Isolation and Characterization of Lignin from Alkaline Pretreatment Black Liquor of Oil Palm Empty Fruit Bunch and Sugarcane Bagasse

M. Adly Rahandi Lubis\(^a,\), Aniva Rizkia Dewi\(^b\), Lucky Risanto\(^a\), Lukmanul Hakim Zaini\(^a\), Euis Hermiati\(^a\)
\(^a\)Research Center for Biomaterial, Indonesian Institute of Science, Jl. Raya Bogor, Km.46, Cibinong, Bogor 16911, Indonesia
\(^b\)Bogor Agricultural University, Jl. Kumbang No. 14, Bogor, Indonesia

Abstract

Lignin is one of potential biopolymers that can be obtained from pretreatment of lignocellulosic materials. In this study, lignin was isolated from alkaline pretreatment black liquor of oil palm empty fruit bunch (OPEFB) and sugarcane bagasse (SB) by precipitation with hydrochloric acid. The isolated lignins were analyzed for their chemical properties, such as chemical compositions, UV absorbances, and FT-IR spectra. The results showed that the ash content of lignin from alkaline pretreatment of SB (16.91%) was lower than from alkaline pretreatment of OPEFB (19.19%), while the Acid Insoluble Lignin (AIL) and Acid Soluble Lignin (ASL) contents were vice versa, 46.23 and 4.00% for the former, and 40.14 and 2.82% for the latter. The empirical formula of the lignin isolates derived from the result of ultimate analysis were \(C_{9}H_{13.8764}O_{5.9318}\) and \(C_{9}H_{14.1112}O_{6.4321}\) for lignin from of OPEFB and SB, respectively. The UV spectra showed that lignin from OPEFB had similar bands with lignin from SB. Both lignin from of OPEFB and SB also had similar FT-IR spectra.

Key words: lignin, oil palm empty fruit bunch, sugarcane bagasse, alkaline pretreatment

I. INTRODUCTION

One of the most challenging topics in material science is to convert biomass waste and feedstock to highly added value-materials. Oil palm empty fruit bunch (OPEFB) and sugarcane bagasse (SB) was the most agro-industrial residues. In the process of extraction of palm oil from oil palm fruit, a lignocellulosic material OPEFB is generated as a waste product. Approximately 15 million tons of this agriculture waste is generated by oil palm milling operation annually and part of it is burned in incinerators [1]. In Indonesia, the national sugar cane production is 33 million tons/year and there are 58 sugar mills with a total milling capacity of 195,622 tons of cane per day [2]. The amount of bagasse produced in sugar cane processing is quite large, which is about 35-40% of the weight of cane with a water content of 48-52%, sugar 2.5 - 6%, and fiber 44-48%. High fiber content and availability of bagasse waste makes great use of bagasse as an alternative to chemical material like lignin is strategic and promising [3].

Together with cellulose, lignin belongs to the most abundantly occurring renewable resources. Lignin (Latin lignum = wood) is the second most common organic natural material and the real lignification substance. High material strength develops by integration of lignin in plant cell walls and association with cellulose fibrils by polyoses, whereby especially trees can stand upright and absorb static and dynamic forces. Lignin components are phenylpropane units with differing amounts of methoxy groups. The percentage of the basic units of coumaryl (H-), coniferyl (G-), and sinapyl (S-) alcohols varies very much depending on the botanical origin (figure 1) [4].

* Corresponding Author.Tel: +62-2187914511
E-mail: adlylubis89@gmail.com
Lignins are renewable and natural polymers. Five million of metric tons of lignins are produced in the world mostly as a non-commercialized waste product per year [5]. In pulp and paper industries, lignins are the main components of residual liquors, notably black liquor from the Kraft process. Their principal application is as fuel to produce energy [6]. The pulp and paper industry estimated that 50 million tons of lignin were extracted in 2010, but only 2% has been commercialized for the formulation of dispersants, adhesives, and surfactants or as antioxidants in plastics and rubbers. In this way, the challenge is then to explore the potential of this renewable resource, producing valuable functional molecules for chemistry [7].

The objective of this research was to characterize lignin obtained from black liquor of alkaline pretreatment of oil palm empty fruit bunch (OPEFB) and sugarcane bagasse (SB).

