Development of Chemical Methods for the Degradation of Lignin
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Joseph McKillip, Dr. Raja Annamalai
University of Wisconsin-Platteville

Abstract

Lignin is an aromatic heteropolymer composed of three different monomers linked in a variety of ways. Encompassing 10-30% of plant lignocellulose, lignin is the second most abundant organic polymer on the planet, second only to cellulose and hemicellulose. As such, there is great interest in using lignin as a chemical feedstock to replace current petroleum-based chemicals. When made accessible, lignin would be an exceptionally renewable resource, but it is currently very difficult to break lignin into useful chemical feedstocks. This report outlines the development of a high-throughput assay to aid in the screening of chemical methods to break lignin down into renewable chemical feedstocks. The assay consists of nitrating lignin and monitoring the breakdown of the lignin polymer by ultraviolet-visible (UV-vis) spectroscopy.

Introduction/Background

A majority of the products encountered in daily life began as crude oil, which is non-renewable and whose burning causes climate change.\(^1\) It would be beneficial for society to develop sustainable, non-polluting sources of chemicals and energy. One example is cellulose, the most abundant naturally occurring organic polymer originating from plants.\(^2\) A polymer is a material that is composed of the same parent compound (known as a monomer) repeatedly connected together to make a long chain. Cellulose is composed of sugar molecules connected end to end and can be broken down to glucose, which can then be fermented to produce bioethanol. The glucose can also be further processed into useful chemicals (Figure 1).\(^2\)

![Figure 1](image.png)

**Figure 1.** Cellulose can be used to produce bioethanol and useful chemicals

During the process of isolating cellulose from various plant products, another polymer, lignin, is present (Figure 2). Lignin is the 2\(^{nd}\) most abundant naturally occurring organic polymer and is made of repeating units of benzene, an organic molecule found in common substances such as polystyrene.
Currently, lignin is separated from cellulose and burned for its intrinsic energy (Figure 3). Some research has been done into utilizing lignin as a renewable source for chemical starting materials but the processes are inefficient.\textsuperscript{3}

**Figure 2.** Atomic-detailed model of lignocellulose of softwoods. Based on experimental data on the structure of cellulose (brown) and lignin (cyan and red).

**Sources of Lignocellulose**

- Forest Waste
- Corn Stover
- Switchgrass
- Lignin burned for heat/fuel
- Cellulose transformed into biofuels

**Figure 3.** Lignocellulose from forest waste, corn stover, and switch grass can be used to make biofuels by isolating the cellulosic sugars and using them in a fermentation process to produce ethanol. In this process, the isolated lignin is burned as a source of heat/fuel. What a waste.

Degradation of this aromatic polymer could provide a renewable resource for a wide array of aromatic products (Figure 4). However, most degradation methods are carried out under extreme basic or acidic conditions at high temperatures and pressures. Also, monitoring lignin degradation involves GPC, LC-MS, or GC-MS, which can be time consuming.
Degradation of lignin is a potential source of aromatic products (polymers, chemical feedstocks, etc.)

Figure 4. Lignin is a high molecular weight polymer formed by biosynthetic polymerization of 3 aromatic alcohols in plants.

Our lab has sought to aid in the process of finding safer, cheaper, and easier methods of turning lignin into commodity chemicals by developing a rapid method to monitor the breakdown of lignin. This reports details those efforts.

Data and Results

As stated above, most methods to monitor the breakdown of lignin are very work intensive. We set out to develop an easier method to monitor lignin breakdown. During a search of the literature, we ran across the work of Bugg and co-workers where they developed a high throughput assay (Figure 5).4

Figure 5. Continuous fluorescent assay developed by Bugg and co-workers

Bugg and co-workers2 reported the development of a continuous fluorescent assay by nitrating lignin. They reported that nitrated lignin had a maximum absorbance at 300 nm which shifted to 430 nm upon degradation of the lignin polymer. They used this assay to screen bacteria for the ability to degrade lignin. We set out to develop the above assay in our labs.
While on paper, the development of the assay seemed straightforward, it was far from it. Lignin, it turns out, is a very difficult material to work with. It is insoluble in almost all solvents necessary to carry out the nitration, which initially led us to believe that we were not able to nitrate the lignin. The presence of the nitro group was validated by ultraviolet-visible (UV-vis) spectroscopy. According to Bugg and co-workers, unnitrated lignin has a characteristic peak at 280 nm, which disappears upon nitration with a new peak appearing at 300 nm corresponding to nitrated lignin. Initially, we never observed these peaks. After much trial and error, we finally observed the presence of the nitrated lignin by UV-vis spectroscopy (Table 1 and Figure 6).

