Autohydrolysis and deligninfication of wheat straw
by Ronald Kurt Nakaoka

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

Abstract:
In an effort to increase the yield of glucose from cellulose in wheat straw, a novel pretreatment consisted of two procedures, autohydrolysis followed by an aqueous ethanol extraction. The anticipated effect of these two processes was a substantial reduction of hemicellulose and lignin present in the lignocellulosic matrix and production of cellulose pulps which hydrolyze readily.

The conditions of autohydrolysis, which include high temperature and a dilute acid environment, serve to disrupt the lignocellulosic matrix. Up to 95% of the hemicellulose originally found in wheat straw was solubilized and removed from the solid during autohydrolysis. In addition, lignin molecules were fragmented by these same conditions. A portion of the lignin fragments were soluble in the autohydrolysis media. Additional lignin fragments were shown to be soluble in the aqueous ethanol media of the extraction step. Thus, by using a combination of an autohydrolysis and an aqueous ethanol extraction step the solid substrate was upgraded from 39.3 weight percent total cellulose (alpha and beta-cellulose) present originally to 76.3 percent in the residue.

Autohydrolysis time at temperature determined the amount of lignin removed by the aqueous ethanol extraction. For a given autohydrolysis temperature the lignin weight percent of the solid residue exhibits a minimum value as a function of time at temperature. If lignin fragments were exposed to autohydrolysis conditions for excessive lengths of time the soluble fragments apparently began to repolymerize. These higher molecular weight polymers were no longer soluble in the extraction media. Thus, for each autohydrolysis temperature there was a specific time at temperature to correspond to a maximum lignin removal.

Dilute acid hydrolyses of cellulose pulps following autohydrolysis alone and combined autohydrolysis and alcoholic extraction were in all cases much slower than expected. Rates were on the order of those obtained using untreated straw. Air drying of pulps is suspected of causing morphology changes that inhibit hydrolysis.

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APPROVAL

of a thesis submitted by

Ronald Kurt Nakaoka

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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TABLE OF CONTENTS

TABLE OF CONTENTS—Continued

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

In an effort to increase the yield of glucose from cellulose in wheat straw, a novel pretreatment consisted of two procedures, autohydrolysis followed by an aqueous ethanol extraction. The anticipated effect of these two processes was a substantial reduction of hemicellulose and lignin present in the lignocellulosic matrix and production of cellulose pulps which hydrolyze readily.

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INTRODUCTION

Motivation for Alternative Energy

On October 18, 1973 the representatives of the thirteen nations that would make up the Organization of Petroleum Exporting Countries (OPEC) met in the small country of Kuwait. At that historic meeting most of the leading oil producing countries of the world decided that it would be in their best interest to limit the number of barrels of oil they produced annually. The news of the OPEC oil embargo swept across the world causing panic, especially in the major industrial nations of the west. In the resulting scramble for OPEC oil, prices skyrocketed from $2.10 per barrel to $10.00 per barrel almost overnight [1]. The dramatic increase in oil prices and limited supply of petroleum products forced the majority of people in the United States to realize just how closely the country was tied to the supply of foreign oil.

The harsh reality of the 1973 oil embargo set in motion a great increase in the search for alternative energy sources. Spurred on by a general fear of dependency on foreign producers, private industry, universities, and government agencies all set out to lessen the country's need for oil. Some of the major research efforts have been in geothermal and solar energy, synthetic fuels and biomass energy conversions.

In the last five years, the energy "crisis" has lessened considerably. Experts credit the decrease in our dependence on foreign oil not to new energy sources, but to simply being able to get along with less. America has reduced its oil consumption from a high of 18.8 million barrels per day in 1978 to 15 million barrels per day in 1983 [2]. Only 4.3 million barrels of oil daily were imported in 1983 compared to twice that number in 1978 [1]. Because of this decline in consumption and a decline in the world price of oil ($34 per

barrel in 1982 to $29 per barrel in 1984 [1]), the interest in alternative energy sources has also declined. Much of the funding for these projects has been cut or discontinued, and very little money for new projects is being allocated by private industry or the federal government.

The state of Montana, on the other hand, recognizes the fact that existing energy resources within the state and the world are slowly being depleted. In an effort to stimulate the utilization of the state's natural resources (other than fossil fuels) the Department of Natural Resources and Conservation (DNRC) provides funding for renewable energy related projects. The main source of revenue for this effort by the DNRC comes from the coal severance tax. These projects are usually undertaken by private individuals, private companies, or universities and colleges.

Crop Residue Availability in Montana

The Montana DNRC foresees an energy related use for the abundant crop residues produced within the state. In 1981, Montana had the fourth largest production of wheat in the United States, 173 million bushels, and also ranked fourth in the production of barley with 57 million bushels [3]. Over 7.5 million acres of land in the state were devoted to cereal grain production in 1981 with six million acres producing some type of wheat [3]. Table 1 uses straw:grain factors developed by Smil [4] for worldwide estimates of crop residue production to approximate residue yields resulting from 1981 grain figures.

As Smil warns, estimates of crop residue production can be off by significant margins because of the wide range of variables that are incorporated in straw:grain ratio factors [4]. However, it is obvious that Montana has an abundant supply of crop residues available for energy uses. For these reasons, the DNRC has funded basic research in the area of developing pretreatments for crop residues that would enhance the potential for cellulose fermentation to ethanol for fuel uses.

Table 1. Montana Crop Residue Estimates for 1981 [3,4].

Residue	Yield (million tons)
Wheat Straw	7.8
Barley Straw	1.6
Oat Straw	0.12
Corn Stover	0.07

Fermentation Ethanol Production

In 1981, the United States produced 415 million gallons of 190 proof ethanol [5]. Of this production, over half was generated using fermentation technology. The resurgence of fermentated ethanol is due to the policies instituted during the Carter Administration. Normally, the capital and production costs of fermentation make the final product non-competitive in the industrial chemical market. The Carter plan was to increase ethanol production in the U.S. in order to lessen the country's dependency on oil. It included tax credits and low interest loans anwhich made ethanol production possible for many companies not involved in the beverage industry. Of the 230 million gallons of ethanol produced from biomass in 1982, 70 to 90 million gallons were used in energy-related areas [5], most notably in gasohol, and 30 million gallons found their way into the industrial market [6].

In 1982, the actual capacity for producing ethanol by fermentation techniques was 600 million gallons [6]. But, because of declining oil prices, lessened demand for gasoline and the abandonment of ethanol policies by the Reagan Administration, fermented ethanol may never reach its full production capacity. The conversion of ethylene from petroleum feedstocks will remain the main source of industrial ethanol until either fermentation production costs decline and/or petroleum prices increase.

Use of Crop Residues as Feedstocks

Current production of ethanol from biomass feedstocks involves the use of grains and natural sugars. The technology for the conversions of sugars and starches to ethanol is well developed. Obviously, it is the high cost of materials, particularly feedstocks (over 65% of the total production costs [7]), that makes this process noncompetitive. On the other hand, the use of agricultural residues as feedstocks for fermentation has just the opposite problems. The starting material itself is very inexpensive. The major costs involve collection and transportation of the residues. Also, unlike grain, there are very few uses for residues other than use as bedding, ground cover and some feed uses which make up only a small percentage of the total amount produced. The problem is one of technology. At present, there are no existing commercial processes that convert the cellulose in agricultural residues to glucose with sufficient yields at competitive prices.

Historical Processes

The idea of using an inexpensive and readily available feedstock such as agricultural residues or wood for a fermentation process is not a new one. During times of crisis many countries have turned to residues for energy. The early techniques were based on the fact that cellulose in lignocellulosic residues is a polymer that consists of a large number of glucose monomers joined by glycosidic bonds. Thus, the most logical process for utilizing cellulose involves an acid hydrolysis; the large cellulose molecule is broken down into single unit sugars, monosaccharides. The monomers are then fermented to produce ethanol.

Table 2 is a summary of the major acid hydrolysis techniques employed in the past. These processes can be split into two major categories, dilute acid and concentrated acid techniques. They were used commercially only during times of war when the economy was under great pressure to produce enough materials to support the war effort. After demand

Table 2. Historical Acid Hydrolysis Processes [8,9,10].

Process	Country	Date of Operation	Type	Acid	Time	Acid Concentration	Temperature	Yield
			Dilute Acid					
American	USA	1913-1926	batch	H_2SO_4	15 min	0.5-2%	155-175°C	14-23%
Scholler	Germany	1920s	percolation	H_2SO_4	12 hr	0.5-0.8%	140-190°C	50%
Madison	USA	WW II	continuous percolation	H_2SO_4	3 hr	0.5%	150-180°C	55%
			Concentrated Acid					
Rheinau	Germany	1932-1959	countercurrent percolation	HCl	3 hr	41%	< 25°C	90%
Noguchi	Japan	1953-1959	absorption of HCl gas	HCl	< 1 hr	60-70%	50°C	85%
Hokkaido	Japan	WW II	spray mixing	H_2SO_4	< 1 hr	80%	25°C	85%

declined with the end of military activity and the economy returned to a more normal level, these processes were unable to compete because of the high cost of production and/or the low yields.

The first commercial processes were instituted during World War I and employed dilute acid techniques. These initial attempts at hydrolysis of wood were carried out in batch reactors (see Table 2). The dilute acid technique was improved during the 1920s in Germany and later utilized in the United States during World War II. In the Madison process (similar to the Scholler process) dilute acid was percolated through a stationary bed of wood chips. This modification limited contact time between acid and sugars and avoided the large decomposition fraction encountered in batch reactions. At the high temperatures used in dilute acid techniques even weak acids tend to take hydrolysis beyond the desired products; glucose and xylose are broken down to furfural and other decomposition products. The major problems with dilute acid techniques included the large volumes of acid solution that had to be recycled, low concentrations of sugars in the resulting fermentation liquor, and low yields due to an inability to dissolve crystalline cellulose.

The concentrated acid techniques utilized stronger acids and lower temperatures than the dilute processes. The lower temperatures resulted in lower decomposition rates and allowed for higher overall conversions of crystalline cellulose. These processes used either hydrochloric acid or sulphuric acid and were used by Germany, Japan and Italy during World War II. Because of high acid concentrations, the capital costs of these techniques were higher than for dilute solutions. Also, because of the need for strong acids, the moisture content of the substrate had to be reduced, and recycling of the acid needed to be very efficient to support the higher yields.

Current Projects

The historical inability to utilize lignocellulosic residues as sources of liquid fuel is the result of the intricate structure of the material itself. The residues being considered as fuels have a high cellulose content. Because the long chain polymer is intertwined with hemicellulose and lignin, hydrolysis rates are slowed. Without pretreatment, the hydrolyzing agents, either enzymes or acids, have limited access into and out of the lignocellulosic matrix.

The majority of current pretreatment projects in the area of converting residues to glucose focus on improving the accessibility of the cellulose polymer. The Iotech process, developed in Canada, makes use of a steam "explosion" of the feedstock and a subsequent enzymatic hydrolysis [11]. The explosion physically separates the constituents of the residue and makes them more susceptible to hydrolysis. Another pretreatment, developed by the paper and pulp industry [12], is being investigated at the University of Pennsylvania with the support of General Electric. This process uses hot aqueous ethanol as a means of removing lignin prior to a combined enzymatic hydrolysis and fermentation [13]. Other research efforts that involve a pretreatment include the use of dilute acid at moderate temperature for a short time in an attempt to hydrolyze only the hemicellulose fraction [14].

Another major effort in the development of residue conversion involves a single-step process that converts lignocellulose to glucose in high yields. The two most successful efforts to date in this area are the Gulf/University of Arkansas and the New York University projects. The former makes use of a fungus developed by Gulf Oil. This fungus produces enzymes that can hydrolyze cellulose to glucose in the presence of lignin. The New York University project employs the old and the new; dilute acid hydrolysis is accomplished in a twin-screw extruder that allows for very precise control of temperature and residence time.

As of yet, none of the projects outlined earlier has yielded a definitive process for the conversion of lignocellulosic biomass to chemical feedstocks or liquid fuels. For this reason the search continues.

Research Objectives

The intent of this investigation is to develop and characterize a pretreatment sequence for enhanced subsequent hydrolysis of wheat straw cellulose to glucose. Experimental apparatus and procedures will be developed to optimize and reproducibly control conditions of the pretreatment sequence. A set of analytical methods for determining the compositions of the resulting products will be developed.

BACKGROUND ON LIGNOCELLULOSE

> The investigator who wishes to analyse plant material for its content of cellulose or hemicellulose must first of all be clear in his own mind about the questions which the analysis is expected to answer. It cannot be too strongly emphasised that "cellulose" and "hemicellulose" are normally determined as the resultants of certain sets of operations, rather than as chemically defined species [17].

Because of the intricate nature of a plant cell the three major constituents, cellulose, hemicellulose, and lignin can be defined on two different levels. They can be described in terms of their ideal chemical nature or as actual physical products of experimental isolation. The ideal chemical nature of a plant cell is a description of the components of the cell in their natural state. These components, when isolated physically, do not necessarily resemble their composition or configuration as they exist in the plant cell. This complication of identification is the result of the complex anatomy of a plant.

