



Asian Journal of Scientific Research

ISSN 1992-1454

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Cocoa Pod Ash Pre-treatment of Wawa (*Triplochiton scleroxylon*) and Sapele (*Entandrophragma cylindricum*) Sawdust: Fourier Transform Infrared Spectroscopic Characterization of Lignin

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ABSTRACT

Effective pre-treatment of lignocellulosic materials, to reduce the recalcitrance of lignin, is critical to achieving high yields of bioethanol. The use of local resources in Ghana for the pre-treatment of bioethanol feedstocks would enhance the feasibility of bioethanol production by reducing the cost of production. The present study was conducted to investigate the effectiveness of cocoa pod ash pretreatment of two important feedstocks in Ghana, wawa and sapele sawdust. Extractive-free milled sawdust samples, containing 25.1 and 27.1% lignin, respectively (dry weight basis), were treated with either 2% cocoa pod ash solution or 2% NaOH at 121°C for 60 min or at 25°C for 24 h. Controls were treated with water. Lignin was isolated from untreated and alkali-treated samples and characterized using Fourier Transform-Infrared (FT-IR) spectroscopy. Differences in spectra were recorded in the low wavenumber region as well as the fingerprint region. Treatment with 2% cocoa pod ash at 121°C completely altered the spectral characteristics of lignin indicating disruption of the structure of lignin contained in wawa and sapele. This was similar to results obtained following treatment with 2% NaOH at 121°C. At 25°C, however, cocoa pod ash treatment had little effect on lignin structure. The findings indicate that cocoa pod ash might be a suitable chemical pre-treatment for the production of bioethanol from sawdust. The use of cocoa pod ash, an inexpensive, abundant and readily available local resource, for the pre-treatment step would reduce cost and thereby enhance the profitability and feasibility of bioethanol production in Ghana.

Key words: Bioethanol, lignin, infra-red, wawa, sapele, sawdust, cocoa pod ash

INTRODUCTION

Owing to the high cost and non-renewable nature of fossil fuel, much attention is currently being paid to renewable energy sources (Akin-Osanaiye *et al.*, 2008; Saratale and Oh, 2012). Cellulosic ethanol production has become particularly attractive because waste lignocellulosic feedstocks are outside the human food chain (Sohel and Jack, 2011; Bailey *et al.*, 2011; Lemee *et al.*, 2012; Vancov *et al.*, 2012). Enzymatic hydrolysis of untreated lignocellulose produces extremely low yields of cellulosic mono- and disaccharides, the precursors for bioethanol production (Chen *et al.*, 2009; Sanderson, 2011). This is largely due to the presence of lignin, a polymer of phenyl propane-based units or monolignols which is highly resistant to enzymatic degradation. By forming close physical associations with cellulose and hemicellulose in plant cell walls, it protects them from degradation (Gibson, 2012).

Lignin is a highly complex macromolecule linked together by different types of bonds, including alkyl-aryl, alkyl-alkyl and aryl-aryl ether bonds (Kumar *et al.*, 2008). It obstructs enzymes from degrading associated structural carbohydrates (Taherzadeh and Karimi, 2008). Therefore, prior treatment of lignocellulosic feedstocks, to remove or denature lignin, is essential for achieving a high yield of fermentable sugars (Yang and Wyman, 2008; Carroll and Somerville, 2009). Plant by-products differ in monolignol constituents of lignins and lignin chemistry can vary depending on its origin. The characterization of lignin is critical to the optimization of pre-treatment parameters in the cellulosic ethanol production process.

Fourier Transform-Infrared (FT-IR) spectroscopy is a useful physico-chemical method for characterizing lignin. It is also a good method for monitoring the efficacy of delignification processes such as chemical treatments (Kotilainen *et al.*, 2000; Lisperguer *et al.*, 2009; Casas *et al.*, 2012). It measures the vibrations of bonds within chemical functional groups and generates a fingerprint spectrum of the sample.

