

Biochemical Modification of Lignin by Fungi Species

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ABSTRACT

Ultraviolet spectra of molecular weight intermediates of lignin degradation of the three lignocellulosic wastes are studied by four strains. The results indicated that the UV spectroscopic of native lignins of three substrates are identical to some extent, the increase in UV absorption intensity at 280 nm which is attributed to the increase in the amount of chromophores structures in degraded lignin. Absorption maximum characteristic for α -Co groups and for β -Co groups was obtained for all samples of lignin degradation. While the polymeric intermediates accumulates as catabolite modified lignin polymer, acid polyphenolic lignin (Appls) in the lignocellulose containing growth medium. Analysis of Appls produced from degradation of lignocelluloses by four organisms were compared to degraded lignin. In most cases, increased levels of carbonyl and carboxylic groups was observed and different new absorption bands appeared in IR spectra of Appls produced from biodegradation of lignocellulosic wastes.

The comparison of the absorption bands at wave lengths characteristic for aromatic ring, shows significant loss of polymer aromaticity, in degraded samples.

INTRODUCTION

Byproduct lignins from microbial or chemical degradation are currently used to make a variety of commercial products, including dispersants, binders, oil well drilling muds, etc., and via chemical degradation to make vanillin, dimethyl sulphoxide and other chemicals (Goheen, 1971; Hoyt and Goheen, 1971; Glasser, 1981). Only minor portions of the by product lignins, however, are converted to commercial products. Biological approaches for utilizing by products lignins are only beginning to be searched 12 years age. Alteration of the functionality of by product lignins by microbial action could

conceivably provide polymers with increased value (Parisi and Parisi, 1989). Therefore, the present work is concerned with the elucidation of the chemistry of biodegradation of lignin by spectral analysis of the catabolic intermediates produced from lignin degradation by four microorganisms.

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MATERIALS & METHODS

1. Ultraviolet Spectra (UV)

The UV spectra of the soluble extractives samples dissolved in dimethylsulphoxide were measured on Beckman spectrophotometer UV

2. Infrared Spectra (I.R)

An acid precipitable polyphenolic lignin "Appl" was recovered from the aqueous extract by acidification to pH 1-2 with 12 M HCl. After collection by centrifugation, washed with distilled water and then air dried or lyophilized. Then the samples were examined by infrared spectrophotometer, Philipis, Pu 9712.

The intensity of the absorption bands was calculated by base-line method. The base-line optical density was calculated from the equation.

$$OD = \log_{10} (AB/CB)$$

Where :

OD = base-line optical density.

CB = distance on the recorded spectrum from zero line to selected absorption peak.

AB = distance from zero line to a straight line.

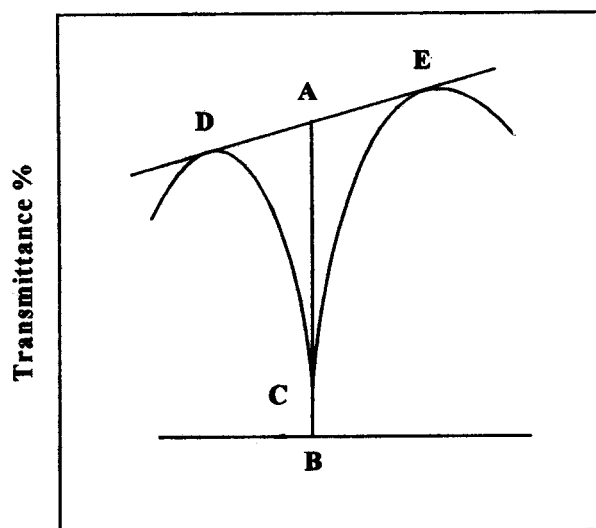


Fig. 1: Measurement of base-line optical density.

The base-line joins two spectral points located on either side (D, E) of the absorption peak. AB was measured at the same wave number as CB. The method used in measuring AB and CB is illustrated in figure (1).

RESULTS

A.1 ULTRAVIOLET SPECTRA

The UV spectra of low molecular weight intermediates of lignin degradation of three lignocelluloses substrates are shown in Figures 2-5 and Table-1.

The UV spectroscopic of undegraded lignin of sugarcane bagasse, *Casuarina equisetifolia* and *Ricinus communis* are identical to some extent 263-280, 300-345 nm. The degraded lignin of sugarcane bagasse gives a new absorption maximum at 315 nm by *Coriolus versicolor* NRRL 6102 and at 363 nm by *Phanerochaete chrysosporium* NRRL 6359. Action of *Coriolus versicolor* NRRL 6102, *Phanerochaete chrysosporium* NRRL 6359 and *Phanerochaete chrysosporium* NRRL 6361 on lignin caused demethoxylation and oxidation to take place with increasing degree of degradation. The ultraviolet spectroscopic studies demonstrated that, the presence of an absorption maximum within the region 350-380 nm which is characteristic for both α -Co-groups and stilbene structures

A.2 INFRARED SPECTRA

Different strains of white rot fungi, *Phanerochaete chrysosporium* NRRL 6359, *Phanerochaete chrysosporium* NRRL 6361 and *Coriolus versicolor* NRRL 6102, and *Streptomyces species*, *Streptomyces viridosporus* NRRL 2414 were grown on lignocellulose substrates such as sugarcane bagasse, *Casuarina equisetifolia* and *Ricinus communis*. The major intermediates of lignin degradation were released into the growth media as an intermediate lignin polymer, acid precipitable polymeric lignin (Appl), this polymer were collected from the cultures of the four ligninolytic organisms, as described in material and method. In this part of investigation IR spectra of Appls produced after degraded by each organism was compared to elucidate the functional groups and chemical differences between Appls produced by four ligninolytic strains on three lignocellulose materials.

