Analysis of Photodegraded Lignin on Cellulose Matrix by Means of FTIR Spectroscopy and High Pressure Size Exclusion Chromatography

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ABSTRACT

A mild acidic dioxane extraction procedure was used to isolate lignin from hardwood bleached chemimechanical pulp (BCMP). The isolated lignin was impregnated on Whatman filter paper and irradiated with ultraviolet (UV) light under various exposure times. Then, photodegraded lignin was recovered and characterized by FTIR spectroscopy and high pressure size exclusion chromatography (HPSEC). Loss of lignin aromatic content induced by syringyl and guaiacyl nuclei degradation, reduction in $C_{aryl-O}$ ether bond caused by demethoxylation and $\beta$-O-4 bond cleavages, and formation of carbonyl and/or carboxyl groups with slight increase in phenolic hydroxyl group, were some of structural changes observed by FTIR spectroscopy studies. Analysis of lignin samples with HPSEC indicated that formation of high molecular condensed structures by radical coupling during 4 h irradiation time, caused to increase lignin molecular weight. However, with continuing irradiation time of up to 120 h, the molecular weight of lignin decreased by 23% of its original value due to predominating photodegradation mechanisms. The results are discussed in relation to the reaction pathways leading to the breakdown of the lignin macromolecular structure.

INTRODUCTION

Lignin, a tri-dimensional phenolic polymer, is formed by a dehydrogenative polymerization of three phenylpropanoid precursors (Figure 1) including (1a) $p$-coumaryl alcohol, (1b) coniferyl alcohol, and (1c) sinapyl alcohol, in a process promoted in peroxidase-$H_2O_2$ system [1]. Lignin constitutes approximately 20% and 30% of hardwoods and softwoods (dry weight basis), respectively.

Lignin-containing papers made of these wood species by mechanical pulping processes tend to become yellow on exposure to light especially UV portion of solar radiation. This phenomenon limits the
utilization of the mechanical pulps to manufacture low quality products like newsprint papers. Photo-oxidative degradation of lignin seems to be the main reason of photo-yellowing of lignin-containing papers [2-4].

Several spectroscopic techniques have been used to assess the degradation of the lignocellulosic materials. Among them, FTIR spectroscopy is a very useful technique for monitoring and analyzing the structural changes of wood components due to different treatments such as natural ageing, artificial ageing, oxidation, and thermal degradation [5,6].

Colom et al. [5] applied FTIR spectroscopy technique to analyze the chemical modifications and structural changes in the cellulose and lignin of two different species as a result of photodegradation. They found that lignin is the most sensitive component towards degradation processes for both woods examined. Kimura et al. [7] studied the structural changes of unbleached and bleached stone-ground wood pulp, irradiated with UV light. Abdulkhani et al. studied the photostabilization of bagasse chemimechanical pulp by acetylation of phenolic hydroxyl groups. They also indicated that the reductive treatment has a little effect in preventing the photo-yellowing [8].

The depth of photodegradation of sugi earlywood induced by UV and visible light was profiled using FTIR microscopy. It was reported that violet light is the component of the visible spectrum that extends photodegradation into wood beyond the zone affected by UV irradiation [9]. Pandey employed FTIR spectroscopy to study the effect of photo-irradiation on the surface chemistry of wood. He reported that colour changes on wood correlate well with degradation of lignin and relative increase in the concentration of carbonyl groups [10].

High pressure size exclusion chromatography (HPSEC) analyses have also been used to determine molecular weight changes and distribution of reaction products formed during irradiation of milled wood lignin (MWL) in solution and in the solid state [11-13].

Hardwoods are usually used to produce high yield pulp under chemimechanical pulping (CMP) process in Iran. The photochemical behaviours of such pulps lignin have not been studied comprehensively yet. Hence, in this study, we made an effort to analyze the chemical changes of dioxane lignin isolated from hydrogen peroxide bleached hardwood CMP pulp applied on cellulose matrix, as a result of UV irradiation by means of FTIR spectroscopy and HPSEC. The advantage of the study of pure lignin is to clarify the photochemical changes occurring without any interference of two other main components of the pulps.

**EXPERIMENTAL**

**Materials**

All reagents and solvents were commercially purchased and used as received. A commercial hardwood BCMP bleached with hydrogen peroxide received from Mazandaran Pulp and Paper Ltd. was used in all experiments. The main properties of BCMP pulp are as follows: brightness: 71% ISO, yield: 83%, yellowness index: 27%, Canadian Standard Freeness (CSF): 400 mL. Whatman No. 1 filter paper was used as cellulose matrix.

