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FRACTIONATION OF LIGNOCELLULOSIC BIOMASS FOR FUEL-GRADE ETHANOL PRODUCTION

Topical Report

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DISCLAIMER

ABSTRACT

PureVision Technology, Inc. (PureVision) of Fort Lupton, Colorado is developing a process for the conversion of lignocellulosic biomass into fuel-grade ethanol and specialty chemicals in order to enhance national energy security, rural economies, and environmental quality. Lignocellulosic-containing plants are those types of biomass that include wood, agricultural residues, and paper wastes. Lignocellulose is composed of the biopolymers cellulose, hemicellulose, and lignin. Cellulose, a polymer of glucose, is the component in lignocellulose that has potential for the production of fuel-grade ethanol by direct fermentation of the glucose. However, enzymatic hydrolysis of lignocellulose and raw cellulose into glucose is hindered by the presence of lignin. The cellulase enzyme, which hydrolyzes cellulose to glucose, becomes irreversibly bound to lignin. This requires using the enzyme in reagent quantities rather than in catalytic concentration. The extensive use of this enzyme is expensive and adversely affects the economics of ethanol production.

PureVision has approached this problem by developing a biomass fractionator to pretreat the lignocellulose to yield a highly pure cellulose fraction. The biomass fractionator is based on sequentially treating the biomass with hot water, hot alkaline solutions, and polishing the cellulose fraction with a wet alkaline oxidation step.

In September 2001 PureVision and Western Research Institute (WRI) initiated a jointly sponsored research project with the U.S. Department of Energy (DOE) to evaluate their pretreatment technology, develop an understanding of the chemistry, and provide the data required to design and fabricate a one- to two-ton/day pilot-scale unit. The efforts during the first year of this program completed the design, fabrication, and shakedown of a bench-scale reactor system and evaluated the fractionation of corn stover. The results from the evaluation of corn stover have shown that water hydrolysis prior to alkaline hydrolysis may be beneficial in removing hemicellulose and lignin from the feedstock. In addition, alkaline hydrolysis has been shown to remove a significant portion of the hemicellulose and lignin. The resulting cellulose can be exposed to a finishing step with wet alkaline oxidation to remove the remaining lignin. The final product is a highly pure cellulose fraction containing less than 1% of the native lignin with an overall yield in excess of 85% of the native cellulose. This report summarizes the results from the first year's effort to move the technology to commercialization.

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EXECUTIVE SUMMARY

PureVision Technology, Inc. (PureVision) of Fort Lupton, Colorado is developing a process for the conversion of lignocellulosic biomass into fuel-grade ethanol and specialty chemicals in order to enhance national energy security, rural economies, and environmental quality. Lignocellulosic-containing plants are those types of biomass that include wood, agricultural residues, and paper wastes. They are a composite polymeric material containing primarily cellulose, lignin, and hemicellulose. Cellulose is a polymer composed of chains of six-carbon sugars (primarily glucose). These chains are bundled into strong fibers that on close inspection are seen to have an organized crystalline structure. Lignin is a complex, three-dimensional polymer composed of linked six-carbon phenolic rings with various carbon chains and other chemical functionalities. Lignin is non-crystalline, and its structure has been described as analogous to a gel or foam. The lignin serves to bind the cellulose fibers. Hemicellulose is a very complex polymer composed of various five- and six-carbon sugars in a highly branched structure. Hemicellulose attaches weakly to both cellulose and lignin and fills the intervening spaces.

For many years, the primary deterrent for economically converting lignocellulosic biomass to sugars via enzymatic hydrolysis has been the high cost of cellulase enzymes (Ramos et al., 1993). Enzymes, in principle, act to catalyze hydrolysis at a multitude of sites by moving from site to site. In this mode, a small amount of enzymes should be able to catalyze a large amount of cellulose so that the cost of the enzymes should not be of great importance. The problem is that when lignin is present, the enzymes are blocked from reaching some sites, and worse, the enzymes become irreversibly bound to the lignin and are no longer able to serve as mobile catalysts. In this situation, the enzymes act more as consumable reagents than as catalysts, and their cost becomes critical. Net enzyme usage in this reagent mode may be as much as ten times what is required in the catalyst mode.

It is generally recognized that enzymatic processes may have advantages in terms of energy requirements and waste management. Lignocellulosic biomass, however, is a complex polymer of cellulose, hemicellulose, and lignin that has evolved over millions of years to resist biological and environmental assaults commonly encountered in nature. Thus, some form of "unnatural" pretreatment is necessary to make biomass more vulnerable to enzymatic attack. To address this pretreatment, PureVision is developing a fractionation process to separate the three components and yield a highly pure cellulose fraction for further processing.

In September 2001 PureVision and Western Research Institute (WRI) initiated a jointly sponsored research project with the U.S. Department of Energy (DOE) to evaluate their pretreatment technology, develop an understanding of the chemistry, and provide the data required to design and fabricate a one- to two-ton/day pilot-scale unit. The efforts during the first year of this program completed the design, fabrication, and shakedown of a bench-scale reactor system and evaluated the fractionation of corn stover. Corn stover was selected as the first feedstock to be used to evaluate the technology. Based on the results using corn stover as the feedstock, the following conclusions have been drawn:

- The batch autoclave experiments demonstrate that hot water/steam hydrolysis of corn stover can achieve a high level of removal of both hemicellulose and lignin from the feedstock.

- The results from the wet alkaline oxidation experiments conducted in the batch autoclaves are inconclusive and additional research is needed to understand this chemistry and develop ways to control the oxidation.

- The preparatory experiment conducted using the washed-bed reactor system followed by wet alkaline oxidation in the batch autoclaves demonstrated that a high yield of pure cellulose could be obtained with this process.

- The results from the preparatory experiment show additional work is needed to optimize each sequence in the process.

