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Characterization of lignin from sugarcane bagasse by Raman spectroscopy and FTIR

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***Abstract*---**Background: The structural information of the lignin samples obtained using various extraction methods have been investigated by FTIR and Raman spectroscopy .In the present work, information regarding the changes in lignin caused by different extraction processes has been reported. The present manuscript appraised various prominent and seclusion methods currently being surveyed in favour of sugarcane bagasse lignin extraction (*Saccharum officinarum*). Methods: The methods used were acid hydrolysis, alkaline pulping, extraction by dioxane and HCL, alkaline KOH. Significant findings: The Raman spectra of lignin calliberated at 532 nm resulted the decrease in the intensity band at 1200 $cm^{-1.}$

***Keywords*---**Sugarcane Bagasse, Lignin, Raman spectra, Spectroscopy, Biodegradable.

Introduction

Last several years have seen tremendous interest in the extraction of lignin from non-wood biomass such as sugarcane bagasse (SB). SB is a dry fibrous residue material remaining after crushing the sugarcane to extract juice and has been used in sugar industries or bioethanol production. It is being exploited as a biofuel for producing heat, electricity and energy etc. and also for pulp and composite materials. SB is a heterogeneous material consisting three polymers as cellulose, hemicellulose and lignin. The lignin extracted from SB has numerous applications such as high heating value biomass fuels, biodegradable polymers for manufacturing wide range of products, pesticide formulation, retarder additive for oil well cement slurry, carbon fiber production etc. due to its outstanding thermal stability and structural strength. Lignin structures are exceedingly

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irregular,making it challenges to define them. Lignins have been studied using a variety of ways over the years [1-3]. Despite the fact that a variety of strategies have been used,Only a few approaches are suitable for in situ analysis of lignins in the solution state. The latter category includes Raman and infrared (IR) spectroscopies category. Raman studies of lignins are less expensive than IR studies.For lignin applications, the process needs to be improved further. Although a variety of approaches have been used to study lignins in the solution state, only a handful are suitable for in situ research. The latter category includes Raman and infrared (IR) spectroscopies category. Raman studies of lignins are less expensive than IR studies.For lignin applications, the process has to be improved further.Raman is a good complement to IR and has the extra benefit of not being affected by water.to the water. Lignins have been studied using a variety of ways over the years. Laser-induced lignin autofluorescence can be a considerable impediment to achieving results.Because the fluorescence intensity can be several orders of magnitude higher than the fluorescence intensity, Raman spectra can be reasonably good. To efficiently eliminate auto fluorescence, two sample approaches have traditionally been used: the water immersion technique and the air immersion technique (usable for woody tissues) [4] and the technique of oxygen flushing [5,6]. The near-IR Fourier transform Raman method can be used to alleviate fluorescence difficulties.Using an NIR laser source with a photon energy well below troublesome low energy electronic transitions, a technique known as NIR-FT was developed. This approach produces high-quality spectra.From several lignin-containing materials, relatively free of fluorescence interference was achieved [7-11]. Raman studies of lignins are relatively new in comparison to IR [12-22]. The phenolic end groups, which are vulnerable to oxidation (they are some of the most significant and reactive lignin moieties), are some of the most important and reactive lignin moieties,have a low redox potential) and consequently play a crucial role in the biosynthesis and breakdown of lignin.

Experimental Details

The sugarcane bagasse used in the present work was collected from National Sugar Institute, Kanpur. It was kept in an oven at temperature of 60^0 C for four days to remove moisture from it. The dried bagasse was then converted into the powdered form. Three grams of bagasse by weight in powder form samples each was further treated with various methods to extract lignin from it. The lignin extraction was made by various methods of extraction such as treating the powder sample with (i) dioxane and HCL (ii) alkali KOH (iii) acid hydrolysis and (iv) alkaline pulping. Analytical grade chemicals Ethanol, Cyclohexane, Hydrochloric acid, Dioxane, Nitrogen gas, Distilled water, Phosphorous anhydride, KOH, Sulphuric acid, Sodium hydroxide. The extraction of the samples is done as follows:

The extraction of bagasse was done in Soxhlet apparatus for two days with a mixture of equal volumes of Cyclohexane-Ethanol, then with water for 1 day and dried until constant weight in an air circulated stove at 60^0C. Then grounded to obtain a powder (1mm diameter). The dry powder treated with a dioxane solution and 0.1 N aqueous hydrochloric acid (8:5:1.5,v:v) with ratio 100 g /1 litre and heated under nitrogen at 100^0 C for two hours, then filtered and washed three times with a fresh dioxane water solution without hydrochloric acid. The filtrates concentrated by evaporation under vacuum to eliminate dioxane in conditions that maintain the pH above 1 to avoid excessive depolymerization of lignin, then centrifuged and the precipitation was dried under vacuum over phosphorous anhydride as purify of bagasse lignin.