**Methods**

Isolation of Lignin

Isolation of lignin from BCMP was carried out in accordance with Wang et al. [14] with slight changes in procedure. Prior to isolation of the lignin, the hardwood BCMP was Soxhlet-extracted with acetone for 24 h. The extracted pulp was then left to air-dry overnight. The dry pulp (75 g) was then refluxed in a 0.01 N HCl dioxane-water (1.5 L, 8.5:1.5) solution under argon atmosphere for 1 h. This pulp was filtered and washed with fresh dioxane (100 mL × 3). The
combined aliquots were neutralized with powdered NaHCO₃, filtered, and concentrated under reduced pressure at 40°C. The concentrated solution was added to 500 mL of a 0.006 N aqueous HCl and the precipitated lignin was twice washed with acidified water (pH 2-3) and then freeze-dried. The lyophilized lignin was subsequently dried under high vacuum at room temperature.

Filter Paper Impregnation
The BCMP lignin was dissolved in a 9:1 dioxane-water (3 mL) solution and applied homogeneously to both sides of Whatman filter paper using a syringe. The impregnated test-sheets were dried overnight under partial vacuum over P₂O₅. Approximately 21% (based on the weight of the sheet) lignin was impregnated on the filter paper. This amount simulates the lignin content of the hardwood BCMP pulp. The advantage of utilization of filter papers is to recover easily the photolyzed lignin samples without introducing any structural changes induced by other severe extracting methods. Consequently, all structural changes in BCMP lignin could be attributable to irradiation effects.

UV Irradiation of Lignin Treated Filter Papers
For accelerated exposure, lignin-impregnated sheets were placed in custom-made photo-reactor. The photo-reactor contains four black lights and equipped with two fans to keep the temperature at 30°C during irradiation. The black lights have spectral distribution between 300 and 400 nm and maximum output at ~350 nm. The amount of UV energy that was emitted from this setup was determined to be 155 J/cm². The impregnated sheets were photolyzed for periods of 0.5, 1, 2, 4, 24, and 120 h. Each side of the paper was photolyzed for half of the desired irradiation time.

Extraction of the Photolyzed Lignin
The photolyzed filter papers were wrapped to fit into test tubes and were extracted with 9:1 dioxane-water mixture (25 mL × 4). The extract was filtered to exclude any cellulose fibres from filter papers and then concentrated under reduced pressure at 40°C. The photolyzed lignin samples were freeze-dried and then further dried using higher vacuum at room temperature. This procedure yielded 80-92% lignin content of test sheets.

FTIR Spectroscopy
FTIR Spectra were obtained utilizing a Bruker Equinox 55 spectrometer. Lignin samples were dispersed in a matrix of KBr, and then compressed to form pellets. The resolution of 2 cm⁻¹ and 16 scans were acquired.

The band at 1032 cm⁻¹, assigned to C-O stretching, was used to normalize the obtained spectra. It is very important to use a spectral band that does not change during the course of treatment, if quantitative comparisons are to be performed. The identification of a reference spectral band that remains completely invariable throughout the whole experiment is difficult. The selected band (C-O deformation associated with primary alcohols in lignin) is one of the bands that remains almost changeless during the treatment [5]. However, the obtained quantitative values by this method, only used qualitatively in discussion of the results and have not been considered as absolute values.

Acetylation of Lignin
According to Lundquist [15], acetylation of lignin samples before HPSEC analysis (100 mg) was conducted with 2 mL of acetic anhydride-pyridine (1:1, v/v) at room temperature overnight in a 50 mL flask. Ethanol (25 mL) was added, and after 30 min, the solvents were removed by evaporation under reduced pressure at 40°C. Addition and removal of ethanol were repeated more than ten times to result in a complete removal of acetic acid and pyridine from the sample.

HPSEC Analysis
The HPSEC analysis of acetylated lignin was performed with Agilent 1100 at room temperature using a set of three PLGEL columns (102, 103, and 104) connected in series which covered the molecular weight range from 500 to 100000. A high sensitivity refractive index (RI) detector was used. Tetrahydrofuran (THF) was used as eluent at a flow rate of 1 mL/min. The injection volume of the lignin-THF solution was 20 μL for all samples. Molecular weight determinations were made using polystyrene as a calibration standard.
RESULTS AND DISCUSSION

Spectral Characterization

Figure 2 shows the FTIR spectra of dioxane lignin isolated from hardwood bleached CMP, before and after 4 h irradiation in the region between 1800 and 800 cm$^{-1}$. In general, no clear differences detected in shapes of bands except a slight shift in the position of some bands. But the absorbance of the lignin samples changed considerably after irradiation. The most representative bands studied within this spectral range are summarized in Table 1.