Cell Wall Organization

The plant cell is made up of a primary wall and three secondary walls (see Fig. 1).

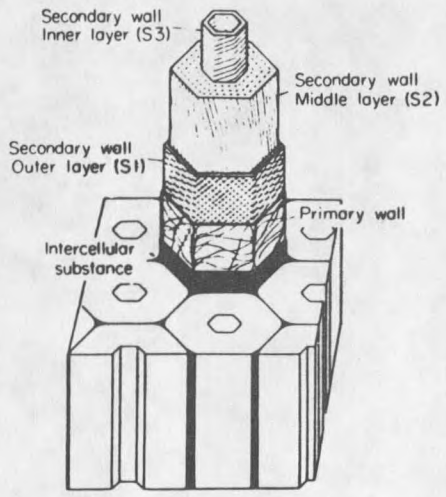


Figure 1. Typical structure of a plant cell wall [18].

Within these four walls there are varying amounts of cellulose, hemicellulose, lignin and other organic compounds. The actual composition of cell walls differ depending on age, history, location within the plant, and a wide variety of other factors [18]. The construction of the cell wall is based on cellulose. The smallest unit of a plant fiber is the elementary microfibril that consists of approximately one hundred cellulose molecules intertwined to form a unit which has a diameter of about 15-35 Å. The elementary microfibrils form a larger bundle, called a microfibril (100 to 300 Å in width) which in turn is part of an even larger unit, the macrofibril. The macrofibril is approximately 0.4 microns in width, and is made up of around 500,000 cellulose molecules in transection [19].

Interspread within all the individual cellulose units in the cell wall are hemicellulose, lignin and other organic compounds. These compounds interact with the cellulose backbone and one another by means of hydrogen bonds, Van der Waals forces and in some cases covalent bonds [8]. Because of this complex organization the extraction and isolation of any one component in a pure, natural state is almost impossible.

Cellulose

Cellulose is the major constituent of the cell wall; the structure is shown in Figure 2. Chemically speaking, cellulose is a long chain polymer that consists of β-D(+) glucose molecules in the pyranose form linked together by 1,4-glycosidic bonds to form the oligosaccharide, cellobiose (4-0-β-D-Glucopyranosyl-D-glucopyranose). Cellobiose is the repeating unit of the cellulose polymer. Cellulose has a degree of polymerization of 10,000 to 50,000 glucose units. This number varies from plant to plant as well as within the fine structure of an individual plant.

Cellulose, as it occurs in plants, is organized in bundles as was discussed earlier. The structure of the smallest unit, the elementary fibril, is arranged in such a way that the glucose units occur at uniform distances apart; a space lattice is formed. Because of this spatial

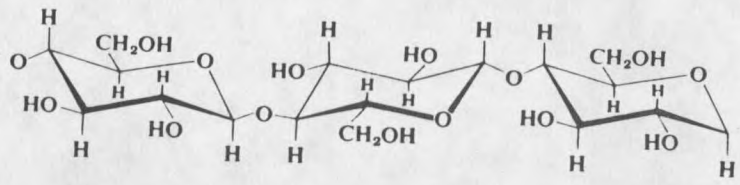


Figure 2. Cellulose molecule [18].

order, cellulose exhibits an X-ray diffraction pattern that is the same for almost all natural cellulosic materials [20]. Crystalline or native cellulose is known chemically as Cellulose I. Cellulose II is cellulose that has been regenerated after being chemically altered or that has been precipitated from solution. Cellulose II has a different X-ray pattern than Cellulose I and has been found to occur naturally only in some marine organisms [20].

A cellulose macrofibril is not completely crystalline in nature, as can be seen from X-ray diffraction patterns. The fiber is made up of crystalline and amorphous regions. These amorphous regions are not really understood but have been explained as being sections of the polymer that are bent or as interfibril molecular connections [21].

In this investigation cellulose is defined by the methods of analysis used in the laboratory. The sequence of operations begins by determining ash content using American Society for Testing Materials method (ASTM) D1102-56, followed by a moisture determination and an ethanol-benzene extraction, which removes waxes, fats, some resins and gums (Technical Association of the Paper and Pulp Industry (TAPPI) standard method T12 os-75). The procedure to remove the lignin was dictated by Browning [22] and is a modification of TAPPI standard T9m. The remaining residue is defined as cellulose. As outlined in TAPPI standard T203 os-74, alpha cellulose is the fraction of the residue that is resistant to 17.5 and 9.45 percent sodium hydroxide solutions under the conditions of the standard test. Beta cellulose is the soluble portion of the residue that is precipitated upon acidification of the solution. Gamma cellulose is defined as that fraction that remains soluble in the acidified solution. In general, the alpha cellulose fraction corresponds to undegraded, high

molecular weight cellulose, while beta cellulose corresponds to broken chain or degraded cellulose and gamma cellulose is mainly hemicellulose.

Hemicellulose

Hemicellulose, unlike cellulose, varies in chemical structure depending on the species of plant. The majority of hemicelluloses are made up of pentose sugars along with a minority of hexose sugars. The hemicellulose fraction of the cell wall is much lower in molecular weight, 100 to 200 sugar units and does not exhibit the crystalline nature of cellulose. For these reasons, hemicellulose is much easier to hydrolyze than cellulose [8].

Wheat straw hemicellulose, like most other plant hemicelluloses, consists of more than one type of monosaccharide. Additionally, wheat straw contains two distinctly different types of hemicellulose, cellulosans and polyuronides [23]. The differences between these two types of hemicellulose are not well understood. Cellulosans are more closely tied to the cellulose fraction of the cell wall and consist of xylans and glucans. This type of hemicellulose is more oriented in terms of structure and is arranged longitudinally, much like the glucose molecules of cellulose. Polyuronide hemicellulose, on the other hand, is more closely linked to the lignin present in the cell wall. This type of hemicellulose is very difficult to separate from lignin using conventional techniques, and many times, portions are removed during a delignification procedure. Polyuronides consist of xylose, arabinose, uronic acids, glucose, and are most likely the side chains or branches of the cellulosan polymer [23].

The constituents of wheat straw hemicellulose have been determined by Reddy et al. [24] using a procedure to break the hemicellulose into monosaccharide units. From their study it was shown that wheat straw hemicellulose is made up of 7.8% uronic acids, 11.6% arabinose, 74.4% xylose, and 6.2% glucose (weight percentages). As was reported by Aspinall [25], the wheat straw hemicellulose is arranged as xylan linear polymers with a

great variety of mono and polysaccharide side chains. In this investigation, hemicellulose is defined by TAPPI standard T203 os-74 and is referred to as gamma cellulose as outlined above.

Lignin

Lignin is the third major component of the lignocellulosic matrix. Unlike some cellulose and hemicellulose, lignin is not easily hydrolyzed and does not show the consistency in structural arrangement that the other components do. Instead, lignin is made up of a highly complex, three dimensional network of phenylpropane units that intertwine and surround the carbohydrate components of the cell wall. The lignin fraction of the cell wall gives the plant added rigidity and serves to protect the plant fiber from natural hydrolysis.

Because of the close association between the materials that make up the cell wall, the isolation and characterization of a large, highly branched molecule like lignin is very difficult. Many theories on structure and techniques for isolating lignin in a more natural state are relatively new and are outlined in detail by Sarkanen and Ludwig [26]. The actual structure of lignin differs from species to species, but many of the woody plant lignins have common features.

The actual interactions that occur within the lignocellulosic matrix have not been fully determined. As was noted earlier, there is a strong relation between the polyuronide hemicellulose and lignin. It has also been postulated that an acetal or hemiacetal linkage exists between the carbonyl groups of lignin and the hydroxyl groups of the carbohydrates [28].

Lignin is defined in this investigation as the material that is removed by a laboratory experimental sequence that includes chlorination, washes with dioxane, 5% monoethanolamine in dioxane and water [22].

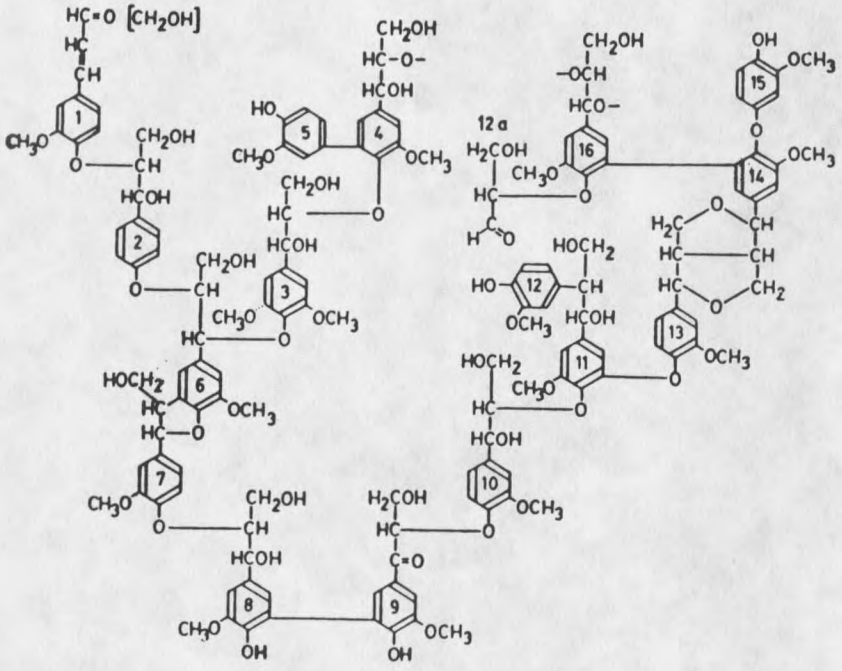


Figure 3. Structure of lignin [27].

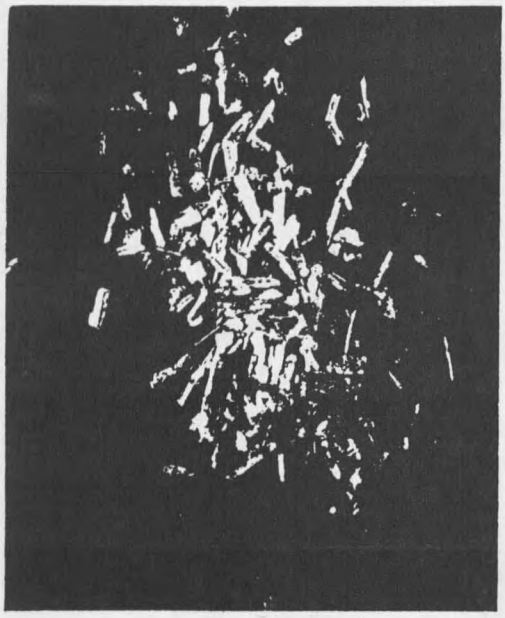
EXPERIMENTAL

Characterization of the Substrate

The wheat straw used in this investigation was spring wheat of the Pondera variety. Two bales of the substrate were obtained from Larry Van Dyke of Manhattan, Montana in November of 1982. After being received, the wheat straw was unbaled, transferred to plastic garbage bags and sealed.

Representative samples of the substrate were subsequently reduced to a more manageable size using a Wiley mill outfitted with a 1 mm discharge screen. Care was taken to avoid excessive heating of the apparatus and the sample in order to maintain the natural integrity of the substrate. The milled straw was separated into three fractions using U.S. Standard Sieves; less than 60 mesh, 35 to 60 mesh and greater than 35 mesh. For all the experiments of this investigation the 35 to 60 mesh fraction was used. This size range was chosen because of TAPPI (Technical Association of the Paper and Pulp Industry) and ASTM (American Society for Testing Materials) requirements in standard analyses. Sample lots of 35 to 60 mesh wheat straw were stored in containers open to the air.

Using a Zeiss Model 1 light microscope under a 10x magnification, the overall structural features of the three milled fractions were investigated (Figure 4). The results of this examination showed that each of the three fractions exhibited the same general physical structure; only the actual dimensions of the particular particles were reduced. However, the larger particles have roughly the same minimum dimension as the smaller particles and should allow for similar accessibility for hydrolyzing agents, such as enzymes or acids. This seems to indicate that the cost of milling could be reduced by using larger particles for this type of process.



