Chemical Studies on Effect of White-Rot Fungi on the Major Chemical Constituents of Eucalyptus Hybrid

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Four white-rot fungi were examined for their ability to remove lignin faster than polysaccharides from Eucalyptus hybrid wood. Quantitative changes in lignin, glucan, araban and xylan during decay by these fungi were determined. All the four white-rot fungi were found ta remove all the major wood components progressively during decay. Lignin removal was always accompanied by removal of polysaccharides, but lignin removal did not correlate with removal of any particular component af the polysaccharides. Araban and Xylan were removed faster than the cellulose. The ratio of Syringaldehyde to Vanillin was higher than in the sound wood. Vanillic and syringic acids were obtained as intermediate products of lignin degradation.

Introduction

White-rot fungi decompose lignin as well as cellulose and hemicelluloses in wood. The fungi of this type vary, however, in the rate at which they remove lignin. Some fungi remove the lignin faster than they do the carbohydrates. The resulting decayed wood has a lower lignin content than that of original sound wood. Studies have been made of the relative rates of removal of the structural components of wood (Cellulose, Hemi-cellulose and Lignin) during decay by white-rot fungi[1]. Quantitative determination of changes in the individual types of structural sugar polymers (glucan, araban and xylan) during the decay of hardwoods have been studied by Cowling[2]. The present study determined changes in the composition of *Eucalyptus hybrid* wood during decay by white-rot fungi.

The specific purpose of obtaining these data were :

(i) to examine in detail the apparent capacity of some selected white-rot fungi to delignify wood;

(ii) to provide information on the importance of hemicellulose decomposition during decay;

(iii) to evaluate variation in the relative rate of removal of the major structural components of wood.

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Experimental

Eucalyptus hybrid wood its blocks of size 2.5 × 2.5 cm. × 1 cm. with shortest diamension parallel to the grain were prepared. To avoid interferenc of extractives with subsequent lignin analysis, the blocks were successively extracted in a soxhlet apparatus, with 95% ethanol-benzene (1:2 by volume) and 95% ethanol. The blocks were then washed with water and dried to a constant weight at 102°C.

Decay Experiment

The blocks prepared as above were exposed to attack by decay fungi in soil-block chambers[3]. Cylindrical 8 OZ. glass bottles, 9 cm. high, 6 cm. mouth diameter, provided with aluminium lids with 1/4 threads were approximately half-filled with air-dry soil of approximately 27% water-holding capacity. Water, equivalent to 13% of the water-holding capacity of soil, was added to each bottle making correction for the amount of moisture already present in the air-dry soil.

Two semul wood (*Bombax Ceiba*) feeder strips, 0.3 × 2.5 × 2.5 cm. in size, with the smallest dimen-

sion across the grain, were placed on top of the soil. The bottles were capped a little loosely and sterilised at 15 lt. pressure for three hours.

Sterilised bottles were then inoculated on the feeder strips with freshly grown cultures (On 2% malt-extract-agarmedium) of the following four white-rot fungi obtained from the Forest Pathologv Branch of the Forest Research Institute, and incubated at 26°C, and 76% relative humidity

1. *Polyporus sanguineus* L.ex fr. No. F.R.I 918
2. *Polyporus versatilis* (Berk) Rom. No. F.R.I 550
3. *Fomes lividus* Kalchbr No. F.R.I. 27
4. *Irpex flavus* Okitzscg No. F.R I. 73

When the feeder strips were fully convered by the mydelia of the respective test fungi. *Eucalyptus* blocks the oven-dry (at-102°C) weights of which had been taken earlier. were introduced into the bottles were then again incubated at 26°C and 76% relative humidity Two blocks per bottle and twelve blocks per fungus were used One-third the number of blocks was removed from the bottles at intervals of 6, 9 and 12 weeks to determine the rate of decay by the test fungi. After removal from the bottles, blocks were freed of adhering mycelium and oven-dried to a constant weight at 102°C. Weight loss in the tested blocks is expressed as percentage loss in weight based on the initial oven-dry weight before test.

Some of the steamed sterilised blocks (untested) were air-dried for use as controls in the analytical works.

