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The Effect of Lignin Structure on Decay Resistance of Some Tropical Woods

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I. Introduction

It is most evident that the natural decay resistance of wood depends on the concentrations of toxic substances in the wood, which are formed during the formation of heartwood. These toxic extractable substances are the principal causes of decay resistance of wood[1-5]. It has been already concluded in the previous paper[6], that Eusiderin, a main compound in the n-hexane soluble fraction of acetone extracts from the heartwood of Ulin was considered to be responsible for the natural decay resistance of this wood.

In addition, although lignin is by far the most important non-toxic factor, but it is well known to be able to limit the growth of microorganisms in the wood biodegradation. As it has been reported previously[7], the chemical structure of lignin in Ulin wood is not typical for hardwood species. It contains a large amount of guaiacyl units. The effect of lignin on decay resistance of wood has been studied by previous investiagtors. They reported that the slow degradation rate of softwoods by white-rot fungi is not due to the inhibitory effect of wood extractives[8, 9], but could be attributed to their high lignin content[8, 25]. Other studies have proposed that the differences in chemical structure and content of lignin between hardwoods and softwoods must be considered as factors which are responsible for the resistance of wood to the wood-decaying fungi[8, 10].

This investigation was addressed the effect of ligin chemical structure on decay resistance of some tropical hardwoods to the white-rot of *Coriolus versicolor*.

II. Materials and Methods

A. Preparation of samples

Thirteen samples of wood species such as Sugi (*Cryptomeria japonica*), Buna (*Fagus crenata*), Ulin (*Eusideroxylon zwageri*), Bangkirai (*Shorea laevis*), Karas (*Aquilaria* spp.), Erima (*Octomeles sumatrana*), Terminalia (*Terminalia* spp.), Nato (*Palaqium* spp.), Koki (*Hopea pierrei*), Malas (*Homalium foetida*), Yellow meranti (*Shorea* spp.), Mersawa (*Anisoptera* spp.), Melapi/White meranti (*Shorea* spp.), were used in this experiment in the forms of 40–60 and 60–80 mesh wood meals which prepared by Willey mill. The first two species which represent a softwood (Sugi) and a hardwood (Buna) of temperate zone were used as comparison.

The wood meals were successively extracted with ethanol:benzene (1 : 2) and pure ethanol. These extracted wood meals were then used for the determination of lignin characteristics and the delignificatin treatment, whereas the methanol extracted wood meals were used for wood decay test.

B. Characterization of ligin

The Klason lignin content was determined according to the TAPPI T-222 so 74[11]. To determine the characteristics of lignin, the procedure of alkaline nitrobenzene oxidation recommended by MESHITSUKA[12] was applied.

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Approximately 50 mg of ethanol-benzene extracted wood meals was oxidized with 0.24 m*l* of nitrobenzene and 4m*l* of 2N KOH in a stainless steel tube for 2 hours at 160℃. At the end of the oxidation period, the stainless steel tube was immediately cooled down in running water to stop the reaction, and filtered. The oxidation product which still remains in the residue was washed by a small amount of 0.1N KOH solution. After removing nitrogenous compounds from alkaline solution with chloroform, the pH of this alkaline solution was adjusted to 2.5 by 1N HCl solution. Finally, the solution was extracted with 30 m*l* of chloroform. This extraction was repeated four times. The extract was then quantitatively analyzed by gas chromatography under the following conditions: glass column 1% OV-1 (1 m), carrier gas He, flow rate 15 m*l* per minute, temperature 180℃.

C. Delignification of wood meals

Delignification by chlorite method has been carried out according to AHLGREN and GORING[13]. The initial chemical charge was 0.3 gram of sodium chlorite and 0.1 m*l* of glacial acetic acid per gram of oven-dried wood meals. The ratio of liquor to wood meals was 15 : 1 and the temperature was 70℃. The wood meal was treated for different lengths of time. At hourly intervals, a fresh charge of chemicals was added without withdrawal of any liquor. At the end of the treatment, the residue was filtered and washed with distilled water. Finally, the Klason lignin content of the chlorite-treated wood meals was determined. The login removal was calculated based on the difference of Klason lignin content between the untreated and chlorite-treated wood meals.