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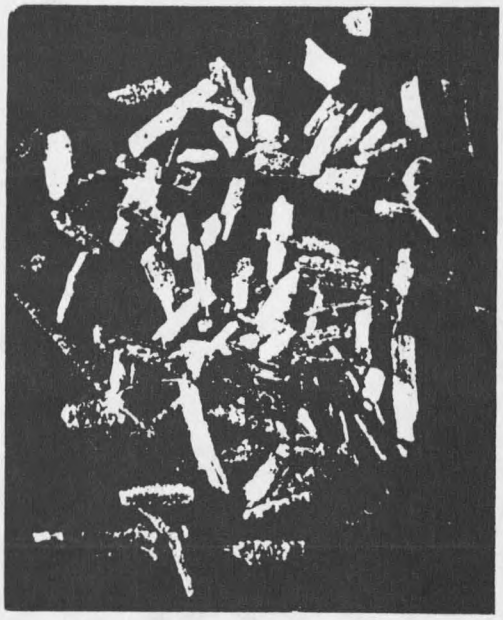
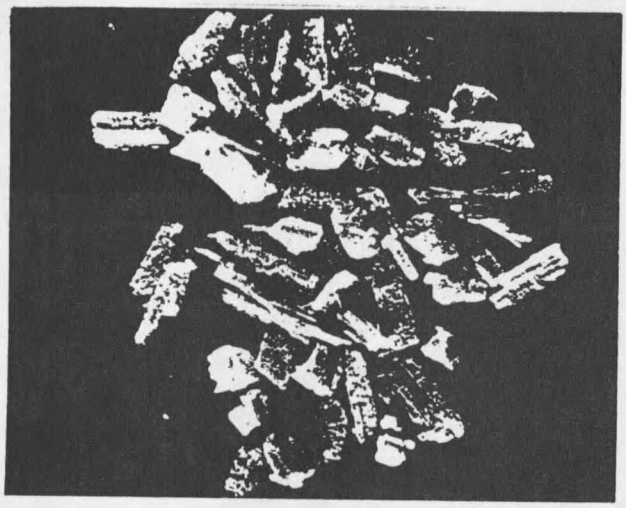


35 to 60 mesh



> 35 mesh

Figure 4. Light microscope photographs (10x) of wheat straw.

Moisture Determination

The moisture content of woody plants, like all the other analyses, depends on the history, preparation, and storage of the substrate. The moisture determination of the wheat straw used in this investigation was performed on substrate that was allowed to come to equilibrium with laboratory air (average temperature = 23° C and humidity = 30%). The conditioned samples were then dried in an atmospheric convection oven at 105-110° C overnight, then cooled in a desiccator and weighed. The percent moisture was obtained by weight difference. Twenty trials were averaged and a moisture content of 5.30 ± 0.48 weight percent was obtained.

Ash Determination

The ash content of raw wheat straw was determined using ASTM D1102-56. The wheat straw was oven dried at 105-110° C, then heated slowly to 600° C and held there for thirty minutes. The samples were then cooled and weighed. The percentage of ash was determined as the weight of ash divided by the weight of the oven-dried sample. Thirteen samples were ashed and the ash percent on an oven-dried basis was 8.34 ± 0.71.

Wheat straw, that previously had been ethanol-benzene extracted (described below), was also ashed to determine if the extraction had any effect on the ash weight percent. Five samples were ashed under the same conditions as the raw wheat straw and a 5.85 ± 0.28 ash percent was obtained. Obviously, by removing extractibles, materials that appeared as ash in the raw wheat straw were also removed by the extraction process.

Ethanol-Benzene Extraction

The ethanol-benzene extraction technique used in this investigation followed TAPPI standard T12 os-75. In this procedure air dried wheat straw was subjected to a six hour

extraction using a 2:1 volume mixture of benzene and 95% ethanol. The process was carried out in a Soxhlet extraction apparatus using five to six siphons per hour. Subsequently, the wheat straw was removed from the extraction apparatus and washed with 200 ml of 95% ethanol in order to remove any remaining benzene. The sample was then placed back in the Soxhlet extractor and the ethanol-benzene was replaced with 350 ml of 95% ethanol. The ethanol extraction was carried out for four hours using the same siphoning rate. Deionized water (200 ml) was used to wash away any residual ethanol following the ethanol extraction. The wheat straw was then split into two fractions and each was added to a flask containing 500 ml boiling, deionized water. This mixture was kept at 85°C for one hour using a Polyscience Corporation immersion circulator water bath (model 73) that maintained the temperature within ±.2°C. The two samples were then combined and washed with 500 ml boiling deionized water. The combined sample was then air dried and the percent extractibles determined by a weight difference. Five trials were done on raw wheat straw with the result being 8.91 ± 1.64 weight percent extractibles.

According to TAPPI standard T12 os-75 the ethanol-benzene extraction removes waxes, fats, resins, and some gums. The hot water removes the rest of the gums, tannins, some starches and sugars, and coloring matter. From this technique, holocellulose is defined. Holocellulose is that fraction that remains after all materials soluble in neutral solvents are removed and consists of cellulose, hemicellulose, lignin, and ash.

The procedure described above, like all the standard methods used in this investigation, was designed for the examination of wood. The ethanol-benzene extraction process when applied to wheat straw did not remove all the neutral soluble components. The final wash of the sample with 500 ml boiling, deionized water removed a significant amount of coloring matter. The filtrate remaining after the wash was a dark yellow color. Because the standard method was explicit in the use of 500 ml boiling water no further attempt was made to remove the remaining color from the sample.

Lignin Determination

The lignin content of the wheat straw was found using a modification of TAPPI standard T9m as explained by Browning [22]. In this procedure air-dried, ethanol-benzene extracted wheat straw was subjected to high purity chlorine gas. This was accomplished using the apparatus shown in Figure 5. The chlorine gas was introduced at the top of the apparatus and bubbled through the sample, which was contained in a 40 ml fritted glass filter, and collected in a 500 ml suction flask that maintained a small vacuum. The sample was exposed to chlorine gas for three minutes, the suction flask and sample were cleared of chlorine using a vacuum filtration and the sample was stirred with a glass rod. The procedure was repeated using an exposure time of two minutes. Again, the sample and flask were cleared of chlorine. After chlorination, the sample was a bright yellow-orange color. The sample was then washed approximately 30 ml of p-dioxane. This wash was vacuum filtered with the filtrate being the same color as the sample. In the next step, the sample was washed with 5% monoethanolamine in p-dioxane that was maintained at 50°C. The sample immediately turned dark brown. The hot solution was allowed to remain for two minutes before being filtered off. The filtrate was, again, the same color as the sample (dark brown). This sequence was repeated one more time and two washes with room temperature p-dioxane followed (the filtrate in this case was clear). The final washes were done with room temperature deionized water. In this step, the wash removed some of the color of the sample. The water wash was done twice. The entire process was repeated, using a single two minute chlorination, until the sample turned white when chlorinated and the final deionized water wash did not change the color of the sample (the filtrate remained clear). The sample was then washed twice with ethyl ether and air dried. The lignin percent was determined by weight difference. The wheat straw used in this investigation was found

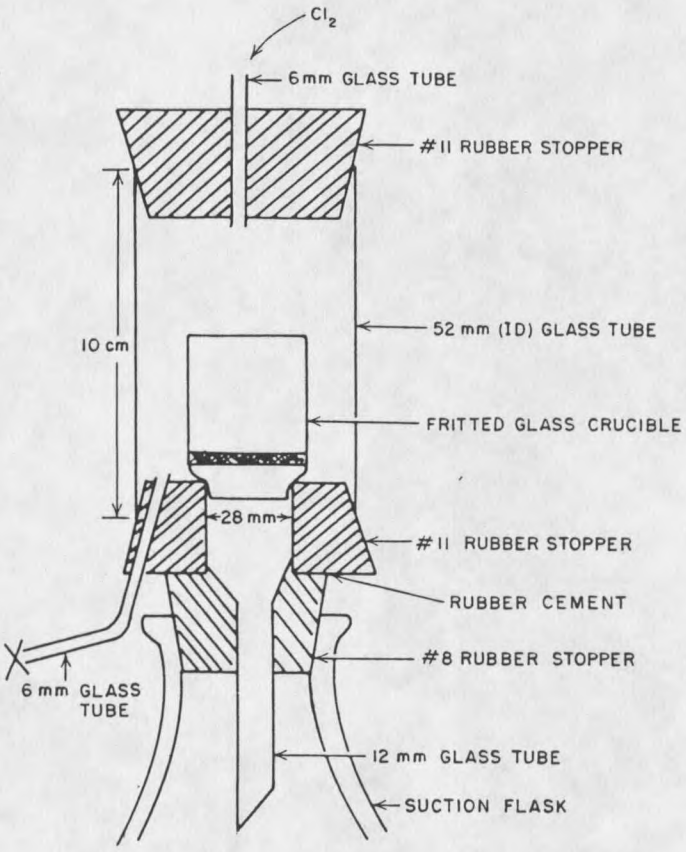


Figure 5. Apparatus used for lignin determination.

to contain 30.02 ± 1.97 weight percent lignin on an air dry, ethanol-benzene extract free basis using six trials.

Cellulose Determination

The cellulose content of wheat straw is defined as the residue that remains after removing materials soluble in neutral solvents (ethanol-benzene extraction) and lignin. In this investigation the composition of the cellulose residue was determined using TAPPI standard T203 os-74.

The standard procedure started with an air-dried, ethanol-benzene extract-free sample of approximately 1.5 grams. The sample was then immersed in 100 ml of 5.21 N sodium hydroxide at 25°C for thirty minutes. Next, the solution was diluted using 100 ml of deionized water at 25°C and allowed to stand for an additional 30 minutes. The solid material was then filtered out and discarded. This solid material is defined as alpha or undegraded, long chain, high molecular weight cellulose. The definition of alpha-cellulose is that fraction of the cellulose residue that is insoluble in the two concentrations of sodium hydroxide mentioned above (17.5 and 9.45 weight percent). Beta-cellulose, or broken chain, lower molecular weight cellulose, and hemicellulose (gamma-cellulose) remain soluble in the final sodium hydroxide solution.

The cellulose/sodium hydroxide mixture (10 ml) was then combined with 10 ml of 0.5 N potassium dichromate and 30 ml concentrated sulfuric acid. The solution remained hot for 15 minutes, 50 ml deionized water was added and the mixture was cooled to room temperature. At the same time a blank sample was prepared in a similar manner using 12.5 ml deionized water and 12.5 ml 5.21 N sodium hydroxide instead of the cellulose/sodium hydroxide solution.

The percent of the cellulose residue that was alpha-cellulose was determined by titrating the two solutions with 0.1 N ferrous ammonium sulfate that had previously been standardized with 0.1 N potassium dichromate. A colorimetric indicator, Ferroin (1,10-phenanthroline monohydrate) was used to distinguish the endpoint of the titration. The indicator turned the solution from blue-green to brown at the endpoint. The alpha-cellulose was determined using the equation:

$$\text{percent alpha-cellulose} = 100 - \frac{[6.85(V2 - V1) \times N \times 20]}{10 \times W}$$

V1 = Volume of pulp filtrate titration (ml)

V2 = Volume of blank titration (ml)

N = Exact normality of the ferrous ammonium sulfate

W = Oven-dried weight of the sample (g)

The beta and gamma-cellulose fractions were separated by acidification of the cellulose/sodium hydroxide solution. The cellulose/sodium hydroxide solution (50 ml) was combined with 50 ml of 3 N sulfuric acid in a 100 ml graduate cylinder. The cylinder was placed in a water bath maintained at 85°C. Under these conditions beta-cellulose coagulated. The precipitate was allowed to settle overnight. The clear solution, that remained over the solid beta-cellulose, was then removed using a pipet (50 ml) and combined with 10 ml of potassium dichromate and 90 ml of concentrated sulfuric acid. The mixture remained hot for fifteen minutes before being cooled to room temperature in a water bath. Like the alpha-cellulose determination, a blank sample was prepared using 12.5 ml deionized water, 12.5 ml sodium hydroxide (5.21 N), and 25 ml 3 N sulfuric acid in place of the cellulose/sodium hydroxide solution. Each of the two solutions were then titrated using 0.1 N ferrous ammonium sulfate and a potentiometeric endpoint indicator. A Beckman Zeromatic SS3 pH meter with an Orion single junction reference electrode (model 90-01)

and a platinum wire electrode were used to detect the endpoint. The gamma-cellulose percentage of the cellulose residue was calculated using the equation:

$$\text{percent gamma-cellulose} = \frac{6.85(V4 - V3) \times N \times 20}{25 \times W}$$

V3 = Volume of solution titration (ml)

V4 = Volume of blank titration (ml)

N = Exact normality of the ferrous ammonium sulfate

W = Oven-dried weight of the sample (g)

The beta-cellulose percentage of the cellulose residue was determined by adding the alpha and gamma-cellulose percentages and subtracting the total from one hundred percent.

In the evaluation of ethanol-benzene extract-free, delignified wheat straw for cellulose percentages the procedure outlined above yielded no beta-cellulose fraction. When 3 N sulfuric acid was added to the cellulose/sodium hydroxide solution no precipitate was formed.

The results of five cellulose determinations were averaged. Alpha-cellulose percent was 73.67 ± 1.86 and the gamma-cellulose percent was 26.33 ± 1.86 based on an oven-dried cellulose residue.

Autohydrolysis

In the present investigation, autohydrolysis was carried out in an Autoclave Engineers one liter autoclave (Zipperclave) pressure rated to 2000 psi at 450° F and equipped with an air drive agitator. To allow rapid heat up of the sample, the autoclave was linked to a 500 ml Parr high pressure bomb (rated to 8000 psi) that served as a steam generator. Also in line with the autoclave was a stainless steel cold trap used to vent the system, collect vapors and assist in cooling the sample quickly. All units were joined by low pressure stainless tubing and Autoclave Engineers low pressure valves (model 6V81U4TG with high temperature packing). The experimental setup is shown in Figure 6.

