FACTORS INFLUENCING PROPERTIES
OF ISOLATED WOOD LIGNIN

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FACTORS INFLUENCING PROPERTIES OF ISOLATED WOOD LIGNIN*

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TEMPERATURE, acid concentration, and length of
time of the reaction must be controlled in order to
obtain, by the sulfuric acid method, lignin as un-
changed as possible.

When lignin was isolated by the Freudenberg meth-
od some of it was lost by the sodium hydroxide and
the copper ammonium solution extractions.

Conditions that produced the lowest yield of lig-
nin by the sulfuric acid method also gave the highest
yield of hydrolyzed carbohydrates.

Lignin isolated by 70 percent sulfuric acid at
10° C. for 16 hours was free from carbohydrates, gave
a higher methoxyl content, and was more readily methy-
lated than lignin isolated by other methods.

One cause of the comparatively slow progress in lignin chemistry
is the difficulty of obtaining lignin free from other plant tissues and un-
changed in the process of isolation. Practically every process that has
been advanced is open to objection. Two general methods are now in use:
The removal of all the constituents of the cell except the lignin; and the
extraction of lignin from the cell. The former, the only one considered in
this paper, depends on the fact that the carbohydrate constituents dissolve
in certain solvents, the most commonly used of which are strong hydrochloric
acid, sulfuric acid, and copper ammonium solution. Upon further hydrolysis
of the dilute acid solutions the carbohydrates are converted into simple
sugars, which may be washed out with water.

The reaction with hydrochloric acid was worked out by Willstatter
and Zechmeister (25) and modified slightly by Kalb (24) and Hagglund (8).
The lignin obtained with this reaction was found by Kalb and Lieser (11) to

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1931.
contain residues of cellulose and by Du Pont (3) to contain pentosans. When the reaction is continued for a longer time a number of side reactions take place, giving an insoluble product incapable of methylation and containing decomposition products of the wood carbohydrates (11).

The copper ammonium solution method of obtaining lignin, that of Freudenberg and Harder (6, 7), gives a tan product uniform in appearance and crumbly. This material represents only the less soluble portion of the lignin present in the wood, as lignin is also soluble in the copper ammonium solution (11, 23). The isolation is such that it is almost impossible to remove all of the carbohydrate material from the sample. A sample of maple lignin prepared by this method at the Forest Products Laboratory gave about 70 percent of the yield obtained by the standard laboratory method, but still contained about 5 percent cellulose and 2.5 percent pentosans and had a much lower methoxy content than was expected.

The sulfuric acid method was developed by Flechaig (5) and others (13, 15) as an analytical method to determine lignin content of wood. It was later modified by Kraso (12), Becker (1), Mahood and Cable (14), Bray (2), and Venter (22). This method has several objections. Palosnik (16) states that long exposure to sulfuric acid causes humus formation and too short a treatment leaves carbohydrates. Euler (4) found a loss of acetic acid and absorption of sulfuric acid. Freudenberg and Harder (6) contend that formaldehyde is split from lignin by the action of acid. On the other hand, the sulfuric acid method has advantages in that the desired concentration is easily obtained by diluting the concentrated acid. It is also easier to duplicate results with the sulfuric acid method than with any other now in use, except perhaps the modification of this method by Ross and Hill (20). The Ross and Hill modified method, however, would never be used for isolating pure lignin because of the possibility of resin formation between formaldehyde and lignin (21). Sulfuric acid also has the advantage of being rapid and requiring only one treatment. Loss of acetic acid and formaldehyde may be reduced by keeping the temperature low.

**Experimental Isolation of Lignin**

Sugar maple was selected as the wood from which to prepare lignin. Wood free from bark and knots was ground to 60-80 mesh fineness and air-dried, then extracted with alcohol-benzene (1-2) for 50 to 60 hours in a Soxhlet apparatus. After drying to remove the alcohol-benzene, the sample was extracted with cold and hot water for several hours. Loss in weight by these extractions was 13 percent. This value is higher than that found by Ritter and Fleck (16) for ether extractive.

Lignin was isolated by each of the three methods described. The Willstatter (25) method gave a dark green product with a yield of 21.2 percent. Boiling with water, according to the method of Rassow and Zickman (17), had no effect on the color of the product. The Freudenberg lignin (6) was light tan in color, gave 14.7 percent yield on the basis of air-dried
LEGEND

- 5° CENTIGRADE
- 10° CENTIGRADE
- 28° CENTIGRADE

TIME: 16 HOURS.

YIELD CALCULATED ON
OVEN-DRIED WOOD.

ISOLATION OF LIGNIN AT VARIOUS ACID CONCENTRATION AND TEMPERATURES

R1021
wood, and contained 15 percent methoxyl as determined by the Zeisel
method. Sulfuric acid lignin, prepared by the standard Forest Products
Laboratory method (2), was dark in color and contained absorbed sul-
furic acid. The average yield in several determinations was 23.5 per-
cent.

