# Infrared Spectra of Jute Stick Treated with White-Rot Fungus

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SYNOPSIS

Jute stick was treated with white-rot fungus for incubation periods of 6 days (MBA) and 12 days (MBB). The infrared spectra of fungus treated samples (MBA and MBB) and control jute stick (MBC) were analyzed and compared. The bands attributed mainly to hemicellulose show an increase in absorbance intensity ratios ($A_v/A_{2900}$) with increase of incubation time. Similarly the bands attributed to lignin show an increase in the absorbance intensity ratios with increase of incubation time. Increase in the intensity of 1635 $cm^{-1}$ band with increase of incubation time was also observed.

## INTRODUCTION

White-rot fungi have been proposed as agents for pretreating wood chips prior to mechanical pulping or for posttreating mechanical pulps.[1-3] These treatments have been suggested as routes, both to reduce fibering and refiner energy requirements[4-5] and to increase strength properties of the resulting paper. An additional benefit is anticipated by way of decreases in the pollution loads resulting from destruction of extractives and other lignocellulosic components of pulping waste liquors. A basidiomycete *Phanerochaete chrysosporium* was found to multiply well on jute stick chips[6] under certain nutritional and physiological conditions, raising the possibility of biopulping.

Although IR spectra of jute stick and alkali treated jute stick[7] and bleached jute stick[8] have been recently reported, no study on the IR spectra of jute stick treated with white-rot fungus is available; however, fairly detailed work on the IR spectra of jute fibre has been reported.[9-11]

In the present investigation, results on IR spectra of jute stick treated with the white-rot fungus *Phanerochaete chrysosporium* for incubation periods of 6 days and 12 days, together with the control sample have been analyzed and discussed.

Journal of Applied Polymer Science, Vol. 44, 1607-1613 (1992)
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## MATERIALS AND METHODS

### Jute Stick

Jute stick (*Corchorus capsularis*, variety JRC 7447) was disintegrated in a Wiley mill, using different sieves. The disintegrated sample was defatted using a mixture of benzene–ethanol (2 : 1, v/v) and passed through 40 and 60 mesh sieves. The 40–60 mesh sample was used to study the major chemical compositions. The (1 × 1 × 3 mm) jute stick sample was used for treatment with the fungus *Phanerochaete chrysosporium*. The 100 mesh fungus-treated samples were used for IR spectral analysis.

### Treatment with White-Rot Fungus

White-rot fungus *Phanerochaete chrysosporium*, Burds (ME 446, ATCC 35540) was obtained from the Centre for Forest Mycology Research, Forest Products Laboratory, U.S. Department of Agriculture, Madison, Wisconsin. The organism was maintained at 40°C on malt agar (2%) slope.[12]

The basal growth medium contained per litre of water, the following minerals: $NaNO_3$ (2 g), $KH_2PO_4$ (1 g), $MgSO_4 \cdot 7H_2O$ (0.05 g), KCl (0.05 g), $FeSO_4 \cdot 7H_2O$ (0.01 g).

Jute stick (7.5 g) was placed in a 1-L conical flask and soaked with 30 mL basal medium containing a suspension of glucose (0.375 g) and yeast extract (0.187 g). The flasks were sterilized by autoclaving

for 15 min under steam pressure (1.06 kg/cm$^2$). Inoculation was made with 10 mL of fungal culture (containing 1.8 mg dry cell obtained from a 7-day-old slope culture), and 30 mL of sterilized distilled water was further added. Jute stick samples were incubated for 6 days (MBA) and 12 days (MBB) at room temperature ($\sim$ 32°C) in still culture. The incubated samples were washed with cold distilled water and finally with absolute alcohol. Defatted jute stick (7.5 g) autoclaved for 15 min with 30 mL distilled water and washed as described for MBA and MBB served as the control sample MBC. Yield of the samples MBA and MBB was 65.74 and 42.8%, respectively.

### Analysis of Jute Stick Samples

Ash content and lignin content were estimated following Technical Association of the Pulp and Paper Industries (TAPPI) methods;[13] $\alpha$-cellulose was estimated by the modified method of Chattopadhyay et al.[14] Pentosans were estimated using Krober's tables following the procedure of Schorger.[15]

### Infrared Spectroscopy

The samples were mixed thoroughly in a mortar with 200 mg of KBr, which was previously dried at 120–150°C for 16 h. KBr pellets were made in a hydraulic press at a working pressure of 8 tons for 10 min pressing time, under vacuum. The spectra were recorded in Shimadzu double beam spectrophotometer, IR-440 under normal slit program and scanning speed of nearly 19 s/100 cm$^{-1}$. A blank KBr pellet was used in the reference beam.