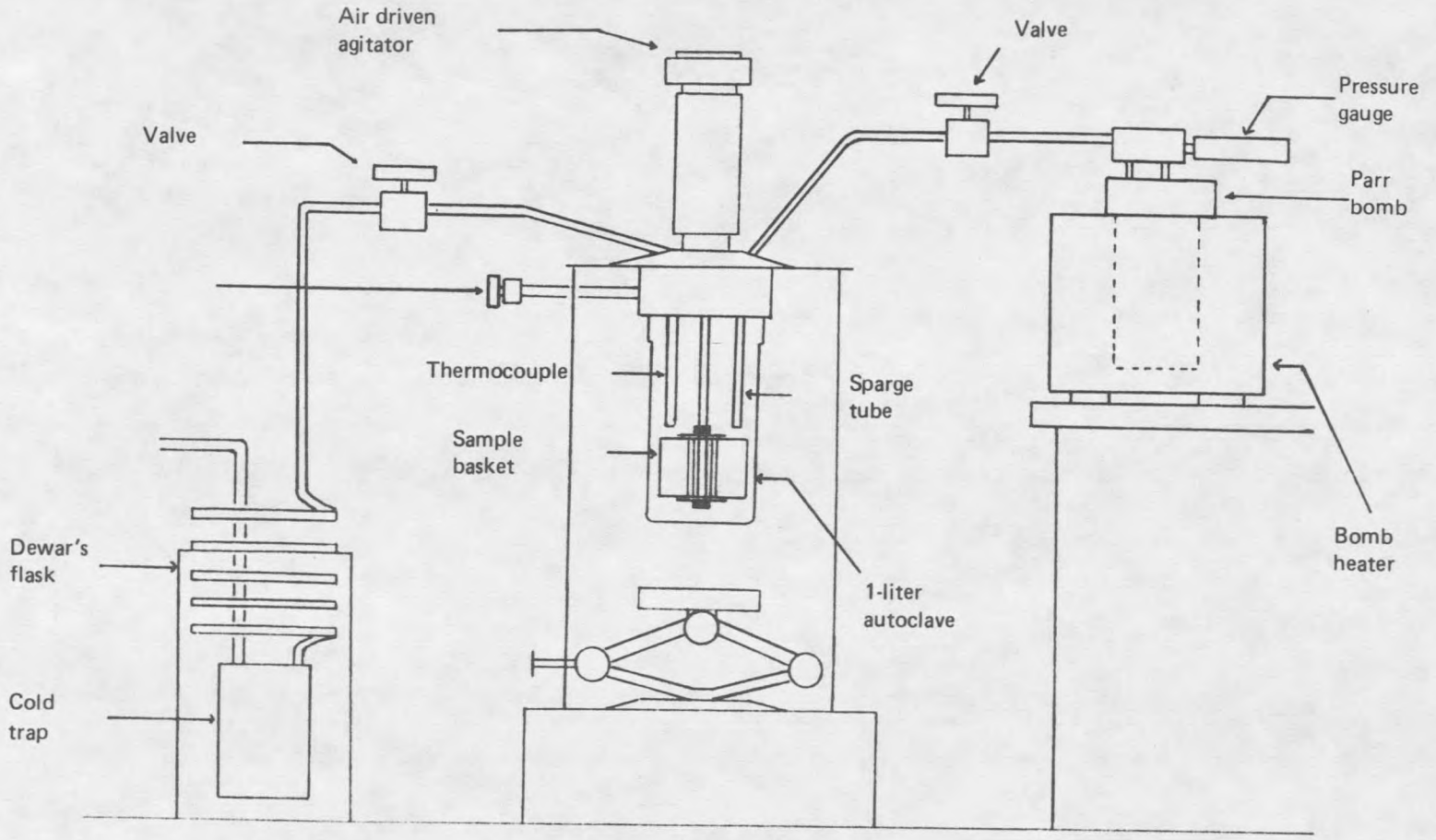


Figure 6. Experimental set up used for autohydrolysis and extraction.

The sample, air-dried ethanol-benzene extract-free wheat straw, was presented for autohydrolysis in a unique manner (Figure 7). A basket, constructed from a 4 inch long, 5/8 inch diameter brass nipple and two 1½ inch diameter steel washers, was used as a support for eight sample envelopes. The envelopes consisted of 200 mesh 316 stainless steel wire cloth sealed closed with two ¼ inch wide aluminum strips secured by two screws. The wheat straw was loaded into the preweighed envelopes through an opening in the top and sealed by folding. The envelopes were then weighed and loaded into the basket support and the whole unit was attached to the agitator shaft of the autoclave.

The initial conditions of the autohydrolysis included a 600 ml charge of deionized water along with the straw sample in the autoclave and a 300 ml charge of deionized water in the steam generator (Parr bomb). The Parr bomb was heated using two 750 watt Parr sleeve heaters. The autoclave contents were preheated to 150°C using the autoclave heating jacket. The average heating time (23°C to 150°C) was 12 minutes. The choice of 150°C as a starting temperature was based on results obtained by Wayman and Lora [30] that suggested that no significant physical or chemical changes occurred at or before this temperature. The final pressure of the steam generator ranged from 900 to 1250 psig which corresponds to 278 and 300°C, respectively. Table 3 shows the average heating (150°C up to autohydrolysis temperature) and cooling times (autohydrolysis temperature down to 150°C) for each autohydrolysis temperature.

The heating of the sample above 150°C was accomplished by releasing the high pressure steam into the autoclave. Once the desired temperature was obtained, the steam was shut off and the temperature was maintained using the autoclave heating jacket. Short releases of reactor vapors served to lower the temperature when it exceeded the desired value. Using these manual adjustments the temperature of the sample was maintained within ±2°C. The reaction system was agitated every ten minutes during autohydrolysis for a period of one minute at less than 250 rpm; the intermittent agitation and low rate was

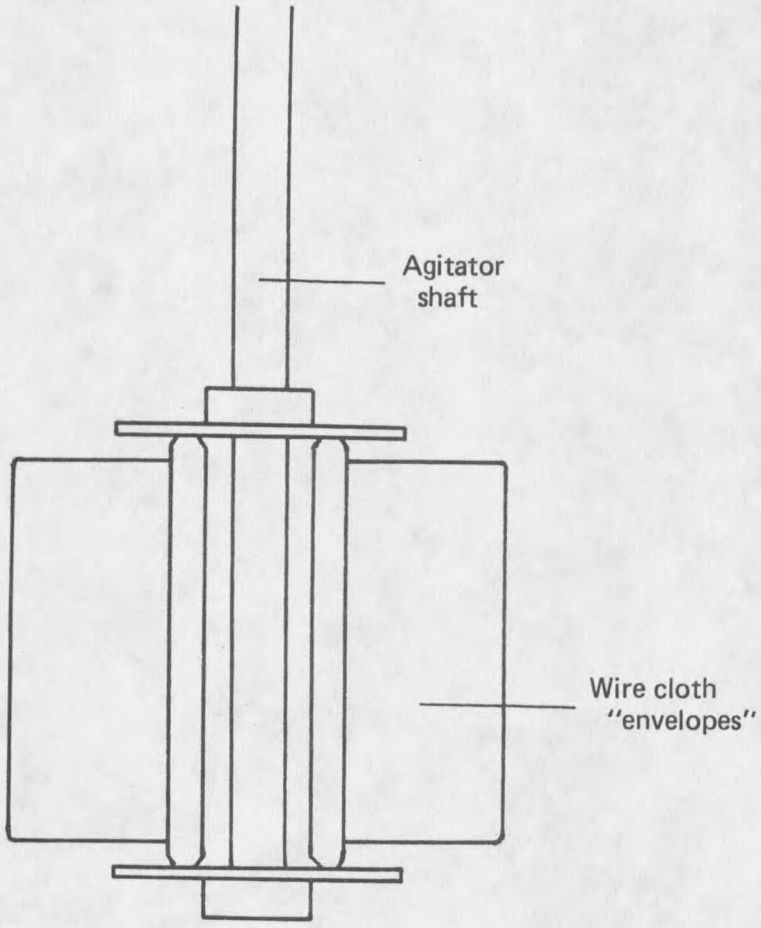


Figure 7. Sample basket

Table 3. Autohydrolysis Heat Up and Cool Down Times.

Autohydrolysis Temperature (°C)	Heating Time (sec)	Cooling Time (sec)
175	42 ± 11	42 ± 11
195	54 ± 11	80 ± 10
205	94 ± 17	99 ± 17

needed to avoid vortexing and maintain reasonable contact between the sample and the liquid. To cool the system following autohydrolysis reactor vapor was released to the cold trap, being careful to condense all the vapors.

Temperatures were monitored using a Cole Palmer Model 8530 digital thermometer and a Type K (Cromel-Alumel) thermocouple inserted into the autoclave stainless steel thermowell. In order to register a reliable sample temperature it was necessary to maintain contact between the liquid and the thermowell. For this reason 600 ml of deionized water was needed as an initial charge in the autoclave. This volume covered the sample basket and contacted the thermowell. The excess liquid also insured that all soluble materials generated during autohydrolysis were washed out of the solid matrix.

Since the cooling process left the autoclave at atmospheric pressure and the Zipperclave allowed easy access, the sample basket could be removed from the autohydrolysis liquor within minutes of pressure release. Autohydrolyzed wheat straw was then removed quantitatively from the envelopes and washed with 500 ml of room temperature deionized water and then air dried. The weight loss was calculated by a weight difference and all the standard analyses were run on a portion of the sample to determine the effects of autohydrolysis on the solid residue. The remaining material was stored in glass sample jars for subsequent experiments.

Extraction

The next step in the present pretreatment sequence is a lignin extraction using a 1:1 volume mix of 95% ethanol and deionized water. Experimentally, the extraction was carried out in the same apparatus as the autohydrolysis. The autoclave was initially charged with 300 ml of 95% ethanol and 300 ml of deionized water. After removing four of the envelopes for examining the effects of autohydrolysis, the remaining four envelopes were reattached to the agitator shaft and the autoclave sealed. The unit was then heated to 150°C using the autoclave heating jacket. The extraction time of one hour started when the 150°C temperature was reached. The manual adjustments used in autohydrolysis were used to keep the extraction temperature within ±2°C. Cooling of the sample was accomplished by releasing reactor vapor as in autohydrolysis. After being removed from the reactor the sample was quantitatively removed from the envelopes and suspended in 50 ml of 1:1 95% ethanol and deionized water then washed twice with 50 ml of the same liquid. The procedure was repeated using deionized water. Finally, the sample was air dried and a weight loss was calculated. Standard analyses were performed on a portion of the autohydrolyzed and extracted material. The remaining wheat straw was stored in glass sample jars for subsequent experiments.

Acid Hydrolysis

To test the overall performance of the pretreatments investigated herein a variation of ASTM D1106-56 was employed as an acid hydrolysis. The standard method is designed to remove all the cellulose and hemicellulose in a solid residue and quantitatively determine the amount of lignin that remains. In an effort to decide how the hydrolyzability of wheat straw was effected by autohydrolysis and extraction, the conditions of the standard method were made less severe. The initial hydrolysis conditions were reduced from 15 ml

of 72 weight percent sulfuric acid at 20°C to a 100:1 weight ratio of 54.5 weight percent sulfuric acid (approximately 34 ml) at 28°C. In the standard procedure the hydrolysis was carried out for two hours. This step was reduced to a hydrolysis of only one hour for this investigation. The next step of the standard method consists of a four hour hydrolysis after a dilution to 3 weight percent acid. This step is carried out at boiling temperature under reflux to maintain a constant volume. In this investigation, the conditions were reduced in severity to consist of a dilution to a total volume of 250 ml (13.5 to 13.9 weight percent) and a temperature of 80°C for two hours. These conditions were intended to hydrolyze a moderate amount of ethanol-benzene extract-free wheat straw and a significant amount of pretreated wheat straw.

Reagents

All the reagents used in the pretreatment sequence and the standard analyses, except the 95% ethanol, were J. T. Baker reagent grade chemicals. The 95% ethanol used in this investigation was a bulk solvent obtained from chemical stocks on campus. Deionized water was generated in-house.

RESULTS AND DISCUSSION

Wheat Straw Composition

Because of the subtle nature of the standard analyses used in lignocellulose research the relative composition of the materials under investigation varies from study to study. The difference in values can be attributed to several different factors. Many of the standard methods are based on judgments of consistency, temperature, or color changes. Also, many times there is more than one method for determining the constituents of lignocellulosic materials. As can be seen in Table 4, the major differences between the results of this investigation and those of other researchers are seen in the values for lignin and hemicellulose weight percentage.

Table 4. Wheat Straw Composition (weight percent - oven dry basis).