D. Determination of specific gravity

Specific gravity of the samples was determined according to the procedure of BROWNING[14]. Wood blocks were used for this determination.

E. Fungi and medium

A species of white-rot fungus, *Coriolus versicolor*, was used in this experiment. The composition of the basal medium for the culture of the fungus are 50 gr/*l* glucose, 5 gr/*l* polypepton, 0.3 gr/*l* K_2HPO_4, 0.3 gr/*l* KH_2PO_4, 0.2 gr/*l* $MgSO_4 \cdot 7H_2O$, 30 gr/*l* agar, and 120 gr/*l* onion extract.

F. Wood decay test

The samples used in this experiment were unextracted and methanol-extracted wood meals. The procedure of this experiment was essentially same as described in the previous paper[6].

III. Results and Discussion

A. Lignin characteristics

The Klason lignin contents of thirteen wood species, 11 of which are tropical hardwoods, one temperate hardwood, and one tmperate softwood, are resumed in Table 1. It can be seen that the lignin contents of the tropical hardwoods are in the range of 23.41% to 29.09% and that those of the woods of Ulin, Bangkirai, and Koki, Malas are the highest ones, whereas the lignin contents of Sugi and Buna are 30.49% and 21.68%, respectively. Several researchers[15, 16] brought out the conclusion that the lignin contents of the tropical hardwoods are generally higher than those of the temperate hardwoods. The results of present study showed the same inclination, but are not sufficient to support the above conclusion, because the number of wood samples used in this experiment is still not sufficient.

Table 1. Nitrobenzene oxidation products (%)*

Wood samples	Klason lignin	Vanillin	Syringaldehyde	Total aldehydes	S/V (molar)
Sugi	30.49	26.1	—	26.5	—
Buna	21.68	7.9	25.9	33.9	2.74
Ulin	29.09	23.3	7.4	30.7	0.26
Mersawa	24.13	13.8	15.0	28.8	0.91
Melapi	25.37	12.1	14.1	26.2	0.97
Bangkirai	28.87	20.1	9.2	29.3	0.47
Karas	23.41	16.0	16.6	32.6	0.86
Erima	25.41	11.4	17.4	28.8	1.27
Terminalia	24.88	15.0	15.2	30.2	0.85
Nato	26.12	16.5	12.3	28.8	0.62
Koki	23.38	20.9	12.7	31.1	0.51
Malas	23.55	16.3	12.3	28.6	0.63
Yellow meranti	24.48	9.7	21.6	31.3	1.33

* Based on the Klason lignin content.

In order to get more detailed information of the lignin characteristics, alkaline nitrobenzene oxidation products from the wood samples were examined. The results of the oxidation studies are given in Table 1. The total aldehyde yields of 11 tropical hardwoods are in the range of 26.2% to 32.6% on the Klason lignin content. In general, the amount of syringaldehyde and vanillin yields from tropical hardwoods are equal, except for the woods of Ulin, Bangkirai, Koki, and Malas. In comparison with the vanillin yield from Buna (7.9%), those from the later four species are extremely high, 23.3% for Ulin, 20.1% for Bangkirai, 20.9% for Koki, and 16.3% for Malas. On the contrary, the yields of syringaldehyde from the four samples are very low, 7.4% for Ulin, 9.2% for Bangkirai, 12.7% for Koki, and 12.3% for the wood of Malas. These syringaldehyde yields are quite different from the yield of Buna wood (25.9%).

Based on the above data, it can be concluded that the lignins of Ulin, Bangkirai, Koki, and Malas woods are primarily constituted of guaiacyl lignin, notwithstanding the fact that the woods are typical hardwood ones. The lignins of these hardwoods are very peculiar.

As described in the previous paper[7], Ulin wood meals have the lower rate of delignification than Sugi and Buna wood meals. It was suggested that the low delignification rate of Ulin wood meals might be correlated to its decay resistance to the wood-decaying fungi. Delignification of tropical hardwoods by chlorite method were then conducted. The results of this experiment are given in Table 2. The results showed that delignification of tropical hardwood samples in one hour treatment was much slower than that of the wood meals of Buna and Sugi. This tendency was particularly clear for the wood meals of Ulin, Bangkirai, Koki, and Malas. The lignin removals from these four wood meal samples are only 15.61%, 17.87%, 20.07%, and 19.40%, respectively. The correlation between delignification rate and weight loss by fungal attack will be discussed later. The slow delignification rate of these four wood meal samples might be due to the physical and chemical factors.