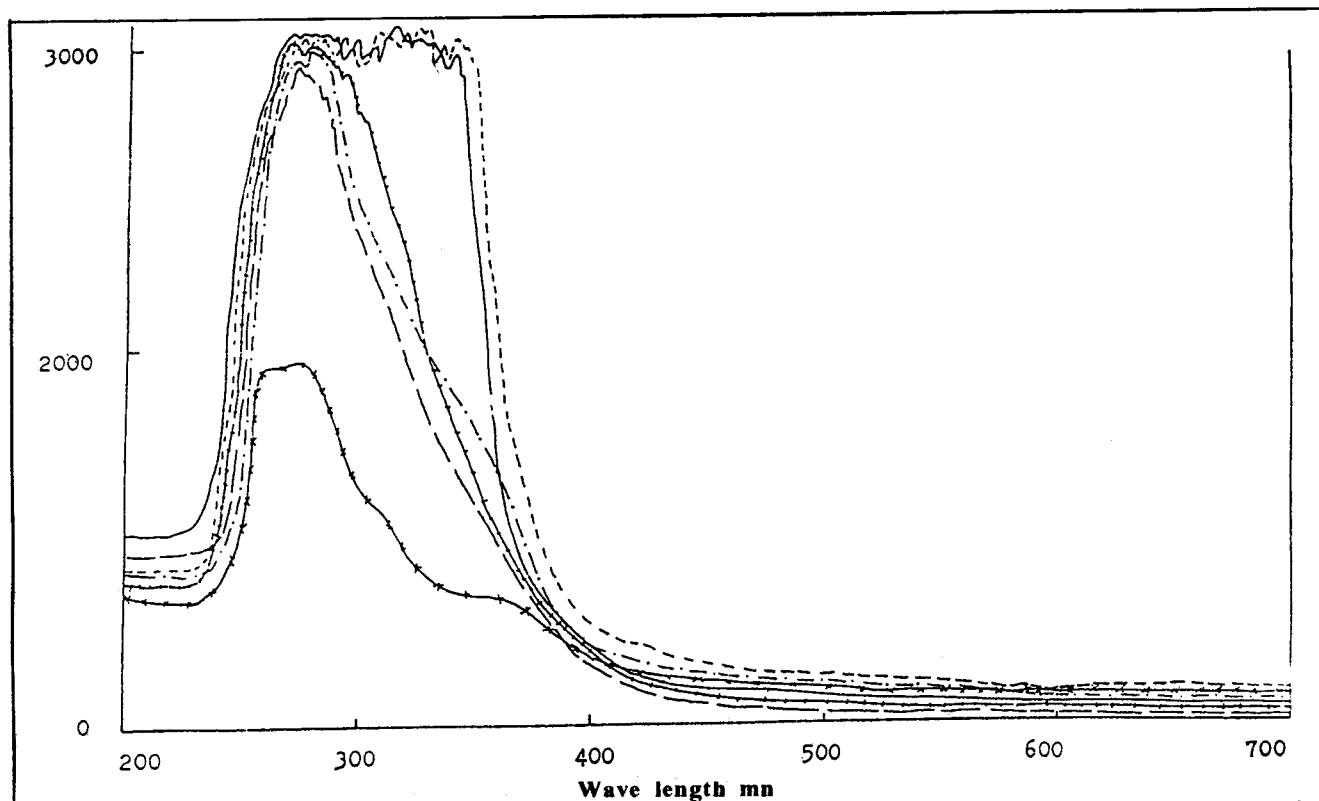


Fig. 2 : UV absorption characteristics of extracted intermediates of degraded lignocelluloses by *C. versicolor* NRRL 6102 (— — —) native of sugarcane bagasse, (—) extractive; (— · —) native of *Casuarina equisetifolia*, (· · · ·) extractive; (+ + + +) native of *Ricinus communis*, (× × ×) extractive.

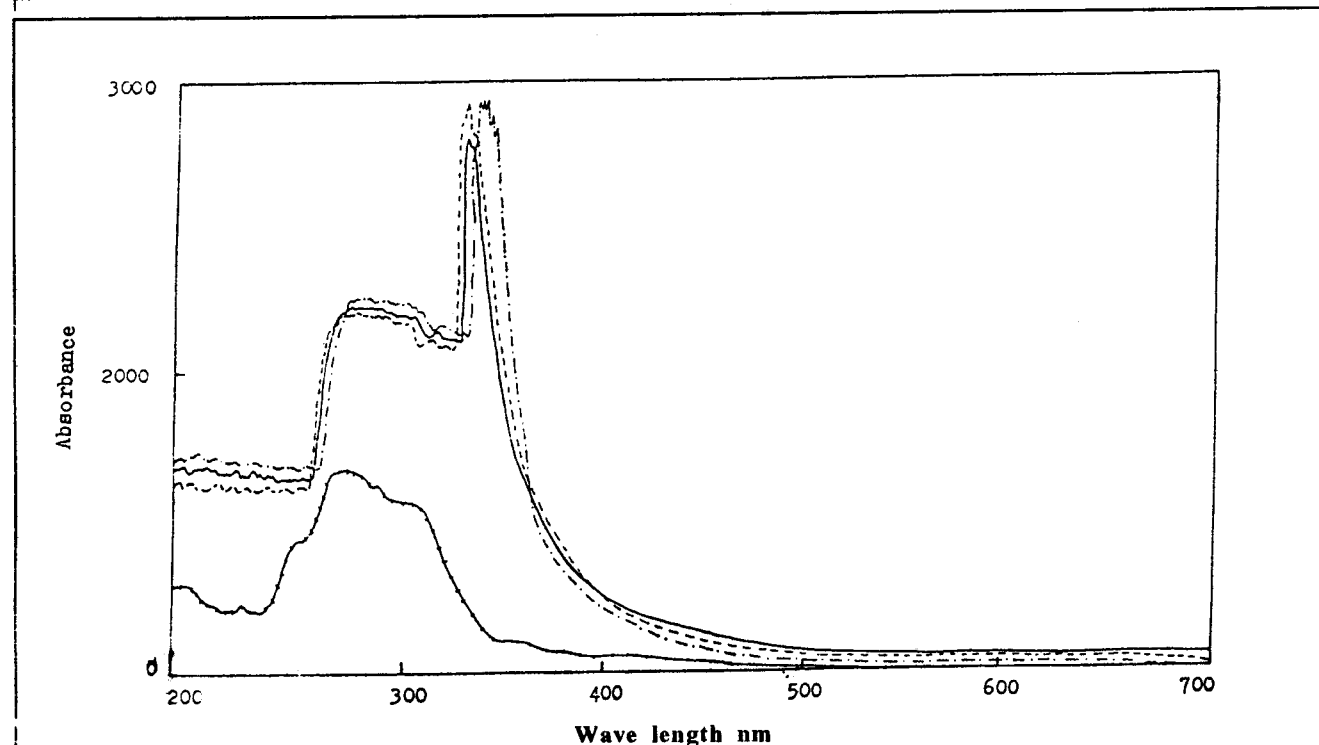


Fig. 3 : UV absorption characteristics of extracted intermediates of degraded sugarcane bagasse by different micor-organisms (— · — ·) control, native (—) *P. chrysosporium* NRRL 6359, (— — —) *P. chrysosporium* NRRL 6163. (× × ×) *S. viridosporus* NRRL 2414.