## RESULTS AND DISCUSSION

In treatments of jute stick with white-rot fungus, the microorganisms utilize lignin as a carbon source and convert it to $CO_2$ and biomass. During the degradation of lignin, numerous simple phenol derivatives have been isolated in small amounts. These include ferulic, syringic, vanillic, protocatechuic, *p*-hydroxycinnamic, and *p*-hydroxybenzoic acids and the aldehyde forms of these acids, dehydrodivanillin, guaiacylglycerol-$\beta$-coniferyl ether, and others. Degradation of jute stick lignin by white-rot fungus can be explained by the degradation of the model compound syringylglycol-$\beta$-guaiacyl ether studied by Kirk et al.[27] and shown in Figure 1. Jute stick lignin was oxidized by *P. vericolor* and *S. fistulatum* to carbonyl compounds giving $\alpha$-guaiacoxyacetosyringone. The alkylphenyl carbon–carbon bond in both the

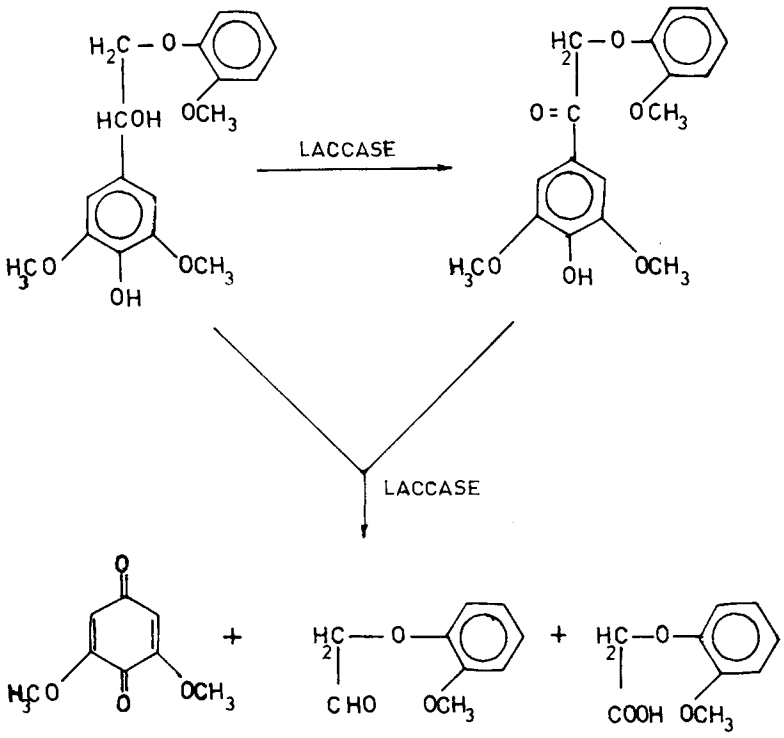


**Figure 1** Cleavage of syringyl compounds by culture filtrates.

**Table I Major Chemical Constituents of Fungus-Treated Jute Sticks and Control Sample[a]**

| Chemical Constituents | Control Sample MBC % | White-Rot Fungus Treated Jute Sticks MBA (6 days) % | MBB (12 days) % |
|---|---|---|---|
| Yield | 92.39 | 65.74 | 42.8 |
| Ash | 0.90 | 0.36 | 0.72 |
| Lignin | 24.8 | 25.57 | 33.82 |
| Pentosans | 23.67 | 21.6 | 21.19 |
| $\alpha$-Cellulose | 41.44 | 40.02 | 34.39 |

[a] Calculated on the basis of 100g oven-dried sample.

syringyl compounds was cleaved by culture filtrates of *P. vericolor* and *S. fistulatum* as shown in Figure 1. Fungal treatments have not yet been comprehensively studied and whether the biochemical mechanism involve lignin removal or lignin degradation are not fully known. Similarly, much remains to be learned regarding the enzymology of lignolytic fungi. Probably the extracellular activity of these microorganisms predominates, this is to be expected considering the size of the lignin macromolecule.

The major chemical constituents of control sample, MBC, and fungus-treated jute stick samples, MBA and MBB, calculated on the basis of 100 g of oven dry (o.d.) samples, are shown in Table I. The major chemical constituents calculated on the basis of 100 g of o.d. defatted jute stick are shown in Table II. The percentage losses of lignin, $\alpha$-cellulose and pentosans after 6 days (MBA) and 12 days (MBB) of incubation, calculated on the basis of total contents in defatted jute stick are shown in Table III. The percentage losses of $\alpha$-cellulose and pentosans are higher than the percentage loss of lignin in both the samples MBA and MBB. The percentage losses of lignin, $\alpha$-cellulose and pentosans of the sample MBB are higher than that in the case of the sample MBA.