INTRODUCTION

A PureVision biomass to value-added-products plant can be referred to as a biorefinery. Instead of refining petroleum to make hydrocarbon derivatives such as fuels, lubricants, chemicals, etc., the PureVision technology refines biomass (wood, agricultural and paper wastes, energy crops, etc.) into fuel, fiber, energy, and sugars. The sugars are raw materials that can be used for making many products such as bioplastics, ethanol, acetic acid, and other chemicals. Over the last 80 years, there has been an economic advantage of hydrocarbons over biomass feedstocks due to large crude oil deposits. However, as world petroleum supplies are dwindling, the demand for energy has increased, resulting in higher oil prices. As prices rise, biomass-derived products increasingly have economic and environmental advantages over products produced from fossil fuels. The overall goal of the PureVision approach is to produce fuel, energy, and specialty chemicals from waste biomass at costs competitive with petroleum in order to enhance and extend the nation's energy and chemical base without adding additional carbon to the environment.

Biorefineries of the future will produce a wide array of products derived from biomass that cannot even be envisioned at this time. These products will include specialty chemicals for the chemical manufacturing industry, polymers, and fuels. However, current markets are not yet ready to accept the majority of the products that will become available through biorefineries. In the near term, biorefineries must rely on products that already have a market in today's economy. Production of fuel-grade ethanol is the key to initial economic survival for biorefineries. Fuel-grade ethanol has a current market for use as an oxygenated additive to motor gasoline and potential for use as an octane booster in near-term applications. For this reason, this project is directed at obtaining a lignin-free, cellulose fraction from lignocellulosic biomass that can be used to produce fuel-grade ethanol.

Lignocellulosic-containing plants are those types of biomass that include wood, agricultural residues, and paper wastes. They are a composite polymeric material containing primarily cellulose, lignin, and hemicellulose. Cellulose is a polymer composed of chains of six-carbon sugars (primarily glucose). These chains are bundled into strong fibers that on close inspection are seen to have an organized crystalline structure. Lignin is a complex, three-dimensional polymer composed of linked six-carbon phenolic rings with various carbon chains and other chemical functionalities. Lignin is non-crystalline, and its structure has been described as analogous to a gel or foam. The lignin serves to bind the cellulose fibers. Hemicellulose is a very complex polymer composed of various five- and six-carbon sugars in a highly branched structure. Hemicellulose attaches weakly to both cellulose and lignin and fills the intervening spaces. The heart of PureVision's biorefinery, the biomass fractionation reactor, is a unique technology that is capable of separating these three major components of lignocellulose into distinct fractions.

It is the unique biomass fractionation technology that distinguishes the PureVision process from competing technologies. The PureVision fractionation process converts lignocellulosic biomass into three distinct fractions, cellulose, hemicellulose, and lignin, each with a wide range of uses and by-products including sugars, ethanol, and other chemical products. The cellulose fraction has potential use as a fiber or as a feedstock for the production of fuel-grade ethanol. The resulting lignin fraction has potential as a source of specialty chemicals, but in the near term, as a fuel for cofiring with coal to produce electricity at the biorefining site. The hemicellulose fraction has potential for being hydrolyzed into five-carbon sugars, which are building blocks for numerous products and industrial chemicals or producing energy by aerobically digesting the sugars to methane.

For many years, the primary deterrent for economically converting biomass to sugars via enzymatic hydrolysis has been the high cost of cellulase enzymes (Ramos et al., 1993). Enzymes, in principle, act to catalyze hydrolysis at a multitude of sites by moving from site to site. In this mode, a small amount of enzymes should be able to catalyze a large amount of cellulose so that the cost of the enzymes should not be of great importance. The problem is that when lignin is present, the enzymes are blocked from reaching some sites, and worse, the enzymes become irreversibly bound to the lignin and are no longer able to serve as mobile catalysts. In this situation, the enzymes act more as consumable reagents than as catalysts, and their cost becomes critical. Net enzyme usage in this reagent mode may be as much as ten times what is required in the catalyst mode.

The development dilemma is between the high cost of producing clean cellulose for lower enzyme usage verses the higher cost of enzymes for use with low-cost pretreatment. The mainstream research effort today is directed at lowering the cost of enzymes so that it will be economical to use the enzymes as reagents. However, studies reported in the literature demonstrate that clean cellulose leads to lower net enzyme consumption. This results in increased enzyme recycle and higher reaction rates (Holtzapple et al., 1992; and Ramos et al., 1993). PureVision’s approach is to produce a clean cellulose product using a unique fractionation technology. The clean cellulose will require enzyme consumption of one fifth or less of that required of competing technologies. This will result in a major breakthrough for the emerging biomass conversion industry.

The chemical structure of lignocellulose can be broken by a variety of conditions not commonly found in nature such as heat, acids, alkali, oxidation, and certain other chemical reactions. The challenge for fractionation of lignocellulose is to break up the larger polymeric structures while preserving the building blocks for various down stream uses. There are dozens of types and strengths of chemical bonds susceptible to modification, and some of these modifications can lead to undesirable products. The PureVision approach to biomass utilization uses a unique process involving mild alkaline hydrolysis and wet alkaline oxidation to sequentially depolymerize hemicellulose and lignin and remove them from the reaction, leaving a nearly pure cellulose fraction (Curreli et al., 1997).

In order to achieve the control needed for fractionation, PureVision has developed a sequential process for the separation of cellulose from other components of lignocellulosic biomass. In the reactive fractionation step, hemicellulose is most easily hydrolyzed, requiring a mild alkaline solution and temperatures in the range of 150 to 200 °C (302 to 392 °F). Next, lignin is removed under more severe conditions requiring a pH above 10, temperatures less than 250 °C (482 °F), the presence of an oxidizing agent, and pressures below 600 psi. The resulting "clean" cellulose is mostly free of lignin and hemicellulose and is a purified feedstock for enzymatic hydrolysis.