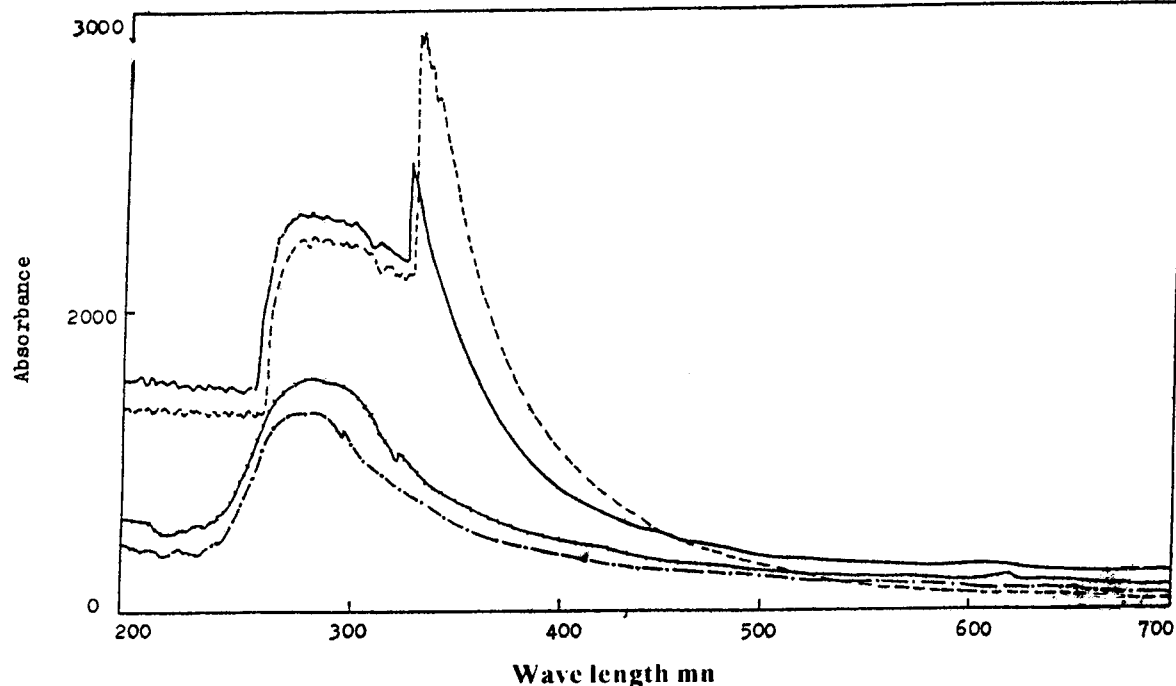


Fig. 4 : UV absorption characteristics of extracted intermediates of degraded *Casiaroma equisetifolia* by different microorganisms (- - -) control, (—) *P. chrysosporium* NRRL 6359 (— — —) *P. Chrysosporium* NRRL 6163 (+++) *S. viridosporus* NRRL 2414.

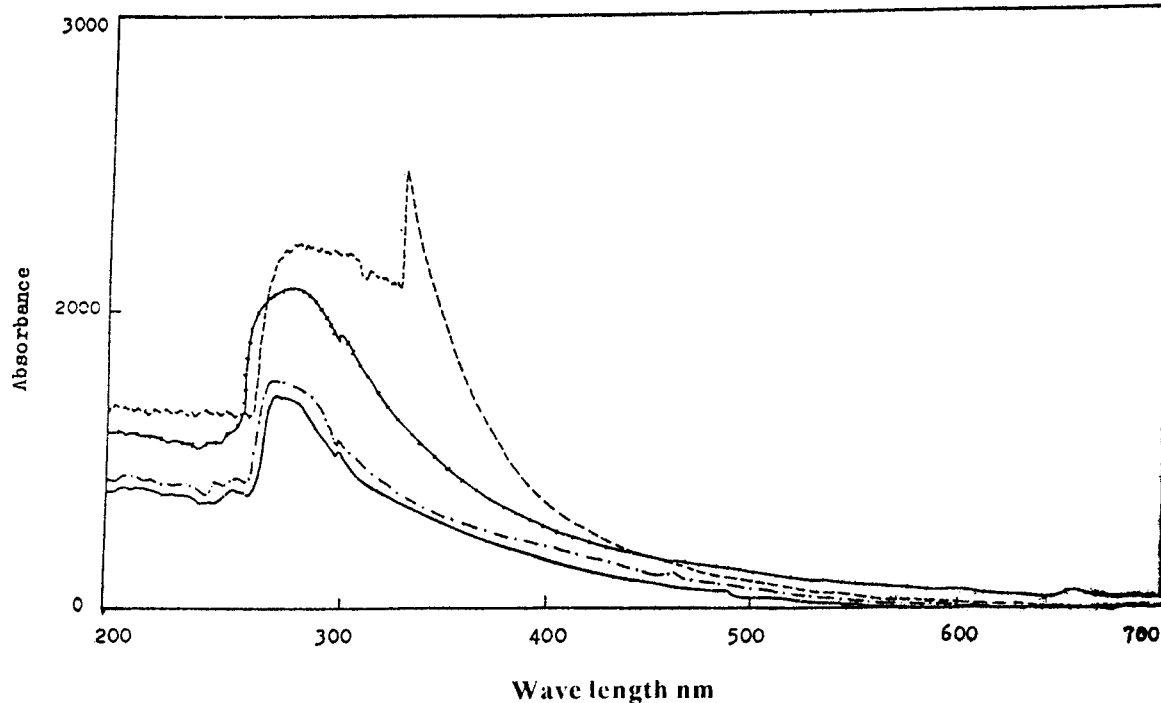


Fig. 5 : UV absorption characteristics of extracted intermediates of degraded *Ricinus communis* by different microorganisms (- - -) control, (—) *P. chrysosporium* NRRL 6359, (— — —) *P. chrysosporium* NRRL 6163. (+++) *S. viridosporus* NRRL 2414.

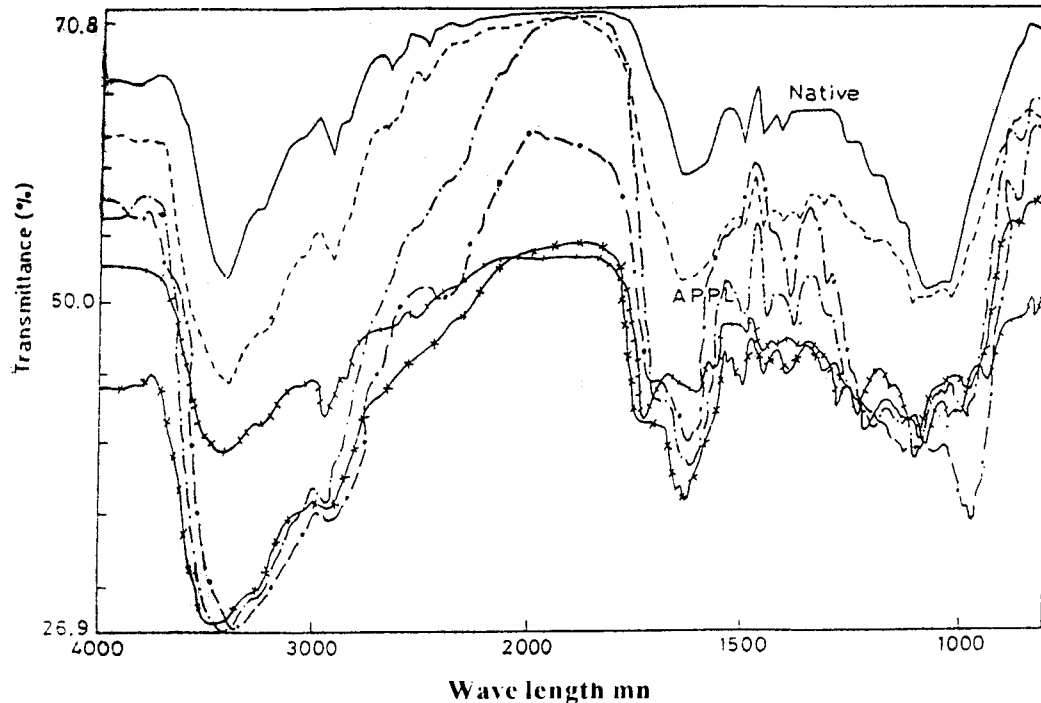


Fig. 6 : IR spectra of native lignin and APPL of degraded lignocelluloses by *Coriolus versicolor* NRRL 6201, (—) native of sugarcane bagasse, (— —) degraded : (— . — .) native of *Casuarina equisetifolia* (- * - * -) degraded : (+ + +) native of *Ricinus communis communis*, (— . — .) degraded.

