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Characterization of Steam-Exploded Wood II[†]

Chemical Changes of Wood Components by Steam Explosion*¹

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Abstract—Chemical changes of main components in wood (cellulose, hemicellulose and lignin) by steam-explosion process have been elucidated by ¹H- and ¹³C-NMR, Gas chromatography, GPC and thermal softening property. By steam-explosion hemicelluloses were rapidly hydrolyzed to lower molecular weight products. Almost all hemicelluloses (27.9%) in Shirakanba wood were hydrolyzed to oligosaccharides to be extracted with water by only one min. steaming at 20 kg/cm², and 53.7% of hemicelluloses were converted to monosaccharides by 8 min. steaming at 28 kg/cm². Monosaccharides obtained by 2 min. steaming at 28 kg/cm² of Shirakanba wood were composed of 61.1% of xylose and only 4.9% of glucose, and the yields were in accord with original composition of hardwood hemicelluloses. Cellulose in non-crystalline area was partially hydrolyzed, and micelle length was decreased to about 2000 Å by 8 min. steaming at 28 kg/cm². However, cellulose was not hydrolyzed to glucose, and non-crystalline cellulose would be annealed and transformed to crystalline cellulose. Thus the crystallinity and micelle width of cellulose were increased by steam explosion treatment. However, when reaction time was increased, the yields of furfural and 5-hydroxymethylfurfural derived from polysaccharides were increased. Lignin was degraded slower than hemicelluloses. The yield of lignin was 29.2% in maximum by 8 min. steaming at 28 kg/cm², and the molecular weight of lignins obtained were decreased to $\bar{M}_w=2100$ and 1100 by 2 min. and 16 min. steaming, respectively. A degradation mechanism of lignin by steam explosion was presumed to be homolytic cleavage of aryl ether linkage.

Keywords: steam explosion, fractionation, homolytic cleavage of lignin, hemicellulose, crystalline cellulose

1. Introduction

Woody biomass is a conglomeration of cell wall constituted with polysaccha-

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rides (cellulose and hemicelluloses) and high molecular weight aromatic polymer (lignin), which could be converted to foods, feeds, liquid fuels and raw materials for chemical industry. Recently, chemical industry of wood on saccharification¹⁻⁴⁾, preparation of cattle feeds⁵⁾ and wood-refinery⁶⁻⁸⁾ have stimulated the development of technology converting wood to energy and chemical raw materials. However, cellulose and hemicelluloses are strongly protected with lignin in wood, and therefore delignification has been recognized as most important step for chemical utilization of wood.

Steam-explosion process will be a useful technique for total utilization of woody resources in the near future.

Thus, chemical characterization of steam-exploded wood is important for utilization of wood. To establish efficient utilization of lignin will become a key point for wood chemical industry. In the previous report we discussed structure and physical properties of steam exploded wood⁹⁾. In the present paper the chemical properties of main components in steam exploded woods are discussed.

2. Experimental

2.1 Preparation of steam exploded wood (EXW)

Four hundred gram chips of Shirakanba (white birch, *Betula platyphylla* Sukatchev var. *japonica* Hara) and Karamatsu (larch, *Larix leptolepis* Gordon) were steamed for 1, 2, 4, 8 and 16 min. with 20, 24 and 28 kg/cm² pressured saturated steam, respectively and released the pressure instantaneously to the atmospheric pressure via a ball valve¹⁰⁾. Steam exploded wood (EXW) was lyophilized for analyses.

2.2 Separation of main components of EXW

Main components of EXW were separated by two methods. 1) The lyophilized EXW (6 g) was extracted with four 200 ml portions of water and the same volume of methanol successively for 48 hr each by a Soxhlet extractor. The water solution was concentrated to about 100 ml and lyophilized (EXS). The methanol solution was evaporated to dryness (EXL). 2) The same weight of EXW was extracted with dioxane-water (9:1 v/v) by the same manner. The obtained solution was concentrated and lyophilized. The extractives was dried over P₂O₅ *in vacuo* and weighted (EXD). One gram of EXD was dissolved in 20 ml of dioxane-water (9:1 v/v) and the solution was dropped into 400 ml of water. The precipitate formed (DL) was filtered and the water solution was extracted with four 100 ml portions of ethyl acetate. The ethyl acetate solution was dried with anhydrous Na₂SO₄ and evaporated to dryness (DWL). Remained water solution was concentrated to about 100 ml and lyophilized (DW). After these extractions the residual wood was dried

in vacuo (EXR).