In September 2001 PureVision and Western Research Institute (WRI) initiated a jointly sponsored research project with the U.S. Department of Energy (DOE) to evaluate the technology, develop an understanding of the chemistry, and provide the data required to design and fabricate a one- to two-ton/day pilot-scale unit. The efforts during the first year of this program completed the design, fabrication, and shakedown of a bench-scale reactor system and evaluated the fractionation of corn stover. The results from the evaluation of corn stover have shown that water hydrolysis prior to alkaline hydrolysis may be beneficial in removing hemicellulose and lignin from the feedstock. In addition, mild alkaline hydrolysis has been shown to remove a significant portion of the hemicellulose and lignin. The resulting cellulose can be exposed to a finishing step with wet alkaline oxidation to remove the remaining lignin. The final product is a highly pure cellulose fraction containing less than 1% of the native lignin with an overall yield in excess of 85% of the native cellulose. This report summarizes the results from the first year's effort to move the technology to commercialization.

BACKGROUND

The U.S. DOE has long been supporting research on the utilization of biomass for the production of fuel ethanol and other chemicals in order to enhance national energy security, rural economies, and environmental quality. It is generally recognized that enzymatic processes may have advantages in terms of energy requirements and waste management. Lignocellulosic biomass, however, is a complex polymer of cellulose, hemicellulose, and lignin that has evolved over millions of years to resist biological and environmental assaults commonly encountered in nature. Thus, some form of "unnatural" pretreatment is necessary to make biomass more vulnerable to enzymatic attack.

The cellulose component of biomass is generally about 40% of the dry weight and is of particular interest. Cellulose is a polymer of glucose, an industrially important sugar that is especially easy to ferment to fuel-grade ethanol. Enzymatic hydrolysis is a process by which cellulose can be decomposed into its glucose building blocks. The more pure the cellulose, the easier it is to decompose, the less enzymes are required, and the lower the cost for expensive enzymes. This is the rationale behind the PureVision pretreatment process that focuses on the production of highly purified cellulose.

The PureVision pretreatment process involves a sequence of increasingly severe steps to decompose the biomass. The first step is a soak and wash with water for approximately five minutes at a temperature of about 200 °C (392 °F). In this step, the more fragile components of hemicellulose, lignin, and extractives are mobilized and removed from the reaction. The second step is a wash with alkali (NaOH) at a concentration of 1 to 4 grams per liter for five to ten minutes at a temperature of about 220 °C (482 °F). This second wash (wet alkaline oxidation) is then continued for about two minutes with the addition of elemental oxygen. Finally, the residual solids are rinsed with water at the elevated temperature, compressed, and released to atmospheric pressure. This process is intended to be carried out in a continuous apparatus with liquids and solids in counterflow, but this continuous process is only simulated in the work reported here.

PURPOSE

The purpose of the work performed during the first year was to conduct the experiments needed to provide the data required for PureVision to move the developmental efforts from batch and semi-batch, laboratory-scale studies to a continuous pilot-plant unit. The first year's research had three principal objectives:

- Generate data necessary for process evaluation and scale up to a continuous-flow demonstration unit;
- Evaluate and understand the reactive fractionation process for biomass feedstocks;
- Demonstrate PureVision's reactive fractionation unit to process lignocellulosic biomass to yield high-purity cellulose.

METHODOLOGY

This section discusses the design and operation of the reactors and analytical procedures used to evaluate the PureVision biomass fractionator.

Washed-Bed Reactor Design and Operation

Experiments were carried out in two types of apparatus, a washed-bed reactor and simple batch autoclaves. The washed-bed reactor system will be discussed in this section. A simplified schematic of the washed-bed system is shown as Figure 1. The actual apparatus includes several thermocouples, pressure regulators, and plumbing for filling, draining, back flushing, and other housekeeping tasks. These are not shown on the schematic in order to simplify the drawing and allow a better understanding of the operation of the unit. The system design was based on utilizing a U-tube reactor immersed in a fluidized sand bath. The U-tube reactor was made of ¾-inch stainless steel tubing with a working volume of 90 ml. The reactor was mounted on a vibrator assembly and connected by 1/8-inch tubing to the remainder of the system. The U-tube reactor was heated by a temperature-regulated fluidized sand bath. Two such sand baths were mounted on a pneumatic lift. At the start of an experiment, the lift carriage is raised to immerse the U-tube reactor in a sand bath at the specified temperature. If an experiment required a change in operating temperature to study another phase of the process, the lift carriage is lowered and moved to position the second sand bath below the U-tube reactor. The carriage is then raised to immerse the U-tube reactor in the second sand bath, thus changing the reaction temperature.

Electrical band heaters were used on the pressure tanks to heat the hot alkali and water and were controlled by temperature controllers through internal thermocouples. Thermocouple data was recorded at five-second intervals using an Omega model OM-220 data logger. A heat exchanger followed by a pressure regulator (not shown) allowed withdrawal of liquid product without flashing while maintaining a system pressure of 500 psi. Water washes were delivered to the reactor system by nitrogen pressure and the alkali wash was fed to the system with a Neptune Chemical Pump Company, series-500, high-pressure pump.

In a typical experiment, the reactor was loaded with 12 grams of corn stover and soaked in water to allow air to escape and wet the feed material. The liquid and sand bath temperatures were set to the appropriate values for the experiment. The reactor was then connected to the system and pressure checked to verify there were no leaks. Raising the first sand bath to begin heating the reactor started the experiment. This initiated auto-catalyzed hydrolysis of the hemicellulose in the corn stover. This was followed by a wash with hot water to remove components dissolved and acids formed. In a matter of a few seconds, the water wash was turned off, the alkali wash was turned on, and the sand baths were switched to provide a higher temperature operation. The experiment was terminated by lowering the sand bath and flushing the reactor with cold water for rapid cooling. Liquid product samples were collected throughout the experiment for analysis, and at the end, the reactor was disassembled and emptied.