ANALYSIS

Decayed wood samples and control samples, ground to pass a 40 mesh screen were analysed (without further extraction) for solubility in 1% sodium hydroxide and benzene—alcohol and for methoxy, lignin, cellulose and pentosan contents. The decayed samples were also oxidised with alkaline nitrobenzene. The decayed samples were also hydroloysed with 72% sulphuric acid and the relative amount of glucan, araban and xylan were determined by paper chromatography.

Results and Discussion:— The results are recorded in Table 1 to 5 and figure 1 to 6.

Loss in wood weight—The results recorded in Table 1 indicate that maximum loss in weight of wood

Table-1

Analysis of Eucalyptus hybrid decayed by various white-rot fungi

Fungus	Loss in weight %	1% NaOH solubility A	1% NaOH solubility B	Benzene-alcohol solubility A %	Benzene-alcohol solubility B
Polyporus Sanguineus	12.89	26.0	22.6	3.15	2.74
	19.74	27.8	22.3	3.45	2.77
	27.91	27.8	20.01	4.70	3.38
Polyporus Versatilis	10.26	24.4	21.90	3.50	3.15
	15.50	27.4	23.10	3.50	2.95
	20.70	27.5	24.70	4.00	3.10
	5.80	25.0	23.5	3.1	2.91
Irpex flavus	11.30	25.9	22.9	3.1	2.94
	21.80	27.9	21.8	4.0	3.12
Fomes lividus	7.90	24.2	22.28	2.75	2.52
	9.82	28.0	25.25	2.90	2.61
	15.50	28.0	23.90	3.1	2.65
Original wood	—	21.2		2.65	

A—Calculated on the basis of the dry decayed wood

B—Calculated on the basis of the dry sound wood

is exhibited by *Polyporus sanquineus* (28%), over a period of twelve weeks, whereas only about 16 percent wood weight loss is caused by *Fomes lividus* during the same period. However, this fungus caused weight loss rapidly in the initial stages. Interpretation of relationship between period of decay and weight loss is shown in figures 1 and 2. A look on figs. 1 and 2 shows that rate of wood weight loss in a case of *Polyporus sanguineus*. *Polyporus varsatilis* and *Irpex flavus* proceeds in two stages. During the first six weeks, rate of weight loss is rapid ank then shows down, whereas in case of *Fomes lividus*. the rate of weight loss during first six weeks is much slower than the rate on prolonger period.

Solvent soluble matter:— The solubility of decayed samples in 1% Sodium hydroxide, and in organic solvents is recorded in Table 1, It does not change much during decay by white-rot fungi. This indicates that the rate of production and utilization of degradation products are approximately equal. So Olysaccharides and lignin remaining in white-rotted wood at various stages of decay are not very different in properties (Table 2) from these substances in sound wood. This suggests that only small parts of the polymers are attacked at any given time and that the affected portions are completely degraded and assimilated before other parts of polymers are attacked.