IR spectra of the control (MBC) and fungus treated jute stick samples (MBA and MBB) are shown in Figure 2. The summary of the results including the significance of bands, the ratios of the absorbance maxima of individual bands and the 2900 $cm^{-1}$ band ($A_\nu/A_{2900}$) has been recorded in Table IV, following the baseline correction method.[16] The 2900 $cm^{-1}$ band has been chosen as an internal standard, as it is present as a prominent band in the IR spectra of isolated lignin, hemicellulose, and $\alpha$-cellulose of jute stick.[7] The internal standard has been adopted for making a comparative study of the spectra.

The 3400 $cm^{-1}$ band is attributed to H-bonded H—O stretching,[17] and the 2900 $cm^{-1}$ band is assigned to C—H stretching in methyl and methylene groups.[12] The band near 1740 $cm^{-1}$, attributed to C—O stretching of the carbonyl and acetyl groups

**Table II Major Chemical Constituents of Fungus-Treated Jute Sticks and Control Sample[a]**

| Chemical Constituents | Control Sample MBC % | White-Rot Fungus Treated Jute Sticks MBA (6 days) % | MBB (12 days) % |
|---|---|---|---|
| Lignin | 22.9 | 16.8 | 14.47 |
| Pentosans | 21.86 | 14.19 | 9.06 |
| $\alpha$-Cellulose | 38.28 | 26.30 | 14.71 |

[a] Calculated on the basis of 100g oven dried original defatted jute stick.

**Table III Percentage Losses of Lignin, α-Cellulose, and Pentosans of Fungus-Treated Jute Sticks[a]**

| | Loss of Lignin % | Loss of α-Cellulose % | Loss of Pentosans % |
|---|---|---|---|
| MBA | 29.4 | 36.47 | 35.08 |
| MBB | 39.2 | 64.46 | 58.55 |

[a] Calculated on the basis of total contents: lignin = 23.8%, α-cellulose = 41.4%, and pentosans = 22.8% in jute stick.

in the hemicellulose of jute stick,[11,18,19] shows a gradual increase in the absorbance intensity ratios. However, since the pentosan contents of the samples MBC, MBA, and MBB show a decreasing tendency, the increase in the absorbance intensity ratios can be explained by the fact that fungal treatment increases the contribution of the carbonyl groups. This may be due to the cleavage of lignin–carbohydrate bonds or some other bonds in the lignin macromolecule.[20–22]

The absorbance band at 1635 $cm^{-1}$, attributed to the vibration of adsorpted water molecules in the noncrystalline regions in cellulose,[17,23] gives a shoulder in the control sample MBC, whereas broad peaks are obtained in the samples MBA and MBB. This can be explained by the fact that fungal action