Batch Autoclave Experiments

Several experiments were conducted in batch autoclaves to gain insight into specific aspects of the fractionation technology. The batch autoclaves were constructed from 150-ml, stainless steel sample cylinders. Each reactor was fitted with a 1/8-inch x 18-inch length of stainless steel tubing and a valve. Stainless steel plugs were used to seal the opposite end of the batch autoclaves. A schematic diagram of the batch autoclaves used in this study is presented as Figure 2. Each bomb was loaded with the desired quantity of reactant solution and 1 to 4 grams of the feedstock designated for the experiment. Air was expelled from the reactor with nitrogen gas, or in the case of the wet oxidation experiments, oxygen gas. The bombs were immersed in the sand bath at the specified temperature and allowed to react for the desired time. The bombs were removed from the sand bath and quenched in cold water.

Analytical Procedures

Several analytical procedures were used to follow the fate of the biomass feedstock through the fractionation technology. Samples of the feedstock and solid products from the different reactions were first hydrolyzed under acid conditions using the method reported by

Templeton and Ehrman (1995). This procedure is based on earlier work reported by Saeman et al. (1945) and provides a direct estimate of the ash content and the concentration of acid-insoluble (Klason) lignin. The procedure also provided an aqueous solution containing the acid-soluble lignin and the free sugars released during the acid hydrolysis. This solution was analyzed for the acid-soluble lignin and sugars content by the procedures discussed below.

The aqueous products from acid hydrolysis of the feedstock and solid products and the different liquid products generated from the experiments studying the technology were analyzed to determine the acid-soluble lignin and sugars concentrations. The acid-soluble lignin concentration was determined using the method described by Ehrman (1996). The sugars were analyzed using the method of Dubois et al. (1956), which reports all of the C-5 sugars as xylose and the C-6 sugars as glucose.

Biomass Feedstock

Corn stover was selected as the first feedstock to be evaluated in the reactor systems discussed above. The National Renewable Energy Laboratory (NREL) in Golden, Colorado provided the corn stover to PureVision. Corn stover was selected as the first feedstock to be evaluated because others have used it extensively as a feedstock and a large amount of analytical data is available for it. Therefore, using it as the first feedstock to be evaluated, it serves as a reference for future work with other feedstocks. The feedstock analysis provided by NREL and the analyses performed by WRI are shown in Table 1. The composition as reported by NREL does not yield 100% because NREL has not yet completed analysis of all of the components present in the sample.

RESULTS AND DISCUSSION

Results from the experiments conducted during this effort are reported and summarized in this section. The discussion includes presentation of the results and their significance to development of the PureVision biomass fractionator.

Severity Index

Many of the results discussed in this report are expressed as a measurable parameter, such as product recovery verses the log of severity (LS). The LS is the logarithm to base 10 of the severity index. The severity index is an expression that combines the independent variables of temperature and time into a single independent variable. This is a mathematical expression of the observation that reaction rates double for every 10 °C increase in temperature. The severity index is expressed mathematically as follows:

$$\int_0^t \exp((T-100)/14.75)\,dt \qquad (1)$$

where: T is temperature in degrees Celsius and
t is time in minutes.

From this equation it is obvious that a temperature of 100 °C (212 °F) for one minute gives a LS value of 1. We have found that using this expression of the effects of reaction time and temperature is a useful tool for evaluation of the data collected during this phase of the project.

Sample and Product Analyses

The lignocellulose biomass used as the feedstock for this study is composed of three biopolymers, cellulose, hemicellulose, and lignin. Direct analysis of the feedstock and the products generated during this study was found to be difficult; therefore indirect analyses of the polymers were performed by analysis of the monomeric units. Cellulose is composed of the six-carbon sugar glucose, while hemicellulose is composed primarily of the five-carbon sugar xylose and small concentrations of arabinose (five-carbon sugar). Hemicellulose also contains small concentrations of the six-carbon sugars mannose and glactose. Lignin is a complex, three-dimensional polymer composed of linked six-carbon phenolic rings with various carbon chains and other chemical functionalities.

The polymers are intact in the feedstock and solid products from the experiments and must first be hydrolyzed in an acid media to generate the monomeric units from cellulose and hemicellulose (Templeton and Ehrman, 1995). The resultant acid solution and the liquid products from the processing experiments contain the free sugars and were analyzed by the spectroscopic method of Dubois et al. (1956). This method reports the C-5 sugars as xylose and the C-6 sugars as glucose. Since hemicellulose is composed primarily of xylose and cellulose is composed of glucose, analysis of the liquids for these two sugars provides a good estimate of the concentrations of hemicellulose and cellulose, respectively.

Only a small fraction of the lignin is soluble in the acid used for the hydrolysis. The concentration of this lignin (acid-soluble lignin) is determined by the method of Ehrman (1996). The acid-insoluble lignin from the acid hydrolysis is collected by filtration of the hydrolyzate and its mass determined gravimetrically.

The liquid products from the experiments contain the monomeric units and oligomers from cellulose and hemicellulose. These solutions were analyzed for five- and six-carbon sugars by the method of Templeton and Ehrman (1995). The method utilizes an acid in the reaction to produce the chromophoric species analyzed by the method. Experience has shown additional acid hydrolysis is not necessary to hydrolyze the oligomers that may be present in the solutions.

The results from this analysis were reported as was discussed for the hydrolyzates from the feedstock and solid products. The liquid products were also analyzed for acid-soluble lignin using the method of Ehrman (1996).

Feedstock Analysis

The results of the analysis of the corn stover used as the feedstock are reported in Table 1. The table includes the results provided by NREL and those generated by WRI. The results provided by NREL are more detailed in terms of the distribution of the components in the feedstock than are the results generated by WRI. NREL reports the individual sugar monomers in cellulose and hemicellulose and the protein and aceyl content. Also, NREL reports only the acid-insoluble lignin.