Researcher	Cellulose	Hemicellulose	Lignin	Ash	Extractives
Wilke and others [30]	54.8		14.5	9.6	7.2
Sloneker [31]	40	29	14		
Porteous [32]	30	50	15		
Montana State	39.3	14.1	28.9	8.3	9.4

The wheat straw compositions reported by the other three investigations included additional components such as proteins. Also, the large differences in lignin and hemicellulose values can be explained by differences in standard analysis methods used. All three of the other studies listed above utilized ASTM D1106-56 or a similar standard method for the lignin determination. These methods use strong acid to dissolve the cellulose and hemicellulose from the solid residue leaving lignin behind. The standard method for lignin determination used in this investigation was exactly opposite. The lignin was solubilized using

chlorine gas (outlined above) leaving cellulose and hemicellulose as a solid residue. The larger lignin value can be attributed to the close association of lignin and polyuronide hemicellulose. Removing the lignin fraction from the solid residue also results in a loss of hemicellulose. Thus, because more than lignin is being removed and less hemicellulose remains for the cellulose standard determination the two values of the standard wheat straw composition reported here are different from other studies done on wheat straw. If the excess lignin weight percent (the difference between Sloneker's value, 14%, and that of this investigation, 28.9%) is added to the hemicellulose value of this investigation, the wheat straw composition reported here agrees closely with that reported by Sloneker.

Autohydrolysis Experiments

Autohydrolysis uses high temperature steam or water to remove the majority of the hemicellulose from lignocellulosic materials. This technique has been used in the paper and pulp industry for many years. More recently, the behavior of lignocellulosic materials in high temperature, aqueous media has been under investigation in an effort to increase the conversion yields of biomass cellulose to glucose.

The conditions of autohydrolysis presumably break hemicellulose out of the lignocellulose branches (polyuronides). The dilute acid and high temperature in turn hydrolyzes the remaining xylan (cellulosan) backbone. The hydrolysis products, unlike hemicellulose, are soluble in water and can be washed out of the solid residue.

Autohydrolysis not only removes the hemicellulose from biomass but also seems to have an impact on the lignin and its relationship with the remaining cellulose. Wayman and Lora [30] have reported over 90% removal of lignin from ethanol-benzene extract-free aspen woodmeal with a 9:1 dioxane and water extraction following autohydrolysis. Without the autohydrolysis pretreatment the dioxane and water extraction has little effect on

the lignin content of the woodmeal. The physical interactions between cellulose, hemicellulose and lignin have been discussed earlier. Thus, when one of the constituents is removed, the rigid structure of the matrix is altered and chemical bonds may be broken. It has been proposed that two different reactions are taking place during autohydrolysis [33]. The first, faster reaction, involves the breaking of lignin-lignin and lignin-cellulose bonds through the formation of carbonium ions in different positions on the side chains of the lignin phenyl propane units [34]. The second, slower reaction is a generation of carbon-carbon bonds through electrophilic substitution that result in a high molecular weight lignin polymer [34]. Because of these competing reactions the longer the autohydrolysis time the increased generation of insoluble polymer products. This fact was observed in Wayman and Lora's investigation of aspen woodmeal [33]. The amount of lignin removed by dioxane extraction following autohydrolysis reached a maximum depending on the temperature and time of autohydrolysis. The higher the temperature, the shorter the autohydrolysis time needed to reach a maximum lignin removal. Also, the amount of lignin removed increased as the autohydrolysis temperature increased (see Figure 8).

"Time at temperature" is a phrase used by Wayman and Lora [33] and the researchers at Colorado State University [35] to describe the actual time the sample was maintained at the desired autohydrolysis temperature. The experimental equipment used by both groups included a number of 25 to 35 ml capacity bombs constructed from iron pipe nipples sealed on one end with an iron cap and the other with a Swagelok stainless steel cap. A typical run consisted of fourteen identically loaded bombs, four of which were equipped with sealed thermocouples. The bombs were immersed in a silicon oil bath at a sufficiently high temperature to heat the samples to the desired temperature. In general, heat up time was 5 to 10 minutes. Once the desired temperature was reached the autohydrolysis time at temperature was started. Cooling the samples was accomplished in a cold water bath

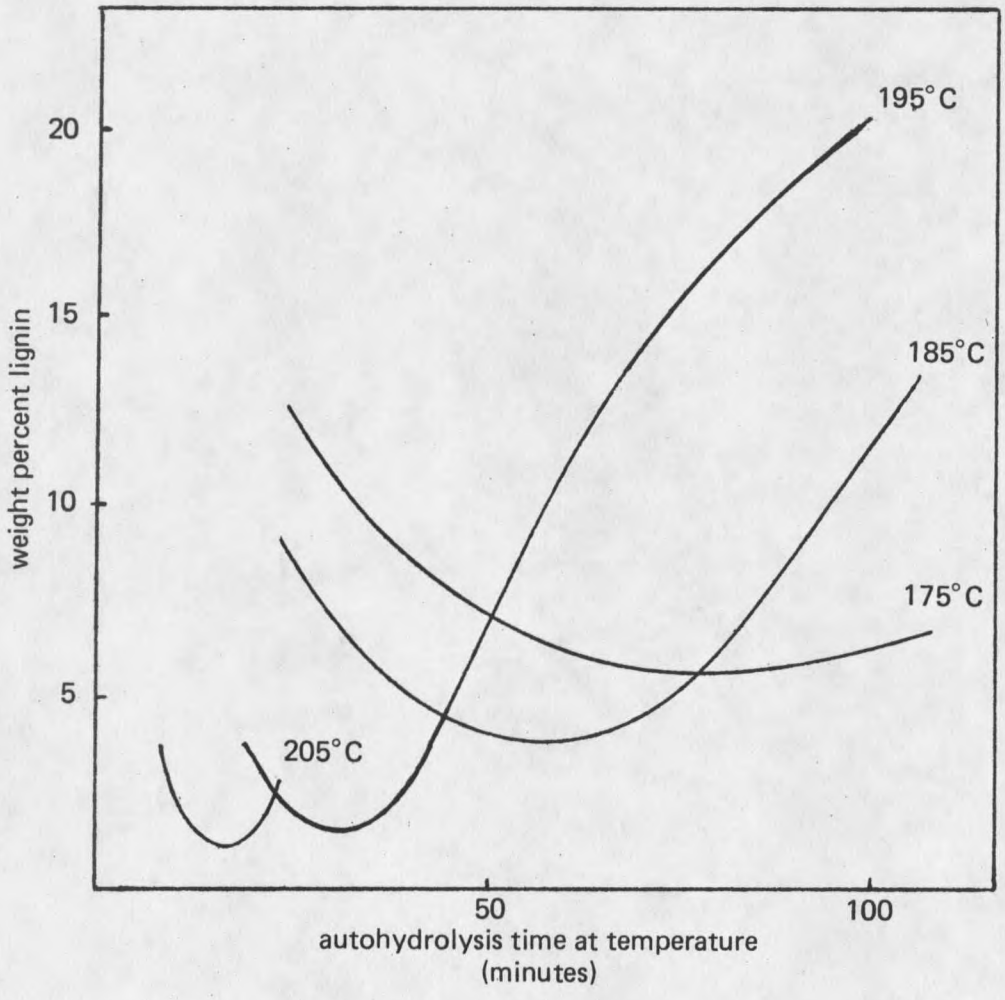


Figure 8. Effects of autohydrolysis and dioxane extraction on aspen woodmeal lignin [33].

with times ranging from 10 to 20 minutes. As can be seen from Figure 8, as the autohydrolysis temperature increases the optimum time at temperature for lignin removal is at shorter times at temperature. The excessive heat up and cool down times incurred using these procedures may cause significant errors in the reporting of an optimum time at temperature for lignin removal using the autohydrolysis pretreatment.

Weight Loss

Autohydrolysis was carried out using air-dried, ethanol-benzene extract-free wheat straw. The sample weight prior to autohydrolysis was determined by first weighing the stainless steel wire-cloth envelopes. The wheat straw was loaded into the envelopes and each envelope was again weighed. The weight of each sample was added and a total initial sample weight obtained. The sample was then subjected to an autohydrolysis (described above). Following autohydrolysis the material from each envelope was combined and washed with 500 ml room temperature deionized water. The sample was allowed to come to moisture equilibrium with the atmosphere and then reweighed. The weight loss due to autohydrolysis was determined by a weight difference. Figure 9 shows that higher autohydrolysis temperatures cause a greater weight loss for shorter times at temperature. But the ultimate weight loss is similar for each of the three autohydrolysis temperatures. This weight loss behavior is explained by the following results of autohydrolysis product compositions.

Hemicellulose

Autohydrolysis is accomplished by subjecting the lignocellulosic material to high temperature in an aqueous medium. These conditions generate a dilute acid environment; the high temperature presumably causes the acetyl groups of the hemicellulose branch groups (polyuronides) to react with the water present to form acetic acid. The hemicellulose is more readily hydrolyzed than cellulose in an acidic environment (due to lower

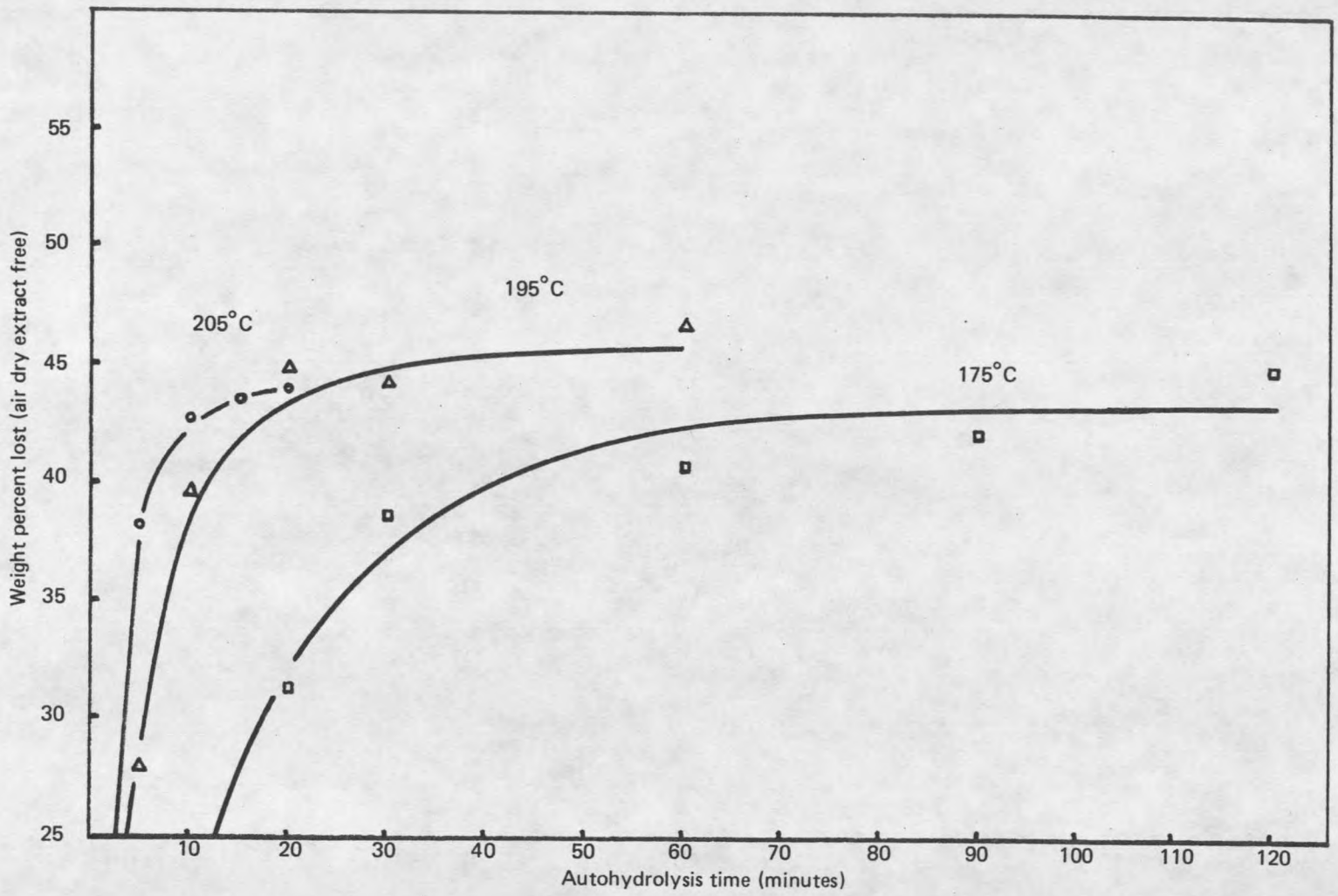


Figure 9. Weight percent lost due to autohydrolysis.

molecular weight and low crystallinity). Thus, the long chain hemicellulose backbone (cellulosans) is broken up into smaller water soluble fractions by the acidic environment. These smaller fractions (primarily xylans) are in turn washed out of the lignocellulosic matrix by the excess water present during autohydrolysis.

The autohydrolysis of wheat straw, much like wood, removes a majority of the hemicellulose from the solid lignocellulosic material (see Figure 10). It is observed that the higher the autohydrolysis temperature the shorter the exposure time needed to remove a majority of the hemicellulose. The time at temperature needed to remove 80% of the hemicellulose was 25 minutes at 175°C and 5 minutes at 195°C. No cellulose determination was done on samples autohydrolyzed at 205°C. It was assumed that autohydrolysis at 205°C removed essentially all the hemicellulose at an autohydrolysis time at temperature of less than five minutes.