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Temperature (°C)</th>
<th>Evidence of nitration by UV-Vis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcOH/HNO₃</td>
<td>Room temp (1 hr)</td>
<td>Yes*</td>
</tr>
<tr>
<td>AcOH/HNO₃</td>
<td>50</td>
<td>Yes</td>
</tr>
<tr>
<td>NO₂ (made from H₂SO₄ and HNO₃)</td>
<td>Room temp</td>
<td>Yes</td>
</tr>
<tr>
<td>H₂SO₄ and HNO₃</td>
<td>Room temp</td>
<td>Yes</td>
</tr>
<tr>
<td>H₂SO₄ and HNO₃</td>
<td>Reflux</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Conditions used to nitrate lignin: *UV-vis spectrum illustrated below

![UV-vis spectrum](image)

**Figure 6.** Sample UV-vis spectrum for successful nitration of lignin for chosen conditions from Table 1
With the successful nitration of lignin, we next set out to break the nitrated lignin into smaller molecules by known procedures to observe the shift of the UV-vis peak from 300 nm to >430 nm. Again, this proved much harder than anticipated, and we were never able to observe that shift, even in the presence of refluxing concentrated acid or concentrated base. So either we were not able to break the lignin down by any of the methods we tried, or there is no shift in the UV-vis for converting between nitrated lignin and nitrated lignin that has been broken into smaller pieces. Also, upon closer perusal of the literature, it is clear that the conditions needed to break lignin down are much harsher than what we have the capabilities of here at the University of Wisconsin-Platteville (UWP). Therefore, to continue this project, investment in some specialized equipment is needed, which is not feasible at this moment.

Conclusion

Lignin is the second most abundant naturally occurring organic polymer after cellulose. If we were able to break lignin down into smaller molecules, these molecules could be used as renewable chemical feedstocks. However, this degradation process is not simple. To develop simpler methods, an easy way to monitor the degradation of lignin is desired. This project developed such an assay through the successful nitration of lignin after much trial and error. It was observed that nitrated lignin has a characteristic peak in the UV-vis spectrum at 300 nm (Figure 6). While we were successfully able to nitrate lignin, we were never able to break it down according to UV-vis spectroscopy. The possibilities are that either there is no characteristic shift in the UV-vis spectrum between nitrated lignin and degraded nitrated lignin, or we were not able to break the nitrated lignin down by any of the conditions we tested and that harsher conditions are needed. Nonetheless, it was concluded that with the facilities we currently have at UWP, degradation of lignin is not feasible. As such, we have moved on into other areas of sustainable chemistry. For example, during the course of this project, one of the methods that was considered for the degradation of lignin was the use of visible light photoredox catalysts. With the conclusion that the developed assay in this project might not be useful, my students and I have moved to exploring the use of visible light photoredox catalysts in other useful transformations. So while the lignin project is currently in hiatus, it led us to explore other exciting areas of sustainable chemistry.

Experimental Procedures

Nitrating Lignin with glacial acetic acid and nitric acid
1) Mix lignin (0.005 g) with acetic acid (1 mL) in small vial.
2) Filter insoluble materials
3) Add nitric acid (0.2 mL)
4) Let sit for 1 hour
5) Add DI water (2 mL)
6) Neutralize to pH 7 with 1 M NaOH
7) Dilute 100 fold with tris buffer
8) Take UV-Vis scan 200-400 nm

Variations attempted: 1) step 4- heat at 50 °C for 1 hour

Nitration of Lignin with Nitrous Acid
1) Add concentrated nitric acid (2.06 mL), concentrated sulfuric acid (2.94 mL), and lignin (0.0515 g) to round bottom
2) Stir and heat to reflux for 1 hour
3) Add DI water (2 mL)
4) Neutralize with 1 M NaOH
5) Dilute with tris buffer 100 fold
6) Take UV-Vis

Variations attempted: 1) step 2- stir at room temp 1 hour

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References