

Lignin Isolation from Pulp

Several different enzymatic, chemical and mechanical methods have been developed for the isolation of lignin from wood and pulp. However, due to the heterogeneous nature of wood and pulp fibers and the heterogeneity that exists between individual fibers, no method is currently available for the quantitative isolation of native or residual lignin without the risk of structural changes during the isolation. Even if the perfect isolation technique could be found, the product would at best represent the average structure of native or residual lignin components. However, the information gained about the chemical reactivity and structure of isolated lignin is valuable. Thus, the three most commonly employed methods for isolating residual lignin are described below.

Lignin Isolation by Enzymatic Hydrolysis of Pulp

Yamasaki *et al.* first developed a technique for isolating residual lignin from kraft pulp by enzymatic hydrolysis.¹ This procedure is based on selective hydrolysis and dissolution of carbohydrates in pulp by commercial cellulolytic enzymes, leaving lignin behind as an insoluble residue. Pulps are typically subjected to several successive enzyme treatments to ensure complete dissolution of the carbohydrates and to increase the amount of recovered insoluble lignin residue. The residual lignin from this isolation method is typically obtained in good yield, especially from unbleached kraft pulps.

Hortling *et al.* used cellulase and β -glucosidase to isolate residual lignin from a series of kraft pulps.² These laboratory-produced kraft pulps varied from 93 to 14 kappa number. The enzymes employed in this enzymatic hydrolysis technique are effective enough to hydrolyze practically all pulp polysaccharides in one enzyme treatment. The insoluble lignin residue remaining after enzymatic hydrolysis was subsequently extracted with 0.5 M sodium hydroxide and the soluble fraction was acid-precipitated, yielding the residual lignin sample. Little or no lignin was water-soluble after the enzymatic treatment of the pulps in the 93-58 kappa number range, while 20-60% of the lignin was dissolved during enzymatic hydrolysis for pulps with lower kappa numbers. The results indicated that the residual lignin samples contained 65-80% lignin, 7-8% carbohydrates, and the remaining impurities from proteins acquired during the enzymatic treatment. The content of protein impurities was higher in the portion of the sample that was soluble in sodium hydroxide than in the insoluble fraction, suggesting that the proteins have an affinity for the residual lignin. Although the molecular weights of the residual lignin were found to decrease with increasing degree of delignification, the authors suggested that the difficulty of removing residual lignin from pulps is due partly to insoluble fractions of the residual

¹ Yamasaki, T.; Hosoya, S.; Chen, C.-L.; Gratzl, J.S. and Chang, H.-M.; Characterization of Residual Lignin in Pulp. In *Proceedings of the International Symposium on Wood and Pulp Chemistry*. Stockholm, Sweden. p. 34-42. (1981).

² Hortling, B.; Ranua, M. and Sundquist, J.; Investigation of the Residual Lignin in Chemical Pulps Part 1. Enzymatic Hydrolysis of the Pulps and Fractionation of the Products. *Nordic Pulp and Paper Research Journal* **5** (1). p. 33-37. (1990); Hortling, B.; Ranua, M. and Sundquist, J.; Investigation of Residual Lignin in Chemical Pulps Part 2. Purification and Characterization of Residual Lignin after Enzymatic Hydrolysis of Pulps. *Nordic Pulp and Paper Research Journal* **3** (7). p. 144-151. (1992).

lignin and/or strong bonds between lignin and carbohydrates rather than to the higher molecular weight of the residual lignin.

There are several drawbacks of utilizing the enzymatic hydrolysis method for isolating residual lignin. All residual lignins isolated with this procedure contain some carbohydrates that cannot be removed by prolonged and repetitive enzymatic treatments or by purification methods that are commonly employed for milled wood lignins. This is believed to originate from the limited ability of enzymes to hydrolyze lignin-carbohydrate linkages.³ Typical carbohydrate contents of residual lignins isolated from unbleached and semi-bleached pulps are in the range of 3-7%. In addition, residual lignin sample obtained by this method contain protein impurities originating from the enzymes used in the hydrolysis stage. Thus, the samples need to be purified to remove most of these protein impurities. Typical nitrogen content values after purification are 0.6 and 2.3% for residual lignins isolated from unbleached and semi-bleached pulps, respectively.⁴ In addition, the time required for the enzymatic hydrolysis procedure is much greater than chemical isolation techniques, such as acid hydrolysis. The carbohydrate and protein impurities from the enzymatic hydrolysis method complicate subsequent analyses of the lignin structure. However, the residual lignin obtained from this isolation technique is considered to be chemically unchanged and the yield is quite good.