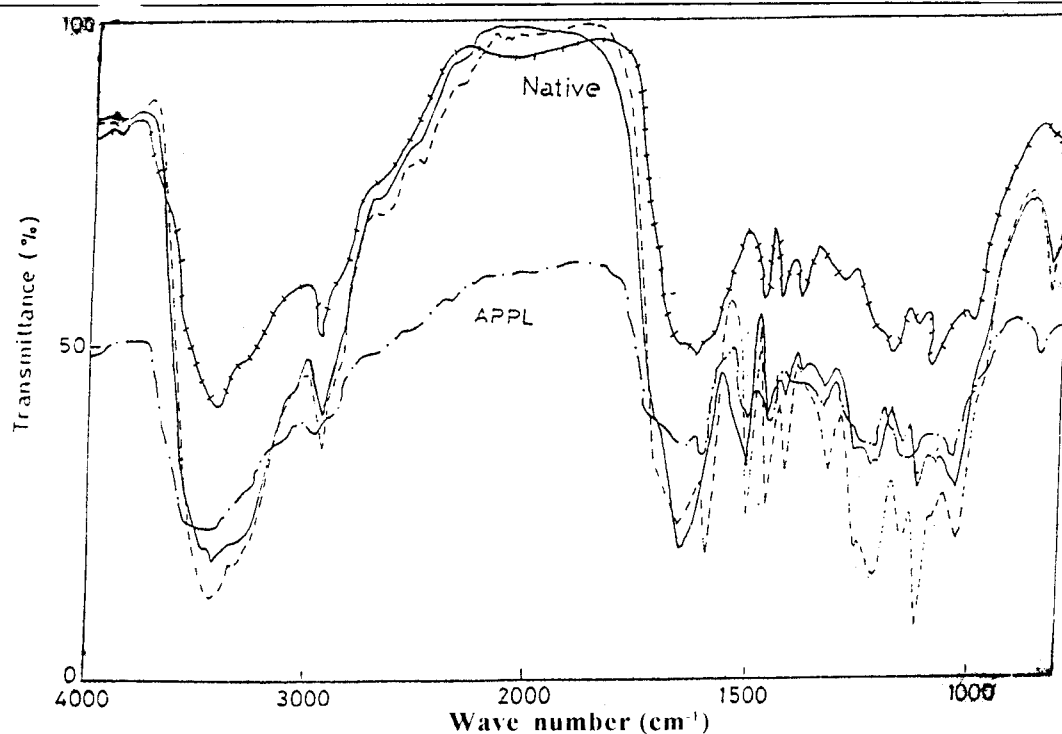


Fig. 7 : IR spectra of native lignin and APPL of degraded sugarcane bagasse by different microorganisms (—) native (— —) *P. chrysosporium* NRRL 6359, (— . — .) *P. chrysosporium* NRRL 6153. (+ + +) *S. viridosporus* NRRL 2414.

Infrared spectroscopy cannot be used to elucidate fully the structure of lignin and its derivatives, but at least some useful information about the various constituent groups can be obtained from it.

The intensity of the absorption bands was calculated by the base-line method, according to Sarkanen *et al.* (1967), the ratio of the optical density of each band to the optical density of the band at 1515 cm^{-1} is calculated.

The spectra of acidified lignins of different lignocelluloses are divided into different regions of absorbance as shown in Tables 2-4 and Figure 7-9.

A. 2.1 REGION $3500\text{-}3300\text{ cm}^{-1}$

The absorption at $3500\text{-}3300\text{ cm}^{-1}$ a broad band, corresponding to the free-OH stretching groups. The broadening of these bands reveal that the OH groups are not free but entering into different modes of hydrogen bonding due to the differences in the energy levels (Burns and Schultz, 1992). It is also obvious that the OH group are chelated since bands are shifted to somewhat longer wavelength, namely 3400 cm^{-1} . This bands is very high intensity in case of Appl of degraded sugarcane bagasse by *C. versicolor* NRRL 6102 reaches 7.05 but in other microorganisms range between 1.7-4.6, and it is very high a Appl of degraded *Ricinus communis* by *C. versicolor* NRRL 6102 and ranged from 2.3-5.1 by other microorganisms. However, in case of Appl of degraded *Casuarina equisetifolia*, it is very higy by *C. versicolor* NRRL 6102, reaches 6 and it is ranged from 2.9-5.6 by other microorganisms.

A. 2.2 REGION $3000\text{-}2900\text{ cm}^{-1}$

In the absorption region $3000\text{-}2900$, strong bands are observed assigning for the stretching vibration of the various C-H bands in the methyl and ethyl group. The band intensity at 2940 cm^{-1} is much higher than at $2870\text{-}2850\text{ cm}^{-1}$ which corresponds to symmetrical C-H stretching vibrations in methoxyl and methylene groups (Friese and Banerjee, 1992). In case of Appl of degraded sugarcane bagasse, it is ranged from 0.5-2 while Appl of degraded *Ricinus communis* it is ranged from 0.7-1.7 and Appl degraded *Casuarina equisetifolia* ranged from 0.8-1.9 by microbial strains.

A. 2.3 REGION $1725\text{-}1580\text{ cm}^{-1}$

The absorption at 1725 cm^{-1} a band characteristic

for carbonyl unconjugated β ketone and conjugated acids or esters appeared. The absorption at this band is comparable to that at 1515 cm^{-1} . This band shifted to $1690\text{-}1654\text{ cm}^{-1}$ in all types of Appl of degraded lignin of different Egyptian lignocelluloses. It is ranged from 0.5-2.8 in case of Appl of degraded lignins of sugarcane bagasse, 0.5-3.7 in case of Appl of degraded lignins of *Casuarina equisetifolia*, and 1-3 in case of Appl of degraded lignins of *Ricinus communis* respectively. The absence of the band at 1580 cm^{-1} in all types of degraded Appl is due to the absence of conjugation with an aromatic ring (Bellamy, 1964).

A further indication for the absence of conjugation of the ring with any double bond $\text{C}=\text{O}$ and $\text{C}=\text{C}$ etc., is the low intensity of the bands in the region.

