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L.T. Fan M.M. Gharpuray Y.-H. Lee

Cellulose Hydrolysis

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L.T. Fan · M. M. Gharpuray · Y.-H. Lee

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With 65 Figures

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Prof. Liang-tseng Fan
Department of Chemical Engineering
Durland Hall, Kansas State University
Manhatten, KS 66506, USA

Dr. Mahendra Moreshwar Gharpuray
Group Operations, Inc.
1110 Vermont Ave, N.W., Suite 500
Washington, D.C. 20005, USA

Prof. Yong-Hyun Lee
Department of Genetic Engineering
College of Natural Sciences,
Kyungpook National University
Taegu 635, Korea

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Foreword

Recent economic trends, especially the worldwide decline in oil prices, and an altered political climate in the United States have combined to bring about major reductions in research on renewable energy resources. Yet there is no escaping the "facts of life" with regard to these resources. The days of inexpensive fossil energy are clearly numbered, the credibility of nuclear energy has fallen to a new low, and fusion energy stands decades or more from practical realization. Sooner than we may wish we will have to turn to renewable raw materials – plant "biomass" and, especially, wood – as significant suppliers of energy for both industry and everyday needs. It is therefore especially important to have a single, comprehensive and current source of information on a key step in any process for the technological exploitation of woody materials, cellulose hydrolysis. Furthermore, it is essential that any such treatment be unbiased with respect to the two methods – chemical and biochemical – for the breakdown of cellulose to sugars.

Researchers on cellulose hydrolysis have frequently been chided by persons from industry, especially those individuals concerned with determining the economic feasibility of various technological alternatives. They tell us that schemes for the utilization of wood and other such resources fly in the face of economic realities. The proper response to such allegations was offered by one of this country's best known "wood" chemists at one of the symposia on "Biotechnology for Fuels and Chemicals" (sponsored by the Oak Ridge National Laboratory). In a free-form discussion on this subject, Professor Irving Goldstein (North Carolina State University) pointed out that it is not the function of the research community to follow the dictates of perceived economic feasibility in choosing subjects for research. Our function is to provide the knowledge base on which technologies may be fashioned. Then, when the times are ripe, the knowledge needed to take advantage of different options will be at hand. This viewpoint provides a cogent argument for the volume which follows.

August 1987

ELMER L. GADEN, Jr.
Wills Johnson Professor and Chairman
Department of Chemical Engineering
University of Virginia

Preface

This monograph covers numerous facets of cellulose hydrolysis. Presentation is expositional so that the book will be useful not only as a reference monograph but also as a textbook. The monograph is comprehensive in that both enzymatic hydrolysis and acid hydrolysis are treated, and their fundamental and applied aspects are discussed as well. Physical and chemical properties and characteristics of cellulose pertaining to these hydrolysis processes are also elucidated. In addition to qualitative elaboration of each subject, every effort is made to describe it quantitatively or mathematically.

Specifically, this monograph furnishes a compendium of the nature of cellulosic materials including their compositions and structures; it elaborates on the nature of lignocellulosic structural resistance, properties and mode of enzymatic action, different pretreatment methods, and a variety of kinetic models for enzymatic degradation; it focuses on the mechanism and kinetics of acid hydrolysis; and finally it describes commercial hydrolysis processes for a variety of cellulosic materials.

Acknowledgement

The authors' research, supported for several years by the U.S. Department of Energy and Kansas State University, has culminated in the present monograph. Besides the authors, Mr. David Beardmore and Mr. Bob Wisecup participated in the research; naturally, they have made significant contributions. While the manuscript was typed by several secretaries, the authors wish to single out Mrs. Janet Vinduska. The first author (LTF) also wishes to acknowledge the aid of his wife, Eva, in preparing the manuscript.