The physical factor is usually related to the wood density. Determination of specific gravity of the samples (Table 2) showed that the specific gravities of the woods of Ulin, Bangkirai, Koki, and Malas, were 1.04, 0.91, 0.74, and 0.78. Ulin has the highest specific gravity among the wood samples tested. It can be stated that the specific gravity of wood sample might be correlated to the delignification rate by chlorite method. Specific gravity is a measure of the weight of wood substances contained in a unit of volume of wood. Cell

Table 2. Lignin removal by chlorite treatment

Wood samples	Lignin removal (%)*		Specific gravity
	1 hour	2 hours	
Sugi	42.97	51.31	0.41
Buna	40.45	59.64	0.59
Ulin	15.61	38.29	1.04
Mersawa	22.71	63.24	0.69
Melapi	27.00	70.44	0.69
Bangkirai	17.87	41.74	0.91
Karas	26.95	61.59	0.45
Erima	27.39	53.60	0.38
Terminalia	27.61	68.73	0.45
Nato	28.26	51.65	0.65
Koki	20.07	51.83	0.74
Malas	19.40	51.10	0.78
Yellow meranti	25.00	76.51	0.45

* Based on the Klason lignin content.

Table 3. Alcohol-benzene and methanol extractives of the wood samples

Wood samples	Alcohol-benzene extractives (%)	Methanol extractives (%)
Sugi	1.97	1.65
Ulin	9.01	6.75
Mersawa	2.44	2.15
Melapi	2.05	1.74
Bangkirai	3.42	3.26
Karas	2.86	2.73
Erima	2.81	2.72
Terminalia	1.76	1.49
Nato	2.33	2.15
Koki	3.41	2.98
Malas	1.87	1.68
Yellow meranti	1.67	1.45
Buna	1.42	0.94

walls of all wood species should have the same specific gravity. Therefore, variation in specific gravity of wood species reflects differences in thickness of cell wall. A wood species with a high specific gravity posseses a thick cell wall, and consequently the cell lumen of this wood is small. In this experiment, the hardwood species of Ulin, Bangkirai, Koki, and Malas, which have high specific gravities, are difficult to be delignified. These results might be explained by the limited impregnation of chemicals into the cell.

The chemical factor which affects the slow rates of delignification of Ulin, Bangkirai, Koki, and Malas wood meals is usualy related to their lignin chemical structure. Previous investigators suggested that the delignification rate increased with the increase in S/V ratio of the wood samples[17]. As it has been described, the lignins in Ulin, Bangkirai, Koki, and Malas hardwoods contain a large amount of guaiacyl units. These four hardwoods also gave the slower rates of delignification by chlorite method. These results suggest that the

Table 4. Percentages of weight loss decayed by *Coriolus versicolor* after two months of incubation

Wood samples	Unexreacted woodmeals (%)	Methanol extracted woodmeals (%)
Sugi	12.48	12.28
Buna	40.51	39.99
Ulin	0.41	1.38
Mersawa	10.84	10.79
Melapi	14.89	15.54
Bangkirai	2.34	2.28
Karas	10.73	10.28
Erima	15.54	14.90
Terminalia	10.93	10.92
Nato	9.44	9.05
Koki	5.07	5.04
Malas	8.34	9.07
Yellow meranti	21.06	21.65

Table 5. Relative growth of fungi with addition of 500 ppm of extracts from Mersawa wood after two weeks of incubation (%)

Fungi	Control	Methanol extract	n-Hexane fraction	Ether fraction	Ethyl acetate fraction
C. versicolor	100	94.6	93.8	91.5	95.3
	100	94.6	89.9	86.0	97.7
	100	96.1	93.8	88.4	89.9
	100	93.8	100.7	93.0	100.7
Average	100	94.8	94.6	89.7	95.9
T. polutris	100	105.3	82.5	111.4	106.1
	100	105.3	80.7	103.5	101.7
	100	112.3	82.5	107.9	117.5
	100	107.0	82.5	108.8	114.0
Average	100	107.5	82.05	107.9	109.8

lignin chemical structure of the sample is correlated to the rate of delinification by chlorite method.