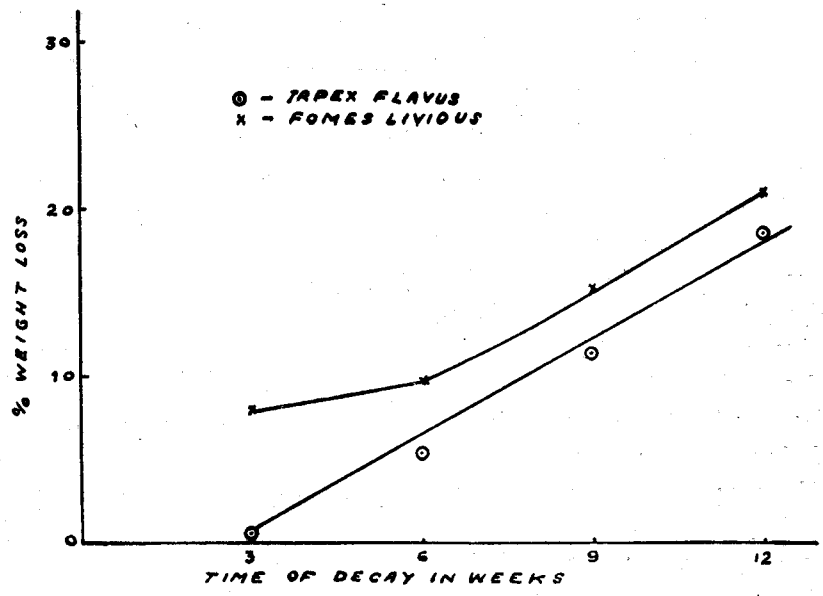

FIG. 1

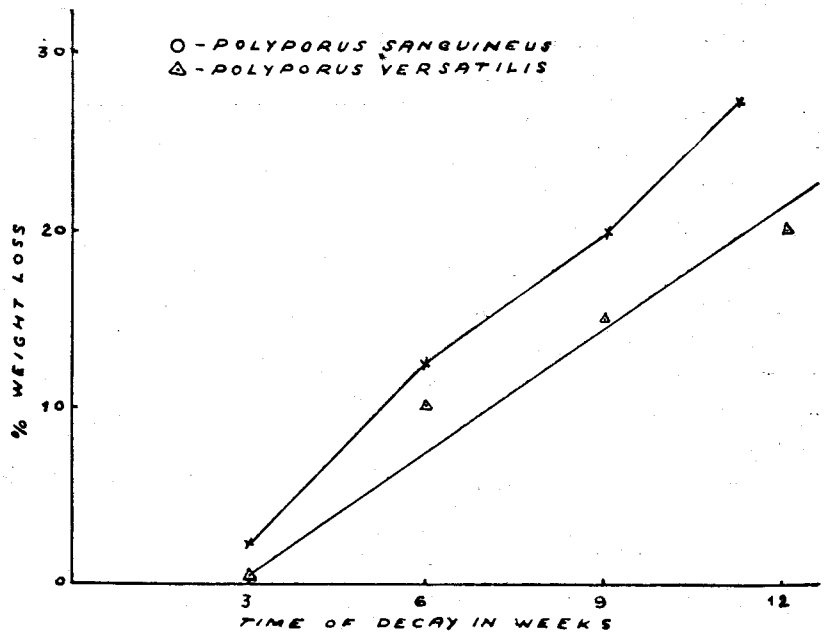

FIG. 2

Removal of lignin and Polysaccharides:— Previous research has shown that much of the lignin in wood can be degraded by certain white-rot fungi without proportional depletion of carbohydrates. This indicates that carbohydrate removal need not accompany ligning degradation. Quantitative changes in lignin, glucan, araban and xylan during decay of *Eucalyptus hybrid* wood by four fungi removes lignin and Polysaccharides simultaneously. However, lignin / carbohydrate ratio remained practically constant throughout the period of decay. *Polyporus versatilis* seems to be slightly different than other three fungi in rate of lignin removal in the initial stages. About 22% lignin was removed by this fungus within the period of six weeks, whereas only 6.3 – 16.5% lignin is removed by other fungi during the same period of decay.

Carbohydrate degradation and assimilation by this fungus was also at the same rate in the beginning as was during subsequent period.

Pentosan type carbohydrates i. e. arabans and xylans were removed at a faster rate by all the fungi than cellulose (Table—4).

In Figs. 3, 4, 5 and 6 graphs are plotted between loss of various major wood components (on original wood basis) and total wood weight loss. It is observed that in case of *Polyporus sagnineus* till the 20% wood weight loss point lignin remains unchan-