2.3 Gas chromatography of alditol acetate of water soluble fractions

Water soluble fraction (DW) (100 mg) was dissolved in 0.22 M-NaBH₄ solution (2 ml) and reduced for 1 hr. Other treatment was conducted according to the standard method¹¹⁾. The reduced DW was dissolved in 1 ml of pyridine and 1 ml of acetic anhydride, and acetylated for 15 min. at 100°C. The solvent was evaporated with benzene. Obtained alditol acetates were dissolved in acetone and analyzed by gas chromatography (column: 3% ECNSS-M chromosorb-W, 2 m, temp: 210°C).

2.4 ¹³C-NMR and ¹H-NMR analysis of exploded lignins

¹³C-NMR spectra of methanol soluble fractions (EXL) and ¹H-NMR spectrum of dioxane soluble fractions were recorded, in deuterated DMSO solutions on Varian XL-200 Spectrometer operating at 50.3 MHz for ¹³C and at 200 MHz for ¹H with 45° flip angle and 1 sec. repeating time at 20°C.

2.5 Determination of molecular weight distribution of exploded lignins

Molecular weight distribution of EXL was determined by GPC: Waters HPLC-6000A, column: microstyrigel 500Å, 10³Å and 10⁴Å, solvent: THF, 1 ml/min, detector: 254 nm and 280 nm UV spectrometer. Molecular weight was calibrated by polystyrene standards.

2.6 Determination of residual lignin of extracted EXR

Residual lignin contents of dioxane-water extracted woods (EXR) were measured by acetyl bromide method according to Jonson et al., (1961)¹²⁾ and the values were calibrated for lignin content of EXRs by Klason method¹³⁾.

2.7 Thermal softening analysis of the fractions of EXW

Lyophilized EXW and respective extractives were used for the determination of thermal properties by a thermomechanical analyzer (TM 1500, Shinku Riko Co. Ltd.) to measure the collapse of a column (4×2 mm) of sample under a constant load of 200 g in a heated glass capillary tube. The measurement was conducted over the temperature range from 20°C to 400°C at a programmed heating rate of 1°C/min^{9,14)}.

2.8 Observation by a transmission electron microscope

Shirakanba wood meal and EXW (28 kg/cm², 8 min. steaming) were suspended in water, and homogenized by a Waring Blender (16,000–18,000 rpm, Teraoka Co., Ltd.) for 8 hr and 1 hr, respectively. Each one drop of supernatant suspension was mounted on a grid and mixed with a drop of aqueous solution of uranyl acetate. The excess solution was removed by filter paper and the materials were

airdried. The preparations were observed by a transmission electron microscope (Hitachi H-500) at accelerating voltage of 100 KV⁹⁾.

3. Results and Discussion

Steam exploded wood was separated to hemicelluloses, lignin and cellulose frac-

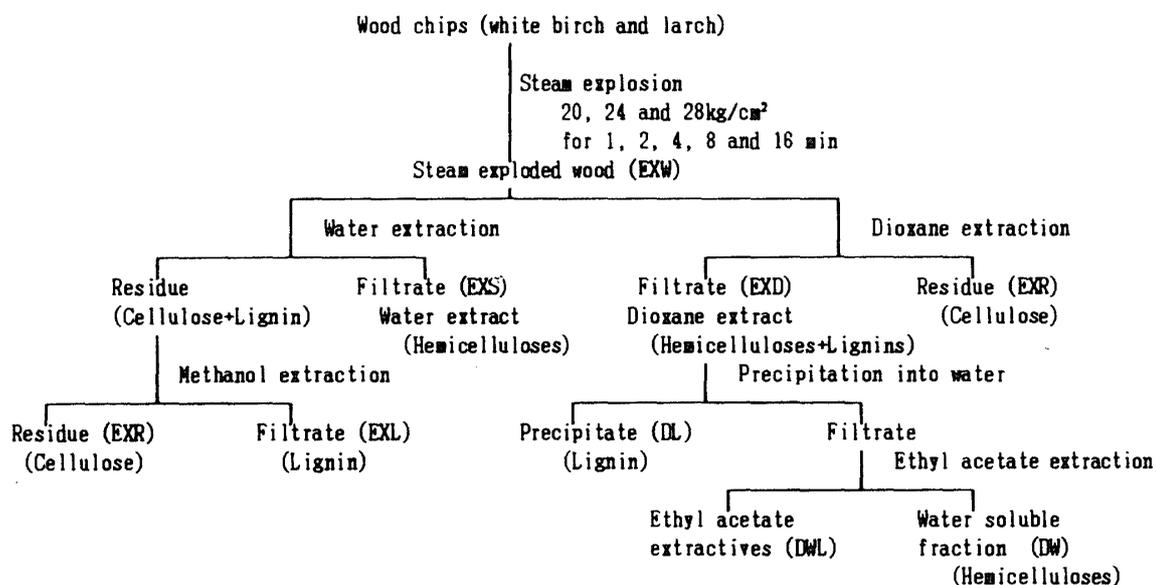


Fig. 1. Fractionation of steam-exploded wood (EXW).