Lignin

The conditions of autohydrolysis, high temperature and dilute acid environment, also serve to disrupt the lignin structure within the solid material. As was outlined above, the three dimensional lignin molecules are broken up into smaller fractions and some of the lignin-cellulose bonds are broken. Unlike hemicellulose, these fractions are not completely soluble in water. Autohydrolysis succeeded in reducing the lignin weight percent from 31.9% in raw, oven-dried, ethanol-benzene extract-free wheat straw to 18.9% at 175°C, 18.8% at 195°C, and 21.5% at 205°C. Also, as can be seen in Figure 11, the lignin weight percent tends to level off as the autohydrolysis time at temperature increases. The autohydrolysis step seems to create a water soluble lignin fraction. At at some specific time at temperature (different times for each autohydrolysis temperature) the lignin weight percent starts to level off. The higher the autohydrolysis temperature the shorter the time at temperature until this occurs.

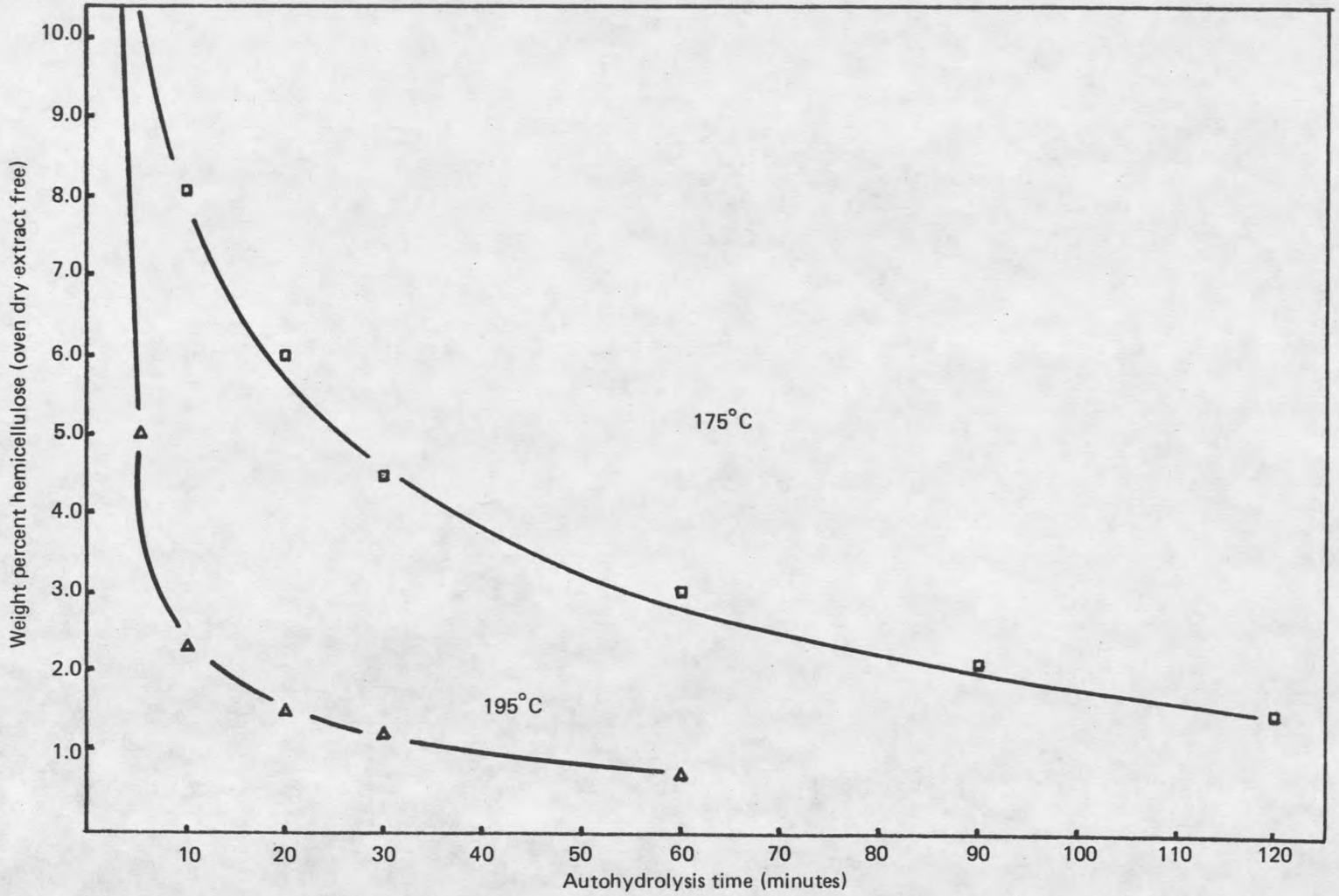


Figure 10. Weight percent hemicellulose after autohydrolysis.

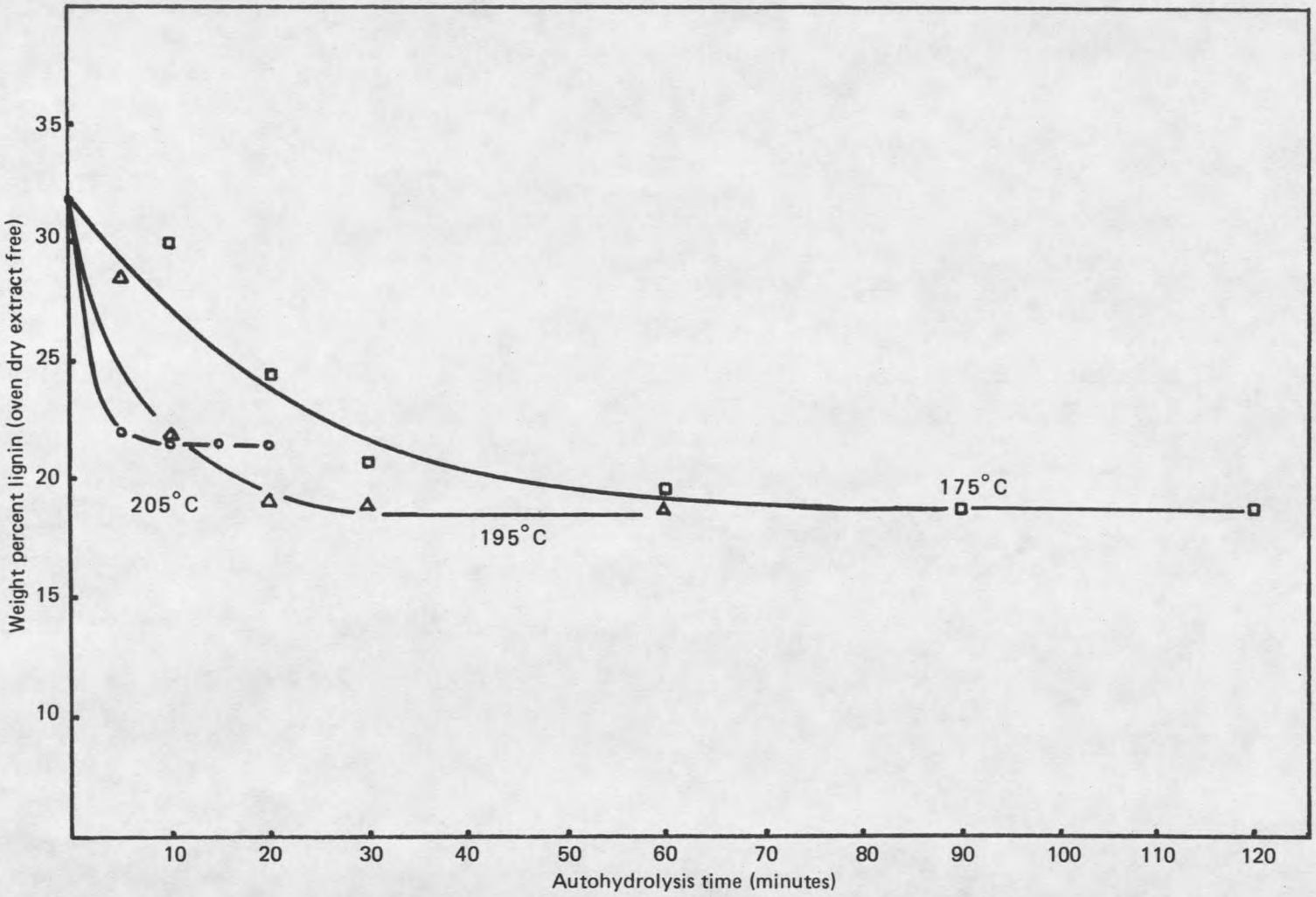


Figure 11. Weight percent lignin after autohydrolysis.

Figure 11 also shows that at an autohydrolysis temperature of 205°C less water soluble lignin is generated than for 175°C or 195°C. At the higher temperature the lignin molecule is disrupted and broken up quickly. Further exposure to the higher temperature does not result in greater lignin removal in the aqueous medium. According to Wayman and Lora [33] the autohydrolysis step causes two lignin reactions to occur. The faster, initial reaction breaks up the lignin polymer while the second reaction reforms some of the carbon-carbon bonds originally broken. At higher autohydrolysis temperatures, each reaction accelerates. Autohydrolysis at 205°C allows only a short span in time at temperatures where the lignin bonds are being broken and the fragments are soluble before repolymerization starts to take over.

Cellulose

Both hemicellulose and lignin fragments are being removed much faster than cellulose during the initial stages of autohydrolysis. For this reason the oven-dried, ethanol-benzene extract-free weight percent of total cellulose (alpha and beta cellulose) increases as the autohydrolysis time at temperature increases (Figure 12). When the lignin weight percent levels off the cellulose weight percent also levels off. Also, since at higher autohydrolysis temperatures hemicellulose is removed faster, the cellulose weight percent at 195°C is greater than for 175°C during the early stages of autohydrolysis. Figure 12 shows that the final cellulose weight percent for both temperatures is around 69%. No cellulose determination was done on the 205°C autohydrolysis products. It is assumed that essentially all the hemicellulose is removed quickly because of the high temperature, yielding a final total cellulose value of 68% (see the Appendix).

Acid Hydrolysis

The utilization of lignocellulosic material as a biomass fuel or a chemical feedstock ultimately depends on the conversion of cellulose to glucose. The goal of a successful

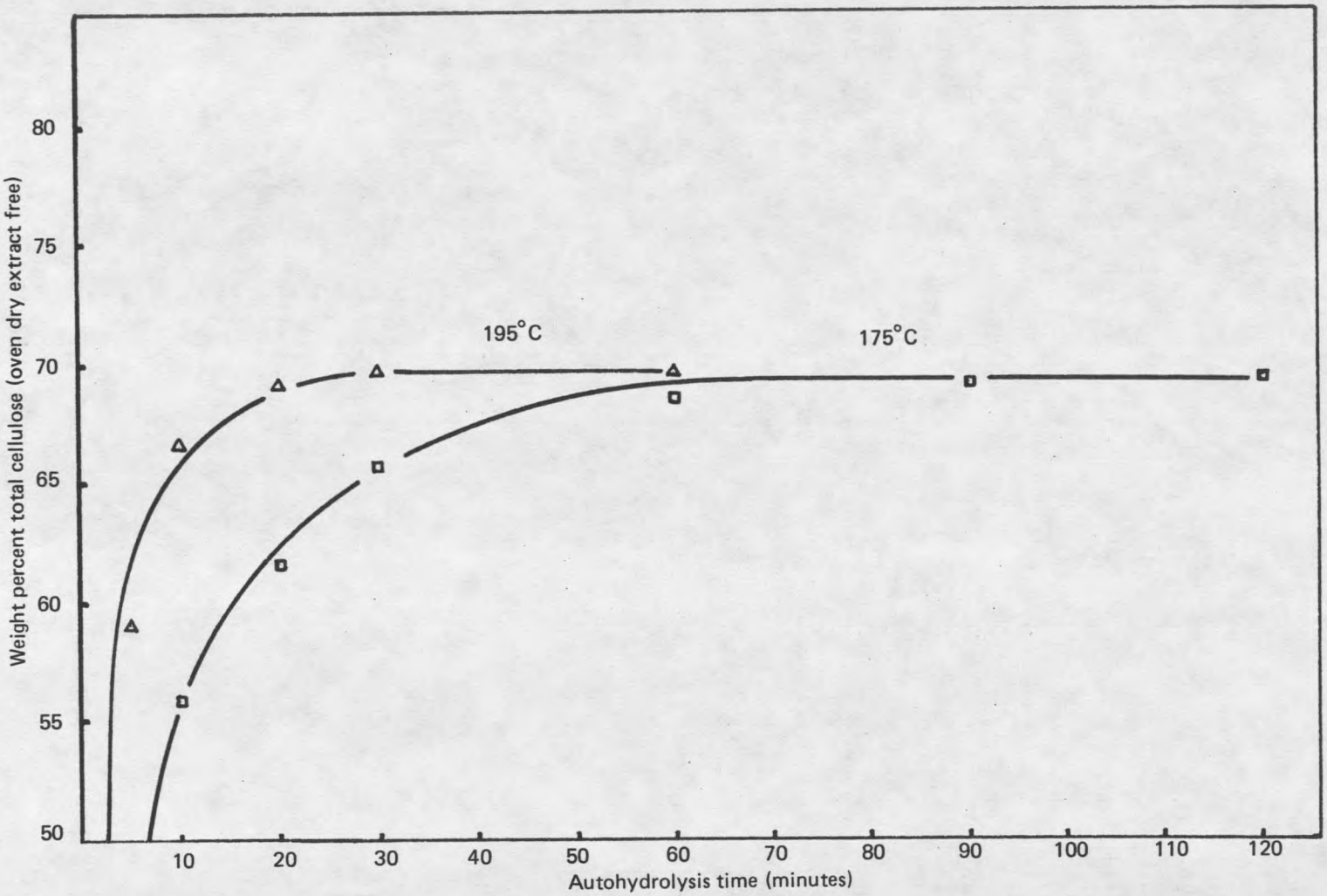


Figure 12. Weight percent total cellulose (alpha and beta) after autohydrolysis.

pretreatment sequence is to increase the low yield normally encountered when lignocellulose is hydrolyzed, either enzymatically or with the use of acids.