A. 2.4. REGION $1660\text{-}1610\text{ cm}^{-1}$

The absorption at 1660 cm^{-1} is characteristic for the stretching of conjugated aryl carbonyl, Sarkanen *et al.* (1967), in Appl of degraded sugarcane bagasse the intensity of this band ranged from 0.5-2.8 for all types of Appl of produced by fungi *C. versicolor* NRRL 6102, *P. chrysosporium* NRRL 6359 and *P. chrysosporium* NRRL 6361. In case of *Casuarina equisetifolia* native separated at 30°C reaches to 0.1, Appl of degraded *Casuarina equisetifolia* by *P. chrysosporium* NRRL 6361 band reaches to 2.9 and by *P. chrysosporium* NRRL 6359 the band reaches to 1.6 respectively. The absorption at 1610 cm^{-1} is characteristic of aromatic skeletal vibration affected by aromatic $\text{C}-\text{O}$ stretching and conjugated with α -carbonyl groups. The A_ν of this band is higher than the sensitivity of this characteristic absorption to structure is reflected by the variation of absorption in different Appl. This band is absent in case of sugarcane bagasse but present only in case of *Ricinus communis* degraded by *C. versicolor* NRRL 6102 1.8, its native separated at 30°C reaches to 1.4 and its Appl produced by *S. viridosporus* NRRL 2414 0.4, Appl of degraded *Casuarina equisetifolia* by *P. chrysosporium* NRRL 6359 reaches to 1.6 while its native separated at 37°C reaches to 3.7.

A. 2.5. REGION $1650\text{-}1595\text{ cm}^{-1}$

The bands at 1995 and 1500 cm^{-1} attributed to the aromatic skeleton ring vibration (Sarkanen *et al.*, 1967). Which is less sensitive to the substituted groups in the aromatic nucleus as observed by various authors (Tables-2-4).

A. 2.6. REGION 1465-1425cm⁻¹

The absorption at 1465cm⁻¹ is attributed to aromatic C-H deformation in methyl or methylene groups considerably affected by methoxyl groups. Ban and Glanser-Soljan (1979), assigned this band to

asymmetric vibration of C-H group in methoxy group. The intensity of the band differ from 0.4-1.1 in case of degraded sugarcane bagasse by different fungi and *S.viridosporus* NRRL 2414. In case of Casuarina equisetifolia this peaks disappear in its native separated at 30°C, and Apples degraded by *P.*

Table-1 The different beaks of UV spectra of extracted intermediates of degraded lignocelluloses by different microorganisms

	Absorbance																			
	250	260	270	280	290	300	310	320	330	340	350	360	370	380	390	400	410	420	430	440
Untreated ligno-celluloses at 30°C																				
Sugarcane bagasse									328										422	
casuarina equisetifolia			275																	
Ricinus cammunis			278																	
Extracted intermediates of degraded																				
Lignocelluloses by																				
<i>C.versicolor</i> NRRL 6102																				
Sugarcane bagasse									315											
Casuarina equisetifolia					290															
Ricinus communis			275									365								
Untreated ligno-celluloses at 37°C																				
Sugarcane bagasse			275																	
casuarina equisetifolia			272																	
Ricinus cammunis			263																	
Extracted intermediates of degraded																				
Lignocelluloses by																				
<i>S.viridosporus</i> NRRL 2414																				
Sugarcane bagasse		260			287															
Casuarina equisetifolia			275																	
Ricinus communis			264																	
Extracted intermediates of degraded																				
Lignocelluloses by																				
<i>P.chrysosporium</i> NRRL 6159																				
Sugarcane bagasse			275									363								
Casuarina equisetifolia		264			286	330														
Extracted intermediates of degraded																				
Lignocelluloses by																				
<i>S.chrysosporium</i> NRRL 6163																				
Sugarcane bagasse					280	335														
Casuarina equisetifolia			278			330														
Ricinus communis			278			330														

chrysosporium NRRL 6359 and by *C. versicolor* NRRL 6102. While in case of Appls degraded by *S. viridosporus* NRRL 2414 and *P. chrysosporium* NRRL 6361, it is slightly higher 0.5-1 as in tables 9-11 than its native separated at 37°C 0.9. The absorption at 1425cm⁻¹ is due to aromatic skeletal vibration, strongly coupled by C-H in plane deformation and sensitive to the nature of ring substituents (Burns and Schultz, 1992). The intensity of the absorption of this band is ranged from 0.1-0.7 in case of Appls of degraded sugarcane bagasse and disappeared in its degraded by *C. versicolor* NRRL 6102. In case of Appls of degraded *Ricinus*; communis ranged from 0.2-0.7 and slightly higher in case of its degraded by *P. chrysosporium* NRRL 6359 0.7 and its disappeared by *C. versicolor* NRRL 6102. Also arranged from

0.01-0.9 in case of Appls of degraded *Casuarina equisetifolia* but disappeared by *P. chrysosporium* NRRL 6359, *P. chrysosporium* NRRL 6361 and its native separated at 30°C.

A. 2.7. Region 1380-1330cm⁻¹

The absorption at 1370cm⁻¹ is assigned by Sarkanen and Ludwig (1971), to phenolic OH bending character Ban and Glanser-Soljan (1979), assigned the region 1330-1380 cm⁻¹ to symmetric vibration of the C-H groups in methoxy the intensity of this band ranged from 0.1-0.4 in case of Appls of degraded sugarcane bagasse and disappeared by *P. chrysosporium* NRRL 6359 and *P. chrysosporium* NRRL 6361. In case of Appls of degraded *Ricinus*

Table-2 Ratio of Ar/A₁₅₁₅ cm⁻¹ of different spectra of native lignin and Appls of degraded sugarcane bagasse by different organisms.

C. versicolor NRRL 6102		Appl of Native at 30°C		S. viridosporus NRRL 2414		P. chrysosporium NRRL 6361		P. chrysosporium NRRL 6359		Appls of Native at 37°C	
Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance
3345	7.05	3363	4.619	3278	3399	3399	3.14	3399	1.75	3399	2.736
2854	2.0	2854	1.238	3272	2.165	2927	1.1	2909	0.519	2927	0.736
2599	0.7	2799	0.952	2927	0.495	2654	0.18	2654	0.075	1645	2.287
2472	0.5	2618	1.0	2618	0.103	2509	0.18	2472	0.083	1509	0.087
1636	4.05	2472	0.429	2527	0.155	1654	2.82	1654	0.594	1445	0.471
1500	0.020										
1445	1.15	2343	0.143	2345	0.144	1500	0.050	1590	0.654	1409	0.126
1390	0.45	2236	0.143	1690	1.113	1463	0.86	1500	0.133	1381	0.126
1354	0.45	1636	2.238	1590	0.732	1418	0.48	1454	0.729	1327	0.161
1290	0.2	1500	0.021	1500	0.097	1327	0.26	1409	0.353	1236	0.575
1245	0.45	1427	0.619	1436	0.773	1218	0.94	1282	0.338	1154	0.207
1227	0.25	1390	0.381	1390	0.454	1172	0.9	1218	0.654	1118	0.471
1209	0.35	1090	5.571	1309	0.392	1036	0.78	1154	0.203	1027	0.805
1109	1.15	1045	5.714	1227	1.371	752	0.36	1099	0.699		
1027	1.0			1145	0.526			1027	0.632		
				1109	0.732			723	0.406		
				1027	0.608						
				709	0.423						

Table-3 Ratio of A/A_{1515} cm^{-1} of different spectra of native lignin and Appls of degraded *Casuarina equisetifolia* by different organisms.