August 1987

L. T. Fan
M. M. Gharpuray
Y.-H. Lee

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Table of Contents

1	**Introduction**	1
	References	3
2	**Nature of Cellulosic Material**	5
2.1	Components of Cellulosic Materials	5
2.1.1	Extraneous Materials	5
2.1.2	Polysaccharides	6
2.1.3	Lignin	8
2.2	Composition of Wood and Straw	11
2.3	Structure of Plant Cell Walls	11
2.4	Structure of Cellulose	14
2.4.1	Chemical and Molecular Structure of Cellulose	14
2.4.2	Structure and Morphology of Cellulose Fibers	16
	References	19
3	**Enzymatic Hydrolysis**	21
3.1	Nature of Lignocellulosic Structural Resistance	21
3.1.1	Degree of Water Swelling of Cellulose Fiber	21
3.1.2	Crystallinity of Cellulose	22
3.1.3	Molecular Structure of Cellulose	23
3.1.4	Extraneous Material – Lignin	24
3.1.5	Presence of Substituent Groups	26
3.1.6	Capillary Structure of Cellulose	26
3.2	Properties and Mode of Cellulase Biodegradation	29
3.2.1	Effect of Cellulase on some Properties of Cellulose Fibers	39
3.2.1.1	Fragmentation of Cellulose Fibers	39
3.2.1.2	Swelling of Cellulose Fibers and the Swelling Factor	40
3.2.2	Mode of Action of the Cellulase	41
3.2.2.1	(C_1–C_x) Concept	41
3.2.2.2	β-1,4-Glucan Cellobiohydrolase	43

3.2.2.3 C_x Components 45
3.2.2.4 β-Glucosidase 47
3.2.2.5 Synergism Among Components 48
3.2.3 Physical Properties of Cellulase 51
3.2.3.1 Molecular Weights of Cellulase Components 51
3.2.3.2 Diffusion Coefficient and Sedimentation Constant of Cellulase . . 52
3.2.3.3 Size and Shape of Cellulase Molecules 52

3.3 Pretreatment Methods 52
3.3.1 Physical Pretreatments 53
3.3.1.1 Milling and Grinding 54
3.3.1.2 Pyrolysis 57
3.3.1.3 High Energy Radiation 58
3.3.1.4 High Pressure Steaming 58
3.3.1.5 Extrusion and Expansion 59
3.3.1.6 Microwave Treatment 60
3.3.2 Chemical Pretreatments 60
3.3.2.1 Alkalis 60
3.3.2.2 Acids 61
3.3.2.3 Gases 63
3.3.2.4 Oxidizing Agents 64
3.3.2.5 Cellulose Solvents 64
3.3.2.6 Solvent Extraction 65
3.3.2.7 Swelling Agents 66
3.3.3 Biological Pretreatments 67
3.3.4 Economic Analysis of Pretreatments 68

3.4 Kinetics of Enzymatic Hydrolysis of Cellulose 69
3.4.1 Kinetic Characteristics of the Heterogeneous Cellulose-Cellulase System 71
3.4.1.1 Mass Transfer Limitation – Diffusion of Enzyme 71
3.4.1.2 Adsorption and Desorption of Enzyme 71
3.4.1.3 Surface Reaction, Fragmentation, and Changes of Surface Area During Hydrolysis 74
3.4.1.4 Product Inhibition and its Mechanism 76
3.4.2 Kinetic Models of Insoluble Cellulose Hydrolysis by Cellulase . . 78
3.4.2.1 Empirical Representations of the Kinetics of Enzymatic Hydrolysis 78
3.4.2.2 Michaelis-Menten Mechanism Applied to an Insoluble Cellulose-Cellulase System 79
3.4.2.3 McLaren's Approach to Enzyme Reactions in a Structurally Restricted System 85
3.4.2.4 Kinetic Models Based on the Combination of the Enzyme Adsorption Equation and the Michaelis-Menten Type Equation . 87
3.4.2.5 Kinetic Model Based on the Depolymerization Mechanism . . . 92
3.4.2.6 Distributed Parameter Kinetic Models 94
3.4.2.7 Mechanistic Kinetic Model 97
3.4.2.8 Miscellaneous Models 102