Table 1. Contents of extractives and residual lignin in EXW

Species	Samples		Extractives/EXW			Contents/dioxane extractives			Residual lignin in EXR (%)
	Pressure kg/cm ²	Time min.	Water EXW (%)	Methanol EXL (%)	Dioxane EXD (%)	DL (%)	DWL (%)	DW (%)	
Shirakanba	20	1	27.9	8.5	27.1	9.6	1.6	88.8	15.1
	24	1	25.8	10.8	41.2	12.2	1.2	86.6	12.8
	28	1	29.3	13.7	47.1	19.4	1.2	78.4	12.2
	28	2	29.2	18.3	58.7	25.7	1.5	72.8	9.7
	28	4	29.4	23.7	58.1	40.1	1.9	58.0	5.0
	28	8	18.8	29.2	56.0	48.0	1.8	50.2	5.0
	28	16	22.0	26.2	54.8	44.9	1.9	53.2	2.7
	0	0	—	—	—	—	—	—	22.3
	Karamatsu	20	1	18.7	4.2	15.8	—	1.9	—
24		1	21.4	6.8	28.9	—	1.8	—	—
28		1	25.2	9.1	32.8	—	2.0	—	30.1
28		2	27.0	10.3	36.3	19.4	2.1	79.5	27.2
28		4	23.1	11.0	35.9	25.8	2.7	71.5	17.7
28		8	18.8	11.3	33.2	26.3	2.8	70.9	11.5
28		16	22.4	10.4	31.9	—	3.0	—	8.7
0		0	—	—	—	—	—	—	34.8

tions by two methods to characterize the chemical properties of EXW. Fig. 1 shows the procedure for separation of main components of EXW. The yields of the separated fractions were shown in Table 1. Water extractives (EXS) and methanol soluble fractions (EXL) were mainly composed of hemicelluloses and lignin, respectively. Dioxane-water (9:1 v/v) extractives (EXD) were mixture of hemicelluloses and lignin which were separated by precipitation into water and extraction of water solution with ethyl acetate to high molecular lignin (DL), water soluble lignin (DWL)