Because of the analytical procedure used by WRI, the protein and aceyl content of the feedstock was not determined. This material is included in the estimate of the hemicellulose concentration. The lignin concentration reported by WRI includes the acid-soluble component, which causes this value to be higher than that reported by NREL.

Considering the differences in the two analytical approaches, the results are comparable. Cellulose is present at the highest concentration and represents about 38% of the feedstock. When the differences in the two methods are considered, the hemicellulose and lignin concentrations reported by the two methods are also comparable. There are differences reported in the ash concentration and may be due to sampling errors, as mineral matter is observed associated with the feedstock.

Water Hydrolysis Studies in Batch Autoclaves

Several experiments were conducted in the batch autoclave reactor system to evaluate the reaction of corn stover with high-pressure water/steam. The chemistry of water under the conditions used for these is unique and has the potential to react with the different polymers in lignocellulose. There are suggestions from the literature that both lignin and hemicellulose can be hydrolyzed under these conditions (Schulman et al., 1999). Figure 3 shows the distribution of the products from water hydrolysis on corn stover at different levels of severity.

Assays of samples were calculated back to the starting material by simple proportioning without any other normalization being applied. In the figure, “Solid” refers to the solid product recovered from the experiment. The values for “Pentose,” “Lignin,” and “Hexose” were determined from analysis of the liquid recovered from each experiment. The “Total” is the sum of each of the components and does not include protein, acetate, furfural, or other degradation products for which no assays were done.

The results in Figure 3 show that the concentration of both pentose and lignin in the reaction liquid increase with increasing levels of severity. Since the pentose concentration is a measure of the removal of hemicellulose from the corn stover, the results show that both hemicellulose and lignin are being removed from the corn stover during this reaction. The concentration of hexose remains relatively constant in the produced liquid with increasing severity. Since the hexose value is a measure of the hydrolysis of cellulose, the results show cellulose is not being removed from the corn stover.

These results are significant for advancement of the PureVision technology. The goal of the pretreatment process is to remove both the hemicellulose and the lignin from the corn stover without affecting the cellulose. These results show that both hemicellulose and lignin are removed and that the cellulose is not greatly affected. Being able to achieve this level of purification of the cellulose with water as the reagent has significant economic benefits to the process.

Alkaline Hydrolysis Studies in Batch Autoclaves Using Lye

Three experiments were conducted in the batch autoclaves to evaluate the reaction of corn stover with an alkaline solution. The alkaline solution used for these experiments was prepared with sodium hydroxide to yield a solution with a pH of 12.2. The results of these experiments are provided in Figure 4. The results displayed in this figure are defined the same way as those provided in Figure 3.

Examination of these results show that both the lignin and hemicellulose (as defined by pentose concentration) are removed from the corn stover during reaction with the alkaline solution. The cellulose polymers are not affected by this treatment, as shown by the low concentration of hexoses in the product liquid. These results imply that an alkaline reaction may have sufficient severity to be used as a final step to remove the remaining hemicellulose from the corn stover.

Wet Alkaline Oxidation Studies in the Batch Autoclaves

Wet alkaline oxidation is being considered as the final step in the pretreatment sequence to remove the final traces of lignin and yield a highly purified cellulose product. However, the benefits of wet alkaline oxidation have not been demonstrated with certainty. The results of a number of bomb experiments are displayed in Figure 5. A composite feedstock for the wet alkaline oxidation studies was prepared from four experiments using the washed-bed reactor. These experiments utilized a LS = 4.6, and a solid product recovery of 38% with an actual lignin content of 1.95%. The bombs were loaded with 1 gram of solid material (roughly 0.006 moles expressed as monomeric glucose) and 100 ml of alkali with a pH of 12.8. Oxygen was then bubbled in to displace air. In a final preparation step, additional alkali liquid was pumped in to establish a desired pressure. Thus, all runs had the same quantity of oxygen (roughly 0.002 moles), but the liquid volume and hence oxygen pressure varied. Oxygen gauge pressure at ambient conditions was varied from 0 to 60 psi for the different experiments. In one experiment (the solid diamond), nitrogen was used as a blank reference instead of oxygen. In contrast to previous charts in this report, "Product Recovery" and "Product Lignin %" are referenced to the material loaded into the bombs and not back to the original feedstock.

No clear trend can be seen from the results in Figure 5, nor can a trend be found in relating the lignin fraction to oxygen pressure or to process severity. Because of the relatively strong alkali used, final pH for all experiments was between 12.5 and 12.6, so there is no relationship to final pH of the product solution. In most of the experiments there was a loss of 12 to 14% of the solid material. Since the molar ratio of oxygen to glucose was about 1:3 it is entirely possible that the oxygen randomly attacked the cellulose polymer, leading to soluble oligomers or other products.

It is not clear why there should have been a loss of material in the experiment with nitrogen. The material had already received a strong treatment with pH-12.8 alkali, and the present treatment was only about one fourth as severe. A possible explanation is that the material had been dried at 105 ˚C (221 ˚F) and may have partially oxidized, thereby becoming more soluble and exhibiting behavior similar to the wet oxidation. Additional research is needed to better understand the wet alkaline oxidation process.

Washed-Bed Reactor Experiments

Experiments in the washed-bed reactor were carried out to verify the results obtained from the batch autoclave experiments, to provide samples for other research groups, and to demonstrate the validity of the pretreatment concept. One very successful washed-bed reactor experiment was conducted for the purpose of preparing samples for tests of enzymatic hydrolysis. To conserve the solid product generated by this experiment, samples were not collected and analyzed at intermediate steps, and only the analytical samples were dried at the end of the experiment.