II. EXPERIMENTAL METHODS

A. Material preparation

OPEFB and bagasse feedstock used in the experiment was obtained from Sukabumi and Subang. Prior to processing, raw material was dried in the open (exposed to sunlight) for one week. The raw material then made into powder using hammer mill. The resulting powder was then filtered with a filter 40-60 mesh. Powder 40 mesh sieve and retained on the 60 mesh is further used as raw material delignification.

B. Isolation of lignin

Two hundred and fifty grams dry matter of sample was treated with 1% NaOH (solid to liquid ratio 1:15), at 170 °C for 1 h. The treatments were carried out in a digester. At the end of the reaction, the digester was cooled for 2 h (temperature below 70 °C). Furthermore, the delignified material was filtered to obtain the black liquor without any fibrous materials. After filtration, the solids are stored in plastic while the black liquor is inserted into the container (such as a bucket) as the insulating material lignin.

Hydrochloric acid (1 M HCl) was added in to the black liquor until reaching pH 2 in order to precipitate the acidified lignin. The precipitate lignin were then inserted into the freezer until frozen (±24 hours). Deposition of lignin that has been frozen removed from the freezer, then air dried at room temperature. The precipitate that had melted filtered using filter paper in a vacuum filter and washed with 250 mL of distilled water. The wet lignin was then inserted to the oven at 45 °C for 24 h. Lignin oven dried was then weighed and analyzed the chemical properties such as, lignin content (ASL and AIL), chemical composition, UV absorption and FT-IR.

C. Lignin Characterization

Ash content of lignin was determined gravimetrically. 0.5 grams of lignin put in porcelain cup. The sample is inserted in a muffle furnace at 525 ± 25 °C for 4 h. After that, put in a desiccator for 1 hour and weighed until constant weight.

Determination of lignin content is done by treating lignin and 72% sulfuric acid, and then stirred with a magnetic stirrer for 4 hours. It is intended that a solution of acid and lignin becomes homogeneous. Due to the color that is too dark blackish brown, then the solution was diluted to 4% by adding distilled water to 84 mL. Furthermore, the solution sterilized by autoclave at 121 °C for 1 h. Solution samples were sterile filtered to separate the precipitate (acid insoluble lignin (AIL)) and the filtrate (acid soluble lignin (ASL)). The precipitate is filtered and then dried in an oven at a temperature of 105 °C in order to analysis % AIL according to NREL LAP-003 [8]. While the resulting filtrate was measured by UV spectrophotometer with a wavelength of 205 nm, which would result in % ASL according to NREL LAP-004 [9].

Centrifuge bottles prepared, each weighed about 5 mg of lignin and inserted into the centrifuge bottle. After that, the mixture was added 5 mL of dioxane:water (9:1), diluted up to 50 times and absorbance was measured by UV-Vis spectrophotometry at wavelength range of 200-400 nm. The same treatment as much as 5 mg of lignin was added NaOH pH 12 by 5 mL, diluted up to 50 times and absorbance was measured with UV-Vis spectrophotometry at wavelength range of 200-400 nm.

Functional group analysis using FT-IR were performed in an Shimadzu IR Prestige 21 instrument by direct transmittance using potassium bromide (KBr) pellet technique. 5 mg of lignin was ground and impregnated in 200 mg
dried KBr. Each spectrum was recorded in the range from 4000 to 400 cm\(^{-1}\).

Total of 6 grams of isolated lignin was prepared to running elemental analysis. The content of carbon, hydrogen, nitrogen and sulfur were analyzed using a Thermo Finnigan model Eager 300 analyzer. The percentage of oxygen was calculated by subtracting 100% with the total number of C, H, N and S. The protein content was calculated as N(%) x 6.25. The average of double bond equivalent (DBE) was calculated based on the elemental composition, C\(_x\)H\(_y\)O\(_z\)S\(_d\) using formula as in Eq. 1 [10].

\[
DBE = C - \frac{H}{2} + \frac{N}{2} + 1
\]

### III. RESULT AND DISCUSSION

Ash Content of lignins isolated from alkaline pretreatment black liquor of OPEFB and SB are shown in table 1. The table shows that ash content in OPEFB lignin (19.19) is higher than SB lignin (16.91), high ash content of both lignin caused by dirty conditions of raw materials as well as methods of delignification and lignin isolation that produces high levels of impurities. The main reason for this is that the alkali lignins, isolated by traditional one step precipitation, that can present high silicate and nitrogen contents [7].