The sugarcane bagasse powder was stirred with 0.5 M aqueous KOH solution with the ratio of 50 g/1.5 L under nitrogen at 35^0 C for 3.5 hours, then filtered and washed with distilled water followed with ethanol. The filtrate is neutralized with 6 M acetic acid solution to pH 5.5. The filtrate was treated by 3 volumes of 95% ethanol and the filtrate was precipitated with water and adjusted with 6M HCL solution to pH 1.5. It was then centrifuged and washed with acidified water to pH 2 and dried under vacuum over phosphorous anhydride.

39.67 ml (98% sulphuric acid) was mixed with 60.33 litre of deionized water to prepare 72% w/w of sulphuric acid solution. The solution was kept to cool by putting in the tray filled with ice cubes.3 grams of the baggse was dipped into the solution for 3 hours. The solution was then filtered with distilled water to extract lignin and dried in the oven at 105^0 C. The filtrate was amounted to 125 ml in a volumetric flask with distilled water and its absorbance was measured at 205 nm. The extracted lignin is shown in the figure below: The right beaker consist of the lignin extracted in solid sample of baggase and left beaker is the lignin extracted in the liquid sample after treatment of bagasse.
Treatment of bagasse by soda solution ,usually after pre treatment by hot water at 100^0 C for 1 hour with different concentration of soda and biomass (solid bagasse) to liquid ratio.The sugarcane was air dried at ambident temperatures for 3 days until humidity was reached till 10% ,then cut into small pieces .The dry powder is treated with hot water at 70^0 C for 2 hours and then it was cooled to 25^0 C,washed with water at same above mentioned ratio and centrifuged for 10 minutes at 2000 rpm.The separated solid was pulped at 99^0C by sodium hydroxide solution with solid to liquid ratio 1:10 -1:18 w/w at 90^0c for 1.5 -4 hours,under constant stirring,then filtered to obtain the black liquor liquid.The filtrate was acidified by sulphuric acid to Ph 2,in order to precipitate the acidified lignin ,then washed by water and collected by centrifugation and air dried. The lignin was extracted from bagasse which was found in both liquid and solid samples of the bagasse after its treatment and extraction by various methods.

Untreated Baggase

The distilled water was boiled on heating plate for 20 minutes with the temperature of around 65 ^{0} C and the bagasse was dipped into the solution.
Samples: The first sample was sugarcane bagasse which was untreated, second sample was The lignin extracted from different methods was transferred into small glass tubes,2mm diameter which were sealed and used in the Raman spectrometer and the Raman spectra of the solid lignin samples was obtained using Acton Research Corporation spectrometer,SPECTRAPRO-2500i,0.500m imaging triple grating monochromator with nm DPSS ,100 mw DPSS 100 mw with the excitation wavelength of 532 nm, Western Core Lab,IITKanpur. The grating used was 600 grove per mm. The lignin was extracted with dioxane and HCL, treatment by alkali KOH, treatment by acid hydrolysis,treatment by alkaline

pulping methods.The untreated bagasse was also subjected to Raman with a excitation wavelength of 532 nm.

Methods

The untreated bagasse is shown below:

Figure1: Untreated bagasse

Treatment by alkali koh:

The image of the lignin extracted is as below:

Figure 2: Lignin extracted from bagasse by alkali KOH

Extraction by dioxane and hcl:

The extracted lignin images are shown below:

Figure 3: The solid and liquid lignin extracted by dioxane and HCL

ACID HYDROLYSIS

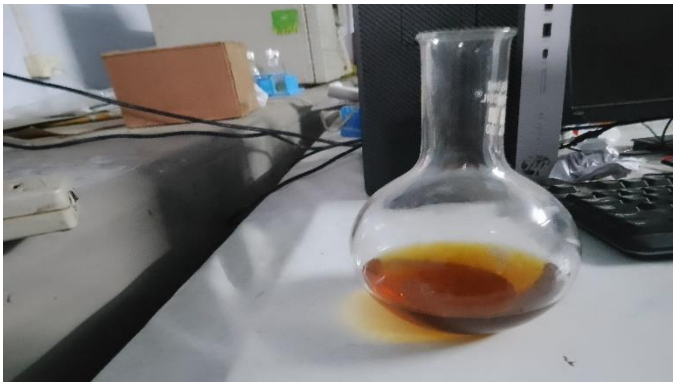

Figure 4: Lignin extracted by acid hydrolysis

Results and Discussions

The raman spectra are from samples that were subjected to the treatment sequence that resulted in the greatest significant decrease in the intensity of the 1200 cm^{-1} band. The relative intensities I_{1200}/I_{520} are displayed in the table below.

Table 1
table showing the relative intensities of raman spectra

Treatment methods	I_{1200} / I_{520}	$I_{1200}/I_{520\ correct}$
Untreated bagasse	0.10	0.13
Treatment by alkali KOH	0.32	0.36
Treatment by dioxane and HCL	0.70	0.64
Treatment by acid hydrolysis	0.69	0.61
Treatment by alkaline pulping	0.30	0.34

The Raman spectra of lignin obtained by various treatment methods such as treatment by alkali KOH,dioxane and HCL,acid hydrolysis,treatment by alkaline pulping in comparison to the untreated bagasse is shown below in the figure 5.The spectrum of the various treatment methods display the intense bands at 500 cm $^{-1}$,520 cm $^{-1}$,1200 cm $^{-1}$ To make the spectral characteristics more visible,the diagram below depicts many spectral regions in the range of 500 cm^{-1} – 3000 cm^{-1} [23,24].Because amorphous hemicellulose/cellulose [25] contributes in small amounts,the Raman features seen in the spectra are entirely due to lignin. The band at 520 cm-1 is assigned associate scientific discipline ring deformation. The weaker band ascertained at 520 cm-1 will likewise be explained by the 120° conformer that constant vibration is expected with a harmonic frequency shift of ~15 cm-1 (freq scaled).The band at 500 cm $^{-1}$ is due to axial and skeletal deformation.The band at 1200 cm $^{-1}$ also corresponds to the axial deformation.

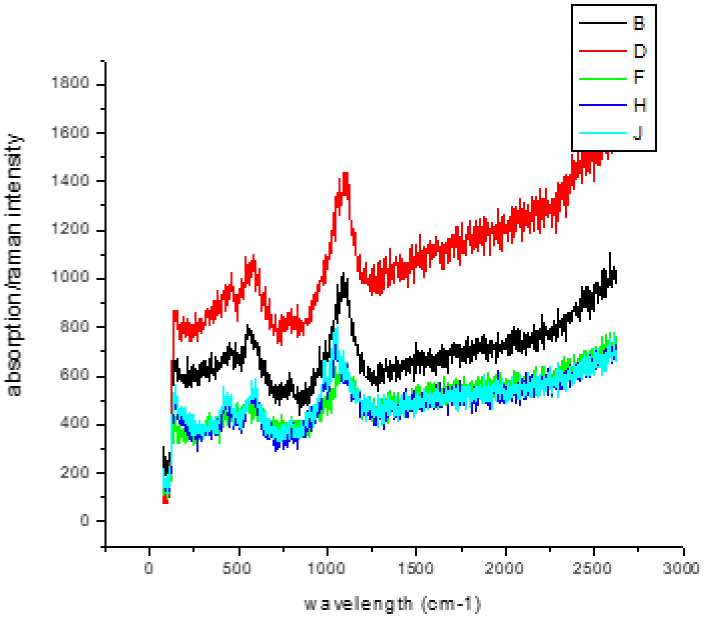

Figure 5: Raman spectra of lignin at 532 nm,B=untreated bagasse,D=extraction by dioxane treatment,F=treatment by alkali KOH,H= treatment by acid hydrolysis,J=treatment by alkaline pulping