Autohydrolysis in this investigation is successful in reducing both the lignin and hemicellulose fractions and increasing the cellulose weight percent. It was expected that if the lignocellulosic starting material could be upgraded to a residue that consisted almost exclusively of cellulose, the resulting material could easily be hydrolyzed using either acid or enzymes. The increase in hydrolysis rate could be attributed to the increased access of the hydrolyzing agent to the substrate.

The results of the acid hydrolysis on the autohydrolysis products obtained in this investigation did not concur with the expected results. The acid hydrolysis was first run on raw, oven-dried, ethanol-benzene extract-free wheat straw. The weight of the sample was reduced by 34.8%. If it is assumed that the ash and lignin portions of the sample were not affected by the concentration of acid used, the total carbohydrate (alpha, beta, and gamma cellulose) weight was reduced by 55.9%. Because the hemicellulose fraction of wheat straw is easily hydrolyzed in acidic solutions the weight loss can be adjusted to 40.0% and reflects the loss of total cellulose (alpha and beta-cellulose).

The acid hydrolysis results for wheat straw autohydrolyzed at 195°C and 205°C are reported in Table 5 (no acid hydrolysis was done on the 175°C autohydrolysis products). The weight loss due to acid hydrolysis only was minimal in all trials. The cellulose residue that results from autohydrolysis appears more resistant to acid hydrolysis than raw wheat straw. When the weight losses of total cellulose (alpha and beta-cellulose) and total carbohydrate (alpha, beta, and gamma-cellulose) that occur during autohydrolysis are combined with those incurred during acid hydrolysis the overall weight loss of carbohydrate is less than if the raw wheat straw was acid hydrolyzed. It is also observed that the overall weight loss of both the total cellulose (alpha and beta) and the total carbohydrate (alpha, beta, and gamma) was consistent over the range of autohydrolysis times. This suggests that only

Table 5. Effects of Acid Hydrolysis on Autohydrolyzed Wheat Straw.

Time at Temperature	Percent Weight Loss Due to Acid Hydrolysis		Percent Weight Loss Due to Overall Process	
	Total Carbohydrate (alpha, beta, and gamma)	Total Cellulose (alpha and beta)	Total Carbohydrate (alpha, beta, and gamma)	Total Cellulose (alpha and beta)
	Autohydrolysis Temperature 195°C			
5 min	29.0	23.6	47.1	28.3
10 min	11.2	8.4	40.1	18.7
20 min	6.3	4.3	40.7	19.6
30 min	5.8	4.2	39.6	17.9
60 min	3.5	2.5	41.2	20.2
	Autohydrolysis Temperature 205°C			
5 min	15.6	No separation of cellulose fraction performed	41.6	No separation of cellulose fraction performed
10 min	11.2		43.5	
15 min	9.0		42.9	
20 min	7.0		42.3	

a specific amount of original carbohydrate was available for removal. The acid hydrolysis rates varied significantly because of the differing amounts of available carbohydrate remaining after autohydrolysis.

The lignocellulosic matrix of wheat straw has been disrupted by the removal of lignin and hemicellulose using autohydrolysis. Because the subsequent acid hydrolysis is less effective on autohydrolyzed wheat straw than on raw wheat straw it must be assumed that the high temperature of autohydrolysis has affected the residual lignocellulosic structure. Also, as was pointed out by the researchers at Colorado State University [36], allowing the substrate to air dry following autohydrolysis substantially reduced the enzymatic hydrolysis yield. The removal of water from the lignocellulosic matrix may cause the remaining fibers to "collapse." The accessibility of air-dried cellulose appears greatly reduced when compared to never-dried cellulose. The material apparently cannot be rewetted to improve the accessibility [37].

The removal of lignin and hemicellulose combined with the high temperature of autohydrolysis and the effects of air drying the substrate may cause major changes in the remaining cellulose fibers. These changes may render the remaining cellulose less susceptible to attack by acid than the raw wheat straw.

Extraction Experiments

The effect of an aqueous ethanol extraction on wood has been investigated by Kleinhart and others as a delignification for the paper and pulp industry [38]. in their work, the extraction was used to remove significant amounts of lignin without disrupting the cellulose fiber structure resulting in a high quality pulp. Many researchers have combined autohydrolysis with a subsequent extraction in an effort to upgrade lignocellulose material and increase the hydrolysis yield of cellulose to glucose. Linden and Moriera et al. [36] used an aqueous ethanol extraction on wood while Wayman and Lora investigated a 9:1 dioxane

and water extraction following autohydrolysis [33]. As was reported earlier, the autohydrolysis step seems to depolymerize the lignin and the extraction step removes the aqueous ethanol-soluble fractions. The interesting aspect of the extraction step according to Wayman and Lora is that the lignin remaining in the solid residue goes through a minimum as the autohydrolysis time is increased. The longer the sample is kept at the autohydrolysis temperature after the optimum the greater the repolymerization of the remaining lignin. Repolymerization yields a higher molecular weight lignin fraction that is insoluble in the aqueous ethanol extraction.

Weight Loss

The autohydrolysis step was followed by an aqueous ethanol extraction as explained earlier. The sample basket consisted of eight stainless steel wire cloth envelopes loaded with wheat straw. During a typical run all eight of the sample envelopes were subjected to autohydrolysis. Following autohydrolysis, the sample basket was removed from the autoclave and four of the envelopes removed. The remaining four envelopes were put back into the apparatus and an aqueous ethanol extraction was performed. The sample was then washed following the sequences described above and air dried. The weight loss was determined by difference. Again, as for autohydrolysis only, combined weight loss occurred faster at higher autohydrolysis temperatures. It was also noted that additional material was washed out of the solid fraction with an extraction step following autohydrolysis (see Figure 13).

Total Carbohydrate (alpha, beta, and gamma-cellulose)

To determine the effects of an aqueous ethanol extraction on the hemicellulose and cellulose fractions of autohydrolyzed wheat straw, a cellulose determination was performed on two samples of material autohydrolyzed at 195°C for 30 minutes. The first determination for alpha, beta, and gamma-cellulose percentages was performed on autohydrolyzed

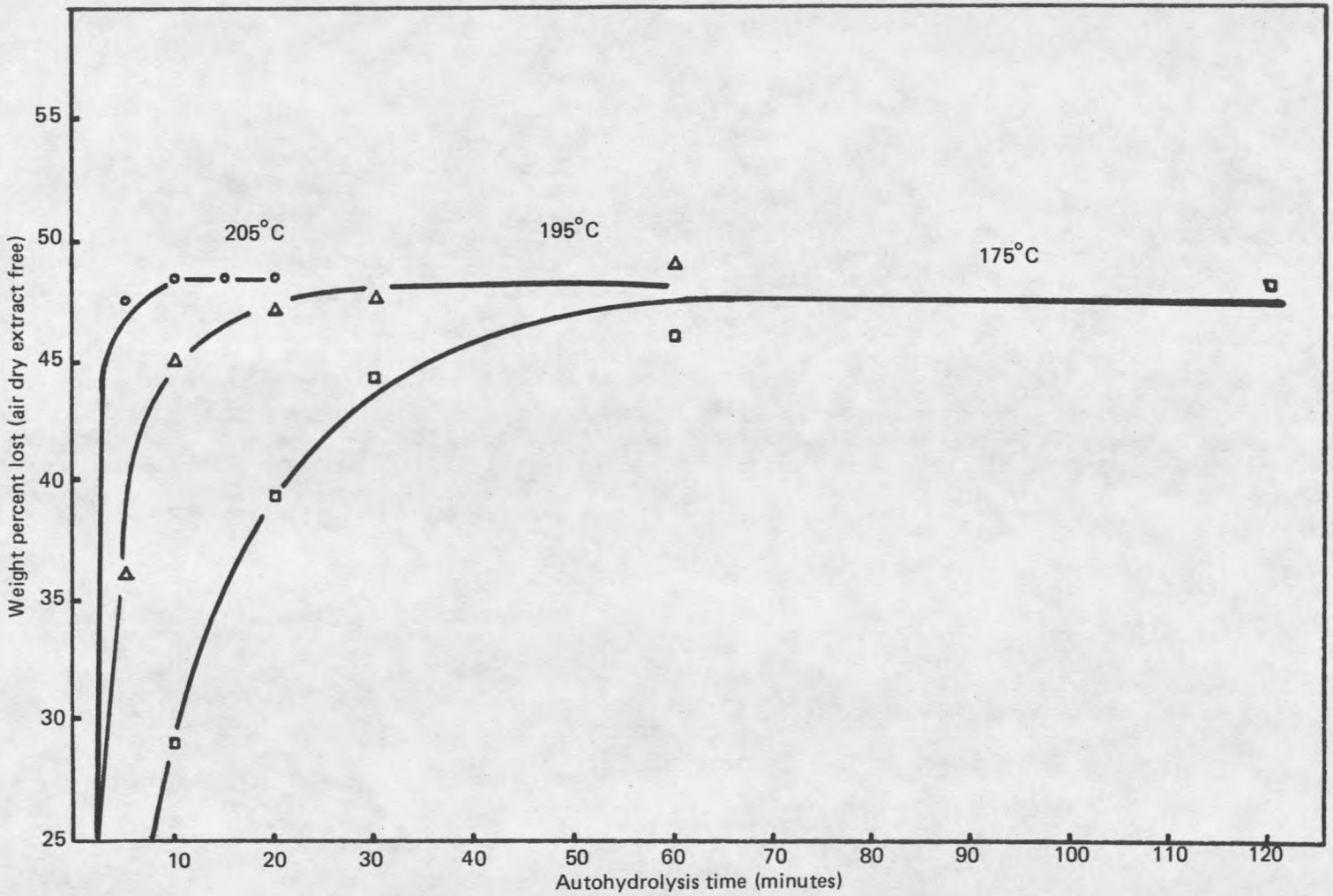


Figure 13. Weight percent lost due to autohydrolysis and extraction.

material. The second sample had been extracted with aqueous ethanol following autohydrolysis using the standard procedure. The values obtained for percentage of alpha cellulose agreed to within less than 1%; the gamma and beta percentages agreed within 10%. It was determined from these results that extraction had very little effect on the total carbohydrate (alpha, beta, and gamma) portions of the autohydrolyzed wheat straw. Cellulose determinations were run only on autohydrolyzed material and the weight percentages obtained were used to determine the weights of carbohydrate after extraction.

Lignin

The extraction of autohydrolyzed wheat straw with a 1:1 volume solution of aqueous ethanol at 150°C for one hour showed the same basic pattern exhibited by aspen extracted with 9:1 dioxane and water [33]. The amount of lignin removed by extraction was dependent on the autohydrolysis time and temperature (see Figure 14). At a specific autohydrolysis temperature the residual lignin weight percent decreased initially until reaching a minimum value. At autohydrolysis times greater than this value the lignin weight percent increased. As autohydrolysis temperature increased the minimum lignin weight percent decreased, as did the time at temperature needed to reach the minimum lignin weight percent on an oven-dried, extract-free basis.

Table 6. Minimum Lignin Weight Percents after Autohydrolysis and Extraction.

Autohydrolysis Temperature	Time of Minimum Lignin Weight Percent	Minimum Lignin Percent
175°C	90 min	15.2
195°C	30 min	13.8
205°C	10 min	12.5

As was discussed earlier, the autohydrolysis of lignocellulosic materials has two effects on the lignin fraction. The extraction step has the effect of removing an additional amount of lignin fragments from the solid residue subsequent to that removed by autohydrolysis.