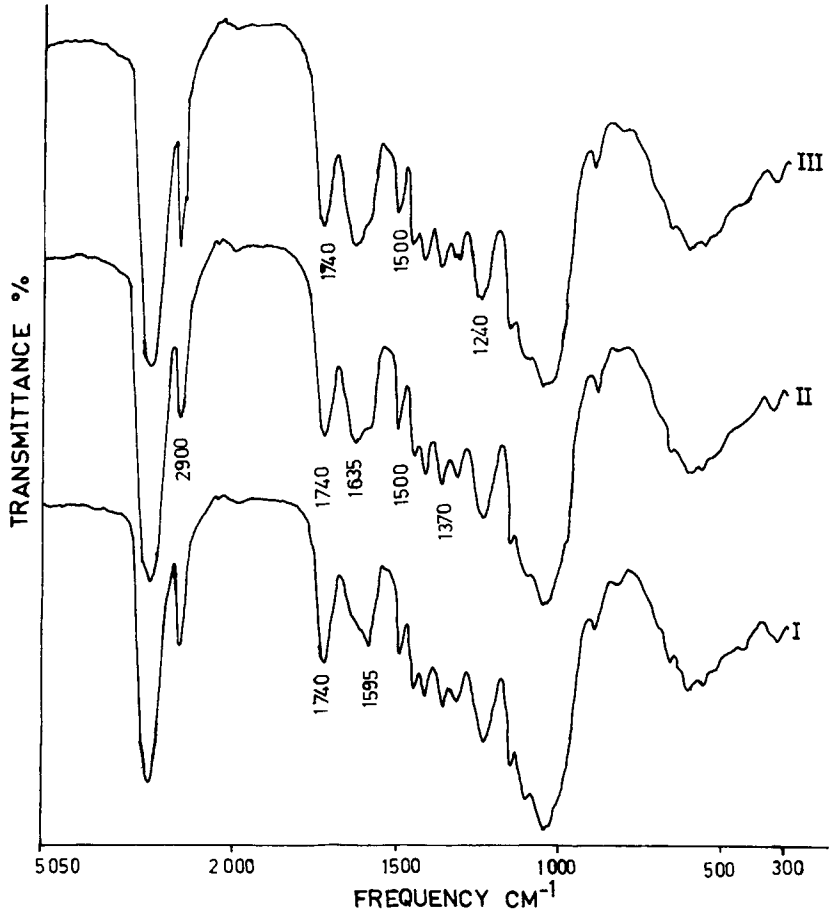


**Figure 2** IR spectra of I - MBC, II - MBA, and III - MBB.

**Table IV Absorbance Intensity Ratios in IR Spectra of MBC, MBA, and MBB Samples**

| Position of Bands ($cm^{-1}$) | Assignments | MBC $A_\nu/A_{2900}$ | MBA $A_\nu/A_{2900}$ | MBB $A_\nu/A_{2900}$ |
|---|---|---|---|---|
| 3400 | H—O stretching (H-bonded) | 0.4079 | 0.2955 | 0.4122 |
| 2900 | C—H stretching in methyl and methylene | 1.0(0.475)[a] | 1.0(0.4225) | 1.0(0.2958) |
| 1740–1735 | C—O stretching in carboxyl and unconjugate β-ketone | 1.0136 | 1.2617 | 1.4997 |
| 1635 | $H_2O$ molecules in noncrystalline cellulose | shoulder | 1.3082 | 1.7675 |
| 1595 | Aromatic skeleton ring vibration | 1.4487 | 1.5106 | 2.229 |
| 1500 | Same as above | 1.6202 | 1.8837 | 3.0297 |
| 1455 | C—H deformation and $CH_2$ bending | 1.4009 | 2.3660 | 2.5843 |
| 1420 | Same as above | 1.4269 | 1.5799 | 2.4380 |
| 1370 | C—H deformation (symmetric) | 1.4714 | 1.6138 | 2.4771 |
| 1320 | Syringyl ring breathing with C—O stretching and H—O in-plane bending | 1.6754 | 1.9097 | 2.9727 |
| 1240 | C—O stretching in acetyl group | 1.5182 | 1.6543 | 2.3774 |
| 1160–1155 | C—O—C stretching (antisymmetric) | 1.3637 | 1.4086 | 2.0353 |
| 1110–1105 | H-bonding on skeletal vibration, involving C—O bond stretching | 0.9999 | 1.0782 | 1.5285 |
| 1050 | Due to hemicellullose | 0.7610 | 0.8207 | 1.1722 |
| 1030 | Aromatic C—H in-plane deformation, guaiacyl type and C—O deformation primary alcohol | 0.7504 | 0.7938 | 1.1240 |
| 990 | Skeletal vibration and C—O stretching in cellulose and xylan | shoulder | shoulder | shoulder |
| 895 | C—H bending β-linkage in cellulose and xylan | 2.3091 | 2.1515 | 3.9441 |
| 830 | Aromatic C—H out of plane vibration | 3.7253 | 2.3667 | Nil |

[a] Values in parentheses indicate intensities at the 2900 $cm^{-1}$ band.

exposes the fibrillar structure, causing exposure of the noncrystalline regions of cellulose, which in turn facilitates the adsorption of water molecules.

The bands at 1595 $cm^{-1}$ and 1500 $cm^{-1}$ attributed to the aromatic skeleton ring vibration[16] show an increasing order of absorbance intensity ratios in accordance with the increasing order of lignin contents in the samples MBC, MBA, and MBB (see Table I). The absorbance bands at 1455 $cm^{-1}$ and 1420 $cm^{-1}$, ascribed to $CH_3$ deformation (asymmetric) in lignin and to $CH_2$ bending in xylan[23] have an increasing order of absorbance intensity ratios for the samples MBC, MBA, and MBB in concurrence with the increasing lignin contents of the samples (see Table I).

The 1370 $cm^{-1}$ band, ascribed to C—H deformation (symmetric) may be attributed to lignin, α-cellulose or xylan.[18,24] The band has an increasing order of absorbance intensity ratios for the samples MBC, MBA, and MBB, and is probably influenced by the increasing residual lignin contents of the samples (see Table I). Similarly, the 1320 $cm^{-1}$ band, attributed to syringyl ring breathing with C—O stretching in lignin[24] and to $CH_2$ wagging in cellulose[18,25] shows an increase in absorbance intensity ratios for the three samples MBC, MBA, and MBB, and is probably also influenced by the increasing residual lignin contents of the samples (see Table I).