<table>
<thead>
<tr>
<th>Analysis Parameters</th>
<th>OPEFB Lignin</th>
<th>SB Lignin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ash Content (%)</td>
<td>19.19</td>
<td>16.91</td>
</tr>
<tr>
<td>Ultimate Analysis (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbon</td>
<td>38.65</td>
<td>41.25</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>5.05</td>
<td>5.30</td>
</tr>
<tr>
<td>Oxygen</td>
<td>36.83</td>
<td>36.25</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>0.28</td>
<td>0.24</td>
</tr>
<tr>
<td>Sulfur</td>
<td>0.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Protein</td>
<td>1.75</td>
<td>1.50</td>
</tr>
<tr>
<td>DBE</td>
<td>2.97</td>
<td>3.08</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AIL</td>
<td>40.14</td>
<td>46.23</td>
</tr>
<tr>
<td>ASL</td>
<td>2.82</td>
<td>4.00</td>
</tr>
</tbody>
</table>

Elemental analysis shows the composition of the carbon, hydrogen, oxygen, nitrogen, and sulfur in lignin based on a percentage of each atom. Both lignins had similar C, H, and O content (table 1). The similarity of the elemental composition may be due to same kind of resources as annual plant and same methods of pretreatment and lignin isolation. Nitrogen content has been detected for both lignin (<0.30 %). The presence of nitrogen content may be due to the formation of protein-lignin complexes during delignification process [11].

The protein residues attached to both lignin suggest a strong chemical bond between precipitated lignin and protein in non-woody plant such as OPEFB and SB. The protein are difficult to remove by acid precipitation and reveals that proteins linked to lignin in the starting material are still attached with lignin fragments during delignification [11].

Alkaline pretreatment is one of sulfur-free delignification process with organosolv process [7]. Sulfur content in the two lignin isolates were very low (≤0.05%), which might come from contamination during delignification and lignin isolation. Lignin has 9 carbon atoms, so the elemental analysis of this can be seen from the empirical formula of the isolated lignin. The percentage of carbon, hydrogen, and oxygen in the two lignin led to empirical formulas of C\(_9\)H\(_{13.876}\)O\(_{5.9318}\) and C\(_{6}\)H\(_{14.1112}\)O\(_{4.4321}\) for lignin from of OPEFB and SB, respectively.

The number of double bond equivalent (DBE) in both lignins implies the degree to which the lignins condenses and the presence of aromatic ring structure. It can be seen that the number of DBEs in the two lignin were almost same, 2.97 and 3.08 for OPEFB and SB lignin, respectively. Alkali lignin have fewer double bonds than the kraft lignin. This is due to the reaction between hydroxide and hydrosulfide with lignin, thus generating more double bonds in kraft lignin [12].

The FT-IR spectra of the two lignins are illustrated in figure 2. FT-IR spectra reflects the chemical structure as well as the purity of lignins [14]. The corresponding assignments and bands for the two lignins are presented in table 2. Both lignins show bands at 1605-1600 and 1515-1505 cm\(^{-1}\) which is corresponding to aromatic rig vibrations of phenyl-propane (C\(_6\)) skeleton. The presence of band at 1470-1460 cm\(^{-1}\), assigned to C-H deformation (asymmetric) in methyl, methylene, and methoxyl groups, confirm that both lignin aromatic structures did not change dramatically during delignification and isolation [15].

In the area from 3450 to 2820 cm\(^{-1}\), similar bands appeared for the two lignin. A wide absorption band that appeared at 3369 cm\(^{-1}\) for OPEFB and 3253 cm\(^{-1}\) for SB lignin can be attributed to phenolic OH and aliphatic OH groups. FT-IR spectra also showed characteristic vibrations of typical lignocellulosic materials groups C-H stretching (2928-2926 cm\(^{-1}\)), C=O stretching unconjugated (1713-1707 cm\(^{-1}\)), and C-C stretching of aromatic ring (1600 and 1506 cm\(^{-1}\)).

Bands at 1330-1325 cm\(^{-1}\) were attributed to syringil with C-O stretching. Bands at 1217 cm\(^{-1}\)
for OPEFB and 1223 cm\(^{-1}\) for SB lignin can be attributed to phenolic OH and ether in syringil and guaiacyl. The bands observed at 1030-620 cm\(^{-1}\) were attributed to hemicelluloses and silicates contribution [16].