C. versicolor NRRL 6102		Appl of Native at 30°C		S. viridosporus NRRL 2414		P. chrysosporium NRRL 6361		P. chrysosporium NRRL 6359		Appls of Native at 37°C	
Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance
3399	6.484	3399	4.054	3399	4.804	3381	2.985	3399	4.3	3399	5.86
2872	1.094	2999	1.966	2927	0.941	2927	0.838	3090	0.567	2927	1.14
1709	2.016	2345	0.362	1636	3.373	2636	0.191	2909	0.9	2654	0.2
1636	3.641	1709	0.570	1509	0.051	2490	0.162	2599	0.367	2509	0.14
1518	0.064	1627	0.149	1445	1.020	2345	0.074	2509	0.3		
1427	0.547	1372	0.483	1409	0.019	1645	2.912	2327	0.2	2327	0.14
1390	0.469	1199	0.235	1372	0.157	1509	0.068	2254	0.133	2218	0.1
1309	0.406	1118	0.215	1318	0.412	1454	0.632	1718	0.4	1618	3.78
1218	0.766	927	2.389	1272	0.373	1399	0.397	1636	1.6	1500	0.050
1118	1.125	7673	0.195	1218	0.804	1318	0.309	1500	0.030	1445	0.92
				1118	1.510	1263	0.147			1399	0.36
				1002	1.137	1209	0.618			1309	0.36
				709	0.294	1118	1.0			1263	0.32
						1027	0.794			1209	0.64
										1118	1.5
										1636	1.32

communis, it ranged from 0.2-0.6. In case of Appls of degraded *Casuarina equisetifolia*, it ranged 0.1-0.4 and disappeared by *P. chrysosporium* NRRL 6359.

A. 2.8. REGION 1330-1220 cm^{-1}

The absorption at 1330 cm^{-1} has been assigned by Ban and Glanser-Soljan (1979), to the asymmetric vibration of the C-H group in methoxy group. The intensity of the absorption ranged from 0.1-1.3 and disappeared in native sugarcane bagasse and ranged from 0.4-1.2 in case of Appls of degraded *Ricinus communis* but disappeared by *C.versicolor* NRRL 6102 and in its native. Appls of degraded *Casuarina equisetifolia*, it ranged from 0.6-0.8 and disappeared by *P. chrysosporium* NRRL 6359 and in its native.

The absorption at 1220 cm^{-1} is due to ring breathing with c-o stretching in quaiacyl and syringly units (Sarkanen and Ludwig, 1971). The absorption at 1270 cm^{-1} relates well with the intensity

of the band at 2900 cm^{-1} stretching vibration of methylene groups. In this work the band at 2940 cm^{-1} showed correlation with the band at 1270 cm^{-1} intensity of absorbance. Both bands at 1335-1235 cm^{-1} are assignable to ring breathing with c-o stretching, for syringyl and quaiacyl unit (Sarkanen and Ludwig, 1971). The intensity of the bands at 1220 cm^{-1} is more higher than 1270 cm^{-1} , in case of Appls of degraded sugarcane bagasse ranged from 0.2-0.9 and is slightly higher by *S. viridosporus* NRRL 2414 and absent in its native separated at 30°C. Appls of degraded *Ricinus colrununis* it ranged from 0.4-1.2 and absent by produced by *C. versicolor* NRRL 6102. But Appls of degraded *Casuarina equisetifolia* it ranged from 0.6-0.8 and absent Appls produced by *P.chrysosporium* NRRL 6359 and its native separated at 30°C.

A.2.9. REGION 1160-1030 cm^{-1}

The absorption at 1160 cm^{-1} is characteristic for

Table-4 Ratio of A_{λ}/A_{1515} cm^{-1} of different spectra of native lignin and Appls of degraded *Ricinus communis* by different organisms.

C. versicolor NRRL 6102		Appl of Native at 30°C		S. viridosporus NRRL 2414		P. chrysosporium NRRL6361		P. chrysosporium NRRL 6359		Appls of Native at 37°C	
Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance	Wave No.	Ratio of Absorbance
3363	6.067	3363	3.018	3381	2.505	3418	2.36	3399	4.344	3418	5.151
2872	1.180	3218	0.696	2909	0.733	3072	0.1	2890	1.781	2909	1.301
1709	1.0	2909	1.446	1654	1.149	2927	0.88	2636	0.219	2490	0.438
1618	1.843	2618	0.393	1590	0.475	2654	0.16	2490	0.125	2345	0.192
1518	0.089	2490	0.375	1509	0.101	2509	0.1	1599	3.5	1727	2.315
1500	0.427	1727	3.0	1454	0.703	2345	0.14	1500	0.032	1645	1.589
1372	0.674	1609	1.411	1409	0.386	1645	2.34	1436	0.75	1518	0.073
1190	0.742	1581	0.056	1327	0.356	1500	0.050	1390	0.25	1445	0.616
1090	1.528	1436	0.321	1272	0.337	1454	0.56	1272	0.281	1354	0.562
1000	0.213	1372	0.429	1227	0.416	1409	0.22	1209	0.719	1136	1.041
767	0.326	1281	1.268	1127	0.832	1327	0.22	1209	0.719	1136	1.041
		1236	1.179	1027	0.772	1272	0.26	1081	1.0	1099	2.0
		1090	2.196	738	0.347	1218	0.54	1018	0.344	1018	2.575
		1027	0.929			1118	0.58			898	1.137
		869	1.018			1027	0.52			796	0.315
		738	0.375			694	0.1			723	0.562

aromatic skeletal vibration (Sarkanen *et al.*, 1967), or to a single or unresolved bands (Sarkanen and Ludwig, 1971). This band appeared to be high intensity ranged between 0.6-1 and very high in native sugarcane bagasse separated at 30°C. Appls of degraded *Ricinus communis*, it ranged from 0.5-2.5 and in Appls of degraded *Casuarina equisetifolia*, it ranged from 0.7-1.3 and absent by *C.versicolor* NRRL 6120, also in its native separated at 30°C, by *P.chrysosporium* NRRL 6359 and by *S. viridosporus* NRRL 2414. The absorption at 1030 cm^{-1} is assigned for the aromatic C-H inplane deformation characteristic of uncondensed guaiacyl (Sarkanen and Ludwig, 1971), and also due to the presence of c-o-c symmetric stretching in dialkylether linkage (Bolker and Somerville, 1963), and c-o deformation of primary alcohol. The disappearance of the band at the absence of c-o deformation of secondary alcohol and aliphatic ether (Fig. 6-9) and (Table-2.4). The intensity of the band at 1030 cm^{-1} is always higher than that at 1130 cm^{-1}