Table–2
Analysis of Eucalyptus hybrid wood decayed by various White-rot Fungi

	Methoxyl A	Methoxyl B	Lignin A	Lignin B	Hollocellulose A	Hollocellulose B	L/C Ratio
Polyporus sanguineus	4.9	4.27	27.9	24.33	53 8	47.0	0.517
	4 2	3.37	25.2	20 22	51.4	41 2	0.490
	1.9	1 36	23.2	19.73	49.4	35.5	0.555
Polyporus versatilis	4.4	3 96	25.3	22.77	56.1	50.49	0.451
	3.6	3 04	23.5	19.85	50.0	42.20	0 470
	3.3	2.50	22.2	17.60	49.9	39.57	0.445
Irpex flavus	4.4	4.13	29.07	27.26	55.7	52.46	0.520
	4.3	3.81	27.6	24.47	51 7	46.75	0.523
	1.08	0.84	25 1	20.33	46.5	36.35	0.559
Fomes lividus	5.1	4.69	27.5	25.32	53.8	49.55	0 511
	3.3	2.97	25.6	23.08	54.2	28 7	0 474
	1.53	1 39	23 8	20.34	49.3	41.82	0 485
Original wood	7,23		29.1		65.2		0.446

A—Calculated on the basis of the dry decayed wood
B—Calculated on the basis of the dry sound wood

TABLE—3
Glucan, Arban and Xylan in sound and decayed Eucalyptus hybrid wood

Fungus	Weight loss	Glucan % A	Glucan % B	Araban % A	Araban % B	Xylan % A	Xylan % B
Polyporus sanguineus	12 78	41.0	35 81	1.86	1.62	12 0	10.48
	19.74	39.4	31.42	1.57	1.25	11.7	9.33
	27.91	37 6	27 23	1.50	1.08	11.6	8.40
Polyporus versatilis	10 26	41.1	36.39	2 1	1.89	14.2	12.78
	15.50	37 0	31.22	1.56	1.31	14.0	10.12
	20.70	36.0	28.52	1.40	1.109	11.8	9.35
Irpex flavus	5 8	42.5	40.02	2 10	1.97	13.8	12.99
	11.3	38.4	34.72	1.56	1.36	12 4	11 21
	12 8	35.0	27.35	1.40	1.12	11.82	9.23
Fomes lividus	7.9	42.2	39.01	2.0	1.84	12.4	11.40
	9.82	41.2	37.01	1.9	1.709	12 3	11.05
	15.5	36.6	30.09	1.83	1.54	12.4	10.47
Original wood		48.4		2.3		16 47	

A=Calculated on the basis of the dry decayed wood
B=Calcuated on the basis of the dry sound wood

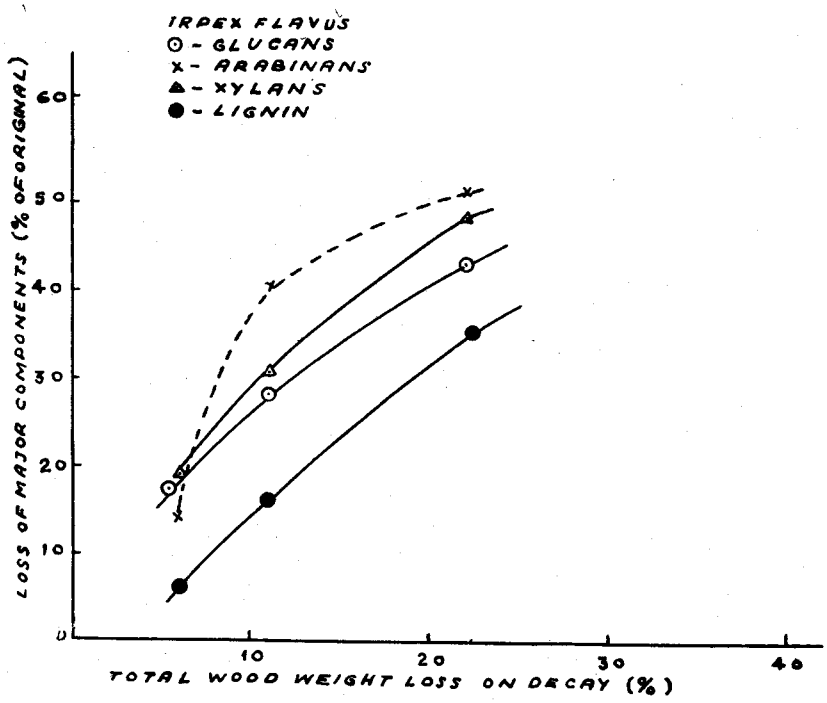
IRPEX FLAVUS
⊙ - GLUCANS
x - ARABINANS
▲ - XYLANS
● - LIGNIN
LOSS OF MAJOR COMPONENTS (% OF ORIGINAL)
0
10
20
30
40
50
60
10
20
30
40
TOTAL WOOD WEIGHT LOSS ON DECAY (%)