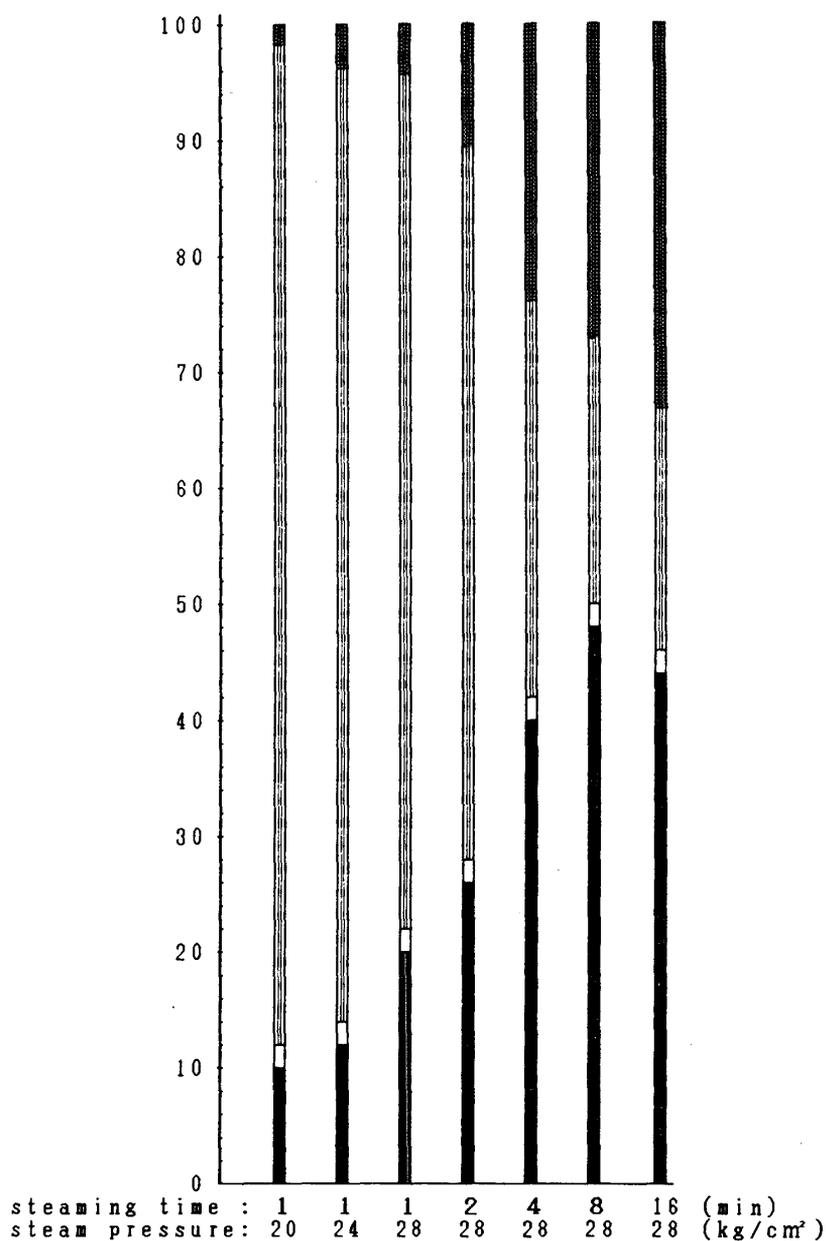


Fig. 2. Contents of lignin and hemicelluloses in dioxane soluble fraction of steam-exploded Shirakanba. ■; lignin, □; water soluble lignin, ▨; oligosaccharides, ▩; monosaccharides.

and hemicelluloses (DW). Hemicelluloses in wood were easily hydrolyzed by steaming to lower molecular weight and converted into almost soluble materials (27.9% of wood) in water by only 1 min. treatment at 20 kg/cm². Severe treatments such as 8 min. and 16 min. steaming at 28 kg/cm² decreased the yields of water soluble fractions and increased those of furfural and 5-hydroxymethyl furfural. Lignin was more resistant than hemicelluloses and was gradually degraded by steaming. The maximum yield of lignin was given in 8 min. steaming at 28 kg/cm² and then the yield was decreased by condensation reaction. Dioxane soluble fractions corresponded to the combined yield of EXL and EXS.

At a steam explosion condition (20 kg/cm², 1 min) oligosaccharides were main components of dioxane extractives but by increasing steaming the contents of lignin and monosaccharides were increased (Fig. 2). By 28 kg/cm² for 8 min. steaming more than a half of hemicelluloses were converted to monosaccharides (Table 2). In 2 min. steaming at 28 kg/cm² 61.1% of monosaccharides was composed of D-xylose and the content of D-glucose was only 4.9% of monosaccharides. The result showed that cellulose in the exploded wood was hardly degraded to glucose (Table 2).

Table 2. Composition of monosaccharides in water soluble fraction of steam-exploded Shirakanba wood (EXW)

Sample	Unknown	Ara.	Xyl.	Man.	Gal.	Glc.	Total
20-1	—	0.8	1.1	—	—	—	1.9
24-1	—	1.0	3.4	—	—	—	4.4
28-1	—	1.5	3.9	—	—	—	5.5
28-2	1.0	2.6	8.8	0.6	0.7	0.7	14.4
(%)	(6.9)	(18.1)	(61.1)	(4.2)	(4.9)	(4.9)	(100)
28-4	1.6	0.3	28.8	1.9	2.5	5.7	40.8
(%)	(3.9)	(0.7)	(70.6)	(4.7)	(6.1)	(14.0)	(100)
28-8	2.1	2.6	37.5	2.5	3.0	6.0	53.7
(%)	(3.9)	(4.8)	(69.8)	(4.6)	(5.6)	(11.2)	(100)

Residual lignin content of EXR was decreased with increasing steam pressure and reaction time. In 8 min. steaming at 28 kg/cm² the content of lignin was decreased to 2.7% of residual wood and the residual wood was 44% of EXW. Then more than 94% of lignin in wood could be extracted by dioxane. In the case of a softwood (Karamatsu) the yield of extracted lignin was lower than in hardwood (Shirakanba) (Table 1). Recondensation of the degraded softwood lignin would be the cause to decrease the yield of methanol and dioxane-water extractives and to disturb enzyme saccharification of the steam exploded softwood.