Ghana's wood industry generates enormous amounts of wood shavings, potential feedstock while the agricultural sector discards huge quantities of cocoa pod husk, a source of alkali (Adamafio *et al.*, 2004). Studies on the characteristics of lignin contained in the most abundant wood shavings in Ghana, as well as the efficacy of local resource-based pre-treatment methods are critical to the establishment of a lignocellulosic bioethanol industry in the country. The present study was conducted to characterize lignin contained in wawa and sapele shavings using FT-IR and to investigate the efficacy of cocoa pod ash treatment in the denaturation of lignin.

MATERIALS AND METHODS

Wawa (*Triplochiton scleroxylon*) and sapele (*Entandrophragma cylindricum*) shavings were obtained locally between March and May 2011. Cocoa pod ash was generously provided by the Cocoa Research Institute of Ghana, Tafo. Sodium hydroxide was purchased from BDH Chemicals.

Alkaline treatment of wood dust and isolation of lignin: Extractive-free milled wawa and sapele samples, containing 25.1 and 27.1% lignin, respectively (dry weight basis), were treated with either 2% cocoa pod ash solution or 2% NaOH in a 1:10 solid:liquid ratio at 121°C for 60 min or at 25°C for 24 h. Controls were treated with water. Thereafter, the samples were neutralized with 2% HCl and washed till pH 7. Lignin was then isolated essentially as described by Phillips and Goss (1934) and stored in sealed vials at -20°C until analyzed.

Fourier transform infrared spectroscopy: Infrared spectra were acquired using a Nicolet Nexus FT-IR spectrometer with a smart endurance single bounce diamond ATR cell that made recording possible without the preparation of KBr pellets. Spectra over the 4000-400 cm^{-1} range were obtained by the co-addition of 512 scans with a resolution of 4 cm^{-1} .

RESULTS AND DISCUSSION

Figure 1 shows the infrared spectrum of lignin extracted from untreated wawa. The FTIR spectrum of a lignin sample gives insight into its structure (Casas *et al.*, 2012). A broad peak characteristic of stretching vibrations of hydroxyl (OH) groups in aliphatic structures and phenolic groups was seen around 3400 cm^{-1} . A weak band at 2900 cm^{-1} was attributed to CH stretching vibrations of aliphatic groups. Carbonyl group stretching was seen at 1650 cm^{-1} . The fingerprint region of the spectrum from 1200-1600 cm^{-1} was dominated by carbon-carbon, carbon-oxygen ether

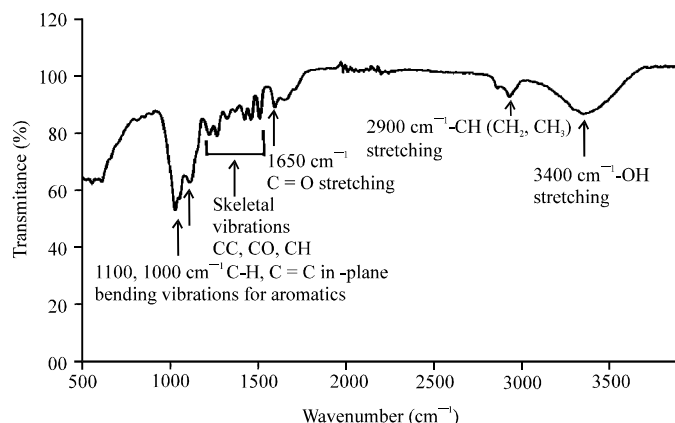


Fig. 1: Infrared spectrum of lignin contained in untreated wawa. Spectrum was acquired for neat solid samples of lignin extracted from wawa

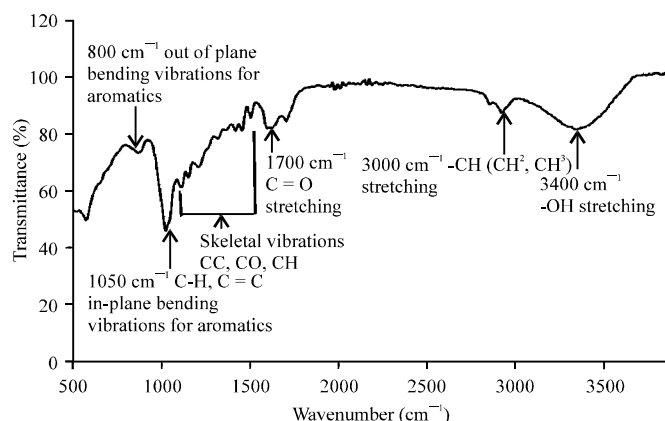


Fig. 2: Infrared spectrum of lignin contained in untreated sapele. Spectrum was acquired for neat solid samples of lignin extracted from sapele