A.2.10. REGION 1000-700 cm^{-1}

The absorption at 920 cm^{-1} attributed to CH out-of-plane deformation of syringyl units this band disappeared in Appls of degraded sugarcane bagasse, it shifts to 1000 cm^{-1} in case of Appls of degraded *Ricinus communis* by *C.versicolor* NRRL 6102 and reaches to 0.2 and 892 cm^{-1} in case of its native and reaches to 1.14. Also disappear in case of *Casuarina equisetifolia* except in its native separated at 30°C, shift to 927 cm^{-1} and is very high intensity reached 2.3. The absorption at 860-820 cm^{-1} , the presence of two bands, one in the range 860-810 cm^{-1} and the other band in the range 765-730 cm^{-1} are characteristic of the 1,3, 5- tri-substituted aromatic (Bellamy, 1964). Colthup proposed the range 770-730 cm^{-1} for the position of the out-of-plane C-H bending absorption of mono-substituted aromatic orthodisubstituted material absorbed in the approximately the same range of frquencies. However, the absence of a mono-substituted

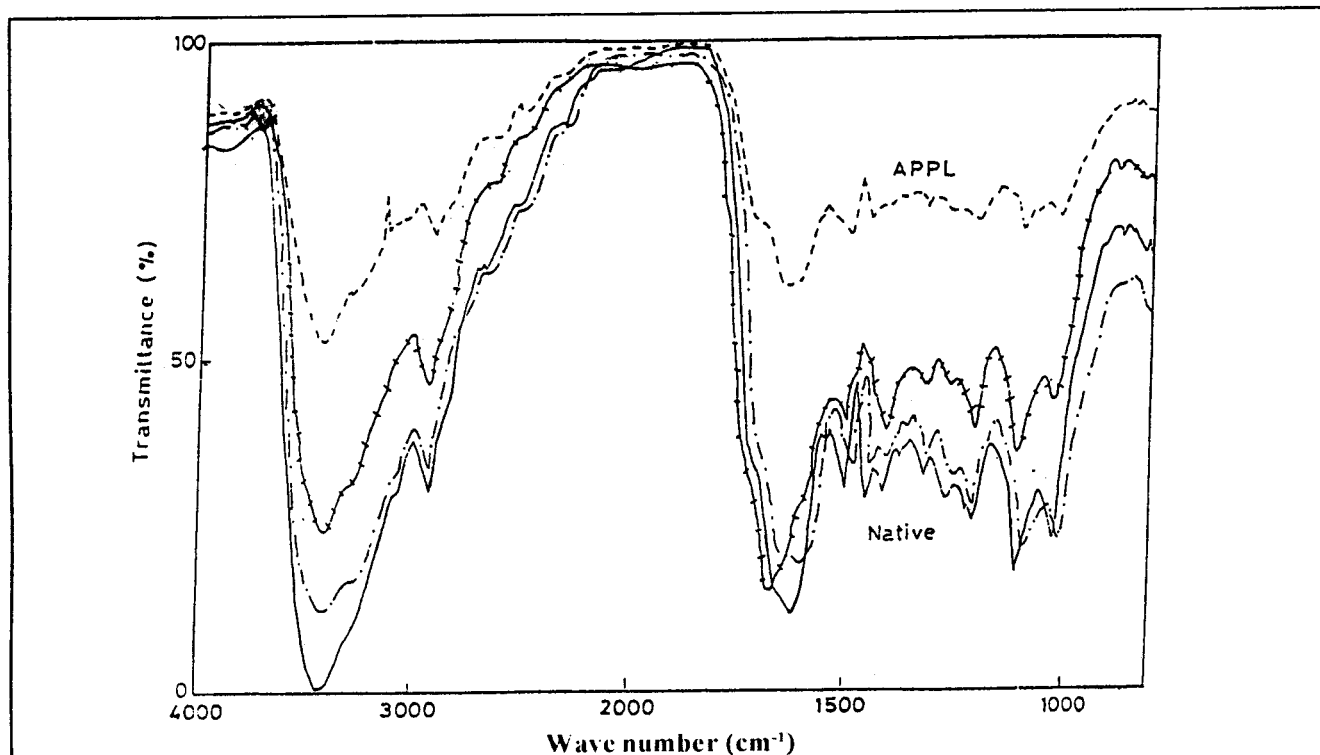


Fig. 8 : IR spectra of native lignin and APPL of degraded *Casuarina equisetifolia* by different microorganisms (—) native (— —) *P. chrysosporium* NRRL 6359. (- . - .) *P. chrysosporium* NRRL 6163. (+ + +) *S. viridosporus* NRRL 2414.

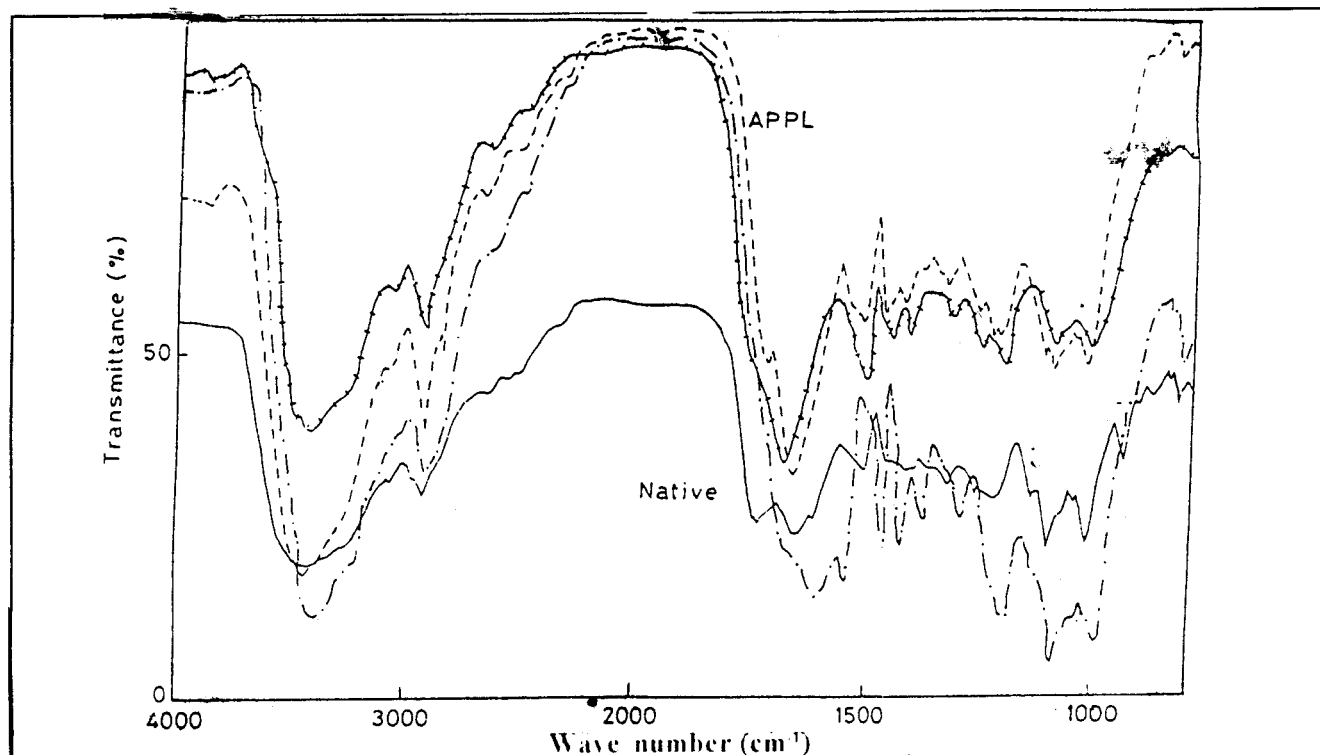


Fig. 9 : IR spectra of native lignin and APPL of degraded *Ricinus communis* by different microorganisms (—) native, (— —) *P. chrysosporium* NRRL 6359, (+ + +) *P. chrysosporium* NRRL 6153. (- . - .) *S. viridosporus* NRRL 2414.