The extracted EXWs with water, methanol and dioxane were subjected to the analysis of thermal softening properties (Fig. 3). The softening points of EXW

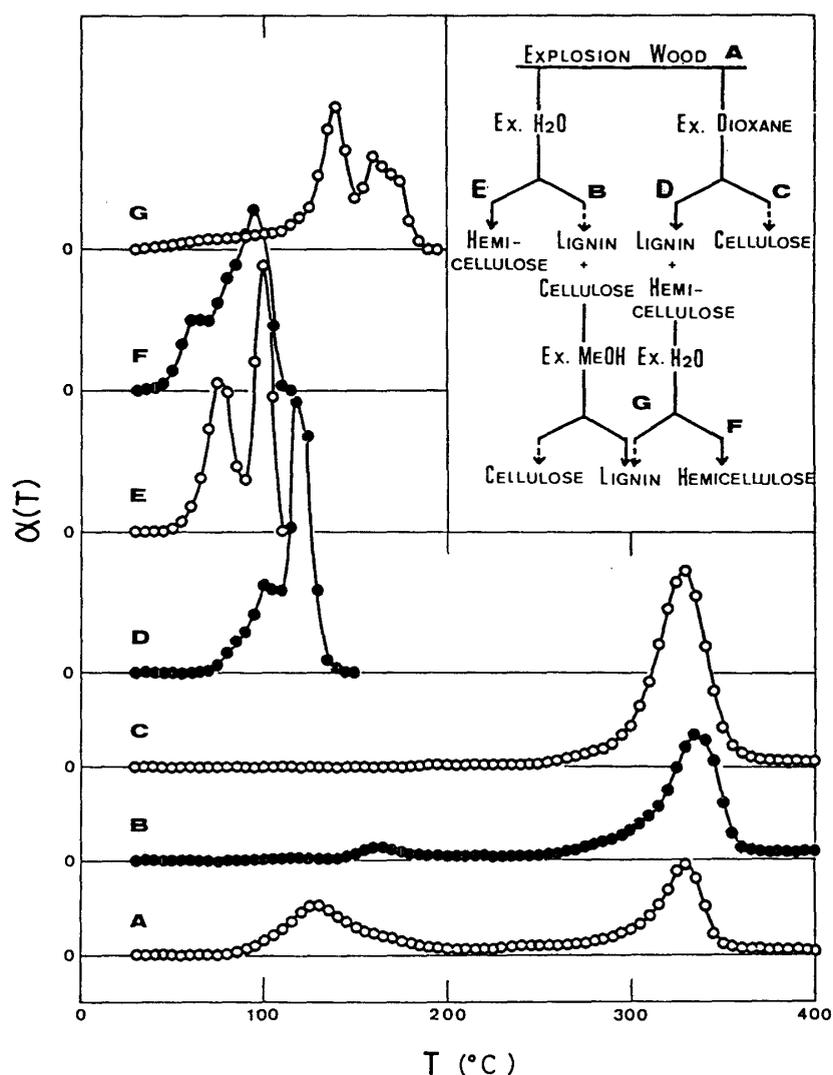


Fig. 3. Thermal softening analysis of the fractions of steam-exploded shirakanba (28 kg/cm², 2 min. steaming). A to G on respective curves denote the fractions separated from EXW shown in the upper right figure. $\alpha(T)$: rate of deformation at each temperature (T)

(28 kg/cm², 2 min) which is a mixture of steam exploded cellulose, lignin and hemicelluloses appeared at 328°C, 160°C and 123°C, respectively (Fig. 3-A). By water extraction hemicelluloses (E) were extracted and the residue was composed of cellulose and lignin (B). By dioxane extraction a mixture of hemicelluloses and lignin (D) were extracted and cellulose remained as residue (C). Lignin fraction (G) was extracted with methanol from water extracted residue (B), and hemicelluloses fraction (F) was separated from dioxane extractives (D) by precipitation from water. The fractions (D, E, F and G) gave two peaks of softening and melting points. The softening point (Ts) and melting point (Tm) of exploded lignin were 138°C and 169°C,

respectively and these of exploded hemicelluloses were 77°C and 100°C, respectively. However, the fraction (D) which is a mixture of exploded hemicelluloses and lignin, was melted at 100°C. Molecular motion of lignin seems to become easy in melted hemicelluloses solution, and the softening and melting points of lignin would be shifted to lower temperature at 123°C. If both hemicelluloses and lignin were more higher molecular weight polymer and not melted, these lower shift of softening points would not be observed¹⁵⁾.

Molecular weight distribution of steam exploded lignin (EXL) was measured by GPC, and weight average molecular weight (\overline{M}_w), number average molecular weight (\overline{M}_n) and a factor of dispersion ($\overline{M}_w/\overline{M}_n$) were calculated using a series of polystyrene standards (Table 3). Molecular weight (\overline{M}_w) of exploded lignin were decreased with increasing steam pressure and increasing time (20 kg/cm²: \overline{M}_w = 2300, 28 kg/cm²: \overline{M}_w = 1900, in constant steaming time for 4 min, and 1 min: \overline{M}_w = 2200, 4 min: \overline{M}_w = 1900, 16 min: \overline{M}_w = 1100, in constant steam pressure at 28 kg/cm²). The molecular weight of softwood exploded lignin was lower than hardwood exploded lignin in contrast to their native lignin and MWL. It seems that almost all exploded hardwood lignins were extracted by methanol extraction but the extraction of exploded softwood lignins with methanol was rather difficult and probably only lower molecular weight fraction of the softwood lignin was extracted with methanol.

