%.[24] These signals appear to be removed and the shoulder for hemicellulose C_1 at 102.5 ppm is at least markedly reduced on steam explosion treatment, which is again consistent with deacetylation and hydrolysis of a substantial amount of hemicellulosic carbohydrate and with the high content of monosaccharides, primarily xylose, in the water soluble extracted material.[15]

(d) The Lignin Component

As with P. radiata, the signals in the region 143-159 ppm can be used to determine lignin/carbohydrate composition and also to examine structural changes in the lignin. In the case of hardwood lignins, the signals in this area are comprised not only of signals for $C_{3,4}$ in guaiacyl units ($G_{3,4}$), but also of signals for $C_{3,5}$ in syringyl units ($S_{3,5}$), which in this Eucalyptus species make a greater contribution to the composition of the lignin (MeO/C_9=1.54),[28] as the syringyl content is high.[29] The signals for $S_{3,5}$ in units with free and etherified C_4 hydroxy-groups are quite well resolved in this type of lignin, in contrast to the poorly resolved band of $G_{3,4}$ signals given by the softwood lignin. They have maxima at 148.0 and 153.3 ppm respectively and although they overlap the broad $G_{3,4}$ band, they quite clearly show changes in etherification at C_4. In contrast, the S_4 signals of non-etherified and etherified units occur in a broad band at 133-139 ppm, which also includes signals for S_1 and G_1 carbon atoms.

The spectrum of the untreated wood shows a prominent signal for $S_{3,5}$ in etherified units, while signals for $S_{3,5}$ in non-etherified units and the $G_{3,4}$ band are only a weak shoulder (Fig.2). This suggests that the lignin in this species of wood has a low content of both non-etherified units and guaiacyl units.

Both explosion treatments result in a similar and dramatic reduction in the intensity of this signal and the appearance of a more intense signal assignable to $S_{3,5}$ in non-etherified units. The relative intensities of these signals in the (EW)'s (ca.2:1), indicates extensive cleavage of the major, β-O-4, interunit linkage, which is supported by the structure of the extensively cleaved extracted lignins,[15] as these also show ratios of ca.2:1.

The spectra of the (EEW)'s show a marked reduction in the intensities of these signals, due to removal of lignin, and they appear to show process-related differences in the structure of the residual lignin and, in the case of (IP.EEW), differences in the etherification of the residual as opposed to the extracted lignin. Whereas the ratio of $S^{free}_{3,5}/S^{eth.}_{3,5}$ in the spectrum of (SP.EEW) appears to be similar to that in the spectrum of (SP.EW), i.e. ca.2:1, in (IP.EEW), it appears to be about half that in (IP.EW), i.e. ca.1:1, and it is consequently about half that in the extracted lignin. This suggests that the residual lignin in (IP.EEW) has a much lower content of free phenolic hydroxy-groups at C_4, than both the extracted lignin, and the residual and extracted lignins of (SP.EEW). A possible reason could be that this lignin has undergone less cleavage or de-etherification than the

residual lignin in (SP.EEW), while the latter underwent greater cleavage, but more repolymerization, to give a more condensed lignin, as was suggested above for P. radiata. Both of these lignins would be expected to have considerably higher molecular weights than the more readily removed, extracted lignins. Structural differences in residual lignins could be a cause of process-related differences in the accessibility to and inhibition of enzymatic hydrolysis of the treated wood substrates.

Structural changes in the lignin of hardwoods, as a result of explosion treatments are much more clearly shown by CP/MAS NMR spectroscopy, than those in the lignin of softwoods. The method can therefore provide valuable information on the effects of explosion treatment and removal of extractable material.

General Conclusion

This study has shown that a single technique, CP/MAS NMR spectroscopy, can give valuable information about the effects of steam explosion treatment on wood structure, i.e. about changes in cellulose crystallinity, hydrolysis of the hemicellulosic component, structural changes in the lignin, carbohydrate degradation and changes in lignin/carbohydrate composition. It has also been shown that this technique is able to provide useful measures of crystallinity and lignin content. The potential to examine some effects in greater detail, e.g. the effect on residual lignin structure and to further study other factors affecting enzyme catalysed reactions of cellulosic substrates also appears to be promising.