The fact that the sulfuric acid lignin was so dark in color
and that the yield was greater than that produced by hydrochloric acid
seemed to indicate that carbohydrate decomposition products were present
in the residue and that decomposition of the sugars, as well as hydroly-
sis of the carbohydrates, took place. The comparative ease with which
carbohydrates may be converted into a lignin-like substance (10) at
elevated temperatures suggested that the same effect may be produced
when sulfuric acid comes in contact with the wood. A series of experiements,
therefore, was made on Cross and Bevan maple cellulose to determine the
rise in temperature, the tendency to char, the residue formed under various
conditions, the relative speed of the reaction of solution and hydrolysis,
and the effect of the concentration of the acid at different temperatures.

Rise in Temperature by Action of Sulfuric Acid

Samples of 2 grams of wood cellulose were treated with 70 per-
cent and 72 percent acid at 0°, 10°, and 25°C. The rise in temperature at
10° and 25°C. with 70 percent acid was about 6 degrees, while that with
72 percent was 8 to 10 degrees. With both concentrations the temperature
rise at 0° was almost negligible because the reaction was very slow. Maple
sawdust treated under the same conditions gave similar results (Table I).

Table 1.—Maximum temperature in treating sawdust with H₂SO₄ at various
temperatures.

<table>
<thead>
<tr>
<th>Concentration of acid</th>
<th>Temp. of reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>°C</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>70</td>
<td>11</td>
</tr>
<tr>
<td>72</td>
<td>16</td>
</tr>
</tbody>
</table>

1 Two grams of sawdust were used.
The greater rise in temperature with the 72 percent acid is thought to be due to the greater speed of the reaction. As shown in Table V, the lowest yields were obtained where the temperature of the initial reaction did not get higher than 17°C.

**Charring Action of Sulfuric Acid**

Solutions of cellulose and 72 percent sulfuric acid were allowed to stand to determine the time when charring began. At 0°C, the solution was still colorless after 72 hours; at 5°C, slight charring took place within 48 hours; at 10°C, charring took place within 16 hours; and at 25°C, charring developed in less than 15 minutes. That this charring produced a lignin-like residue was determined by hydrolyzing the solutions. The sample at 0° for 36 hours gave 0.08 percent residue; at 25° for 16 hours, 0.30 percent; and at 25° for 36 hours, 3.0 percent residue. These residues, after chlorination, were soluble in sulfite solution. This indicated that the dark color and higher yield of lignin with sulfuric were caused by decomposition of the carbohydrates in the wood.

**Speed of Solutions and Hydrolysis**

As charring developed under some conditions from the action of sulfuric acid on cellulose, it was desirable to know the time required for the solution of the cellulose. To determine this the action of sulfuric acid on maple cellulose was studied with various acid concentrations.

Table 2.—Time required for cellulose to lose gelatinous property at different temperatures.

<table>
<thead>
<tr>
<th>H₂SO₄ Conc.</th>
<th>5°C.</th>
<th>Length of Time at:</th>
<th>10°C.</th>
<th>25°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>Minutes</td>
<td>2</td>
<td>Minutes</td>
<td>2</td>
</tr>
<tr>
<td>80</td>
<td>302</td>
<td>2</td>
<td>202</td>
<td>102</td>
</tr>
<tr>
<td>75</td>
<td>50</td>
<td>30</td>
<td>60</td>
<td>152</td>
</tr>
<tr>
<td>72</td>
<td>120</td>
<td>90</td>
<td>252</td>
<td>35</td>
</tr>
<tr>
<td>70</td>
<td>180</td>
<td>90</td>
<td>60</td>
<td>35</td>
</tr>
<tr>
<td>68</td>
<td>250</td>
<td>90</td>
<td>90</td>
<td>252</td>
</tr>
<tr>
<td>65</td>
<td>500</td>
<td>210</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td>60</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

1One gram in 15 cc. H₂SO₄.
2Charring.
3No solution.
The higher concentrations and temperatures produced charring and, therefore, were unsatisfactory, while the lower concentrations did not dissolve the cellulose. The lower solutions, if diluted with water and boiled, gave a precipitate of unhydrolyzed cellulose. Therefore, it was necessary to let the solution stand until the hydrolytic products remained in solution on dilution and boiling (Table 3).

Table 3.—Time necessary to give a solution in which no cellulose remains after boiling 4 hours with 3 percent sulfuric acid.

<table>
<thead>
<tr>
<th>Acid Conc.</th>
<th>5°C.</th>
<th>10°C.</th>
<th>25°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>Hours</td>
<td>Hours</td>
<td>Hours</td>
</tr>
<tr>
<td>68</td>
<td>16-20</td>
<td>10-16</td>
<td>7-10</td>
</tr>
<tr>
<td>70</td>
<td>16-20</td>
<td>10-16</td>
<td>7-8</td>
</tr>
<tr>
<td>72</td>
<td>8-16</td>
<td>8-10</td>
<td>1</td>
</tr>
</tbody>
</table>

Charring.