Lignin Isolation by Acid Hydrolysis of Pulp

A common method for chemically isolating residual lignin involves extraction via acidic dioxane. Prior to using this lignin isolation technique, kraft pulps are typically extracted with acetone to remove extractives. The extracted pulp is then refluxed under an inert atmosphere (i.e., nitrogen or argon) with 0.1 M HCl in 9:1 dioxane:water (azeotrope boiling point of 88°C). The solubilized lignin is then recovered from solution. This technique offers a residual lignin that is free from carbohydrates and other impurities. The acid hydrolysis isolation method is a comparatively rapid way of obtaining pure residual lignin for further analysis when compared to enzymatic hydrolysis.

The mechanism believed to be responsible for the liberation of lignin is the hydrolysis of covalent linkages between lignin and carbohydrates. Repeating the acid hydrolysis procedure in the absence of an acid catalyst failed to release any lignin into solution thereby supporting the proposed lignin-carbohydrate complex acid hydrolysis

³ Gierer, J. and Wannstrom, S.; Formation of Alkali-Stable C-C Bonds between Lignin and Carbohydrate Fragments During Kraft Pulping. *Holzforschung* **38** (4). p. 181-184. (1984); Minor, J.L.; Chemical Linkage of Polysaccharides to Residual Lignin in Loblolly Pine Kraft Pulps. *Journal of Wood Chemistry and Technology* **6** (2). p. 185-201. (1986).

⁴ Jiang, J.-E.; Chang, H.-M.; Bhattacharjee, S.S. and Kwoh, D.L.W.; Characterization of Residual Lignins Isolated from Unbleached and Semibleached Softwood Kraft Pulps. *Journal of Wood Chemistry and Technology* **7** (1). p. 81-96. (1987).

mechanism.⁵ One drawback encountered in this method is the rather strong acidic conditions that are necessary to liberate the lignin from pulp fibers. The acid hydrolysis conditions can be expected to cause some structural modifications to the lignins including the cleavage of some α -aryl and α -alkyl ethers as well as β -aryl ethers in benzyl alcohol units.⁶ The cleavage of the aryl ethers would result in the creation of phenolic hydroxyl groups, causing the content of this functional group to be higher than the actual amount present in the wood or pulp. Lignin condensation reactions under acidic conditions are also possible but believed not occur during the isolation, at least for residual lignin in kraft pulp.⁷ One reason for the apparently low tendency to form a condensed lignin under these strongly acidic conditions could be the low amount of residual benzyl alcohol and benzyl ether structures in the lignin after a completed kraft cook. Jiang and Argyropoulos⁸ compared residual lignins from the acid hydrolysis process using flow-through and batch reactors. These researchers found that the structure of lignin isolated from a flow-through process was similar to the residual lignin isolated from a batch process. These results provide additional evidence that the structure of residual lignin in kraft pulps is not significantly altered during the acid hydrolysis procedure. Another drawback to this isolation technique is that the yield is rather low compared to enzymatic hydrolysis (40-60%) and is dependent on the severity of the isolation conditions. Increasing the yield requires that more severe conditions be used (i.e., higher acid concentration and longer reaction times), suggesting that optimal isolation conditions are necessary.

Experimental Procedure for Acid Hydrolysis Isolation of Lignin

Residual lignin was isolated from the *Pinus taeda* kraft pulps by the acid hydrolysis procedure, in accordance with standard literature methods.⁵ This technique is frequently employed since it is relatively rapid and yields a lignin of relatively high purity, free of carbohydrate contamination. Figure 1 shows the experimental apparatus used for the acid hydrolysis procedure. The pulps were thoroughly washed with deionized water and air-dried prior to Soxhlet extraction with acetone for 24 hours. A 3000-mL three-necked round-bottom flask equipped with a Friedrichs condenser was charged with 30.0 g o.d. pulp (air-dried). The pulps were then refluxed with 0.100 N HCl in *p*-dioxane at 4.00% consistency for two hours under an argon atmosphere. The 0.100 N HCl solution was a 9:1 *p*-dioxane:acid water solution. The solutions were then cooled and filtered through a coarse-sintered glass Büchner funnel covered with filter paper to avoid plugging. The resulting filtrate was passed through a fine-sintered glass Büchner funnel packed with a

⁵ Froass, P.M.; Ragauskas, A.J. and Jiang, J.E.; Chemical Structure of Residual Lignin from Kraft Pulp. *Journal of Wood Chemistry and Technology* **16** (4). p. 347-365. (1996).

⁶ Lundquist, K.; Acid Degradation of Lignin. (8). Low Molecular Weight Phenols from Acidolysis of Birch Lignin. *Acta Chemica Scandinavica* **27** (7). p. 2597-2606. (1973).