FIG. 3

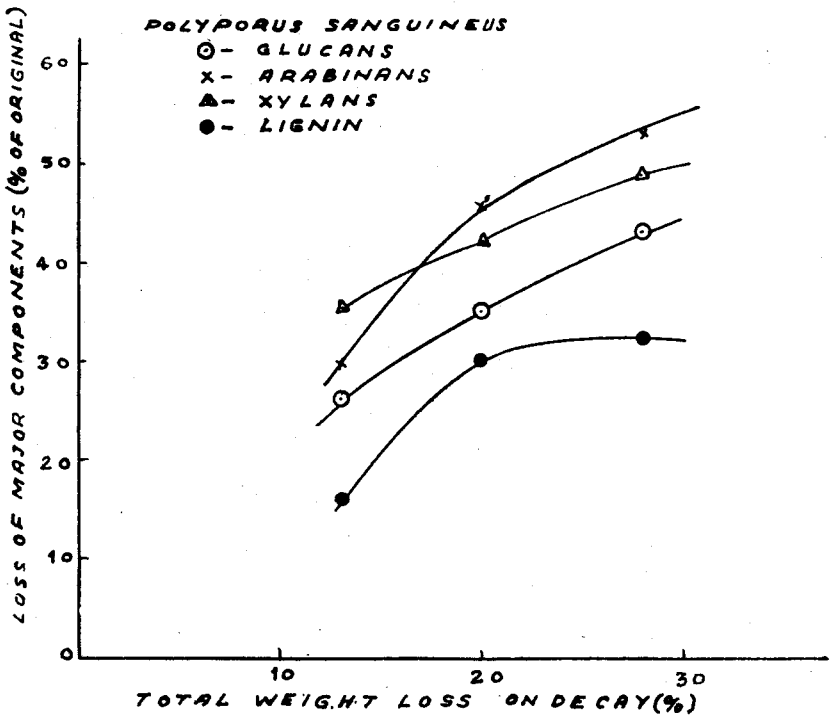
POLYPORUS SANGUINEUS
⊙ - GLUCANS
x - ARABINANS
▲ - XYLANS
● - LIGNIN
LOSS OF MAJOR COMPONENTS (% OF ORIGINAL)
0
10
20
30
40
50
60
10
20
30
TOTAL WEIGHT LOSS ON DECAY (%)

FIG. 4

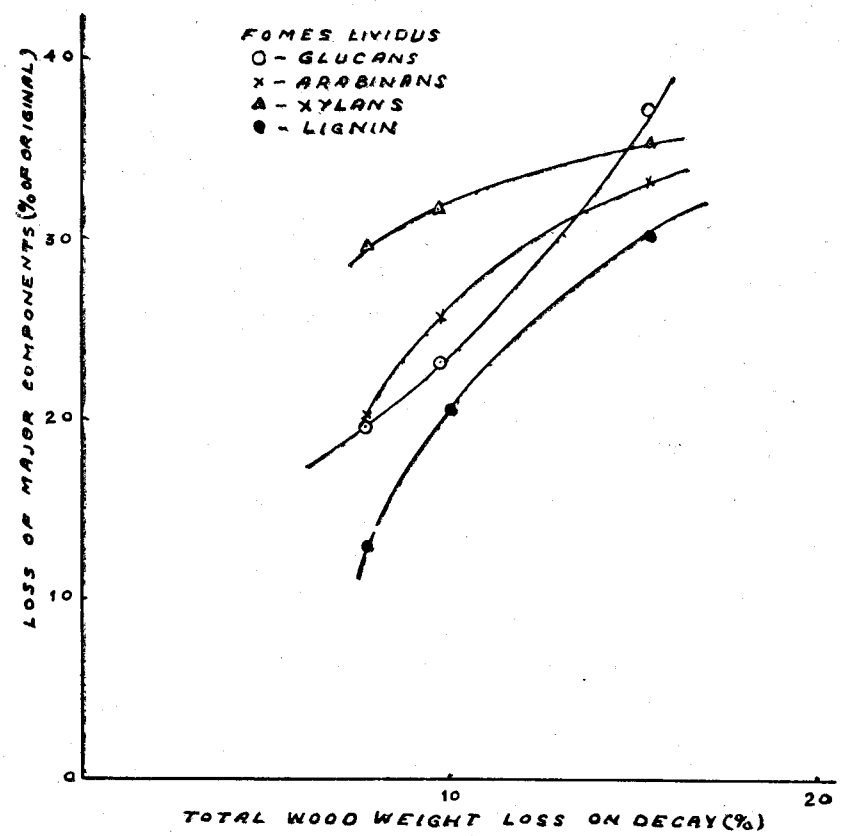
FOMES LIVIDUS
O - GLUCANS
x - ARABINANS
Δ - XYLANS
● - LIGNIN
LOSS OF MAJOR COMPONENTS (% OF ORIGINAL)
0
10
20
30
40
10
20
TOTAL WOOD WEIGHT LOSS ON DECAY (%)