EXPERIMENTAL

Steam explosion of New Zealand Pinus radiata and Eucalyptus regnans wood was performed by the Iotech Corporation Ltd, Canada (IP) and the Division of Chemical and Wood Technology, CSIRO, Australia (SP).[1-5] In both processes, wood chips are heated with high pressure steam to a temperature between 200 and 250°C, cooked at that temperature for a period of time ranging from seconds to minutes and then the pressure is rapidly released and the digester contents are discharged through a nozzle or die. The exact conditions used by Iotech Corporation for treating our samples were not divulged, but were presumably in the range normally used, i.e. a steam pressure of about 5.5 MPa and cooking at about 230°C for about 60 sec.[1,3] In contrast, the Siropulper process used steam at 1.7 MPa to heat the chips to 205°C. For Eucalyptus regnans chips, the pressure was then raised to 6.9 MPa with a 60/40 volume mixture of CO_2/N_2. After 15 min., the pressure was released and the digester contents were discharged through a specially designed nozzle.[4] In the case of Pinus radiata chips, the pressure was raised to 13.8 MPa with N_2. SO_2 pretreatment involved exposing the chips to SO_2 gas at ambient temperature and atmospheric pressure before steam explosion.[5] Treatment by each process involved a single set of conditions and the effect of varying these was not examined.

Samples of exploded wood were prepared by oven drying the wet exploded wood at 60→40°C. Samples of extracted exploded wood were prepared by stirring the wet exploded wood in acetone for 2 hr. and removing the aqueous acetone solubles by filtration. The

acetone content of the E. regnans filtrate was about 85%, and of the P. radiata filtrate, about 90% (by volume). The ratio of dry exploded wood to acetone was 90 g/l.

Lignin yields were 7-10% of dry wood weight for P. radiata samples and 13-16% for E. regnans samples and corresponded to about 90% of the soluble lignin. The samples of extracted exploded wood were air dried at low temperature and milled to 40 or 60 mesh using a Wiley mill.

NMR Spectra

CP/MAS ^{13}C NMR spectra were run on a Varian XL-200 spectrometer. The ^{13}C NMR frequency was 50.3 MHz and proton radiofrequency fields of 0.5 and 1.0 mT were used during cross-polarisation and data acquisition (respectively). Samples (0.3g) were packed in Kel-F rotors and spun at 2.3-2.5 kHz. Each 1 ms contact time was followed by 50 ms of data acquisition and a pulse delay of 0.5 s, for all samples except Whatman filter paper cellulose (10 s). Transients were averaged for 30-100 minutes.

Proton spin-lattice relaxation rates were measured for the P. radiata samples to check that the chosen pulse delay was adequate for full recovery of the proton magnetisation. The same pulse delay was assumed adequate for the other samples. Cross-polarisation rates and rotating-frame relaxation rates were also measured for the P. radiata samples. The results showed that the 1ms contact time was adequate for full cross-polarisation of all of the signals. Rotating-frame relaxation rates were similar for protons associated with lignin and cellulose, although the differences were sufficient to cause significant

overestimation of cellulose crystallinity for contact times greater than 1ms. Detailed results of these spin relaxation measurements will be published elsewhere in an assessment of the reliability of CP/MAS NMR for quantitative analysis.

The NMR analysis is based on measurements of relative signal areas and therefore rests on the assumption that all of the carbon in the wood contributes to the NMR spectrum. This was tested for E. regnans wood by running spectra of wood mixed with reference substances. These preliminary experiments showed that signals assigned to the wood were as strong as expected for the known weight and carbon content, within experimental uncertainties.

Expanded scale spectra were used to examine in detail the structure of the lignin band at 140-160 ppm.

Measurement of Signal Areas

Signal areas were measured by cutting out and weighing the signals. Perpendiculars were dropped to the boundary of area B for carbohydrate and methoxyl signals and the baseline for lignin and acetyl methyl group signals. (See Fig.1).

The error in C/L ratios (see below) is estimated to be 3-4% and in C_4 signal ratios, e.g. C_4^{ci}/C_4^{as}, 6-8%. An error of 50% in estimating the area of the weak acetyl methyl group signal given by P. radiata is possible.

Lignin Content (C/L)

Except for minor contributions from other types of carbon, the signals occurring between 141 and 159 ppm are given by the two oxygen-linked aromatic carbon atoms ($C_{3,4}$) of softwood (guaiacyl) lignin units and,

in the case of hardwood (guaiacyl/syringyl) lignins, by the two methoxyl-linked aromatic carbon atoms ($C_{3,5}$) of syringyl units. A portion of these signals resides in spinning side bands (SSB's). Integration of these signals and multiplication by the SSB correction factor (1.6) therefore measures one-third of the lignin aromatic carbon. Total aromatic carbon is therefore given by the formula: ArC = 4.8A, where A is the area of the centreband, (see Fig.1).

