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Understanding Factors that Limit Enzymatic Hydrolysis of Biomass

Characterization of Pretreated Corn Stover

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Abstract

Spectroscopic characterization of both untreated and treated material is being performed in order to determine changes in the biomass and the effects of pretreatment on crystallinity, lignin content, selected chemical bonds, and depolymerization of hemicellulose and lignin. The methods used are X-ray diffraction for determination of cellulose crystallinity (CrI); diffusive reflectance infrared (DRIFT) for changes in C–C and C–O bonds; and fluorescence to determine lignin content. Changes in spectral characteristics and crystallinity are statistically correlated with enzymatic hydrolysis results to identify and better understand the fundamental features of biomass that govern its enzymatic conversion to monomeric sugars. Models of the hydrolysis initial rate and 72 h extent of conversion were developed and evaluated. Results show that the hydrolysis initial rate is most influenced by the cellulose crystallinity, while lignin content most influences the extent of hydrolysis at 72 h. However, it should be noted that in this study only crystallinity, lignin, and selected chemical bonds were used as inputs to the models. The incorporation of additional parameters that affect the hydrolysis, like pore volume and size and surface area accessibility, would improve the predictive capability of the models.

Index Entries: AFEX; corn stover; multilinear regression; statistical model.

Introduction

Lignocellulosic biomass feedstocks typically contain 55–75% by dry weight of carbohydrates that are polymers of five- and six-carbon sugar units *(1,2)*. These carbohydrate polymers that exist mostly in the plant cell wall must be broken down to their respective low-molecular-weight sugar components before microorganisms can complete the conversion to ethanol or other products.

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Plant cell walls and structural tissues are primarily composed of cellulose, a polymer of β(1,4)-linked cellobiose residues, hemicellulose and lignins (Fig. 1A)*(3)*. Currently, no definitive model of the cell wall exists, particularly one that relates the cell wall composition to its mechanical properties. However, the architectural features of the primary cell wall are the following. The fundamental framework of cellulose and cross-linking glucans lies embedded in a second matrix of pectic polysaccharides. An additional independent network consists of the structural proteins or a phenylpropanoid network. Cellulose and pectin networks are largely independent or only interact weakly through hydrogen bonding. Hemicellulose interacts much more strongly with cellulose and makes the network more rigid.

Success in the modification of various cell wall constituents helps in understanding the molecular basis for mechanical and structural properties of plant-derived materials. A better understanding of plant structures and the effects pretreatment has on these structures will help identification of specific variables (e.g., crystallinity, acetyl content, types of bonds) that can be used to tune the pretreatment parameters (e.g., pretreatment time or moisture content) to obtain the desired products and/or manipulate product yields in such a way as to obtain the optimum distribution.

Cellulose in lignocellulosics is composed of crystalline and amorphous components. The amorphous component is digested more easily by enzymes than the crystalline component. The crystalline cellulose exists in the form of microfibrils, which are paracrystalline assemblies of several dozen (1,4) β-D-glucan chains hydrogen-bonded to one another along their length. The (1,4) β-D-glucan chains are tightly linked by numerous hydrogen bonds, both side-to-side and top-to-bottom in a lattice like manner. The glucan chains in the core of the microfibril have a precise spacing (Fig. 1B)*(3)*. The arrangement of atoms in the unit structure of the microfibril core has been determined by X-ray diffraction *(4)*.

Cross-linking glycans are a class of polysaccharides that can hydrogen-bond to cellulose microfibrils. They may coat microfibrils but are also long enough to span the distance between microfibrils and link them together to form a network. Most cross-linking glycans are called "hemicelluloses." Hemicelluloses are largely composed of aldopentoses (arabinose, xylose, galactose), which are in either pyranose or a furanose form. Hemicelluloses also link the polyphenolic portion of the plant cell in the three-dimensional structures, known as lignin–carbohydrate complexes (*3*).

The most distinguishing feature of secondary walls is the incorporation of lignins. These are complex networks of aromatic compounds called phenylpropanoids. After cellulose, lignins are the most abundant organic natural products known and account for as much as 20–30% of all vascular plant tissue. The phenylpropanoids, hydroxycinnamoyl alcohol and "monolignols" (*p*-coumaryl, coniferyl, and sinapyl alcohols), account for most of the lignin networks *(3)*. Non-woody plants contain lignins