3.4.3 Kinetics of Cellulases on β-1,4-Oligoglucosides 104
3.4.4 Kinetics of β-Glucosidase 108

Nomenclature 109

References 112

4 Acid Hydrolysis of Cellulose 121

4.1 Mechanism of Acid Hydrolysis 121
4.1.1 Course of Hydrolysis Reaction 121
4.1.2 Identification of Mechanism 127
4.1.3 Degree of Polymerization 128
4.1.4 Recrystallization 131

4.2 Kinetics of Acid Hydrolysis of Cellulose 133
4.2.1 Homogeneous Kinetics 133
4.2.2 Heterogeneous Kinetics 138

Nomenclature 144

References 145

5 Design and Economic Evaluation of Cellulose Hydrolysis Processes 149

5.1 Enzymatic Hydrolysis 149
5.1.1 Pure Cellulose Substrate 149
5.1.2 Lignocellulosic Substrate 157

5.2 Acid Hydrolysis 164
5.2.1 Dilute Sulfuric Acid Processes 164
5.2.2 Concentrated Sulfuric Acid Processes 170
5.2.3 Hydrochloric Acid Processes 172
5.2.4 Hydrofluoric Acid Process 174

Nomenclature 185

References 186

6 Epilogue 189

Author Index 191

Subject Index 197

1 Introduction

Cellulose is an abundantly available carbohydrate polymer in nature. This polymer is continually replenished by photosynthetic reduction of carbon dioxide catalyzed by sunlight. The estimated volume of existing cellulosic resources is 324 billion cubic meters. Furthermore, the annual net yield of photosynthesis is 1.8 trillion tons of biodegradable substances, about 40% of which is cellulose. The abundance coupled with the renewability renders cellulose to be the most promising feedstock for production of energy, food, and chemicals. In addition, the nature's balance and aesthetic value are not diminished by its use as feedstock [4]. Only a small fraction of cellulosic resources are currently utilized to manufacture products such as lumber, fuel, textiles, paper, plastics, films, foils, explosives, varnishes, thickeners, and glues. Moreover, easily harvestable trees, which are rich in cellulose, tend to be exploited for this purpose [2].

In addition to cellulose present in trees, vast amounts of cellulose exist in cellulosic wastes, such as municipal waste, agricultural wastes, and animal manure. Municipal wastes include paper and packaging materials; they have served as landfills or fuel sources to generate steam through combustion or through pyrolysis. Combustion and pyrolysis are attractive alternatives for utilizing cellulose; however, these processes can not handle excessively wet wastes such as municipal sewage or pulping wastes. Agricultural wastes generally arise from food production where food grains are harvested and associated plant materials are discarded. Food wastes, including coffee grounds, oat hulls, potato and fruit peelings, are also generated from food processing. Presently, animal manure is either returned to the land as fertilizer or digested by anaerobic fermentation to produce methane gas. The production of methane is hampered by drawbacks including the high retention time (10–30 days) and instability of the microbial system involved in the fermentation.

In order to utilize cellulose as a feedstock for production of energy, food and chemicals, its degradation to glucose is essential. Cellulose is resistant to degradation since a cellulose molecule serves largely as a structural molecule rather than as a molecule to store energy [4]. It is reported that theoretically about 77 kcal of incident radiational energy is stored in each gram of pure cellulose. Unfortunately, the majority of existing processes utilizing cellulose extract only a small fraction of this energy due to their high energy consumption.