bands and C-H skeletal vibrations (Malutan *et al.*, 2008). A band at 1067 cm^{-1} was attributed to carbon-oxygen deformation of aliphatic ethers and secondary alcohols. Guaiacyl absorbance might account for a band seen at 1275 cm^{-1} (Jahan *et al.*, 2012). It has been reported that guaiacyl type aromatic C-H in-plane deformation and C-O vibrations in primary alcohols account for the band at 1030 cm^{-1} (Mao *et al.*, 2012). The bands between 1000 and 1100 cm^{-1} were attributed to in-plane carbon-carbon aromatic skeleton vibrations. Wawa lignin clearly showed the two guaiacyl bands at 855 and 815 cm^{-1} which is characteristic of softwoods (Obst, 1982).

The spectrum for Sapele lignin which is presented in Fig. 2, was strikingly similar to that of wawa in the high wave number region between 1780 and 4000 cm^{-1} . As shown in Fig. 3, the FT-IR spectra of lignins from the two species clearly reflected differences between them in the fingerprint and low wavenumber regions. Bands at 1123 and 1330 cm^{-1} might be attributable to syringyl absorbances (Rodrigues *et al.*, 1998; Jahan *et al.*, 2012). A distinct out-of-plane bending vibration band was seen at 800 cm^{-1} for sapele lignin but was absent in the spectrum for wawa samples. These results underscore the differences in functional groups and skeletal structural features of lignin fractions from different wood species.

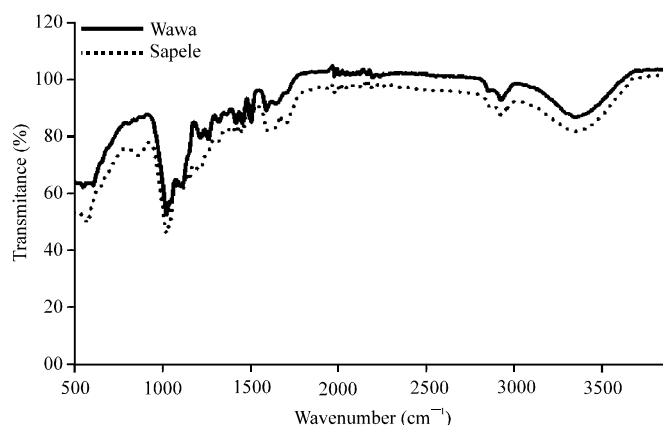


Fig. 3: Overlaid infrared spectra of lignin contained in untreated sapele and wawa samples. Spectra were acquired for neat solid samples of lignin extracted from sapele and wawa

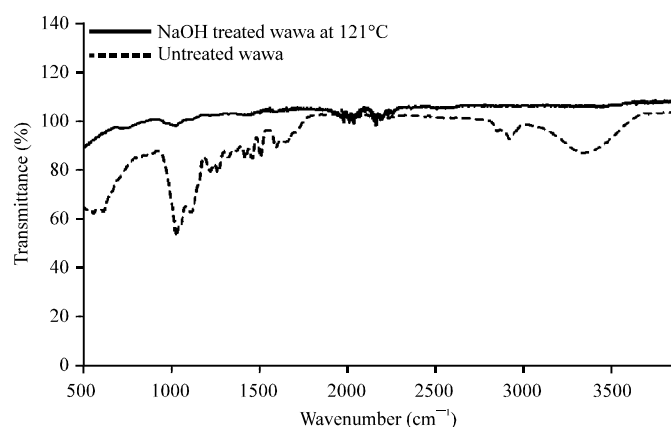


Fig. 4: Overlaid spectra for lignin from untreated and NaOH-treated wawa at 121°C