The broad medium intensity band having a maxima near 1240 $cm^{-1}$, ascribed to the C—O linkage in the acetyl group in xylan,[19,20,25] shows an increase in absorbance intensity ratios for the three samples MBC, MBA, and MBB, and this can be explained in the same way as the changes in the 1740 $cm^{-1}$ band of the above samples.

The 1160 $cm^{-1}$ band, attributed to both cellulose and hemicellulose,[19,23,24] shows an increase in absorbance intensity ratios due to the fungal action on jute stick similar to the case of the band 1740 $cm^{-1}$ of the samples under discussion. Similarly, the 1110 $cm^{-1}$ band, assigned to H-bonding on the skeletal vibration involving stretching of the C—O bond in cellulose and hemicellulose,[18,19,25,26] shows an increase in the absorbance intensity ratios for the three samples MBC, MBA, and MBB, and is ascribable

to the same reason as in the changes of the 1740 $cm^{-1}$ band.

The 1050 $cm^{-1}$ band, assigned to skeletal vibration involving C—O stretching in hemicellulose,[18] records an increase in the absorbance intensity ratios in the three samples MBC, MBA, and MBB, and the increase can be explained on the basis of increase in the contribution due to the carbonyl group as in the case of the 1740 $cm^{-1}$ band of the above samples.

The 1030 $cm^{-1}$ band is assigned to aromatic C—H in-plane deformation (guaiacyl type), C—O deformation for primary alcohol groups in lignin,[24] and to skeletal vibration involving C—O stretching in hemicellulose.[18] The band shows an increase in the absorbance intensity ratios in the three samples MBC, MBA, and MBB and is in concurrence with the increase in the residual lignin contents of the three samples under discussion (see Table I).

The shoulder at 990 $cm^{-1}$ is attributed to skeletal vibration involving C—O stretching in cellulose and hemicellulose.[18] The 895 $cm^{-1}$ band is assigned to antisymmetric out-of-plane stretching due to $\beta$-linkage in hemicellulose.[18] The 830 $cm^{-1}$ band ascribed to aromatic C—H out-of-plane vibration in lignin[24] appears to have shifted to the lower frequency 795 $cm^{-1}$ in the case of the MBB sample.

Bhattacharyya et al.[28] showed that paper sheets made from jute stick pretreated with the same white-rot fungus (*P. chrysosporium*) are very brittle and are unsuitable for making paper. This observation was supported by Roy et al.,[29] who showed by scanning electron microscopic study that jute stick pretreated with the above mentioned white-rot fungus (*P. chrysosporium*), which is not a cellulase-less mutant, results in the opening of protoxylem along with a varying degree of cell wall damage, and that this increases with the increase in incubation time.

The above observations are consistent with the increase in the intensity of the 1635 $cm^{-1}$ band, attributed to opening-up of fibrillar structure as a result of fungal action. Damage to the cell wall is also consistent with the increase in the absorbance intensity ratios of the bands attributed to lignin which is increased (see Table I) as compared to the control sample.

## CONCLUSION

Although the IR spectra of the three samples appear to be similar, changes introduced in the chemical structure of the fungus treated samples are reflected by the increase in the absorbance intensity ratios of the IR bands, attributed mainly to lignin and hemicellulose. The bands at 1740 $cm^{-1}$, 1240 $cm^{-1}$, 1160 $cm^{-1}$, 1105–10 $cm^{-1}$, 1050 $cm^{-1}$ and 895 $cm^{-1}$, attributed mainly to hemicellulose show an increase in absorbance intensity ratios with increase of incubation time, when compared with that of the control sample. Although the pentosan contents of the fungus treated samples show a tendency to decrease, cleavage of lignin–carbohydrate bonds or some other bonds in the lignin macromolecule, increases the absorption intensity ratios of the above bands with increase of incubation time.

The bands at 1595 $cm^{-1}$, 1500 $cm^{-1}$, 1455 $cm^{-1}$ and 1420 $cm^{-1}$ attributed mainly to lignin show an increasing order of absorbance intensity ratios with increase of incubation time, in concurrence with the increase in lignin contents of the fungus treated samples. Increase in the intensity of the 1635 $cm^{-1}$ band reveals the opening-up of fibrillar structure as result of fungal action causing exposure of the non-crystalline regions.

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*Accepted May 22, 1991*