Acid and enzymatic hydrolysis of autohydrolyzed lignocellulosic substrates
by David Allen Lamar

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University

Abstract:
Four biomass residues (barley straw, wheat straw, lodge pole pine, and Douglas fir) were analyzed for effects of an autohydrolysis pretreatment on lignin extractibility and cellulose hydrolysis, both acid and enzymatic catalyzed. A pure cellulose substrate, Chromedia, was also used for hydrolysis tests.

The conditions used for the autohydrolysis were 205 °C and 10 minutes. These conditions were those found to be optimal for lignin extractibility from wheat straw during previous work at this laboratory.

The extractibility of lignin (by an ethanol-water solvent) following pretreatment was very similar for barley straw and wheat straw. A total of about 75% of the lignin was removed during the autohydrolysis and subsequent solvent extraction. The amounts of lignin removed from the two woods were also very similar with about 30% of the lignin being removed.

Experiments were performed to determine the effects of lignin content and substrate morphology on acid hydrolysis of lignocelluloses. These experiments involved hydrolyses on lignin-free substrates and substrates containing lignin. The delignification procedure resulted in a substrate that was no more hydrolyzable than a non-pretreated substrate with the lignin intact. Acid hydrolyses on ball-milled Chromedia revealed that as the amorphous content of the substrate increases the rate of hydrolysis also increases.

Pretreated and non-pretreated substrates were hydrolyzed using both sulfuric and hydrochloric acids and enzymes to determine the pretreatment effect on degree of hydrolysis. Pretreatment of wood substrates resulted in only slightly increased carbohydrate conversion via acid hydrolysis over that observed for non-pretreated woods. No increase in hydrolysis rate was observed for pretreated straw substrates when acids were used as the catalytic agents. When mixed enzymes were substituted for acids in wheat straw hydrolysis, the cellulose conversions increased dramatically for pretreated substrates, with values in excess of 90% observed.

A theory based upon the solubility of reaction products is presented to explain higher cellulose conversions with mixed enzymes versus acid hydrolysis results. This theory leaves open the possibility that autohydrolysis pretreatment renders all substrates investigated more subject to hydrolysis.

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APPROVAL

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David Allen Lamar

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citation, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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Date May 27, 1987

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ABSTRACT

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INTRODUCTION

The realization that petroleum supplies are not limitless has awakened the U.S. to the need of an alternative hydrocarbon source for fuel and chemical feed-stocks. A search has been focused on determining a hydrocarbon source that does not compete with food supplies or other valuable raw materials. An ideal source of hydrocarbon might be waste or by-product biomass from agricultural operations or wood product industries. The utilization of this biomass would benefit the above industries by converting low value materials into more valuable commodities.

The energy requirement of the U.S. is roughly 76 quadrillion BTU's (Quads) per year. The generation of this energy would require 40 million barrels of oil per day. A major goal is to replace expensive petroleum used in energy production with low cost biomass [1].

Presently, two to three Quads of the U.S. energy requirements are supplied by biomass utilization. Combustion of forest products is the primary source for this energy. A conservative estimate of the energy that will be supplied by utilization of biomass by the end of this century is 15 Quads [1].

Biomass for energy could be supplied from direct and indirect sources. Direct sources might include farms developed to grow plants solely for energy uses, while indirect sources would include agricultural and forest waste or by-products mentioned above. The indirect sources are of primary interest in this study.

It is estimated that 278 million dry tons of agricultural by-products and 108 million dry tons of unused mill and logging residues are produced annually [1]. Theoretically, if these materials were converted to glucose and the glucose fermented to alcohol, approximately 30 billion gallons of ethanol could be produced per year. This ethanol would meet the entire current industrial demand and provide ethanol for gasoline blending

as well. Most of the present industrial grade ethanol is now produced from petroleum-based feedstocks. This use of biomass would therefore reduce the demand for petroleum [1].

The above estimate of ethanol from lignocellulose requires a 90 percent conversion of the cellulose to glucose. Barriers exist in lignocelluloses that prevent such high conversion of cellulose to glucose. Existing technologies only provide about a 50 percent cellulose conversion. A higher conversion is desirable to economically produce cellulose-derived products.

Research Objectives

The first objective of this investigation is to test three lignocellulosic substrates for degree of lignin removal and enhanced hydrolysis of their cellulose to glucose after a novel pretreatment. The substrates to be investigated are barley straw, lodge pole pine, and Douglas fir. These materials will be pretreated by autohydrolysis at conditions found to be optimum for lignin removal from wheat straw during previous work at this laboratory.