The reactor was loaded with 12 grams of corn stover previously soaked in water. In the washed-batch phase there was a five-minute soak time with a temperature rise to 195 ˚C (383 ˚F) and a LS = 3.2. This was followed by a 370-ml water wash at 200 ˚C (392 ˚F) for four minutes reaching a cumulative LS = 3.6. The material in the reactor was then subjected to an 865-ml, pH-12.8 alkali wash at 216 ˚C (420 ˚F) for 11 minutes reaching a cumulative LS = 4.47. Finally, the material was rinsed with cold water to end the washed-bed phase. The solid wet product was divided and transferred to three batch autoclaves, to each of which was added 100 ml of pH-12.8 sodium hydroxide solution. The bombs were purged with oxygen and pressurized to 50 psig with 12.8-pH solution, as described previously. The wet alkaline oxidation stage of the experiment was conducted for 10.5 minutes reaching a peak temperature of 215 ˚C (419 ˚F) and a LS = 3.85.

The results from this experiment are presented in Table 2. As discussed, the solid product from the washed-bed reactor portion of this test was not collected for analysis. It was used intact for the wet alkaline oxidation portion of the experiment. Solid product recovery was

33.4% by weight of the original material with a slightly negative lignin concentration. The closure for this experiment was 92.7%, which is considered good in light of the amount the products were handled to demonstrate the process sequence.

The results in Table 2 show that the hot-water flush removed about one third of the lignin and little of the hemicellulose from the raw corn stover. These values are lower than was observed in the hot-water experiments conducted in the batch autoclaves. It is believed the desired level of severity was not achieved during this phase of the experiment to fully utilize the hot-water treatment. The liquid from the alkaline wash was collected as two samples. The initial liquid accounts for the majority of the lignin and about one third of the hemicellulose removed from the feedstock. The final alkaline wash liquid contains the majority of the products from the hydrolysis of the hemicellulose and the remaining lignin products. Analysis of the solid product from the wet alkaline oxidation phase of the pretreatment shows it is composed primarily of hexose with a trace of pentose sugars. Based on these results, the solid product is composed primarily of the desired cellulose. The lignin value from the analysis was slightly negative and is reported as zero. These results show that the goal of the pretreatment process can be achieved. The final solid product is primarily the glucose polymer cellulose and it is essentially free of lignin contamination.

CONCLUSIONS

Based on the results discussed in this report, the following conclusions can be drawn:

- The batch autoclave experiments demonstrate that hot water/steam hydrolysis of corn stover can achieve a high level of removal of both hemicellulose and lignin from the feedstock.

- The results from the wet alkaline oxidation experiments conducted in the batch autoclaves are inconclusive and additional research is needed to understand this chemistry and develop ways to control the oxidation.

- The preparatory experiment conducted using the washed-bed reactor system followed by wet alkaline oxidation in the batch autoclaves demonstrated that a high yield of pure cellulose could be obtained with this process.

- The results from the preparatory experiment show additional work is needed to optimize each sequence in the process.

REFERENCES

Curreli, Nicoletta, et al., 1997, Mild Alkaline/Oxidative Pretreatment of Wheat Straw, Process Biochemistry, 32, pp. 665-679.

Dubois, Michel; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, Fred, 1956, Colorimetric Method for Determination of Sugars and Related Substances, Anal. Chem., 28, (3), pp. 350-356.

Ehrman, Tina, 1996, NREL CAT Task Laboratory Analytical Procedure #004, Determination of Acid-Soluble Lignin in Biomass.

Holtzapple, Mark T., et al., 1992, Pretreatment of Lignocellulosic Municipal Solid Wastes by Ammonia Fiber Explosion (AFEX), Applied Biochemistry and Biotechnology, 34/35, pp. 5-21.

Ramos, L. P.; C. Breuil; and J. N. Saddler, 1993, The Use of Enzyme Recycling and the Influence of Sugar Accumulation on Cellulose Hydrolysis by *Trichoderma* Cellulase, Enzyme Microb. Technol., 15, pp. 19-25.

Saeman, Jerome F.; Bubl, Janet L.; Harris, Elwin E., 1945, Quantitative Saccharification of Wood and Cellulose, Ind. Eng. Chem. Anal., 17, pp. 35-37.

Schulman, D., S. Allen, J. Lichwa, and M. J. Antal, 1999, "Comparison of Steam and Hot Water Pretreatments for the Bioconversion of Lignocellulosics to Ethanol," Division of Fuel Chemistry, Preprints, Vol. 44 (2), pp. 219-223.

Templeton, David; and Ehrman, Tina, 1995, NREL CAT Task Laboratory Analytical Procedure #003, Determination of Acid-Insoluble Lignin in Biomass.

Table 1. Composition of the Corn Stover Used as the Feedstock as Determined by NREL and WRI

Component	Weight Percent (NREL)	Weight Percent (WRI)
Cellulose	37.5	38.4
Glucose	37.5	38.4
Hemicellulose	25.9	34.2
Xylose	20.8	34.2
Mannose	0.8	
Glactose	1.6	
Lignin	17.6	24.9
Protein	2.9	
Aceyl	2.2	
Ash	6.7	4.3
Total	92.8	99.9

Table 2. Results of the Experiment to Demonstrate the Combined Effect of the Different Pretreatment Steps

Run PVT-19 (12.01 gm)	Mass In Product, g					
Sample	Hexoses	Pentoses	Acid-Sol. Lignin	Acid-Insol. Lignin	Ash	Total Mass
U-Tube Product	NA	NA	NA	NA	NA	NA
Hot-Water Flush Liquid	0.03	0.09	0.09	0.36	0.00	0.57
Initial Alkaline Wash Liquid	0.32	1.47	0.15	0.71	0.01	2.66
Final Alkaline Wash Liquid	0.67	3.29	0.52	0.19	0.01	4.69
Oxidation Solid Product	3.11	0.03	0.00	0.00	0.07	3.21
Product Assay Total, g	4.13	4.88	0.77	1.27	0.09	11.14
Percent of Feed, %	34.4	40.6	6.4	10.5	0.8	92.7