The F^T-IR spectra presented in Fig. 4 show that treatment of wawa samples with NaOH at an elevated temperature (121°C) completely disrupted the structural characteristics of lignin. This is consistent with the documented effects of NaOH on lignin fractions of other lignocellulosic materials (Lewis and Yamamoto, 1990; Gupta and Lee, 2010). Spectra of untreated, NaOH-treated and cocoa pod ash treated wawa at 121°C, presented in Fig. 5; show clearly the ability of cocoa pod ash to hydrolyse, to a large extent, lignin contained in wawa samples. The similarity in the chemical shifts caused by cocoa pod ash and NaOH gives an indication that both alkali species disrupted the structure of lignin in a similar fashion. The disruption in lignin structure is inextricably linked to the dissociation of lignin from structural polysaccharides (Chen and Dixon, 2007; Hendriks and Zeeman, 2009) and should increase the yield of fermentable sugars from wawa sawdust during cellulosic ethanol production (Hu *et al.*, 2008; Kumar *et al.*, 2009). Similar results (not shown) were obtained following cocoa pod ash-treatment of sapele samples, suggesting that cocoa pod ash is effective in modifying lignin contained in both softwood (Wawa) and medium-hardwood (Sapele). The present findings suggest that pre-treatment with cocoa pod ash should be an effective means of achieving delignification of sawdust for purposes of bioethanol production.

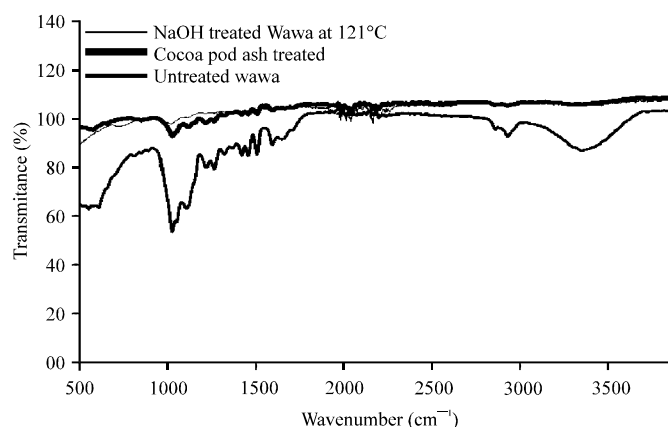


Fig. 5: Infrared spectra for lignin from untreated, NaOH-treated at 121°C and cocoa pod ash-treated wawa at 121°C

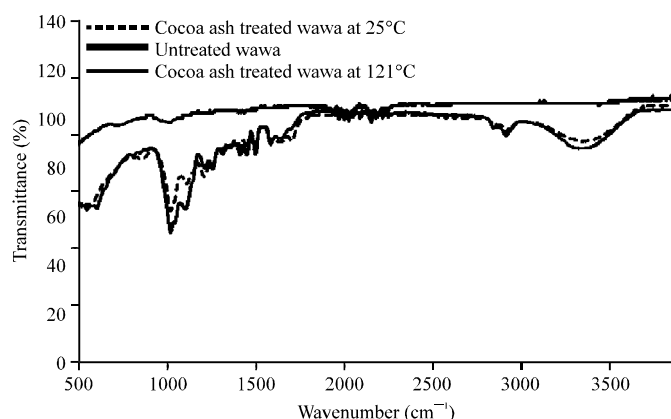


Fig. 6: Overlaid infrared spectra of untreated, Cocoa pod ash-treated at 25°C and cocoa pod ash-treated at 121°C wawa samples

The effect of temperature on the hydrolysis process is depicted by the overlaid spectra in Fig. 6. The findings show a minimal spectral shift following alkaline treatment at 25°C, indicating that, as has been observed with other sources of alkali, the degradation of lignin is temperature-dependent (Kindsigo and Kallas, 2006; Yang and Wyman, 2008).

CONCLUSION

In conclusion, the present findings provide clear evidence of the efficacy of cocoa pod ash pretreatment of wood dust for biofuel production. From an economic point of view, discarded cocoa pod husks are inexpensive, abundant and readily available for ashing. By avoiding the use of imported alkali, the cost of production is reduced, enhancing the feasibility and profitability of local production of cellulosic biofuel from wood shavings. The wood industry in Ghana generates enormous amounts of wood shavings that could serve this purpose.

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