Cellulose is a polymer composed of anhydroglucose monomer units. Its degradation to glucose is accomplished by addition of a water molecule for each glucose molecule produced. The potential of this reaction, termed hydrolysis, has been well stated by Hajny and Reese [5]. "Cellulose is the major constituent of all

vegetation, comprising from one-third to one-half of dry plant material. As such it is one of the world's most plentiful resources. ... Most vegetation is unused by man or animals and undergoes natural decay by microorganisms capable of producing cellulases. ... Among the many problems facing the world today, two of the most important are pollution and the threat of famine. ... With the tremendous world-wide activity in seeking foodstuffs from conventional and unconventional sources, it is surprising that cellulose and lignocellulose have received so little attention. ... (the) amount of scientific research devoted to finding practical means to produce a digestible nutrient from cellulose bearing materials is negligible. Now is a propitious time for attacking this problem. Success can not only alleviate hunger, but by increased utilization of residues, it can assist in pollution abatement."

The hydrolysis of cellulose to glucose can be catalyzed by an enzyme or an acid. Enzymatic hydrolysis has been investigated extensively in the last decade or so. At present, no commercial plant appears to exist for enzymatic hydrolysis. A pilot plant to enzymatically consume dried bagasse and rice straw has been built in Japan [1]. Acid hydrolysis has been industrially practiced in the U.S. since 1913 when a plant was constructed in South Carolina to hydrolyze waste from pine mills. The plant eventually became uneconomical due to a decrease in prices of other sugar sources. In Germany, acid hydrolysis was performed using dilute sulfuric acid and dilute hydrochloric acid during World War II [7].

Enzymatic hydrolysis has several advantages over acid hydrolysis. The enzymes hydrolyzing cellulose are very specific and do not produce undesirable by-products; at the end of hydrolysis, neutralization of the product is not required; capital and operating costs are substantially lower since enzymatic hydrolysis takes place at mild temperatures, and expensive corrosion-proof equipment is not required. Furthermore, recent advances in genetic engineering have yielded highly effective strains of cellulose enzymes, thereby bringing the enzymatic hydrolysis process nearer to commercially viability. The advantages of acid hydrolysis over enzymatic hydrolysis include short reaction time and the ability to utilize cellulose without expensive pretreatment [3].

The main product of hydrolysis, namely glucose, commands a wide range of usage.

(a) Its refined form can be consumed directly as a food source in the food industry.
(b) Glucose subject to isomerization becomes a sweetner.
(c) It can be a substrate for alcohol fermentation.
(d) It can be a substrate for single cell protein production.
(e) It can be a substrate for biopolymer production [6].
(f) It can be a substrate for other fermentations in the production of chemicals and drugs, such as Penicillin, gluconic acid, L-amino acids, citric acid, pectin, aetone, and butanol.

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2 Nature of Cellulosic Material

The hydroysis of native lignocellulosics, especially that catalyzed by enzyme, is a slow process. The heterogeneous degradation of lignocellulosics is governed primarily by their structural features since (1) cellulose present in biomass possesses a highly resistant crystalline structure, (2) lignin surrounding the cellulose forms a physical barrier, and (3) the reactive sites available are limited. The cellulose present in lignocellulosics is composed of crystalline and amorphous components [7]. The amorphous component is usually more reactive than the crystalline component, and thus any means that will increase the amorphous content will enhance the hydrolysis rate [12, 16, 17, 50]. The presence of lignin forms a physical barrier for attack by either enzyme or acid molecules; therefore, treatments causing disruption of the lignin seal will increase the accessibility of cellulose to enzyme or acid molecules and eventually its hydrolysis rate. The limitation of available reactive sites stems from the fact that the average size of the capillaries in biomass is too small to allow the entry of reactive molecules, especially the large enzyme molecules.

This chapter furnishes a compendium of the nature of cellulosic materials including their compositions and structures.

2.1 Components of Cellulosic Materials

Cellulosic materials are composed of three major components, extraneous substances, polysaccharides, and lignin, as illustrated in Fig. 2.1 [28]. These components are delineated below.