NA is not analyzed

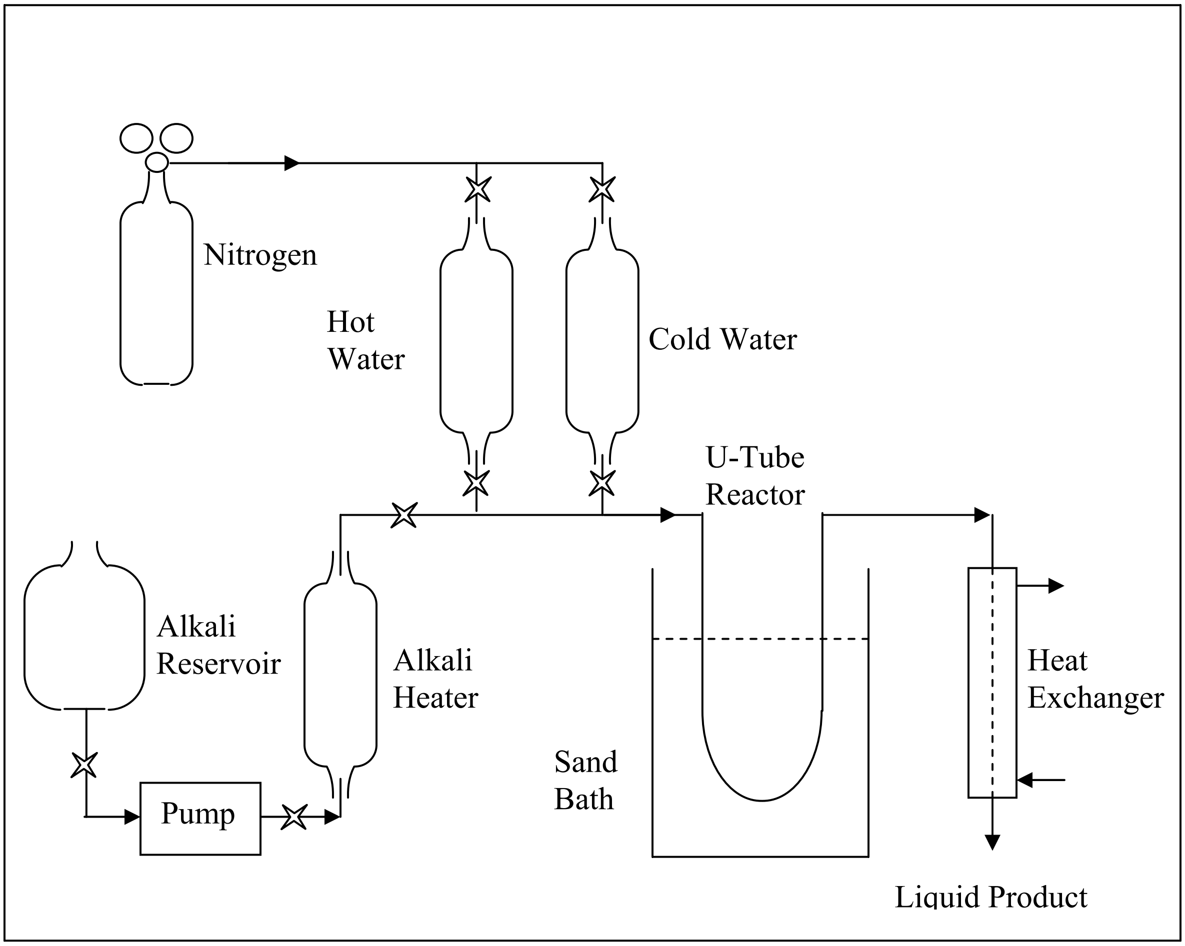

Figure 1. Simplified Schematic of the Washed-Bed Reactor System

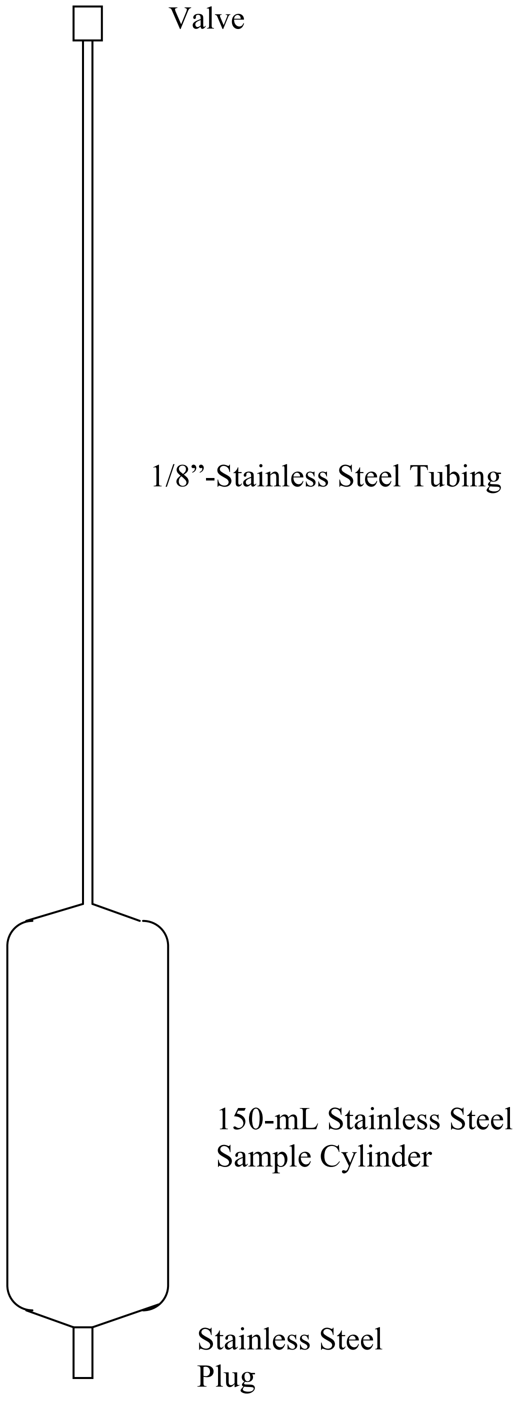

Figure 2. Schematic Diagram of Batch Autoclave Reactor System

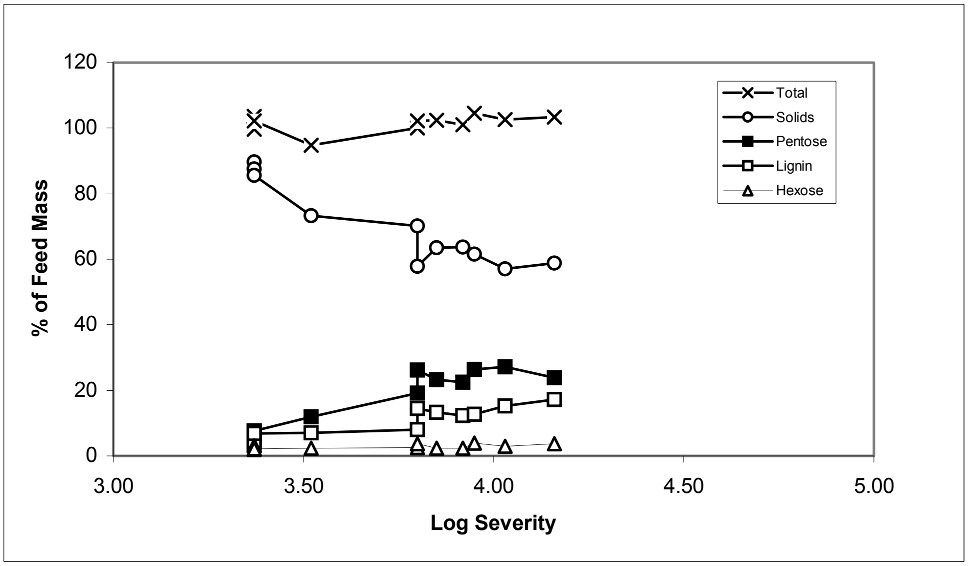

Figure 3. Product Distribution as Functions of Severity From Water Soak Experiments with Corn Stover