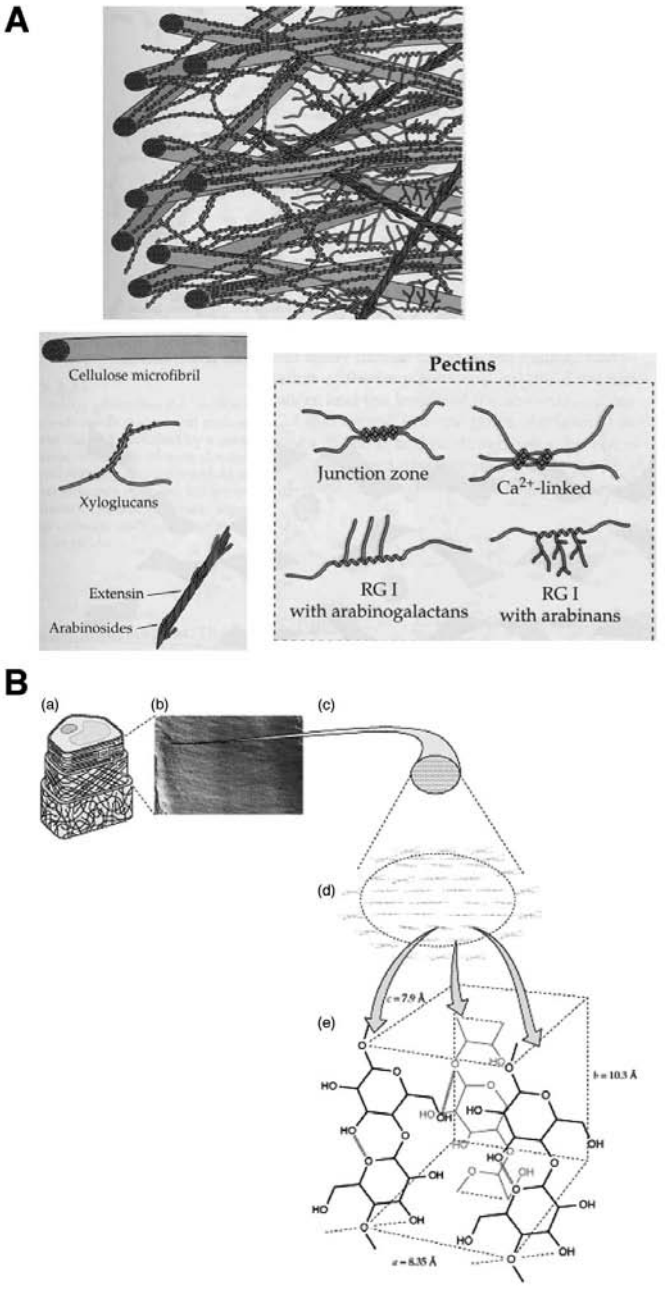

Fig. 1. Cell wall composition . **(A)** Three-dimensional molecular model of a cell wall; **(B)** cellulose microfibril. Copyrighted by the American Society of Plant Biologists (reprinted with permission).

that appear to be formed from mixtures of monolignols and hydroxycinnamic acids. The monolignols are linked by way of ester, ether, or carbon–carbon bonds. Lignin is covalently linked to cellulose and xylans in ways that indicate that the orientations of polysaccharides may serve as a template for the lignin patterning. A range of cross-linking possibilities exists including hydrogen bonding, ionic bonding with Ca+ ions, covalent ester linkages, ether linkages, and van der Waals interactions *(3)*. Lignin–carbohydrate interactions exert a great influence on digestibility of forage crops by animals.

Owing to the location of the cellulose fraction within the cell wall, enzymatic access is restricted by the lignin and hemicellulose interference. As a result, pretreatment of the biomass is necessary. Numerous pretreatments have been studied through the years *(5–8)*, each having their advantages and disadvantages. Strong acids can break glycosidic linkages of polysaccharides, freeing the individual monosaccharide components, but also tend to degrade monomeric sugars. Some alkaline pretreatments yield highly digestible cellulose and produce liquid streams rich in extracted lignins and polymeric hemicellulose. Pretreatment affects many characteristics of the plant material that impede digestion including (1) cellulose crystallinity, (2) lignin content, (3) acetyl linkages, and (4) the complex hemicellulose–lignin shield that surrounds cellulose in the plant cell wall. For industrial applications, a pretreatment must be effective, economical, safe, environmentally acceptable, and easy to use. This study emphasizes the use of AFEX. A comparison between aqueous ammonia recycle percolation (ARP), uncatalyzed hydrolysis, dilute acid hydrolysis, controlled pH, lime, and ammonia fiber explosion (AFEX) is also presented. A detailed explanation of all these pretreatments processes, equipment, and effects is presented elsewhere *(5–7,9–12)*.

The ammonia fiber explosion treats lignocellulosic biomass with high-pressure liquid ammonia, and then explosively releases the pressure (*6*). The ammonia can then be recovered and recycled. The small amount of ammonia that remains in the biomass (less than 1% by weight of the biomass) might serve as a nitrogen source for the microbes that use the sugars enzymatically hydrolyzed from the lignocellulose *(9)*. AFEX uses moderate pressures (up to 280 psi) and moderate temperature (60–100°C) to treat the biomass.