FIG. 5

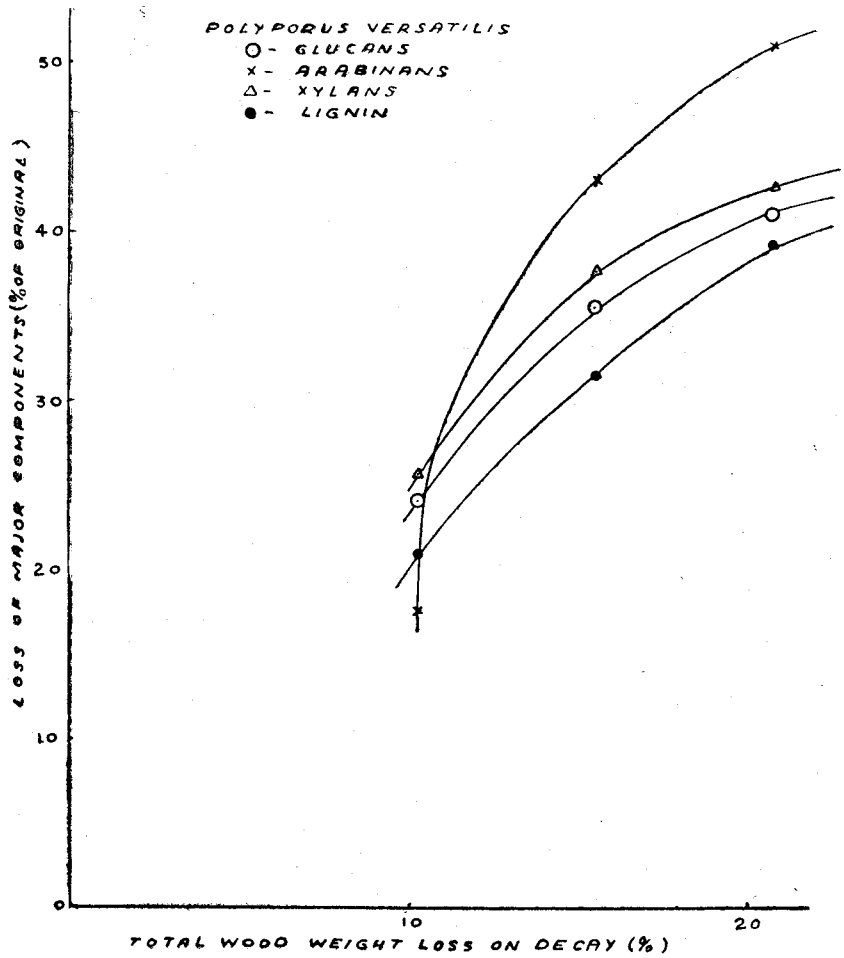
POLYPORUS VERSATILIS
O - GLUCANS
x - ARABINANS
Δ - XYLANS
● - LIGNIN
LOSS OF MAJOR COMPONENTS (% OF ORIGINAL)
0
10
20
30
40
50
10
20
TOTAL WOOD WEIGHT LOSS ON DECAY (%)

FIG. 6

ged, whereas in case of other wood components, of all the sugars arabinose seem to be the first to be effected by the fungus whereas glucan and xylans degrade simultaneously and at a constant rate.

