Catalytic hydrolytic cleavage and oxy-cleavage of lignin linkages

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ABSTRACT

In this work, new strategies involving organic bases were evaluated to depolymerize lignin to reduced molecular fragments in aqueous medium. NaOH as an inorganic base was also investigated as a reference. Full nature lignin samples were used for the study. As research tools to unravel the complexity of the macro lignin structure and bulky molecular size under this study, size exclusion chromatography and high resolution mass spectrometric analysis, typically used for protein characterizations, were used to follow the progress of lignin depolymerisation by measuring the molecular weight distribution of the products and determining the key molecular mass fingerprints, respectively. The results show that sodium phenoxide and guanidine carbonate are effective catalysts for lignin depolymerization. It is observed that the organic bases enhance the oxy-cleavage effect of H$_2$O$_2$, which is strongest with guanidine carbonate.

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1. Introduction

Plant biomass is an important source of feedstock for the production of renewable energy. This class of biomass can be fractionated into major components like cellulose, hemicelluloses and lignin [1]. Lignin is a complex and high molecular weight polymer, which is formed via photosynthetic pathways involving de-hydrogenation of p-hydroxy-cinnamyl alcohols such as p-coumaryl (I), coniferyl (II), and sinapyl (III) alcohols [2]. Because a typical lignin structure has nearly half the oxygen content as that in cellulose and hemicellulose, the heat value of lignin is over 50% higher than that of cellulose and hemicellulose on equivalent weight basis. Therefore, 30 wt.\% lignin in a typical softwood biomass has nearly equivalent energy as 45 wt.\% cellulose [3]. However, research in lignin conversions has received considerably less attention than that in cellulose conversions even though the energy content of lignin is much higher than that of cellulose [4]. This shortage of research activity in lignin conversions may be partially due to the complexity of lignin structures that make it considerably challenging to follow the progress of a depolymerisation process as well as to obtain unambiguous molecular information from conventional research tools. Recently, some research reports appeared in converting lignin into small molecules in water or other solvents by base catalyzed depolymerisation [5,6]. However, selective bond cleavage remains a major challenge for converting lignin into value-added chemicals. Thermochemical methods for lignin depolymerization and conversion into lower molecular weight components have been reviewed [7].

The relative abundance of lignin and its relatively high resistance to microbial degradation during early diagenesis left it selectively preserved and concentrated in some sedimentary deposits. The macromolecular structures of lignin have been extensively studied in the past, but most analysis was made on lignin fragments following pyrolystic degradation of lignin. Because severe conditions were applied prior to the analysis (mostly by NMR), multiple chemical transformations during pyrolysis can be expected that make the structural information less reliable. It is generally accepted that lignin is mainly composed of phenylpropane monomeric structural units that link together primarily through the C–O linkage of α and β-ether bonds [8].

Because β-O-4 linkage is a dominant bond connecting phenylpropyl based molecular building units in lignin structure, efficiently breaking the β–O–4 linkage is envisioned a potential strategy for degradation of lignin. Acid and base catalysts have been found effective in lignin depolymerisation [5,6]. Metal chlorides in certain ionic liquids have also been found to be effective catalysts for cleaving β–O-4 linkage [9]. Another strategy for depolymerization of lignin is oxidative cracking reaction because the presence of hydroxyl group in lignin. Alkaline oxidation of softwood lignin can produce vanillin, which is a low-molecular-weight chemical [10,11]. Hydrogen peroxide is a very weak acid and can be used to degrade and solubilize lignin. Fenton’s reagent (mixture of hydrogen peroxide with ferrous sulfate) is also highly effective in depolymerization of lignin by oxidation [12].
In this work we focus on the performance of organic base catalysts, such as alkali phenoxy and guanidine carbonate, for lignin hydrolytic cleavage as well as for lignin oxy-cleavage in the presence of hydrogen peroxide. These catalysts, together with process condition variation, would allow the cleavage at controlled mild conditions without affecting the structural integrity in untargetted part of the lignin. As research tools to unravel the complexity of the macro lignin structure and bulky molecular size under this study, size exclusion chromatography and high resolution mass spectrometric analysis, typically used for protein characterizations, were used to follow the progress of lignin depolymerisation by measuring the molecular weight distribution of the products and determining the key molecular mass fingerprints, respectively.