Table 3. Average molecular weight of steam-exploded lignin (EXL)

Sample	Pressure kg/cm ²	Time min.	\overline{M}_w	\overline{M}_n	$\overline{M}_w/\overline{M}_n$
Shirakanba	28	1	2200	780	2.8
	28	2	2110	800	2.6
	28	4	1890	880	2.1
	28	8	1870	900	2.1
	28	16	1130	780	1.5
	20	4	2300	860	2.7
	24	4	1900	730	2.6
	28	4	1890	880	2.1
Karamatsu	20	4	1630	740	2.2
	24	4	1330	690	1.9
	28	4	1460	690	1.2
	28	16	1220	630	1.9

¹³C-NMR spectra of EXLs were shown in Fig. 4. EXL of 1 min. steaming at 28 kg/cm² of Shirakanba gave a similar spectrum to that of MWL. However, ether linkages of lignin (152, 110, 86, 72, and 60 ppm) were gradually degraded with increas-

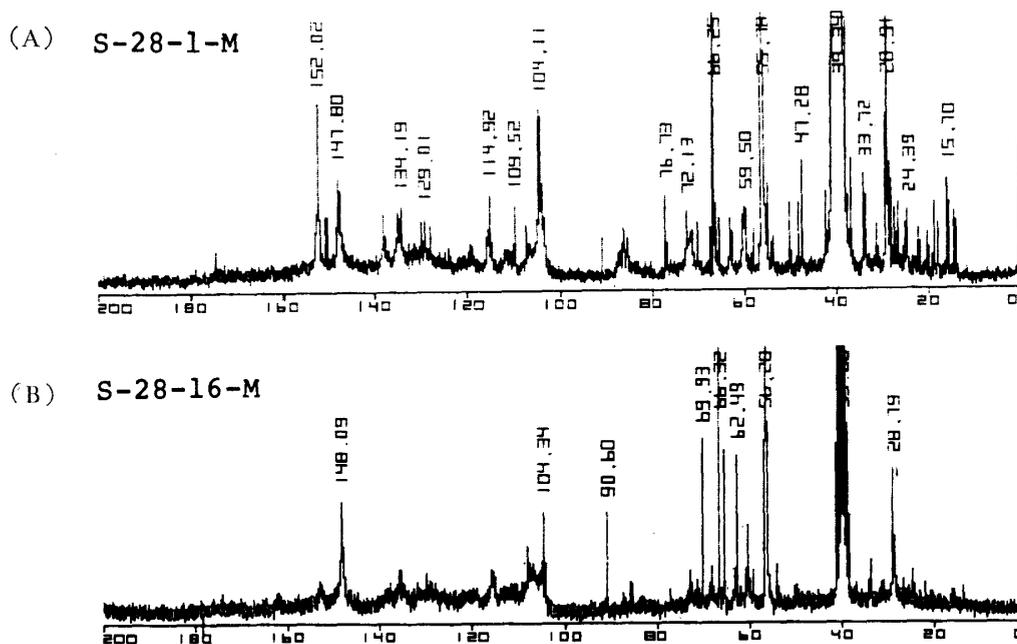


Fig. 4. ^{13}C -NMR spectra of methanol soluble fractions (EXL) from steam-exploded Shirakanba. (A): 1 min, and (B): 16 min steaming at 28 kg/cm^2 .

ing steaming time and the spectrum of 16 min. steamed EXL showed that aryl ether bonds were almost degraded but intensity of the carbonyl groups in the spectrum of EXL was very weak. If lignin degradation by steam explosion occurred through acidolysis reaction, carbonyl groups would be more increased followed by increasing of phenolic hydroxyl groups in EXL. The spectrum of the lignin from 16 min. steamed EXW showed the increase of resinol ($\text{C}\alpha$; 86.9 ppm and $\text{C}\beta$; 54.8 ppm) and phenyl coumaran structures ($\text{C}\alpha$; 88.1 ppm and $\text{C}\beta$; 54.8 ppm) compared with the amounts of both structures in 1 min. steamed EXL.