The bands at 1505 cm$^{-1}$ and 1595 cm$^{-1}$ are assigned to aromatic skeletal vibration. The intensity of these bands, as observed in Figure 3, decreases considerably with proceeding irradiation time indicating degradation of lignin polymer structure and pronounced loss of lignin aromatic content. The main photodegradation process of phenolic structural elements of lignin is demethoxylation of the guaiacyl and syringyl rings; however, simple phenols might be photo-oxidized into quinones and muconic acids [18]. The photo-oxidative conversion of aromatic to non-aromatic structure could also contribute to the drop in these bands intensity. However, Kimura et al. [19] with monitoring the change in height of the few bands at 1505, 1267, and 1235 cm$^{-1}$ against irradiation time proposed that the destruction of the guaiacyl structure is mainly attributable to ring cleavage and not for example to demethoxylation.

Guaiacyl nuclei (1505 cm$^{-1}$) during 4 h and 120 h irradiation of lignin samples were reduced about 17% and 55% of its original value respectively, while syringyl nuclei (1595 cm$^{-1}$) decreased 10% and 37%, respectively. This result is consistent with other researchers’ findings [3,7,20] that guaiacyl nuclei are more sensitive to the artificial ageing degradation process than syringyl nuclei.

The increase of C=O stretch absorption at 1737 cm$^{-1}$ after UV irradiation (Figure 4) is attributed to the formation of carbonyl and/or carboxyl groups. Agarwal et al. [21] have noted similar trends when using FTIR spectroscopy to characterize photo-yellowed thermomechanical pulps (TMPs). These carboxyl groups are thought to be mostly of vanillic acid origin, forming via C$_\alpha$-C$_\beta$ bond scissions. It seems that during photo-exposure of lignin, C$_\alpha$-C$_\beta$ scission results in the formation of benzyl alcohols that are subsequently oxidized to aldehydes and then acids in the presence of oxygen [22-24]. In addition, syringaldehyde and its corresponding syringic

Table 1. Characteristic bands of FTIR spectra of BCMP dioxane lignin [6,16,17].

<table>
<thead>
<tr>
<th>Wavenumber (cm$^{-1}$)</th>
<th>Assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1226</td>
<td>C-O stretching of syringyl</td>
</tr>
<tr>
<td>1328</td>
<td>Syringyl/guaiacyl ring breathing with C-O stretching</td>
</tr>
<tr>
<td>1462</td>
<td>Asymmetric bending in CH$_3$</td>
</tr>
<tr>
<td>1505</td>
<td>Aromatic skeletal vibration</td>
</tr>
<tr>
<td>1595</td>
<td>Aromatic skeletal vibration</td>
</tr>
<tr>
<td>1737</td>
<td>C=O stretching of carbonyl or carboxylic acid</td>
</tr>
<tr>
<td>3445</td>
<td>O-H stretching</td>
</tr>
</tbody>
</table>

Figure 2. FTIR Spectra of dioxane lignin isolated from bleached CMP before irradiation and after 4 h UV irradiation.

Figure 3. Loss of aromatic content of BCMP lignin as a function of irradiation time.
Acid are two oxidation products of hardwood lignin [25], which can contribute to absorbance at 1737 cm⁻¹.

Ether bonds are relatively abundant and important in lignin macromolecule and their dipole moment gives relatively intense IR bands. Methoxyl groups and β-O-4 interunit bonds are two main aryl-alkyl ether linkages in lignin. Each linkage contains Caryl-O bond. Coiler et al. [16] have assigned Caryl-O vibrations to 1262-1224 cm⁻¹ region, except syringyl derivatives which Caryl-O vibrations appear at 1328-1295 cm⁻¹ and 1254-1204 cm⁻¹. Faix [26] also has assigned the bands at 1330-1325, 1275 and 1235-1230 cm⁻¹ to guaiacyl and/or syringyl ring breathing with C-O stretching.

However, there is another evidence that Caryl-O ether bond is the main contributor of mentioned bands. For example, Haw et al. [27] observed a decrease in 1225 cm⁻¹ band relative to 1510 cm⁻¹ band during cleavage of aryl-alkyl ethers by low-temperature pyrolysis.

Therefore, as shown in Figure 5, we used the relative intense changes at 1326 cm⁻¹ and 1226 cm⁻¹ to monitor Caryl-O ether bond during irradiation. In addition, the band at 1462 cm⁻¹ assigned to C-H deformation of methyl and methylene groups was used as relative criterion of methoxyl group changes in lignin. Decreasing the intensity of these bands with increasing irradiation time as shown in Figure 5, indicates that demethoxylation reactions and cleavage of Caryl-O ether bonds occur as a result of lignin photodegradation.

During UV irradiation of hydrogen peroxide bleached CMP lignin, the absorbance of the OH group vibrations at 3445 cm⁻¹ increase slightly as the irradiation time is increased to 120 h, as shown in Figure 6. This result is consistent with the small increase in phenolic group content in lignin during photodegradation. The irradiation of lignin caused to scission of
\(\beta\)-O-4 resulted in the formation of free guaiacyl and syringyl phenolic units. Some of the quenching of these phenoxy free radicals proceed via hydrogen abstraction yielding new phenolic units [12,22]. The relatively small increase in phenolic group content is due to photo-oxidation of most phenolic groups formed during photolysis.