2. Experimental

2.1. Materials

Guanidine carbonate (Gu$_2$CO$_3$), sodium hydroxide and sodium phenoxide trihydrate (PhONa), hydrogen peroxide (H$_2$O$_2$), hard wood lignin (HW), and lignin (alkali, carboxylated), were purchased from Sigma–Aldrich. The alkali lignin was acidified and washed before use for hydrolysis. 10% of lignin in water (pH 9.3) was titrated by 6M HCl until precipitation occurred at pH < 3.0. The acidified lignin was filtered and washed with water (pH 2.0), then dried at 37°C.

2.2. Depolymerization of lignin

Acidified lignin and hard wood lignin (HW lignin) were dissolved into water for 1% (w/v) solutions and were put into autoclave and subsequently heated at 100°C for 4h. The depolymerization of lignin in presence of the base catalyst, or together with the presence of hydrogen peroxide, was carried out in same conditions.

2.3. Analytical thin layer chromatography

The hydrolyzed products after autoclave were dried and dissolved in 1:1 water and ethanol solution and analytical thin layer chromatography (TLC) was performed using silica gel with methanol for elution.

2.4. Size exclusion chromatography

A low-pressure liquid chromatography system was employed that used Sephadex G100 (Sigma–Aldrich) packed in a Kontes FlexColumn (Fisher Scientific). The Sephadex gel was swollen in liquid phase at 25°C for 24h before packing and was then equilibrated with 50mM NH$_4$HCO$_3$ (pH 7.8). The column was calibrated with 2',3'-O-(2,4,6-trinitrophenyl) adenosine 5'-triphosphate (TNP-ATP, molecular weight 790Da) and 2',3'-O-(2,4,6-trinitrophenyl) adenosine 5'-monophosphate (TNP-AMP, molecular weight 579). Acidified lignin and HW lignin (0.5ml) after autoclave were filtered and loaded on the column for elution with 50mM NH$_4$HCO$_3$ (pH 7.8). The eluent was monitored at 280 nm.

2.5. Mass spectrometry analysis of lignin hydrolysis

Electro-Spray Ionization Mass Spectrometry (ESI-MS) was used to measure the model lignin compound, sinapyl alcohol, and hydrolyzed lignin [13,14].

Fig. 1. Elution profile of unconverted lignin, in reference to (a) elution point of TNP-ATP (M$_r$ = 790) and (b) elution point of TNP-AMP (M$_r$ = 579).

3. Results and discussion

3.1. Catalytic cleavage of model lignin by alkaline base and organic base

Research into the depolymerization of model acidified lignin was focused on catalytic cleavage by alkaline base and organic base. The effectiveness of strong organic bases, sodium phenox-ide (PhONa) and guanidine carbonate (Gu$_2$CO$_3$) are selected in this study. Size exclusion chromatography (SEC) shows that the model lignin has a sharp peak with retention time at 14 min with average molecular weight about 175 kDa (Fig. 1). In the present work, 1% of acidified lignin was subjected to autoclave treatment at 100°C for 4h in the presence of NaOH, PhONa and Gu$_2$CO$_3$ and the products were analyzed by SEC. The elution spectra of SEC are depicted in Fig. 2. Striking differences in elution profiles were observed that indicate significant changes in molecular weight distribution resulted from the different efficiencies of the catalysts. The elution profiles are significantly shifted toward lower-molecular-weight components when NaOH (B in Fig. 2), PhONa (C in Fig. 2) and Gu$_2$CO$_3$ (D in Fig. 2) were used as catalysts, indicating the chemical components in original lignin sample were fragmented to different degrees depending on the nature of base catalysts. The dominant distribution of molecular weight is ranged about 600–800Da as indicated by calibration markers a and b in Figs. 1 and 2.

The conversions of lignin in presence of the selected bases as catalyst after autoclave were compared with lignin in water (pH 5.0) (Table 2) and the hydrolyzed media at high pH is beneficial for cleavage of lignin. At the same initial pH (pH 11.5) the ability for lignin hydrolysis follows the order of PhONa ~Gu$_2$CO$_3$ > NaOH (Table 2). It has been reported that there exists competition between lignin depolymerization and repolymerization when aspen wood and isolated lignin from aspen were subjected to steam explosion treatment in acetic acid media, but addition of reative phenol and 2-naphthol was shown to inhibit the repolymerization reaction strongly [15]. Therefore, the increased cleavage products and the stability of hydrolyzed lignin due to the presence of organic base in this work may have provided dual functions: (1) catalyzed hydrolytic cleavage of lignin oxygen linkages by the base and (2) stabilized the lignin fragments by the organic base group that also functions as radical scavengers.