2.1.1 Extraneous Materials

The extraneous component refers to all the non-cell wall materials. This component consists of an astonishingly wide variety of chemicals. Based on their solubilities in water and neutral organic solvents, these chemicals can be classified as extractives or non-extractives [28].

The extractives can be crudely divided into three groups, namely, terpenes, resins, and phenols [44]. The terpenes are regarded as isoprene polymers and are a source of turpentine in industrial processes. Related to terpenes are terpene alcohols

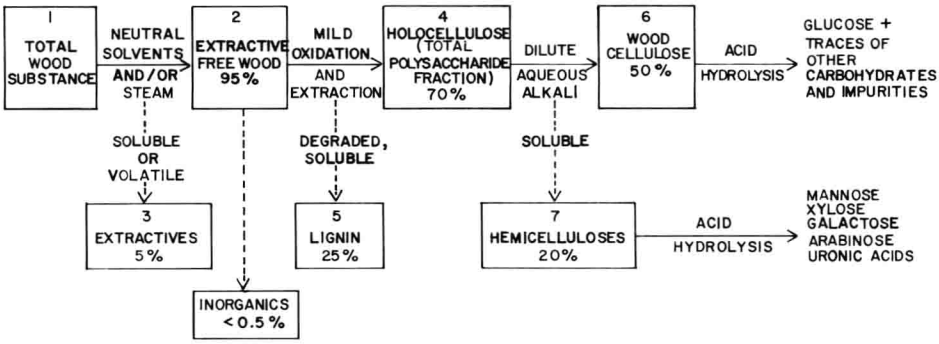

Fig. 2.1. Classification of the major components of wood [28]

and ketones. The resins include a wide variety of non-volatile compounds, including fats, fatty acids, alcohols, resin acids, phytosterols, and less known neutral compounds in small amounts. The phenols consist of numerous compounds which have not been explored adequately; the most important among them are tannins, heartwood phenols, and related substances. Other extractives include low molecular weight carbohydrates, alkaloids, and soluble lignin.

The non-extractives mainly consist of inorganics mostly present in ash minerals. The dominating components are alkali and alkali earth carbonates and oxalates. Silica deposited as crystals is especially abundant in straws; furthermore, small amounts of non-cell wall substances, such as starch, pectin, and protein, are not extractable [28].

In spite of the fact that they exist in small quantities, extraneous compounds play a very significant role in that they render cellulose not only resistant to decay and insect attack but also inhibitive to pulping and bleaching.

2.1.2 Polysaccharides

The polysaccharide component comprises high molecular weight carbohydrates, namely, cellulose and hemicellulose, which amount to 60 to 80 % of the total wood. Cellulose is the major component of cell walls of wood fiber, and it is a linear polymer of D-glucose with a high molecular weight of approximately half a million. Individual glucose molecules are linked together by β-1,4 linkages to form a highly crystalline material that is resistant to enzymatic hydrolysis [16]. The number of chain units, the so-called degree of polymerization or DP in short, varies for different cellulosic materials (Table 2.1). The glucosidic bonds in chain molecules, along with the hydroxyl groups, mainly determine its chemical properties [16]. The innumerable hydrogen bonds, holding the chains together, are not broken by water; cellulose is completely insoluble in water. However, strong acids, strong alkalis, concentrated salt solutions, and various complexing reagents can swell or disperse and even dissolve the cellulose [23].

Table 2.1. Molecular weight and degree of polymerization of cellulosic materials

Cellulosic material	Molecular weight	Degree of polymerization
Before hydrolysis [43]		
Native cellulose	600,000–1,500,000	3,500–10,000
Chemical cottons	80,000– 500,000	500– 3,000
Wood pulps	80,000– 340,000	500– 2,100
Rayon filament	57,000– 73,000	
After hydrolysis with 2.5 N HCl at 105 °C for 15 min [6]		
Natural fibers:		
Ramie, hemp		350–300
Cotton, purified		250–200
Unbleached sulphite wood-pulp		400–250
Bleached sulphite pulp		280–200
Mercerized cellulose (18% NaOH at 20 °C, 2 h)		90– 70
Vibratory milled wood cellulose		100– 80
Regenerated fibers:		
Fortisan, fiber G		60– 40
Textile yarns		50– 30
Tire yarns		30– 15