Water soluble lignin (DWL) was a mixture of low molecular weight lignin degradation products, phenolic extractives, 5-hydroxymethylfurfural and organic acids. From this fraction vanillin, syringaldehyde, vanillic acid, syringic acid, coniferyl aldehyde, sinapaldehyde, coniferyl alcohol, sinapyl alcohol and a mixture of *d*, *l*-epi- and *d*, *l*-syringaresinols were separated by TLC and determined by ^1H -NMR (Fig. 5). However, acidolysis monomers were not detected from DWL, and therefore the degradation of lignin by steam explosion would not occur through acidolysis. We suppose that the degradation of lignin by steam explosion occurs through homolytic cleavage of aryl ether linkage of lignin. Detailed study of the degradation mechanism of lignin by steam explosion will be reported in the following paper with results on the steam explosion of lignin substructure model compounds (guaiacylglycerol- and syringylglycerol- β -guaiacyl ethers)¹⁶⁾.

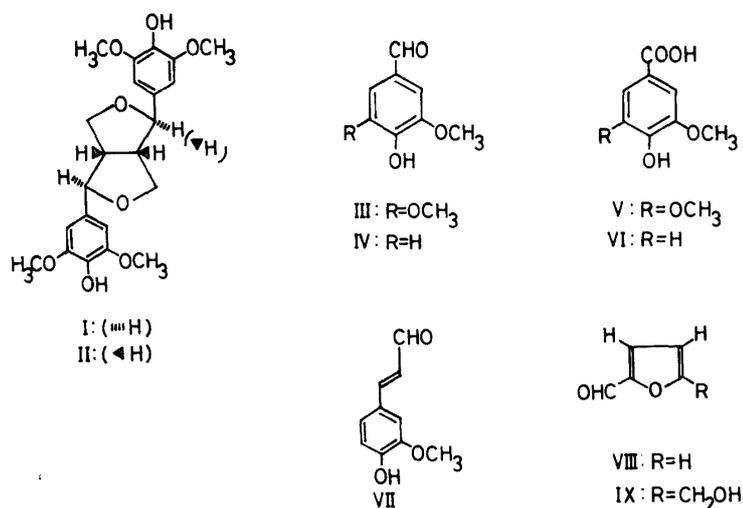


Fig. 5. Identified degradation products in ether soluble fraction of exploded Shirakanba lignin.

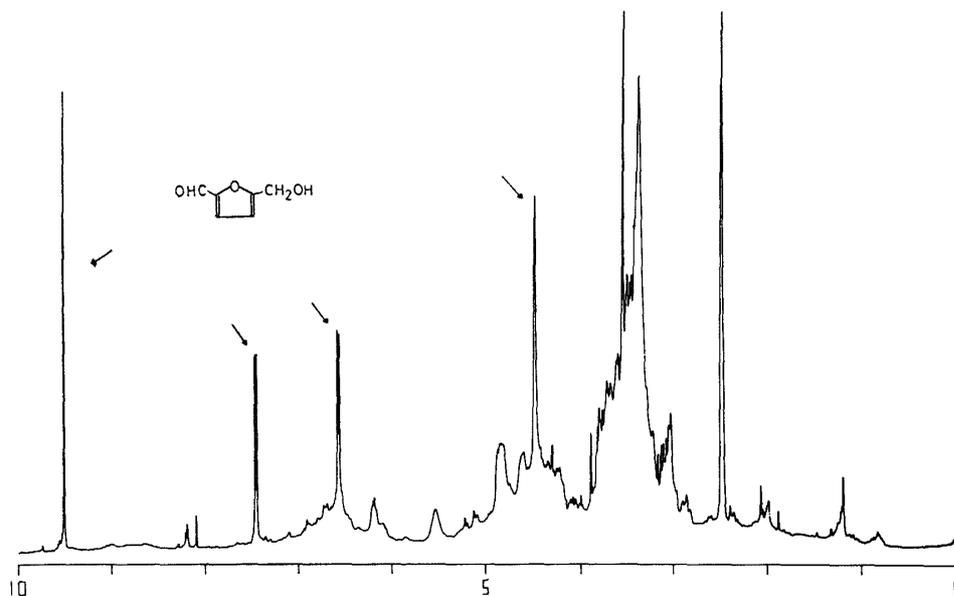


Fig. 6. ¹H-NMR spectrum of dioxane extractives of steam-exploded Karamatsu (28 kg/cm², 8 min. steaming).

¹H-NMR spectrum of dioxane extractives of exploded Karamatsu (28 kg/cm², 8 min) was shown in Fig. 6. Distinct peaks at 9.5 ppm (singlet), 7.45 ppm (doublet, J=3.8 Hz), 6.60 ppm (doublet, J=3.8 Hz) and 4.5 ppm (singlet) were assigned to the protons of 5-hydroxymethylfurfural produced from hexosan by steaming. Intensity of these peaks increased with increasing time of steaming and yields of the compound from the softwood were larger than that from the hardwood at the same steaming time.