B. Wood decay test

The lignin structure of 13 wood species tested in this study have already been described. The distinct differences in lignin chemical structure of the wood samples may influence the decay resistance caused by wood-decaying fungi. Here, the experiment is mainly aimed to know the influence of the lignin chemical structure on decay resistance of the wood samples. Before this experiment was conducted, it is necessary to know whether the extractives of the wood has an influence on decay resistance of wood or not. Contents of alcohol-benzene and methanol extractives of the wood samples are given in Table 3. The results given in Table 4 showed that except for the wood meal of Ulin, any difference in weight loss after fungal attack has not been found between unextracted wood meals and methanol extracted wood meals. It means that the methanol extractives did not influence

Table 6. Relative growth of fungi addition of 500 ppm of extracts from Melapi wood after two weeks of incubations (%)

Fungi	Control	Methanol extract	n-Hexane fraction	Ethyl ether fraction	Ethyl acetate fraction
C. versicolor	100	94.4	100.0	88.9	107.1
	100	96.0	96.8	88.9	104.8
	100	92.1	98.4	93.7	108.7
	100	92.1	92.1	101.6	103.2
Average	100	93.7	96.8	93.3	105.9
T. polutris	100	93.1	82.8	96.6	101.7
	100	98.3	87.1	90.5	103.4
	100	100.0	80.2	101.7	100.0
	100	98.3	79.3	111.2	101.7
Average	100	97.4	82.4	100.0	101.7

Table 7. The correlation between the characteristics of wood samples and the weight loss by *Coriolus versicolor*

Wood samples	Lignin removal (%)*	S/V ratio (molar)	Weight loss (%)**
Sugi	42.97	—	11.33***
Ulin	15.61	0.26	0.41
Bangkirai	17.87	0.47	2.34
Koki	20.07	0.51	5.07
Nato	28.26	0.62	9.44
Malas	19.40	0.63	8.34
Terminalia	27.61	0.85	10.93
Karas	26.95	0.87	10.73
Mersawa	22.71	0.91	10.84
Melapi	27.00	0.97	14.89
Erima	27.39	1.27	15.54
Yellow meranti	25.00	1.33	21.06
Buna	40.45	2.74	40.51

* Treated with chlorite for one hour.
** After two months of incubation.
*** After four months of incubation.

the durability of wood meals of the 12 wood species tested.

The methanol extractives of the woods of Mersawa and Melapi were then successively fractionated into n-hexane, ethyl ether, and ethyl acetate soluble fractions. Each fraction was then subjected to the fungal bioassay. As shown in Table 5 and Table 6, none of the soluble fraction inhibited the mycelial growth of both *C. versicolor* and *T. polutris*.

The results collected in Table 7 also indicated the possibility of suspected relation between slow rate of delignification and weight loss by fungal attack, which were previously reported[7]. The wood meals of Ulin, Bangkirai, Koki, and Malas, which have lower delignification rates (Table 2) also gave smaller weight losses by fungal attack, as shown in Table 4. From the results of this experiment, it can be stated that delignification rate of wood meals by chlorite method is correlated to the weight loss decayed by wood-decaying fungi.

The data listed in Table 7 shows a correlation between the characteristics of wood samples and the weight loss by white-rot fungus of *C. versicolor*. Here, a high S/V ratio corresponds to a syringyl-rich lignin, and to the contrary, a low S/V ratio corresponds to a guaiacyl-rich lignin, though the ratio was used as a tentative indicator of lignin nature. Table 7 indicates that a S/V ratio correlates to a weight loss by fungal attack. In other woods, the higher guaiacyl content in lignin wood almost coincide with the higher weight loss by fungal attack.

The wood meals of Ulin, Bangkirai, Koki, and Malas, in which the guaiacyl units are predominating in the chemical structure of lignin, were decayed far less than the wood meal of Buna which contains higher amount of syringyl units. The wood meals of Buna suffered the highest weight loss after two months of incubation.