Methoxy content in decayed samples is less than in the sound wood due to the lignin degradation.

Alkaline nitro-benzene oxidation

Alkaline nitro-benzene oxidation of decayed samples was carried out and the results are recorded in Table 5. Vanillin and syringaldehyde are obtained as main oxidation products. The results so obtained show that lignin was decomposed substantially by white-rot fungi. The nitrobenzene oxidation of decayed wood resulted in significantly reduced yields of vanillin and syringaldehyde, although the syringaldehyde to vanillin ratio in the oxidation products mixture was higher than that obtained from sound wood. These results suggest that those units of micromolecule that will yield vanillin or syringaldehyde on subsquent oxidation are degraded more readily than syringyl units.

TABLE—4

Loss of major structural components of Eucalyptus hybrid wood after various extent of decay by white-rot fungi

Fungus	Loss % Lignin		Glucan		Araban		Xylan	
	A	B	A	B	A	B	A	B
Polyporus sanquineus	4.12	16.39	15.30	26.01	25.0	29.56	26.0	35,30
	13.4	30.51	10.20	35.08	31.7	45.65	27.7	42.40
	20.2	32.19	22.3	43.73	34.7	53.04	28.0	48.14
Polyporus versatilis	13.05	21.75	15.5	23.57	8.7	17.82	12.4	25.90
	19.20	31.78	23.5	35.49	32.1	43.04	26.5	37.53
	23.36	39.51	25.6	41.07	89.1	51.78	27.1	42.28
Irpex flavus	0.10	6.32	12.1	17.31	8.7	14.34	14.8	19.81
	5.15	15.91	20,6	28.26	34.0	40.86	23.4	30.80
	13.70	30.13	27.6	43.49	27.3	51,30	27.0	43.02
Fomes lividus	5.49	12.08	12.4	19,40	15.0	20.00	24.5	59.62
	12 03	20.68	14.7	23.53	17.3	25.82	24.0	31.79
	12.21	30.10	22.3	37.83	20.0	33.04	24.5	35.37

A=Calculated on the basis of the dry decayed wood
B=Calculated on the basis of the dry sound wood.

TABLE—5

Alkaline nitrobenzene oxidation of Eucalyptus hybrid wood decayed by various white-rot fungi

Species	Vanillin* (V) %	Syringaldehyde* (S) %	S/V
Polyporus sanquineus	0.75	3.75	1 : 5.0
Polyporus versatilits	1.04	6.44	1 : 6.2
Irpex flavus	0-56	4.20	1 : 7.5
Original wood	2.0	8.0	1 : 4.0

*Calculated on the basis of recovered aldehyde mixture.

Conclusions:—

1. White-rot fungi degrade and metabolise all the structural polymers of wood, the cellulose, the hemicelluloses and the lignin. However, they differ in relative rate of removal of the major structural components. The components remaining in the wood at any stage of decay are relatively intact.
2. Cellulose and hemicelluloses depolymerisation occurs by splitting apart of thc monomeric units. The action of enzymes from white-rot fungi in depolymerisation of polysaccharide molecules is mainly hydrolytic, producing oligosaccharides.
3. The formation of vanillic and syringic acids as intermediate products of lignin decay, shows that white-rot fungi are of hydrolytic and oxidative nature.

4. All four white-rot fungi removed araban and usually oxylan faster than the glucan.

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3. Bakshi, B. K., Puri, Y. N., & Sujan Singh Natural decay resistance of Indian Woods. I. Introduction and Method. II. Decay resistance of sal (*Shorea robusta* Gaertn) and teak (*Tectona grandis* L. F.) *Indian Forester* 93, 305-328.