To determine the conditions for the most complete hydrolysis of the carbohydrates in the cellulose solutions, samples of the boiled, diluted solutions were analyzed for reducing sugars. With 68 percent acid at 10°C. for 16 hours, the value was low, indicating incomplete hydrolysis. With 70 percent acid at 10°C., the value was very near that expected from theory. With 72 percent acid at 10° and 20°C., in which charring was obtained, the value was again low, indicating decomposition of the carbohydrates. At least 16 hours, therefore, were required for solution at 10°C. for the 70 percent sulfuric acid.

Two reactions were considered as taking place, namely, hydrolysis and decomposition. At the higher concentrations of acid both occurred at the same time. Later experiments were carried out to determine the conditions for the most complete hydrolysis and the least decomposition. This condition should give a high sugar-reducing number and a low lignin residue. Both of these occur with 70 percent acid at 10°C. for 16 hours. Table IV shows the effect of temperature and time of reaction on the yield of lignin with 72 percent sulfuric acid. After hydrolysis the solution in each case was diluted to 3 percent acid concentration, boiled for 4 hours, and filtered through an alundum crucible.
Table 4.—Lignin by 72 percent acid at various temperatures and intervals.

<table>
<thead>
<tr>
<th>Interval</th>
<th>-20°C.</th>
<th>0°C.</th>
<th>10°C.</th>
<th>25°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hours</td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>21.2</td>
<td>21.6</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>23.8</td>
<td>21.8</td>
<td>22.72</td>
</tr>
<tr>
<td>36</td>
<td></td>
<td>21.1</td>
<td>23.64</td>
<td>28.03</td>
</tr>
<tr>
<td>48</td>
<td>1</td>
<td>27.03</td>
<td>40.03</td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1—Gelatinization. 2—Dark. 3—Charred. 4—Dark. 5—Incomplete solution.

The material prepared at 0° for 36 to 40 hours, or at 10° for 7 to 16 hours, was easily filtered, was light tan in color, settled out to form a clear solution, and, on drying, formed a dry amorphous powder. These observations are contrary to the findings of Harlow (9), who reported that hardwood lignin filtered with difficulty and dried to a hard cake. Lignin prepared at higher temperatures or over a longer time, however, did have the properties described by Harlow. This may be due to the decomposition products of the carbohydrates that remain in the residue. The results of an experiment similar to that just described, but with the acid concentration varied and the time constant, are shown in Table V and Figure 1.

Table V. Lignin isolated at various acid concentrations and temperatures.

(Time, 16 hours; yield calculated on oven-dried wood)

<table>
<thead>
<tr>
<th>H2SO4 Conc.</th>
<th>0°C.</th>
<th>0°C.</th>
<th>0°C.</th>
<th>0°C.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td>80</td>
<td>21.4</td>
<td>29.0</td>
<td>29.5</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>21.3</td>
<td>22.3</td>
<td>21.2</td>
<td>22.8</td>
</tr>
<tr>
<td>72</td>
<td>21.1</td>
<td>21.0</td>
<td>30.8</td>
<td>30.8</td>
</tr>
<tr>
<td>71</td>
<td>21.1</td>
<td>20.9</td>
<td>20.0</td>
<td>21.7</td>
</tr>
<tr>
<td>70</td>
<td>24.2</td>
<td>21.4</td>
<td>21.0</td>
<td>22.0</td>
</tr>
<tr>
<td>69</td>
<td>30.3</td>
<td>21.4</td>
<td>21.7</td>
<td>21.7</td>
</tr>
<tr>
<td>68</td>
<td>37.5</td>
<td>21.4</td>
<td>21.7</td>
<td>21.7</td>
</tr>
<tr>
<td>67</td>
<td>40.4</td>
<td>21.7</td>
<td>21.7</td>
<td>21.7</td>
</tr>
<tr>
<td>65</td>
<td>48.8</td>
<td>22.0</td>
<td>21.7</td>
<td>21.7</td>
</tr>
<tr>
<td>60</td>
<td>59.5</td>
<td>55.5</td>
<td>54.8</td>
<td>59.2</td>
</tr>
<tr>
<td>55</td>
<td>62.8</td>
<td>60.0</td>
<td>60.0</td>
<td>60.0</td>
</tr>
</tbody>
</table>

The product from the higher concentrations and temperatures was black, filtered very slowly, and, on drying, formed a hard brittle mass. With the lower concentrations (55 and 60 percent) the appearance of the wood changed very little. The product isolated at 10°C, with 70 percent acid seemed the most desirable because it gave the lowest yield.
was freest from charred decomposition products, and, as determined by solubility with chlorine and sulfite solution, was free from cellulose. The sample isolated at 10°C, with 68 percent acid contained a small amount of cellulose. A sample prepared at room temperature and at the same concentration as used by Klassen (12) was not so satisfactory because the yield was almost 1 percent higher and the product was darker than in the case of that obtained at 10°C and 70 percent acid. More recently, Ritter and Seborg (19) have applied these effects of time and temperature with 72 percent sulfuric acid on the quantitative yield of lignin from wood and have suggested the reduction of time from 16 to 2 hours.