AFEX is thought to affect both the chemical and physical characteristics of the biomass. The chemical effects include cellulose decrystallization, hemicellulose prehydrolysis, and lignin alterations. The physical effects include the increase of accessible surface area and decrease in bulk density. These effects increase the susceptibility of the biomass to enzymatic hydrolysis.

Several structural and compositional factors affect the enzymatic digestibility of lignocellulosic materials. The most generally cited factors are (1) cellulose crystallinity *(1–3,13)*. The degree of crystallinity of cellulose

is expressed in terms of the crystallinity index (CrI) as defined by Segal et al. (*13*); this is determined by the ratio of the crystalline peak to valley (amorphous region) in the diffractogram based on a monoclinic structure of cellulose. (2) cellulose protection by lignin *(13–17)*. Lignin is covalently bonded to polysaccharides in the intact plant cell wall, thus reducing accessible surface area of cellulose. The mechanism that explains the protective effect of lignin against polysaccharide hydrolysis remains uncertain although a number of factors; such as the degree and type of cross-linkage to polysaccharide, the diversity of structures found in the lignin component, and the distribution of phenolic polymers through the cell wall are important. (3) Hemicellulose sheathing and degree of hemicellulose acetylation *(5,18,19)*. The bonds between lignin and carbohydrates are predominantly ester-linked to arabinose side chains of arabinoxylans. Xylans are extensively acetylated. Analytical methods have been developed through the years to measure these biomass properties in an effort to identify the effect these factors have on enzymatic hydrolysis. A spectroscopic approach is presented here.

Analysis of the different spectra (DRIFT, XRD, fluorescence) for these pretreated and untreated samples creates an enormous amount of data that must be analyzed and correctly interpreted in order for it to be useful. Multivariate analysis allows us to relate and model different variables simultaneously *(20,21)*. With multivariate calibrations, empirical models are developed that relate the spectral data for multiple samples to the known sugar concentration of the samples. These empirical relationships can then be used in multivariate predictive analyses of spectra of unknown samples to predict their sugar concentrations. The goal of the calibration is to produce a model that relates the data from the instrument to the results by an independent method. The prediction then uses the model to predict the value for an unknown sample. The model that has been used in this study is multiple linear regression (MLR) *(20,21)*. The goal of MLR in this study is to find a linear combination of the variables such that the rate of enzymatic hydrolysis value estimated by the model is as close to the known value as possible. The criterion of closeness for MLR is defined as minimizing the sum of the squares of the deviations of the predicted values from the true values.

Materials and Methods

The feedstock used by all the collaborating institutions, corn stover, is milled (to pass a 6-mm screen) and dried (about <10% moisture content). Corn stover and its composition were provided by National Renewable Energy Laboratory (NREL), Golden, CO. The composition is presented in Table 1.

Auburn University, Dartmouth University, Michigan State University, Purdue University, and Texas A&M University pretreat the corn stover with their treatment of expertise (ARP, Steam, AFEX, controlled pH and

Table 1
Composition of the Corn Stover

Component	Mass percent (dry basis)
Glucan	36.1
Xylan	21.4
Arabinan	3.5
Mannan	1.8
Galactan	2.5
Lignin	17.2
Protein	4.0
Acetyl	3.2
Ash	7.1
Uronic acid (est)	3.6
Nonstructural sugars	1.2

lime, respectively). The biomass is then provided to the other universities to be analyzed. β-Glucosidase (Novozymes188, lot number 11K1088) was obtained from Sigma, St Louis, MO. Cellulase (lot 301-00348-257) was provided by NREL. A Sigma α-cellulose (cat. no. C-8002, lot number 11K0246) provided by Auburn University was used as a standard in the analyses. Anhydrous ammonia was obtained from AGA (Lansing, MI).

AFEX Treatment

The AFEX process treats lignocellulosic materials with liquid ammonia under pressure and then the pressure is rapidly released. The moisture of the material treated ranges from 20 to 60% moisture (dry weight basis). The ammonia to biomass ratio used ranges from 0.7:1 to 1.3:1 (mass:mass). Fifteen grams (15 g) of previously chopped and cleaned corn stover provided by NREL in Golden, CO, is prewetted in order to obtain the desired moisture content, and loaded in a 300-mL stainless-steel vessel (PARR Instrument Co., IL). The vessel is topped with stainless-steel pellets (approx 1 mm diameter) to occupy the void space and thus minimize transformation of the ammonia from liquid to gas during loading, and then the lid is bolted shut. The predetermined amount of liquid ammonia is delivered using precalibrated ammonia cylinders to the vessel to reach the desired ammonia to biomass ratio. The temperature of the vessel is increased using a 400 W PARR heating mantle until the target temperature is obtained. After reaching the target temperature/pressure, the reactor is held at these conditions for 5 min and then the pressure is suddenly released. A scheme of the AFEX experimental setup is presented elsewhere *(23)*. The pretreated corn stover is then removed from the vessel and left under a fume hood until the remaining liquid ammonia evaporates (approx 24 h). The treated samples were kept in plastic bags at 4ºC for further analysis.