**Molecular Weight Measurements**

To supplement further our knowledge on the molecular changes induced on dioxane lignin during irradiation, the molecular weights of original lignin and photolyzed samples were measured.

The chromatogram curves in Figure 7 show that irradiation of dioxane lignin isolated from bleached CMP, formed both high and low molecular weight fractions. Formation of the light-induced condensation products is attributed to the coupling of phenyl propane units in the lignin macromolecule. Evidence of this coupling has been found by analysis of the products of oxidative degradation of lignin-rich pulp irradiated with UV light [24].

The term condensed structures, refers to lignin phenolic units that bear substituents at the C₅ carbon of the phenyl propane units, i.e. 5-5', \(\beta\)-5, 4-O-5. Formation of the C₅-related condensed phenolic units like phenicoumaran (\(\beta\)-5) has been demonstrated during irradiation of milled wood lignin of bleached thermomechanical pulp (BTMP) [22] and lignin model compounds studies [28]. It seems that syringyl units, due to limited possibilities of other kinds of condensation, frequently form \(\beta\)-\(\beta\) bonds.

There are two proposed reaction pathways leading to breakdown of the macromolecular structure of lignin. One is the cleavage of the phencyl-O-4-aryl ether bond of the singlet and triplet excited states of aromatic carbonyl groups [29,30], and the other is abstraction of benzylic hydrogen from a guaiacyl-glycerol \(\beta\)-O-4 guaiacyl ether group to produce ketyl and phenoxy free radicals [31]. The products of both of these reaction pathways are phenols and the guaiacyl propyl residue. It is obvious that syringyl propyl residue also constitutes portion of photo-degraded products of hardwood lignin. The phenoxy free radicals resulting from \(\beta\)-O-4 bond cleavage are prime candidates for the formation of radical coupling and rearrangement products. Further fragmentation of lignin may also be caused by C\(\alpha\)-C\(\beta\) bond cleavage as reported in our work.

According to Table 2, molecular weight of dioxane lignin isolated from hardwood bleached CMP changes differently during various irradiation times. Average molecular weight (\(\bar{M}_w\)) of lignin after 4 h irradiation, increased by 27% of its original value, while upon irradiation time up to 120 h, a reduction of 23% in molecular weight was observed. Molecular weight enhancement, during 4 h short time irradiation is probably due to the high rates of radical coupling reactions leading to formation of higher molecular weight of C₅-related condensed structures such as 5-5', 4-O-5, \(\beta\)-5 products than reactions leading to degradation. However, during long term irradiation

| Table 2. The effect of UV irradiation on \(\bar{M}_w\) and \(\bar{M}_n\) of dioxane lignin isolated from peroxide bleached CMP. |
|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | 0 h             | 4 h             | 24 h            | 120 h           |
| \(\bar{M}_w\)   | 6631            | 5120            | 8424            | 4820            |
| \(\bar{M}_n\)   | 5120            | 94              | 127             | 3628            |

**Figure 7.** Molecular weight distribution of dioxane lignin isolated from bleached CMP, before and after UV irradiation.
like 120 h, photochemical reaction rates leading to lignin degradation predominate.

β-O-4 Ether linkages constitute approximately 60% of the total phenylpropane units in hardwood lignin [1,32]. Therefore, cleavage of these linkages due to photooxidative degradation of lignin causes formation of phenoxy free radicals [31], oxidation of which leads to the formation of coloured quinones [4]. Presence of the quinones even in low concentration, gives yellow colour to papers. It is expected that lignin-rich papers lose considerably their brightness upon increasing irradiation time and formation of more intermediate phenoxy free radicals. Thus, the results confirm the dependency of observed molecular degradation and the formation of photo-yellowing precursors.

CONCLUSION

The results clearly demonstrate that UV irradiation with $\lambda_{\text{max}} = 350 \text{ nm}$ under ambient condition, caused photooxidative degradation of dioxane lignin isolated from peroxide bleached hardwood CMP. The photo-degradation exhibits itself as the loss in aromatic content of lignin, decreasing $C_{\text{aryl}}$-O ether bonds resulting from demethoxylation reactions and β-O-4 bond cleavage, increasing carbonyl and/or carboxyl groups during irradiation.

Molecular weight measurement and its distribution of lignin isolated from hardwood bleached CMP indicated that the rate of radical coupling reactions leading to formation of higher molecular condensed structures, during 4 h short time irradiation is far more than 120 h long term irradiation. In addition, pronounced loss of lignin molecular weight at long term irradiation implies severe degradation of lignin macromolecular structure via β-O-4 bond cleavage and consequently is related to formation of coloured products such as quinones from oxidation of resulting intermediate phenoxy free radicals.

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REFERENCES