In the literature, the fact that controversial results were reported for lignin depolymerisation illustrates the complexity of such chemistry occurred to lignin, which is limited by the lack of reliable analytical tools and mechanistic understanding. For example, treatment of lignin with hydrogen peroxide in the presence of peroxidise suggests that peroxideases catalyzed
Guanidine acidified and presence of guanidine carbonate. References: (a) elution point of TNP-ATP (M₄ = 790) and (b) elution point of TNP-AMP (M₄ = 579).

Table 1
Depolymerization of acidified lignin in the presence of organic base catalysts (1%).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>H₂O₂ (%)</th>
<th>pH before hydrolysis</th>
<th>pH after hydrolysis</th>
<th>Conversion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>-</td>
<td>5.0</td>
<td>4.0</td>
<td>41</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>4.1</td>
<td>42</td>
</tr>
<tr>
<td>NaOH</td>
<td>-</td>
<td>11.5</td>
<td>4.8</td>
<td>57</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>11.5</td>
<td>4.0</td>
<td>69</td>
</tr>
<tr>
<td>Sodium phenoxide</td>
<td>-</td>
<td>11.5</td>
<td>9.7</td>
<td>89</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>7.3</td>
<td>89</td>
</tr>
<tr>
<td>Guanidine carbonate</td>
<td>-</td>
<td>11.5</td>
<td>9.6</td>
<td>71</td>
</tr>
<tr>
<td>1</td>
<td></td>
<td></td>
<td>8.7</td>
<td>88</td>
</tr>
</tbody>
</table>

H₂O₂ enhanced lignin depolymerisation, while a very significant drop for the NaOH system was observed, in which the pH was dropped from 11.5 to an acidic level (Table 1). In comparison, for the organic base catalyzed systems, the drop in pH was moderate while the lignin depolymerisation was much higher. There was also a drop in the pH as compared to that in the absence of H₂O₂; the end pH remained about the neutral level. The decrease of the intense SEC peak at lower retention time and the increase the profiles at the higher retention time in C and D of Fig. 2 indicates the reduction of heavier lignin polymer concentration together with the formation of smaller lignin molecules. Interestingly, the end pH (8.7) of the Gu₂CO₃ catalyst system was higher than that (7.3) of the PhONa catalyst system, at similarly high lignin conversion level. It can therefore be concluded that using H₂O₂ without an effective catalyst does not induce appreciable lignin depolymerisation. While the organic bases showed strong effect for catalytic hydrolytic cleavage of lignin, addition of H₂O₂ further enhances the depolymerisation. There appears a synergy between

Table 2
Difference in pH between unhydrolyzed and hydrolyzed hard wood lignin in different reaction media (1% catalyst).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Reagent</th>
<th>pH before conversion</th>
<th>pH after conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>-</td>
<td>n.a.</td>
<td>5.5</td>
</tr>
<tr>
<td>Sodium phenoxide</td>
<td>1% H₂O₂</td>
<td>n.a.</td>
<td>3.3</td>
</tr>
<tr>
<td>Guanidine carbonate</td>
<td>1% H₂O₂</td>
<td>11.1</td>
<td>9.8</td>
</tr>
<tr>
<td></td>
<td>1% H₂O₂</td>
<td>11.1</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>1% H₂O₂</td>
<td>11.3</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>1% H₂O₂</td>
<td>11.3</td>
<td>7.8</td>
</tr>
</tbody>
</table>

* Not measured.
Fig. 3. Elution profile of hard wood lignin in presence of base catalysts with or without H₂O₂ after autoclave at 100 °C for 4 h. (A) Water. (B) 0.005 M NaOH. (C) 1% Sodium phenoxide. (D) 1% Guanidine carbonate. (a) Elution point of TNP-ATP (Mᵣ = 790). (b) Elution point of TNP-AMP (Mᵣ = 579). (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

Fig. 4. Mass spectrometry analysis of model lignin hydrolysis products near references a and b. Refer to Fig. 5 for assignment of the key mass numbers.
the organic bases and the H₂O₂ for the depolymerisation of the lignin, as indicated by the treatments illustrated in C and D of Fig. 2.

