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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MONTANA STATE UNIVERSITY
Bozeman, Montana

July 1985

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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ACKNOWLEDGMENTS

I would like to thank the faculty and staff of the Chemical Engineering Department at Montana State University for their help and support. I also extend a special thanks to my advisor, Dr. Daniel Shaffer, whose guidance and insight have proved to be invaluable. The great deal of assistance provided by special friends, Jeffrey Surma and David Lamar, is greatly appreciated. And to my wife, April, whose patience and encouragement made this effort possible, I give my love. Finally, I would like to thank the Montana Department of Natural Resources and Conservation for their insight in funding this project.

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).

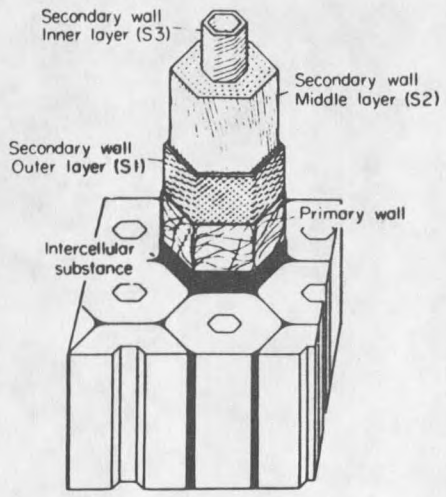

Figure 1. Typical structure of a plant cell wall [18].

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

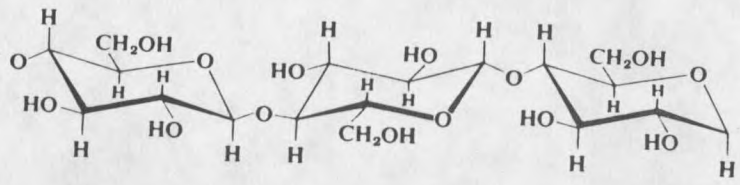

Figure 2. Cellulose molecule [18].

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].

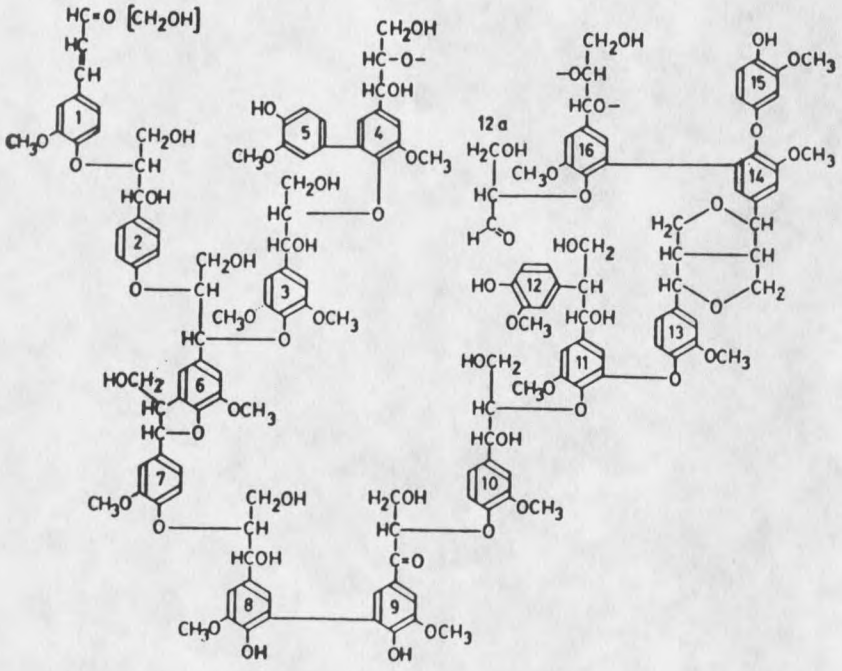

Figure 3. Structure of lignin [27].

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.