FTIR Discussion

The figure 6 shows the FTIR spectra of the lignin obtained by acid hydrolysis, alkaline pulping, extraction by dioxane and HCL,treatment by alkali KOH, untreated bagasse in the range of 500-4500 cm $^{-1}$. The FTIR spectra of the five lignins investigated exhibit distinct variances.The carboxyl group in lignin are represented by carboxyl variations between 1750-1550 cm $^{-1}$. The carbonyl stretching coupled with aromatic ring skeleton appears at 1460 cm^{-1} in lignin, and the C-H deformation appears at 1600 cm^{-1}. The aromaticity of lignin structure is found in aromatic skeletal vibrations of guaiacyl and syringyl units in the wavenumber range of 1600 cm-^{1} to 1000 cm-^{1}.The peak at 1100 cm^{-1} corresponds to the C-O deformation of the secondary alcohol and aliphatic ethers and the peak at 1086 cm^{-1} corresponds to the C-O deformation in secondary alcohol.The peak at 1513 cm^{-1} corresponds to the aromatic skeletal vibration.The peak at 3500cm^{-1} corresponds to the O-H stretching.The peak at 2600 cm^{-1} corresponds to the C-H stretch in the methyl and methylene group.The peak at 650 cm^{-1} corresponds to CH deformation and the OH out of the plane bending.The table 2 is showing the FTIR table of lignin extracted by the above methods with the peak obtained.

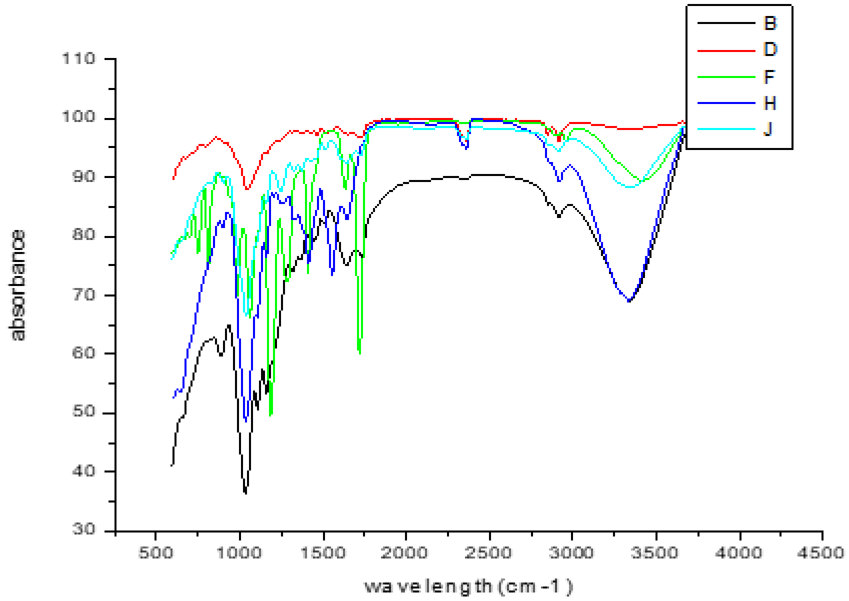

Figure 6: Figure showing FTIR spectra of lignin:B=acid hydrolysis,D=alkaline pulping,F=extraction by dioxane and HCl,H=treatment by alkali KOH,J=untreated bagasse

Table 2
FTIR table of lignin showing the obtained peaks with its various assignments.

Band	Assignment
1750-1550 cm $^{-1}$	Carboxyl group
1460 cm-^{1}	Carbonyl stretching
1600 cm-^{1}	C-H deformation
1600 cm-^{1} to 1000 cm-^{1}	skeletal vibrations of guaiacyl and syringyl units
1100 cm^{-1}	C-O deformation
1086 cm^{-1}	C-O deformation
1513 cm^{-1}	Skeletal vibration
3500 cm^{-1}	O-H stretching
2600 cm^{-1}	C-H stretch in the methylene group
650 cm^{-1}	C-H deformation

Conclusion

The absorbance of O-H bands was reduced after acid hydrolysis of lignin.The acid hydrolysis treatment of lignin enhances the phenolic O-H group, which increases its reactivity toward other processes. Raman at 532 nm specifically probes lignin end groups such as cinnamaldehydes and cinnamyl alcohols. The maxim that IR should always be utilised in conjunction with Raman for extensive chemical investigation is reiterated here, with the addition that Raman spectra at preresonant and nonresonant regimes are valuable for gaining more insight into lignin.

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