3.2. Alkaline catalytic cleavage of hard wood lignin

HW lignin is a less water-soluble product in low pH (<7.0) as compared with the model lignin. SEC shows that only the fragment with molecular weight small enough is able to elute with the elution buffer for the autoclave-treated HW lignin. Neither molecular weight distribution nor the solubility of HW lignin was changed in presence of H₂O₂ (sample A in Fig. 3). The low peak intensity in the SEC of the sample A is attributed to a small soluble fraction of the HW lignin after the treatment in autoclave. The two peaks observed from hydrolysis of the HW lignin in a 0.02% NaOH solution correspond to fragment of larger and lower-molecular-weight components, respectively (blue trace in B, Fig. 3). The total soluble fraction of the HW lignin after this treatment remains small as indicated by the low peak intensities. The addition of H₂O₂ is not able to change the distribution of HW lignin in the NaOH medium (red trace in B, Fig. 3). Remarkably, using PhONa as catalyst resulted in significant increase of the peaks in the elution profile (sample C, Fig. 3), indicating that PhONa effectively catalyzed the cleavage of the HW lignin to reduced molecular sizes that became soluble in water. The relative distribution of lower-molecular-weight products from cleavage of the HW lignin was not markedly changed in the presence of H₂O₂ (C in Fig. 3). The presence of Gu₂CO₃ catalyst was also found to significantly enhance the cleavage of HW lignin and the solubility of the cleaved products (D, Fig. 3). Remarkably, the molecular weight distribution was dominantly shifted toward smaller molecules when H₂O₂ is also present. The pH values are compared for unhydrolyzed and hydrolyzed HW lignin in different reaction media (Table 2). Gu₂CO₃ is a better catalyst to keep the medium at higher pH during hydrolysis in presence of H₂O₂, an observation consistent with that for the model lignin depolymerisation as shown in Table 1. The results therefore strongly suggest that (1) the organic bases are effective lignin hydrolytic cleavage catalysts for the HW lignin and (2) there is a stronger synergism for the system containing both Gu₂CO₃ and H₂O₂. It is particularly important to note that the high pHs were better maintained in the organic bases in the absence of H₂O₂. The presence of H₂O₂ resulted in an accelerated drop in the pH, which may be rationalized on the basis that oxidative cleavage took place, leading to the formation of carboxylic acids in the formed lignin fragments. The mechanism involved by the organic bases, particularly the observed synergism between the organic base and H₂O₂ is a subject for continued future study.

3.3. Mass spectrometry analysis of hydrolytic products of model lignin

It is generally accepted that an ideal lignin is mainly composed of oxygen functionalized phenylpropane monomers that are linked together primarily through the C-O linkage of α and β-ether bonds. However, deviations from this ideal unit have been frequently reported. Generally, the cleavage of β-O-4′ linkage is the most important depolymerization reaction in lignin. In the present study, mass spectrometry method is used to understand the cleavage of model lignin after hydrolysis. Due to the high complexity of the large number of products, it is not practical to determine all the products after the organic base catalyzed cleavage. For the purpose of gaining preliminary understanding of the pathways involved in the cleavage, the molecular mass distribution for a fraction with predominant molecular mass at reference elution points a and b as shown in Figs. 1 and 2 was measured by mass spectrometry analysis. Fig. 4 depicts fragmentation of hydrolysed lignin cation

![Mass Spectra of Lignin Fragmentation](image-url)
and fragmentation pathway of selected lignin hydrolysis product. Possible structures are reconstructed and multiple isomers may exist for the ions studied (Fig. 5). The result from mass spectrometric measurement is consistent with molecular weight distribution from SEC, e.g. base as catalyst efficiently cleaves the C–O linkage of α and β-ether bonds and results in predominant molecular mass around 600 Da. It is noted that the mass spectra resolved structures in Fig. 5 indicate non-ideal phenylpropane basic units, which further confirms the complexity of the lignin.

4. Conclusion

Both sodium phenoxide and guanidine carbonate are shown to be highly effective catalysts for the cleavage and dissolution of acidified lignin and hardwood lignin. Such catalysts also promote depolymerization of lignin by hydrogen peroxide by showing synergism between H₂O₂ and the organic bases. Guanidine carbonate appears to be a more efficient catalyst than sodium phenoxide for lignin depolymerisation. The synergism between Gu₂CO₃ and H₂O₂ is strongest among the base catalyst studied. The presence of H₂O₂ causes a larger drop in pH, possibly due to oxo-cleavage of some lignin o-linkages. Size exclusion chromatography marker and high resolution mass spectrometric measurement are consistent in showing predominant molecular weight distribution at 600 Da for hydrolyzed lignin under the current experimental conditions.

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