The second objective is to investigate reasons that might explain the low acid hydrolysis yields observed with pretreated wheat straw. Meeting this objective entails applying new hydrolytic catalysts and/or conditions to the hydrolyses in an attempt to increase yields.

STRUCTURE OF LIGNOCELLULOSE

Forest and agricultural residues consist of several primary components. These components include cellulose, hemicellulose, lignin, protein, and miscellaneous extractibles. Table 1 summarizes the percent composition of individual components by weight.

Table 1. Major Component Composition of Lignocellulose [2].

Component	% Composition
Cellulose	45-50
Hemicellulose	20-25
Lignin	20-30
Extractible and Protein	0-10

Together these components make the basic structural unit of biomass, the plant cell. A cell, in simple terms, is composed of two basic parts, the lumen and the cell wall. The lumen contains the living matter of the cell. Once dead the cell's lumen is either void space or filled with extractibles [2]. The cell wall serves as a mechanical divider between individual cells. Rigidity of a plant stalk is the direct result of its cell walls.

The cell wall also consists of two parts, the primary wall and the secondary wall. The primary wall is very thin in comparison with the secondary wall. The secondary wall consists of three distinct layers termed the outer (S_1), middle (S_2) and inner (S_3) layers [3]. Surrounding the primary wall and separating adjacent cells is the middle lamella.

Figure 1 [1] is a representation of the basic structure of a plant cell. Cellulose is mainly in the microfibrils, shown as lines in the diagram. Orientation of the fibrils is different in the respective portions of the cell wall.

The amount of cellulose in the plant is highest in the secondary wall and decreases toward the middle lamella. Hemicellulose has its

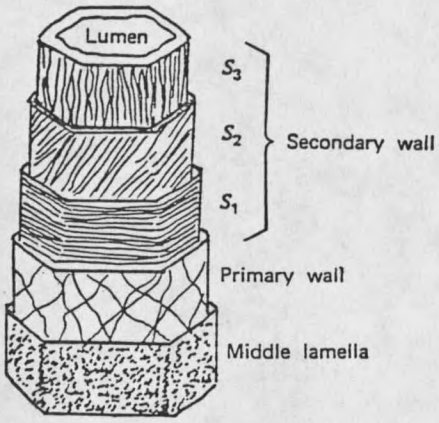

Figure 1. Basic Structure of a Plant Cell [1].

highest percentage in the middle lamella and decreases toward the lumen. Hemicellulose and lignin form a matrix that surrounds the cellulose fibrils. Lignin and hemicellulose are also found in the spaces between the crystalline regions of the microfibrils, the amorphous (non-crystalline) regions [2].

Cellulose

Cellulose is a linear polymer of D-anhydroglucose molecules serving as the monomer. These monomers are linked by β-1-4-glucosidic bonds. Figure 2 is a schematic of a cellulose molecule. Cellulose degree of polymerization ranges from 3,500 to 14,000 glucose units when in a native form. The average length of cellulose molecules range from 2,500 nm to 5,000 nm [2,5].

The linear molecules lay one on another forming bundles of molecules, fibrils, that are held together by lateral hydrogen bonding. The large number of hydrogen bonds result in crystalline regions of about 60 nm in length that comprise 67 to 90% of the cell wall. Since the cellulose molecule is longer than 60 nm, the molecules pass through several crystalline and amorphous regions [2]. Fibrils are surrounded by a sheath of hemicellulose and lignin [5].

The apparent morphology of cellulose depends on the methods of analysis and also the source of the cellulose. At present cellulose is

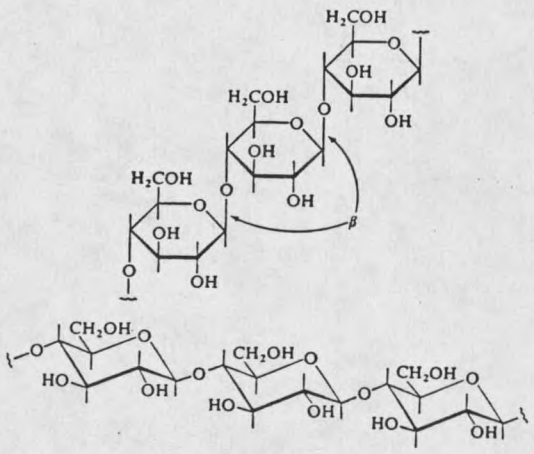

Figure 2. Chemical Structure of Cellulose.

categorized into four distinct types, cellulose I, II, III, and IV. Each group is based on the aggregation of molecules in the crystalline solid. Degree of crystallinity decreases with cellulose type (i.e., Cellulose I is more crystalline than Cellulose II, and so forth). The class to which a particular cellulose belongs depends on the method used to produce the pure cellulose. The class is identified by the x-ray diffraction pattern of the sample [4].