Hemicellulose is composed of shorter chain polysaccharides, and it is the principal non-cellulosic fraction of polysaccharides. The role of this component is to provide a linkage between lignin and cellulose. In its natural state, it exists in an amorphous form and can be divided into three groups, namely, xylans, mannans and galactans; these groups can exist separately as single components or collectively [4, 9, 29, 37]. The xylans are present as arabinoxylans, glucuronoxylans, or arabinoglucuronoxylans; the mannans are present in wood as glucomannans and galactomannans; and the galactans are relatively rare but are often found in the form of arabinogalactans [29, 41].

Xylan is a polymer of 4-O-methylglucuronoxylan and 4-O-methyl-glucuronoarabinoxylan linked by a β-D-(1 $\rightarrow$ 4)-bond, similar to the linkages of the glucose units in cellulose. In xylan, however, the hydroxyl groups are substituted with 4-O-methylglucuronic acid, arabinose and acetyl groups [35].

The mannan groups in the glucomannans from deciduous wood and coniferous wood are different; the former is characterized by the absence of galactose units. Thus, it is assumed that the glucomannans have a linear structure so that there is no branching at either of the two carbon positions, namely, (C-2) or (C-3). The presence of short side-chains of galactose units in the glucomannans from coniferous woods may be considered to be certain. Linnell and Swenson [32, 33] also have proposed that the configuration of the glucomannan from black spruce has a linear polysaccharide structure and that the glucomannan and lignin occur in the fiber in the form of a cross-linked matrix with a considerable number of lignin-carbohydrate bonds.

A possible structure of the arabinogalactan from tamarack had been proposed by Adams [1], based on the evidence of methylation and periodate oxidation. Later,

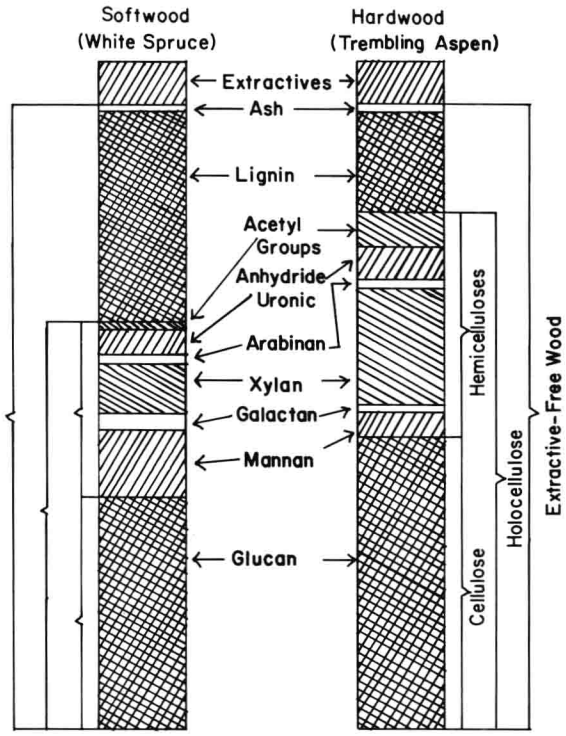

Fig. 2.2. Comparison of the compositions of hardwood and softwood [8]

Haq and Adams [25] have proposed a further modification to its structure. A possible structure for repeating units in galactans from other larch species has been proposed by White [54] and Aspinall et al. [5]. According to White [54], this structure is characterized by a main chain of 1, 6 linked galactose units substituted in position 3 by a secondary chain of three $1 \rightarrow 3$ linked D-galactose residues. According to Aspinall et al. [5], the structure consists of a $1 \rightarrow 3$ linked D-galactose main core with two $1 \rightarrow 6$ linked D-galactose units attached to it through position 6. Hardwood hemicelluloses are rich in xylan polymers with small amounts of mannan, whereas softwood hemicelluloses are rich in mannan polymers and contain significant quantities of xylans, as shown in Fig. 2.2 [8].