⁷ Gellerstedt, G.; Pranda, J. and Lindfors, E.; Structure and Molecular Properties of Residual Birch Kraft Lignins. *Journal of Wood Science and Technology* **14** (4). p. 467-482. (1994).

⁸ Jiang, Z.-H. and Argyropoulos, D.S.; Isolation and Characterization of Residual Lignins in Kraft Pulps. *Journal of Pulp and Paper Science* **25** (1). p. 25-29. (1999).

Celite[®] filter aid to remove fines. The lignin/water/dioxane mixture was then neutralized with an aqueous saturated solution of sodium bicarbonate to an apparent pH value of 5.0–5.5 and concentrated at approximately 35°C under reduced pressure to approximately 10% of the original volume. Deionized water (ca. 400 mL) was added and the mixture was concentrated again under reduced pressure to remove the last traces of *p*-dioxane. The resultant aqueous lignin solution was transferred to a 1 L beaker, diluted with deionized water to a volume of approximately 750 mL, acidified to a pH of 2.0–2.5, and transferred to 250 mL capacity centrifuge bottles. The centrifuge bottles were frozen, thawed, and centrifuged. Freezing promoted lignin coalescence, which improved the yield. The supernatant was siphoned and fresh acidified water was added to wash the lignin. This step was repeated four times, each time combining the lignin into fewer centrifuge bottles. During the third wash, all the lignin was contained in one centrifuge bottle. The lignin was then freeze-dried for 3–5 days, after which it was characterized by NMR, UV/vis, and methoxyl content. The yield of residual lignin was calculated by determining the original pulp’s lignin content by kappa number and acid-insoluble lignin content. The lignin concentration for kappa number was estimated using the relationship in Equation 1.

$$\text{Lignin Weight \%} = (0.15) \times (\text{kappa number})$$

Equation 1. Calculation to determine the weight percent of lignin in a pulp based on the kappa number.

Equation 1 is based on the studies by Tasman and Berzins in their studies on the relationship between the pulp kappa number and the lignin content of pulp. The lignin yields were then calculated as in

Equation 2.⁹

$$\% \text{ Lignin Yield} = \frac{(\text{mass of lignin isolated})}{(\text{initial kappa number}) \times 0.15} \times 100$$

Equation 2. Calculation to determine the lignin yield after residual lignin isolation by the acid hydrolysis technique.

The lignin yields ranged from 31.6–61.9%, with the lower yields arising from relatively high lignin content kraft pulps (~160 kappa number).

⁹ Tasman, J.E. and Berzins, V.; The Permanganate Consumption of Pulp Materials: The Relationship of the KAPPA Number to the Lignin Content of Materials. *Tappi* **40** (9). p. 699-704. (1957).

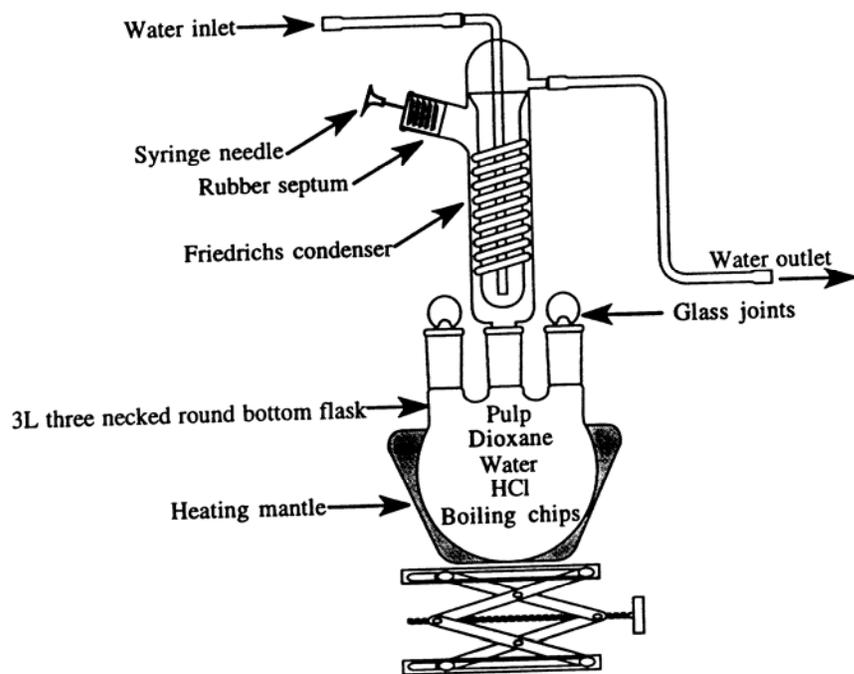


Figure 1. Experimental apparatus for the acid hydrolysis procedure