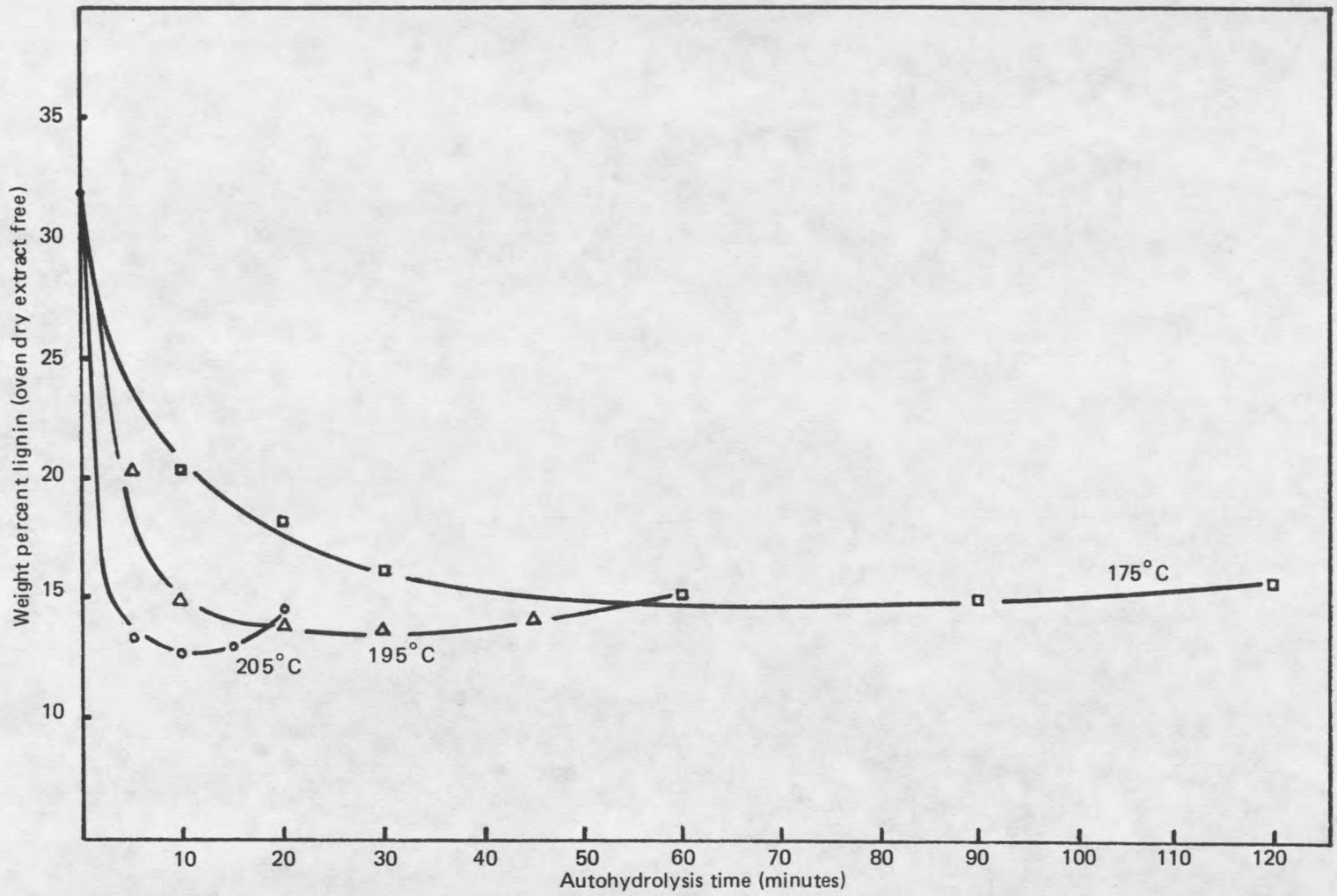


Figure 14. Weight percent lignin after autohydrolysis and extraction.

There is a point in the autohydrolysis sequence where most of the water soluble lignin fractions have been removed but the repolymerization of lignin is not yet significant. At this point, there is still a substantial amount of the lignin that is fragmented. This fraction is soluble in the aqueous ethanol extraction solution. At longer autohydrolysis times the repolymerization takes over and the lignin percent removed by extraction decreases.

Acid Hydrolysis

Because of the increased deligninification that was obtained when the extraction step was coupled with autohydrolysis, the resulting residues had an even greater concentration of cellulose than with authohydrolysis alone. Again, it was expected that the solid residue that resulted from the pretreatment sequence would be highly susceptible to attack from enzymes or acids. The results of the acid hydrolysis tests done on the solid residues are shown in Table 7. The percent of total carbohydrate (alpha, beta, and gamma-cellulose) and total cellulose (alpha and beta) that were hydrolyzed by acid are approximately the same as for acid hydrolysis of autohydrolyzed wheat straw (no extraction). Thus, the hydrolysis of total carbohydrate and total cellulose for the overall process is, again, about the same as for the hydrolysis of the autohydrolyzed wheat straw without the extraction step.

The pretreatment sequence succeeds in removing the hemicellulose and lignin as was reported by Wayman and Lora [33] for aspen woodmeal. The resulting residue is a highly concentrated cellulose solid, but its hydrolytic characteristics are not enhanced over those for raw wheat straw. As was the case for autohydrolyzed wheat straw without extraction, the removal of lignin and hemicellulose coupled with possible effects of air drying results in a highly cellulosic residue that is not readily broken down by acid in the concentration used in this investigation.

Table 7. Effects of Acid Hydrolysis on Autohydrolyzed and Extracted Wheat Straw.

Time at Temperature	Percent Weight Loss Due to Acid Hydrolysis		Percent Weight Loss Due to Overall Process	
	Total Carbohydrate (alpha, beta, and gamma)	Total Cellulose (alpha and beta)	Total Carbohydrate (alpha, beta, and gamma)	Total Cellulose (alpha and beta)
	Autohydrolysis Temperature 195°C			
5 min	22.1	16.2	43.0	22.6
10 min	7.9	5.0	38.7	16.8
20 min	3.0	1.0	37.4	15.0
30 min	4.2	2.6	38.7	14.0
60 min	3.9	2.9	41.8	21.0
	Autohydrolysis Temperature 205°C			
5 min	10.5	No separation of cellulose fraction performed	42.5	No separation of cellulose fraction performed
10 min	5.4		39.6	
15 min	5.1		39.7	
20 min	4.9		40.3	

SUMMARY

The results from each step of the pretreatment sequence investigated are presented here.

Autohydrolysis

The exposure of wheat straw to the conditions of autohydrolysis results in the removal of a majority of the hemicellulose from the lignocellulosic matrix without greatly affecting the cellulose. Also, the lignin fraction is fragmented by autohydrolysis. A certain portion of the fragments are soluble in the autohydrolysis liquor.

Aqueous Ethanol Extraction

The extraction step removes additional lignin fragments from the cellulose pulp produced by autohydrolysis. The amount lignin soluble in the extraction media is a function of the autohydrolysis time and temperature. Cellulose and hemicellulose are not affected by the extraction step.

Acid Hydrolysis

The cellulose pulps produced by autohydrolysis or autohydrolysis followed by extraction are not significantly broken down by moderately strong acid. The hydrolysis technique used in this investigation seemed to solubilize the remaining hemicellulose and any short chain of degraded cellulose without affecting the majority of the remaining cellulose. The final residue is a concentrated, but hydrolysis-resistant cellulose pulp.

CONCLUSIONS

1. The experimental apparatus and procedures developed in the course of this research can be successfully utilized to produce pulps rich in cellulose from lignocellulosic residues. Novel autoclave heating and cooling techniques apparently yield better control of reaction time at temperature than widely used techniques reported in the literature.
2. The standard methods and analytical procedures used in this investigation are appropriate for use in determining the composition of wheat straw residues and pretreatment products.
3. Autohydrolysis and ethanol extraction can be used as a pretreatment to remove substantial amounts of hemicellulose and lignin from wheat straw residues. However, the concentrated cellulose pulps generated are not readily converted to glucose using the acid hydrolysis techniques of this investigation.
4. The morphology of wheat straw residues, altered by the removal of key structural components, seems to contribute to the ineffectiveness of acid hydrolysis on the final pretreatment product. The loss of components, coupled with the air drying of the concentrated cellulose pulp, may lead to a collapse of the remaining fibers. The collapse can, in turn, inhibit the access of the hydrolyzing agent to the substrate and limit the conversion of cellulose to glucose.

RECOMMENDATIONS FOR FURTHER RESEARCH

1. Because of the low acid hydrolysis conversions obtained in this investigation, the effects of substrate drying on the pretreatment results should be studied. The pretreatment sequence used in this investigation should be performed on wheat straw in three different stages of dryness; fresh from the fields, allowed to come to moisture equilibrium with the atmosphere, and oven-dried. In this manner the overall effectiveness of the pretreatment could be determined.
2. Different hydrolysis techniques should be investigated. The removal of lignin and hemicellulose should allow an enhanced hydrolysis of cellulose. Enzymes or different acid hydrolysis techniques could improve the yields of cellulose to glucose obtained with this pretreatment sequence.
3. The conditions of the extraction step utilized in this investigation were held constant. The effects of extraction media, temperature, and duration on lignin removal should be investigated.
4. An analysis of the liquid products of the pretreatment sequence should be done to determine the effects of the process on the carbohydrate components. Substantial degradation of the carbohydrate fractions could overshadow the improved yields ultimately obtained using a pretreatment.
5. The separation of components in the autohydrolysis and extraction liquors could alter the economic feasibility of this process. An investigation of the solubility of lignin in both the autohydrolysis and extraction liquors would yield an insight into this aspect of the process.

6. The ultimate utilization of the pretreatment would be as the front end of an ethanol fermentation process. For this reason, and the fact that the conditions of the pretreatment could be easily controlled, an attempt should be made to adapt the pretreatment outlined in this investigation to a continuous process.

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APPENDIX

Table 8. Wheat Straw Composition (weight percent).

Basis	Air Dried	Oven Dried	Air Dried Extract Free	Oven Dried Extract Free
H_2O	5.3	—	5.8	—
Ash	7.9	8.3	5.5	5.8
Extractive	8.9	9.4	—	—
Lignin	27.4	28.9	30.0	31.9
Total cellulose	50.5	53.4	58.7	62.3
α cellulose	37.2	39.3	43.2	45.9
β cellulose	13.3	14.1	15.5	16.4

Table 9. Run Summary—Autohydrolysis.

Autohydrolysis Temperature (°C)	Autohydrolysis Time (min)	Run Number	Ash	Composition				
				Lignin	Total Cellulose	α Cellulose	β Cellulose	γ Cellulose
				(oven-dried, extract-free weight percent)				
175	10	2	7.1	30.0	62.9	52.0	3.8	7.1
	20	3	8.4	24.4	67.2	58.5	3.2	5.6
	30	1	9.4	20.7	69.9	61.3	4.4	4.3
	60	4	9.1	19.5	71.4	63.5	5.0	2.9
	90	6	10.0	18.9	71.2	64.2	4.9	2.0
	120	7	10.4	18.9	70.7	64.5	4.7	1.4
195	5	11	8.0	28.4	63.6	54.9	4.2	4.5
	10	8	9.6	21.7	68.8	63.0	3.7	2.2
	20	9	10.5	19.0	70.5	65.1	4.0	1.5
	30	10a	10.4	18.8	70.9	65.5	4.2	1.2
	60	12	10.8	18.9	70.3	65.1	4.4	.8
205	5	13	9.3	21.9	68.7	No analysis done		
	10	14	10.1	21.6	68.4	"		
	15	15	10.2	21.5	68.3	"		
	20	16	10.3	21.5	68.2	"		

Table 10. Run Summary—Autohydrolysis and Aqueous Ethanol Extraction.

Autohydrolysis Temperature (°C)	Autohydrolysis Time (min)	Run Number	Ash	Composition				
				Lignin	Total Carbohydrate	α Cellulose	β Cellulose	γ Cellulose
				(oven-dried, extract-free weight percent)				
175	10	2	7.9	20.4	71.7	59.3	4.3	8.1
	20	3	9.5	18.2	72.3	62.9	3.4	6.0
	30	1	10.4	16.3	73.3	64.2	4.6	4.5
	60	4	10.0	15.3	74.7	66.5	5.2	3.0
	90	6	10.8	15.2	74.0	66.8	5.1	2.1
	120	7	11.2	15.9	72.9	66.6	4.9	1.5
195	5	11	8.0	28.4	63.6	54.9	4.2	4.5
	10	8	10.5	15.0	74.5	68.2	4.0	2.3
	20	9	10.9	13.9	75.1	69.4	4.2	1.5
	30	10a	11.0	13.8	75.2	69.5	4.2	1.5
	45	10b	11.3	14.4	74.4	No analysis done		
	60	12	11.4	15.3	73.3	67.9	4.6	.8
205	5	13	11.1	13.5	75.5	No analysis done		
	10	14	11.2	12.5	76.3	"		
	15	15	11.2	12.9	75.9	"		
	20	16	11.1	14.5	74.4	"		

@ 195°C, 5 MIN
18.8% LIGNIN LOSS
80% HEMICELL

Nakaoka, R. K.
Autohydrolysis and
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