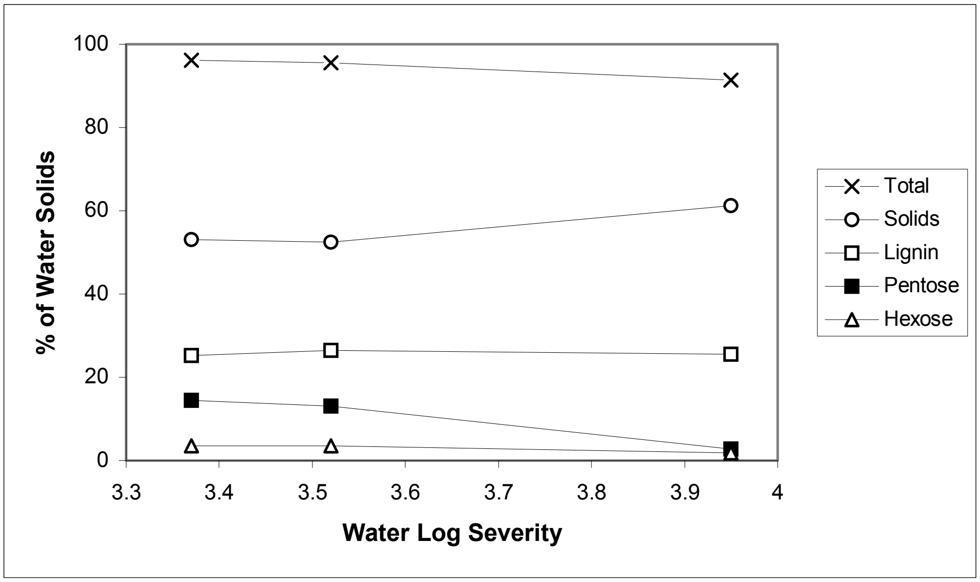

Figure 4. Product Distribution as a Function of Severity for Corn Stover Reactions in Lye Solution

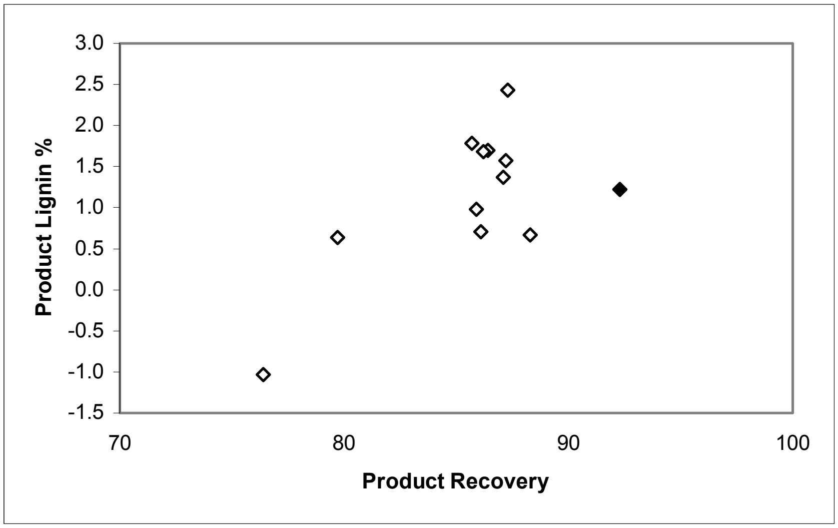

Figure 5. Percent Lignin in the Solid Product as a Function of Product Recovery From Wet Alkaline Oxidation (Solid Diamond is Nitrogen Gas Blank)