product, but the converse is not necessarily true, and confirmation must be sought from the 750cm^{-1} region the meta distributed aromatic structure shows $710\text{-}690\text{cm}^{-1}$. The absence of bands in the range $910\text{-}700\text{cm}^{-1}$ may indicate that the aromatic ring in hexa-substituted (Bellamy, 1964). Based on these assignments, one or more of the above mentioned may be found in one of the lignins considered, taking into consideration that some of the bands may undergo some shift in their positions due to complex structure of lignin. However, some of the degraded Appls showed the absence of band in the range $910\text{-}700\text{cm}^{-1}$, which indicates the absence of hexa-substitution in these lignins.

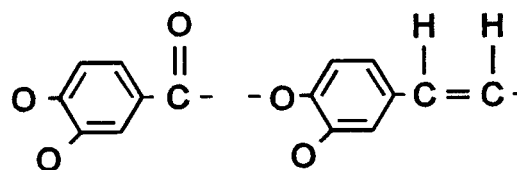
DISCUSSIONS

Ultraviolet spectra of the low molecular weight intermediates of lignin degradation of three-cellulosic substrates are studied. The results indicated that the UV spectroscopic of native lignins of sugarcane bagasse, *Casuarina equisetifolia* and *Ricinus communis* are identical to some extent. However, differences in UV spectra of degraded samples between the organisms were observed.

The results indicate an increase in UV absorption intensity at 280 in most cases which is attributed to the increase in the amount of chromophores structures in degraded lignin. This increase in UV absorption intensity at 280 nm which is attributed to the increase in the amount of chromophores structures in degraded lignin (Solar *et al.*, 1987; Mittar *et al.*, 1992). Absorption maximum characteristic for α -co groups and for β -co groups was obtained for all samples of lignin degradation. The presence or absorption maximum 365 in degraded *Ricinus communis* by *C. versicolor* NRRL 6102, in degraded sugarcane bagasse by *P. chrysosporium* NRRL 6359 indicates the presence of these groups in these degraded lignin. The absorption in the region 260-280 nm in all samples of lignin degraded by the four tested microorganisms had to conclusion of the presence of β -co-groups structures and this attributed at least in part to a small amount of aromatic acid moieties (Kirk and Chang, 1975).

The degraded lignin of sugarcane bagasse gives a new absorption maximum at 315 nm by *Coriolus versicolor* NRRL 6102 and at 363 nm by *Phanerochaete chrysosporium* NRRL 6359. It is well known that absorption within the region 250 to 290 nm is due to electronic oscillations of oxygen

substituted phenyl ring in lignin. Several investigators, reported that lignin have shown the absorption in the spectral region 300-350 nm can be assigned to the influence of a carbonyl or ethylenic double bond in conjugation with a phenyl ring. In a report Pearl and Beyer (1951), the ultraviolet absorption spectra of the ethyl esters and parent aldehydes of various ethers of 3,4-dihydroxycinnamic acid were presented. Their spectra are very similar to that obtained by our lignin samples. Therefore, it can, at least, be assumed that undegraded lignins of sugarcane bagasse, *Ricinus communis* and *Casuarina equisetifolia* contain one or both of the following building stones.



The polymeric intermediates accumulate as a water-fraction in the lignocellulose containing growth medium. Appls were collected from cultures of the tested microbial strains on different lignocellulosic substrates. IR analysis of Appls produced from degradation of the three lignocellulosic substrates showed that it significantly changed in chemical structure as compared to undegraded lignin. In agreement with the present results, Crawford *et al.* (1983), indicated that Appls derived from lignin by *S. viridosporus* T7A significantly changed in structure from a standard undegraded corn stover lignin. The IR measurement revealed remarkable changes in chemistry of Appls produced depending on the strain and/or the degraded substrate. This indicates that different mechanisms of lignin metabolism may exist in these four microbial strains. The present results are in accordance with the findings of Borgmeyer and Crawford (1985), working with two lignin-olytic streptomyces strains, *S. viridosporus* T7A and *S. badius* 252. They observed differences between Appls produced by the two strains. They concluded that different mechanisms of lignin metabolism exist between these two ligninolytic streptomyces species strains. Crawford *et al.* (1984) found that when *S. viridosporus* T7A or *S. badius* 252 grown in lignocellulose, both lignin and carbohydrates are extensively degraded by oxidative mechanisms, as shown by chemical characterization of *Streptomyces* species-degraded soft wood lignin.

Several single ring aromatic, intermediates have been shown to be released by *S. viridosporus* T7A from hard wood, soft wood and grass lignin (Crawford et al., 1982). However, the most important intermediate of degradation by *S. viridosporus* was Appl. (Crawford et al., 1983).

Recently, lignin degradation by bacteria has received increasing attention (Vicuna, 1988), and peroxidase activity has been reported to be found in *S. viridosporus* (Ramachandra, 1988).

Results on IR spectra analysis revealed differences in Appls produced *S. viridosporus* NRRI 2414 and the three White-rot fungal strains which may indicate different mechanisms of lignin metabolism exist between these microbial strains. In agreement with this view, Leisola and Garcia (1989), reported that bacteria, however, decompose lignin slowly and incompletely compared with that by white-rot fungi. Moreover, they reported that the mechanism of bacteria lignin degradation differs from that fungi, the major effect being solubilization of lignin to high molecular weight carbohydrate complexes.

IR spectra of Appls of all degraded cellulosic samples indicate an increase in numbers of carbonyl groups and carboxylic groups. These carbonyls are almost of C α -carbonyl groups and would result from oxidative attack on the lignin side chain (Kirk and Chang, 1974 and 1975). Similar results on Appls produced from the degradation of lignin by *Aspergillus flavus* was obtained by Betts and Dart (1988).

A lignin degrading enzyme was discovered in 1983, (Glenn et al., 1983 & Tion and Kirk, 1983), in ligninolytic culture of *P. chrysosporium*. The enzyme was identified as peroxidase (Harvey et al., 1985; Kuwahara et al., 1985) and the catalytic activity of those lignin peroxidases have been extensively studied (Kirk and Farrell, 1987). Kuwahara et al., (1984), discovered another lignin peroxidase in extracellular culture medium of *P. chrysosporium* which catalyze oxidation of phenolic structure in lignin.

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