Cellulose in its native form is classed Cellulose I. Cellulose II is a cellulose that has been regenerated from solution at ambient temperatures or one that has been mercerized with caustic solution in excess of 15% sodium hydroxide. Cellulose I and II are the most common morphologies. Treatment of cellulose with anhydrous ammonia or one of several different amines produces cellulose III. Heat treatment of cellulose II or regeneration of cellulose from solutions at elevated temperatures results in cellulose IV [4].

Hemicellulose

Hemicellulose is a polymer of simple sugar molecules like cellulose, though it consists of more than one type of sugar. The backbone of the polymer is a linear chain containing D-xylose sugar units linked together by β-1-4-glucosidic bonds. Unlike cellulose, hemicellulose is not a linear homopolymer. It contains side chains branching from

the main chain of D-xylose sugars. The branches are usually bonded to the xylose molecules via 1-3 glycosidic links, but they can also contain 1-4 and 1-6 glycosidic bonds. The side chains can contain glucose, xylose, galactose, mannose, arabinose, and uronic acids of glucose and galactose. Composition of a particular hemicellulose varies from source to source, not only with plant species, but also with climate and location of the particular plant. Degree of polymerization of hemicellulose ranges from 100 to 200 molecules but rarely exceeds 200 [1,2].

Hemicellulose does not form crystals like cellulose and is found only in an amorphous state. It is usually in intimate contact with plant lignins. It is thought that the hemicellulose and lignin are chemically bonded together.

Lignin

Lignin is a highly complex, three-dimensional polymer of various phenolic acids connected by ether linkages. Unlike the other components discussed so far, lignin has no set pattern of structure. Figure 3 [1] presents a possible structure of lignin.

Lignin acts as the cement that holds the cellulose fibrils together. It is an integral part of a system that gives plants their strength and rigidity. This polymer not only acts as a cement but also as a protective shield against elements that would otherwise destroy the plant by attacking the cellulose. Investigations have shown that lignin can limit the microbial degradation of plant polysaccharides [6].

Structure as It Relates to Hydrolysis

A lot of work has been done to determine how the chemical and physical structure of lignocellulose inhibits hydrolysis of the carbohydrate components. The following is an overview of this work and some of the conclusions that have been drawn.

Many different aspects of plant structure and chemical make-up

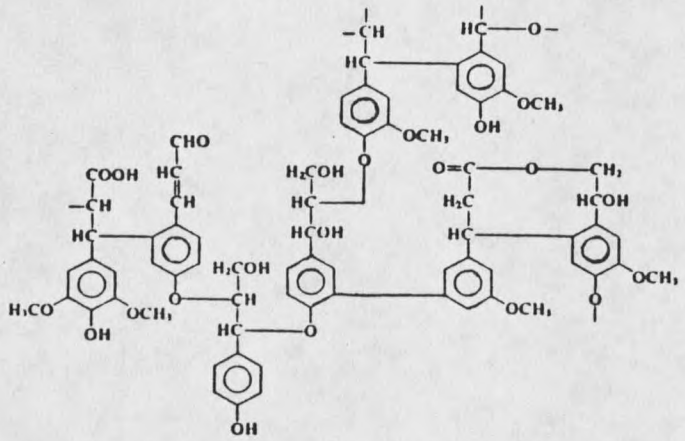

Figure 3. Structure of a Portion of Lignin [1].

have been identified as detrimental to the hydrolysis of biomass. These are the hemicellulose-lignin barrier, crystallinity of the cellulose, surface area, degree of polymerization of cellulose, and pore size distribution. Some of these properties act synergistically to prevent hydrolysis. The ramifications of individual aspects will be discussed here.

Hydrolysis of cellulose can be catalyzed by either acids or enzymes. Although chemically the results are the same, the mechanisms are quite different. A successful acid catalyzed hydrolysis (see Figure 4) takes place when the oxygen atom of the β-1,4-glycosidic bond is attacked by a hydrogen ion. This attack results in a positive charge on the oxygen which then pulls electrons from the oxygen-carbon bond resulting in a partial positive charge on the carbon atom. Non-bonding electrons of a water oxygen atom are attracted to this partial positive charge. Electrons from the original carbon-oxygen bond form a bond with the attacking hydrogen ion, and hydrogen-oxygen bonding electrons from the water molecule form a bond between the carbon atom and the attacking oxygen atom releasing a hydrogen atom. This series of events results in the breaking of the β-1,4-glycosidic bond by the addition of a water molecule in between two glucose monomers of the cellulose molecule. A proposed mechanism for an enzyme catalyzed hydrolysis is presented later in this work.