Many investigators payed attention to the effect of lignin chemical structure on decay resistance of wood against wood-decaying fungi. But only a small number of wood samples were used in their experiment. In the present study 13 woods species were used in the experiment, 11 of which are tropical hardwoods. In a study of lignin degradation of Birch wood by the white-rot fungus of *Coriolus versicolor*, it had been found that *C. versicolor* degraded the syringyl-rich lignin first, and then the guaiacyl-rich lignin[18], though most of the syringyl type of lignin was found in the fibre secondary wall, containing over half of the whole lignin in the xylem[19]. In a similar study, HIGHLEY[20] tested the influence of type and amount of lignin on decay resistances of some hardwoods and softwoods by the white-rot of *C. versicolor*. The hardwood of Ceibo (*Erythrina crista-galli*), one of the wood samples used in the study, is known to contains a high amount of guaiacyl ligin[21]. The results of their study concluded that a hardwood containing guaiacyl-rich lignin was degraded in a manner similar to a softwood. It indicates that the slower decaying rate of a softwood by fungi is ascribable to the type of lignin rather than to the amount of lignin. An attractive argument was also put forward by NILSSON and BUTCHER[22] concerning the effect of lignin and soft-rot susceptibility amongst hardwoods species. It was suggested that wood species containing guaiacyl lignin were more resistant to soft-rot fungi than those of wood containing syringyl-guaiacyl lignin. This argument is largely supported by LEIGHTLEY[23], who studied on the chemical identification of some decayed and undecayed tropical wood species. Their study indicated that the white-rot fungus of *Trametes lactinea* degraded both of *Alstonia scholaris* (hardwood) and *Pinus elliottii* (softwood). However, *A. scholaris* suffered more than *P. elliottii* even though guaiacyl lignin contents appear to be similar. They suggested that the greater susceptibility of *A. scholaris* might be due to te presence of syringyl lignin. Recently, FAIX[24] studied on the degradation of gymnosperm (guaiacyl) and angiosperm (syringyl-guaiacyl) lignins by the fungus of *Phanerochaete chrysosporium*. The results of the study were interpreted to indicate that syringyl-guaiacyl lignin is more readily degraded to low-molecular weight substances than the guaiacyl lignin by *P. chrysosporium*. This interpretation is in accord with the greater susceptibility of angiosperm woods to decay by the fungus.

Amongs 12 hardwoods species used in the present study, the wood meals of Ulin, Bangkirai, Koki, and Malas, were decayed far less than the wood meal of Buna. Based on the previous investigators, it can be interpreted that the slower decaying rates of the wood meals of Ulin, Bangkirai, Koki, and Malas by *C. versicolor* might be due to the chemical structure of lignin, in which the guaiacyl units are predominating in the lignin composition.

Summary

This study examined the effect of lignin struture on decay resistance of some tropical hardwoods to the white-rot fungus of *Coriolus versicolor*. Among the wood samples tested,

the lignin of Ulin wood (*Eusideroxylon zwageri*), Bangkirai (*Shorea laevis*), Koki (*Hopea pierrei*), and Malas (*Homalium foetida*), are primarily constituted of guaiacyl units, notwithstanding the fact that the wood are typical hardwoods. To assess the influence of lignin structure on the rate of decay, these peculiar hardwoods were then subjected to decay by *C. versicolor*. From the present investigation it can be interpreted that the hardwoods containing guaiacyl-rich lignin are more resistant than those of hardwoods containing syringyl-rich lignin.

Key words: Delignification, Basidiomycetes, Decay, Guaiacyl-rich lignin, Syringyl-rich lignin

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熱帯材の耐極性におけるリグニン構造の役割

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和文要旨

11種の熱帯材の耐久性と含有リグニンの化学構造との関係を追究した。リグニンのニトロベンゼン酸化によればグアシルリグニン含量が大きいものはウリン *Eusideroxylon zwageri*, バンキライ *Shorea laevis*, コキ *Hopea pierrei*, マラス *Homaliumm foetida* であった。またこれらの樹種では脱リグニンの速さも小さいことがわかった。カワラタケに対する腐朽実験によると重量減少率の小さい木材もこれら4種であった。近時グアシルリグニン含量の大きい木材は白色腐朽菌に大きな耐久性を示すと言われているが, 本研究における11種の熱帯材でも同傾向の事実を示した。