Table 2

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3450-3400</td>
<td>O-H stretching (phenolic OH and aliphatic OH)</td>
<td>3369</td>
<td>3253</td>
</tr>
<tr>
<td>2940-2820</td>
<td>C-H stretching (CH(_3) and CH(_2))</td>
<td>2926</td>
<td>2928</td>
</tr>
<tr>
<td>1715-1710</td>
<td>C=O stretching (unconjugated ketone, carbonyl, and ester groups)</td>
<td>1707</td>
<td>1713</td>
</tr>
<tr>
<td>1605-1600</td>
<td>C-C stretching (aromatic ring)</td>
<td>1603</td>
<td>1599</td>
</tr>
<tr>
<td>1515-1505</td>
<td>C-C stretching (aromatic ring)</td>
<td>1506</td>
<td>1506</td>
</tr>
<tr>
<td>1470-1460</td>
<td>C-H deformation (asymmetric in -CH(_3) and -CH(_2))</td>
<td>1454</td>
<td>1450</td>
</tr>
<tr>
<td>1330-1325</td>
<td>C-O stretching (Syringil)</td>
<td>1325</td>
<td>1323</td>
</tr>
<tr>
<td>1220</td>
<td>C-O(H) + C-O(Ar) (phenolic OH and ether in Syringil and Guaiacyl)</td>
<td>1217</td>
<td>1223</td>
</tr>
<tr>
<td>1115</td>
<td>Ar-CH in plane deformation (Syringil)</td>
<td>1113</td>
<td>1120</td>
</tr>
<tr>
<td>1085-1030</td>
<td>C-O(H) + C-O(C) (first order aliphatic OH and ether)</td>
<td>1034</td>
<td>1039</td>
</tr>
</tbody>
</table>

Figure 2. FT-IR spectra of OPEFB and SB Lignins.

The most significant functional group in lignin structure is the free phenolic group because in most chemical reactions involving lignin, phenolic phenylpropane units are preferentially attacked [12]. UV Absorbance measurements on lignin based on the absorption of lignin conjugation. Lignin is a good UV absorber so that energy is transferred to be in the range of 200-240 nm to initiate the degradation process.

Both of lignin has similar UV absorbance in dioxane-water solution, with maximum absorption at 240 nm and weak absorption at 280 nm (figure 3). However, in alkaline medium (pH 12 NaOH) SB lignin showed higher absorbance than OPEFB lignin. In alkaline solution, the maximum absorption appears at 210 nm and weak absorption at 280 nm (figure 3). Absorption occurs in the elements of the network structure of chromophore in the lignin molecules. Chromophore elements in lignin will appear at different wavelengths, for example at the phenolic hydroxyl group at \(\lambda\) 220-240 nm, conjugated double bond at \(\lambda\) 200-240 nm, \(\alpha\) carbonyl group at \(\lambda\) 350-353 nm, and biphenyl at \(\lambda\) 489-493 nm. UV absorbance of lignin correlates with phenolic hydroxyl group in lignin. The more phenolic hydroxyl group, the higher the UV absorbance intensity [17].

IV. CONCLUSION

The lignin from alkaline pretreatment of OPEFB and SB has similar properties. Both contain high ash content, and low lignin content due to the alkaline pretreatment and one step precipitation. The percentage of carbon, hydrogen, and oxygen in the two lignin isolates led to empirical formula of \(\text{C}_9\text{H}_{13.876}\text{O}_{5.9318}\) and \(\text{C}_9\text{H}_{14.1112}\text{O}_{6.4321}\) for lignin from of OPEFB and SB, respectively. UV analysis showed that both lignin has a strong

Adopted from MEV Journal at www.mevjournal.com © 2012 RCEPM - LIPI All rights reserved
absorbance at 240 nm and weak absorbance at 280 nm in neutral conditions (dioxane water), and a strong absorbance at 210 nm and weak absorbance at 280 nm in alkaline conditions (NaOH pH 12). The more phenolic hydroxyl group, the higher the UV absorbance intensity. The FT-IR spectra of the two lignin isolates showed that the intensity of the bands in the spectra is similar. Further studies on lignin isolation method is needed to determine how to produce lignin with high grade and purity.

REFERENCES