**Isolation of Lignin with Sulfuric Acid**

These experiments showed that the following procedure for the isolation of lignin with sulfuric acid is satisfactory:

Add a sample of alcohol-benzene and water-extracted sawdust (60-80 mesh) weighing 8 grams (equivalent to 8.65 grams of original sawdust) to 80 cc. of 70 percent acid previously cooled in a stoppered flask to 10°C. Shake the flask until gelatinization occurs (about 3 minutes) and return to the cooling bath. After about an hour, when the mixture begins to thin, shake again for a few minutes to insure contact of the acid with all the sawdust. After 16 hours in the cooling bath at 10°C, pour the contents of the flask into a 4-liter flask containing 2 liters of cold water. Wash the sulfuric acid mixture remaining into the flask with cold water and fill the flask up to 3 liters. Then mix the contents of the flask well to prevent superheating of the residue in contact with strong acid which otherwise would cause the loss of the formaldehyde group (6). Boil the dilute acid mixture for 4 hours under a reflux condenser, allow it to settle, and decant through an alundum crucible. Wash the residue into the crucible with 1 liter of hot water to remove adsorbed sulfuric acid. When the filtering and washing are complete (usually in 15 minutes) dry the residue at room temperature to about 9 percent moisture content. For experimental work it was found more satisfactory to work with the product at 9 percent moisture content than at lower moisture content values because oven-drying gave a less reactive and darker product. The average yield from several runs was 20.8 percent of lignin (oven-dry basis).

The product thus isolated was found to contain 21.0 percent methoxyl by Meisel. After two methylations with dimethyl sulfate the product was very near the original sawdust in color and contained 32.5 percent methoxyl.

The product isolated at room temperature with 72 percent acid contained 17.1 percent methoxyl and, on being methylated twice, contained 22.8 percent methoxyl. The methylated product was still dark brown. Further methylation produced no increase in methoxyl content.
The original wood contained 5.3 percent methoxyl. The isolated lignin contained 82.4 percent of this, which, as far as the authors are aware, is as high a yield of methoxyl as any reported. Lignin isolated by the Freudenberg method contained only 41 percent of the methoxyl of the original wood. Some of the methoxyl-containing material was removed by the extraction with cold 5 percent sodium hydroxide in the Freudenberg method, as this extract gave a residue similar to lignin when treated with sulfuric acid and contained methoxyl groups. Wood sawdust previously treated with cold 5 percent sodium hydroxide before the isolation by 70 percent acid yielded 17.3 percent lignin of approximately the same methoxyl content. This indicated a loss of almost a fifth of the lignin by sodium hydroxide extraction alone.

**Discussion**

In the isolation of lignin by sulfuric acid in as unchanged form as possible, it is necessary to control conditions to prevent secondary reactions which bring about decomposition of dissolved carbohydrates. Temperature is one of the most important factors, as a few degrees greatly change the speed of the two reactions, decomposition apparently being hastened more than hydrolysis. At low temperatures very little decomposition occurs, even in long periods. Moderate acid concentrations have advantages over higher concentrations in that the rise in temperature when the acid comes in contact with the sawdust is less, and the charring action therefore less, while the speed of hydrolysis is but slightly retarded. If the acid is allowed to remain in contact with the hydrolyzed carbohydrates for long periods decomposition takes place. If the time is too short, hydrolysis is incomplete. The highest yield of sugar from hydrolysis should indicate the point where the greatest amount of cellulose is hydrolyzed and the smallest amount of sugars decomposed. It should also be the point where the lowest yield of lignin is obtained. A low lignin yield, therefore, is considered an index to the extent of decomposition.

In the method for isolating lignin used by Freudenberg (7), the extraction of the sawdust with alkali is open to question because lignin is dissolved, as shown by the fact that this extract gives a lignin-like residue that contains methoxyl, and the lignin yield by sulfuric acid from alkali-extracted sawdust is decreased by almost a fifth, while the methoxyl ratio is unchanged.

If the percentage of methoxyl in the isolated lignin may be considered as a check on the purity, sulfuric acid under controlled conditions produces lignin of higher quality than that produced by other methods. Sulfuric acid lignin better represents the lignin of the wood because it contains a high percentage of the total methoxyl found in the wood.
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