The SSB correction factor was estimated from relative areas of centrebands and sidebands in CP/MAS spectra of pure compounds. Values of 1.48 and 1.57 were found for $S_{3,5}$ in syringic acid and 2,6-dimethoxy-4-methylphenol, while values of 1.61 and 1.74 were found for G_3 and G_4 in vanillyl alcohol, and of 1.52 and 1.58 for G_3 and G_4 in dihydroconiferyl alcohol. This range is so small that a single mean value (1.57) was used for both syringyl and guaiacyl units in lignin. Correction factors based on crystalline model compounds can be used for NMR of wood, since SSB intensities are determined mostly by intramolecular effects rather than molecular packing effects. Preliminary experiments involving simulated spectra of solid lignin have confirmed that the spinning side band intensities are very close to those observed for crystalline monomers. These results will be published elsewhere. Cellulose signals showed insignificant SSB's and required no correction.

The signals occurring between 50 and 110 ppm are given primarily by carbohydrate carbon atoms, but include contributions from lignin side-chains, lignin SSB's and lignin and hemicellulosic carbohydrate methoxyl groups. We have drawn a boundary between

areas B and D (see Fig.1) to approximate the relative contributions from carbohydrate and other signals. The boundary was drawn at the level of the low-field lignin SSB signals (G in Fig.1). The validity of this assumption is discussed below. Area C represents signals from both lignin and hemicellulose methoxyl groups and area H signals from acetyl methyl groups.

The ratio of carbohydrate carbon to lignin aromatic carbon is therefore given by B/4.8A and this ratio can be converted to (a) the ratio of carbohydrate to lignin monomer units (C/L) and (b) the weight percentage of lignin, by making the following assumption: as wood carbohydrate consists primarily of hexosans, a C_6 monomer repeating unit was assumed to simplify estimation of carbohydrate content. A lignin monomer unit also contains six aromatic carbon atoms, therefore the above ratio, B/4.8A gives directly the C/L monomer ratio.

The pentosan content is typically between 7 and 10% in softwoods and 16 and 23% in hardwoods and values of 8.0% for P. radiata and 16.5% for E. regnans have been reported.[24,30,31] Therefore the error in C/L resulting from assuming a C_6 carbohydrate repeating unit is 4% for E. regnans, while the wt.%L values (see below) are almost independent of hexosan/pentosan composition.

The C/L ratio was converted to wt.% lignin using the formula $C_6H_{10}O_5$ (MW 162) for the anhydrohexose repeating unit. The formula of a typical softwood milled wood lignin, Norway Spruce, ($C_9 H_{7.92} O_{2.40} (OMe)_{0.92}$), MW 183 was used for the MW of the softwood lignin monomer unit.[32] In the case of the hardwood, as the composition of hardwood lignins is more variable,

the formula reported for E. regnans MWL, ($C_9 H_{7.72} O_{2.75}(OMe)_{1.54}$), MW 207 was used for the MW of the hardwood lignin monomer unit.[28] Therefore:

$$wt.\%L = 10^2/[1 + (B \times MW\ carb.)/(4.8A \times MW\ lignin)]$$

The MW's of the exploded wood extracted lignins are lower than the MW's of the milled wood lignins, but the 6-7% error in MW involved in using the latter for exploded wood samples is not large enough to justify making the method of calculation dependent on additional data, or the loss in the simplicity of the calculation for comparing a number of substrates.

Carbon atom balances were determined to test the validity of the boundary chosen for areas B and D. The sum of the estimates of lignin carbon content (7.9A for P. radiata and 8.4A for E. regnans) and carbohydrate carbon content, given directly by area B, comes to 101% for P. radiata wood and 105% for E. regnans wood. These balances are satisfactory in view of the experimental uncertainties and thus show that area B is a reasonable measure of carbohydrate carbon and that lignin side-chain signals and SSB's contribute a background signal approximated by area D.

A method of estimating carbohydrate by allowing for side-chain contributions to area B+D would not be accurate for exploded wood samples, which lose side-chain signals from this region. The chosen procedure has the advantage of simplicity and giving satisfactory carbon balances. Also, as the C/L monomer ratio is B/4.8A and area B is much greater than area D, the exact level of the boundary is relatively unimportant. Area E includes signals from extractives and SSB's in the case of untreated woods and may, in the case of treated wood samples, include signals from modified

lignin side-chains and carbohydrate degradation products. Signals in area G are primarily lignin SSB's but include carbonyl carbon.

Cellulose Signal Area Ratios C_4^{ci}/C_4^{as} etc.

Experiments with contact times varying from 0.30 to 1ms detected little or no change in the C_4^{ci}/C_4^{as} ratio. Ratios are therefore average values determined from two spectra acquired using a 1ms contact time.

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