In the previous report we showed that crystallinity and micelle width of cel-

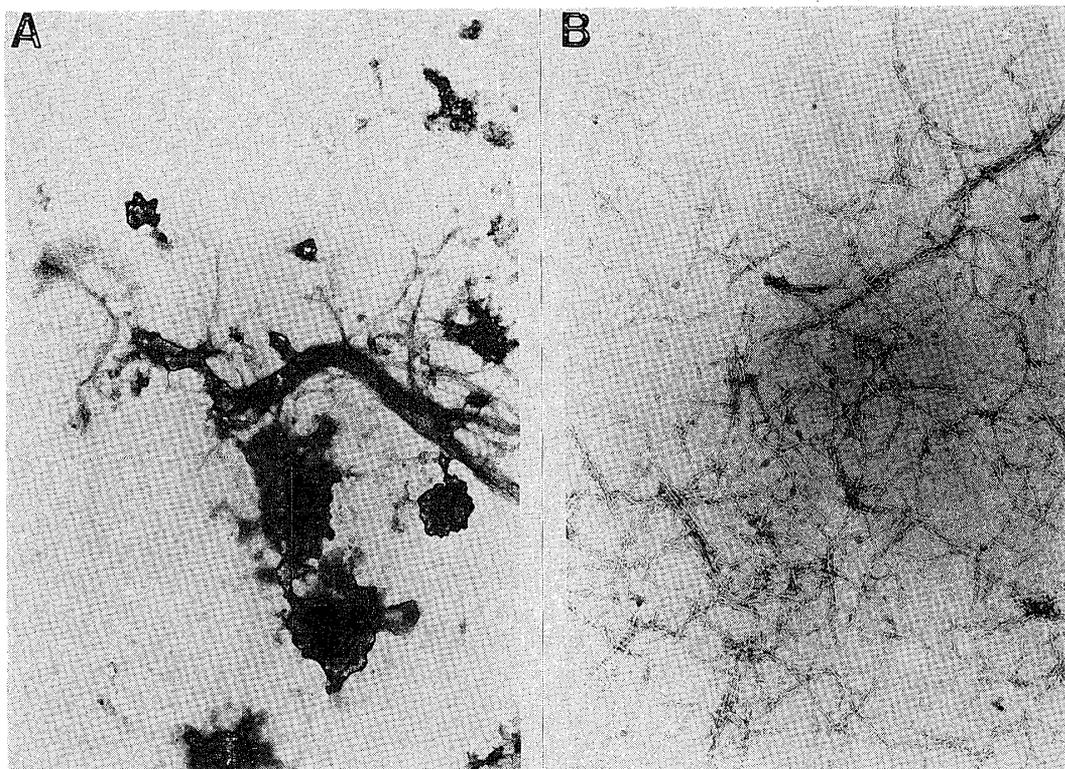


Fig. 7. Photographs of scanning electron microscope of homogenized wood (8 hr) (A), and homogenized EXW (28 kg/cm², 8 min. steaming and 1 hr homogenized) (B).

lulose in wood determined by X-ray diffraction analysis were increased by steam explosion. Microfibril width determined by the electron microscopy analysis was increased about two times⁹⁾. In the present investigation microfibril length was measured on a photograph of transmission microscope of homogenized EXW (Fig. 7B). EXW (28 kg/cm², 8 min. steaming) was easily homogenized by a Waring Blender for only 1 hr but wood meal (unexploded) whose microfibrils were strongly covered with lignin matrix, was hardly homogenized to separate cellulose microfibrils. And the length of original microfibril could not be measured (Fig. 7A). However, microfibrils in 1 min. steamed EXW at 28 kg/cm² were observed, but the length was longer than the observing screen. Thus, we concluded that the length of original microfibrils were enough longer as compared with these of EXW. The average microfibril length of 8 min. steamed EXW was 1800 Å. From this value the average DP of cellulose was calculated to be about 360. The result showed that cellulose was partially hydrolyzed at noncrystalline regions, and micelle length and molecular weight were decreased to about 1800 Å and DP=360 by 8 min. steaming at 28 kg/cm². However, cellulose was not converted to monosaccharide (glucose) by the steaming. Hydrolysis of cellulose by the steaming was slower than that of hemicelluloses, and in partially hydrolyzed cellulose physical stress could

be released and noncrystalline cellulose would be annealed and transformed to crystalline cellulose by high temperature steam. Then the crystallinity and micelle width were increased by steam explosion. Characterization of crystalline change of exploded cellulose will be reported in detail in next paper of this series¹⁷⁾.

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