2.1.3 Lignin

Lignin is probably the most complex and least well characterized molecular group among wood components [48, 52]. It is essentially a three dimensional phenylpropane polymer with phenylpropane units held together by ether and carbon-carbon bonds [20]. The amount of lignin constitutes 20–35 % of the wood structure. Lignin possesses a high molecular weight and is amorphous in nature. In wood, the lignin network is concentrated between the outer layers of fibers as illustrated in

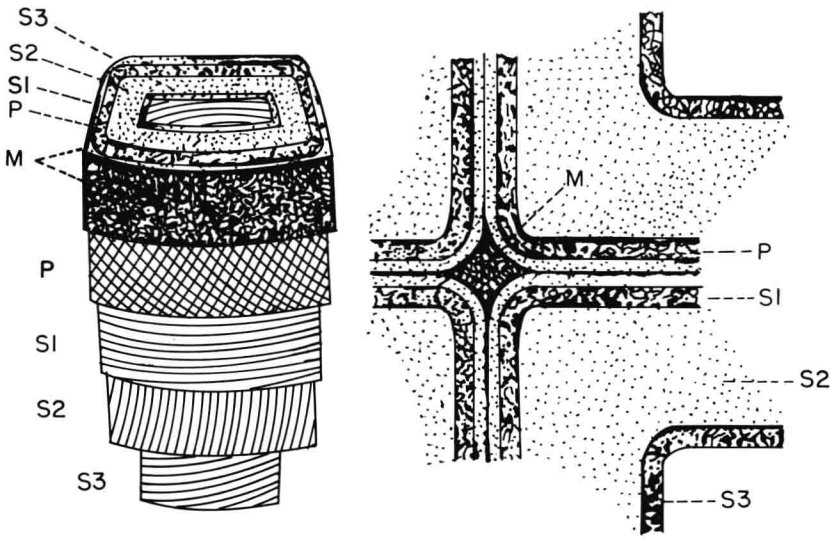

Fig. 2.3. Diagrammatic sketch of the intricate structure of wood cell wall [12]

Fig. 2.3. The lignin gives structural rigidity by stiffening and holding the fibers of polysaccharides together [13].

The lignin from grasses, softwoods, and hardwoods differs somewhat in composition, mainly in methoxyl substitution, and the degree of linkage between phenyl groups. Nevertheless, their common structural features predominate [27]. Figure 2.4 shows the schematic structure for spruce lignin [3].

The arylglycerol-β-aryl ether structure (Units 1–2, 2–6, 6–7, 7–8, etc. in Fig. 2.4) has been found to be the most abundant interphenylpropane linkage in lignin [2, 15, 26, 30, 45]. It plays a dominant role as a connecting link between the phenylpropane structural units. The phenylcoumaran structures (Units 4–5 and 15–16 in Fig. 2.4) have been identified by Sakakibara [45] and Freudenberg [21], respectively. The diarylpropane structures (Units 3–4 in Fig. 2.4) have been isolated as hydrolysis products of coniferous [47] and hardwood lignin [34, 38].

Ericksson et al. [15] and Larsson-Miksche [30] have estimated the amount of biphenyl structure (Units 11–12 and 16–18 in Fig. 2.4) in lignin as being approximately 0.095–0.11 per C_6–C_3 unit. Diphenyl ether compounds, containing the 4-O-5 linkage (Units 8–9 and 13–16 in Fig. 2.4), have been isolated [22, 39, 46]. The amount of this structure has been estimated to be approximately 0.035–0.04 per C_6–C_3 unit [15].

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