Analytical Methods

In order to perform the analytical procedures, both the untreated and treated samples must be prepared. The samples treated with ARP, controlled pH, and dilute acid were washed before we received them. The wet samples received were dried at 45°C overnight. Once the samples were dry, they were ground using a mortar and pestle and sieved through a 140-mesh screen with 106 μm openings. The fine powder obtained is analyzed by the following techniques.

Diffusive Reflectance FT-IR (DRIFT)

The DRIFT results presented are performed in a Perkin Elmer FT-IR system 2000 with the Diffusive Reflection Accessory. Spectra were obtained using 32 scans of the powdered sample (no dilution), triangular apodization, a resolution of 4 cm^{-1}, and an interval of 1 cm^{-1}. The sample is loaded in the holder and analyzed. The equipment was calibrated using KBr for water and air background. Peaks are identified following Stewart et al. *(24)*.

Sample Crystallinity

For crystallinity, each spectrum was collected using the θ–2θ method in a Rigaku Rotaflex 200B diffractometer at 45 kV and 100 mA with slits sized 0.5°, 0.5°, 0.3° and 0.5°, respectively. The powdered sample was placed vertically in the slide using double-sided tape to hold it in place and analyzed using the horizontal goniometer. Duplicate samples were scanned at 1°/min from 2θ = 10–26° with a step size of 0.05°:

$$\text{Crystallinity index}(\text{CrI}) = \frac{(I_{200} - I_{am})100}{I_{200}} \quad (1)$$

Where I_{200} is the intensity of the peak at 22.30°, and I_{am} peak is the intensity of the peak at 18°.

CrI results were calculated using the method of Segal et al. (*13*) and Eq. (1). The CrI was calculated taking into consideration the most prominent peak and the amorphous "peak."

Fluorescence

Fluorescence spectra were recorded using a SPEX-3 Fluorolog. Autoemission spectra were obtained at an excitation wavelength of 350 nm with an interval of 0.5 nm. The excitation and emission slits were set at 3 and 5 nm, respectively. The solid sample holder was filled with powdered sample and was held in place with a quartz cover slip. Sample was placed 45° from the incident beam. The mode of detection was front face.

Enzymatic Hydrolysis

The digestibility of the treated and untreated corn stover was determined using NREL Laboratory Analytical Procedure (LAP)-009 *(6)*.

Simultaneous Saccharification and Fermentation (SSF)

SSF experiments were conducted according to NREL standard protocol LAP-008 *(26)*.

Acid Hydrolysis

The lignin content (soluble and insoluble) and determination of carbohydrates in biomass were obtained by following the NREL LAP-002, -003, and -004 *(26)*.

Statistical Analysis

The statistical analysis was performed using the software Unscrambler® v8.0. Spectroscopic data were entered along with their respective hydrolysis data at 3 and 72 h in order to obtain the MLR coefficients. A model was created and from this a prediction for the unknown was obtained.

Results and Discussion

Figure 2 presents the DRIFT results for the different pretreaments studied. The most prominent peaks are identified and labeled. In general, all the pretreatments show an increase in the OH and amorphous cellulose peaks as compared with the untreated sample, because of depolymerization of biomass polymers and crystalline structure disruption. In addition, all the pretreatments show a reduction or disappearance of the carbonyl peak and a decrease in lignin peaks. This implies a decrease in the number of bonds associated with hemicellulose (hydrolysis of hemicellulose) and a disruption in the lignin polymer, respectively. Lime pretreatment shows an apparent increase in the aldehyde peak, while the other pretreatments show a decrease. All the pretreatments except controlled pH and dilute acid show an increase in the C–H peak as compared to untreated samples.

Figure 3 shows the difference in peak intensities of the different pretreatments as compared to the untreated sample. To permit easier comparison of trends, the intensity of the untreated sample peaks (Fig. 2A) has been subtracted from the intensity of the respective peaks in the treated samples (Figs. 2B–G). For example, the intensity of the O–H stretch peak in the untreated sample is 1.037 and for the ARP-treated sample it is 1.471. The figure shows a difference of 0.434. A positive value indicates an increase in the peak as compared to the untreated sample and a negative value represents a decrease.

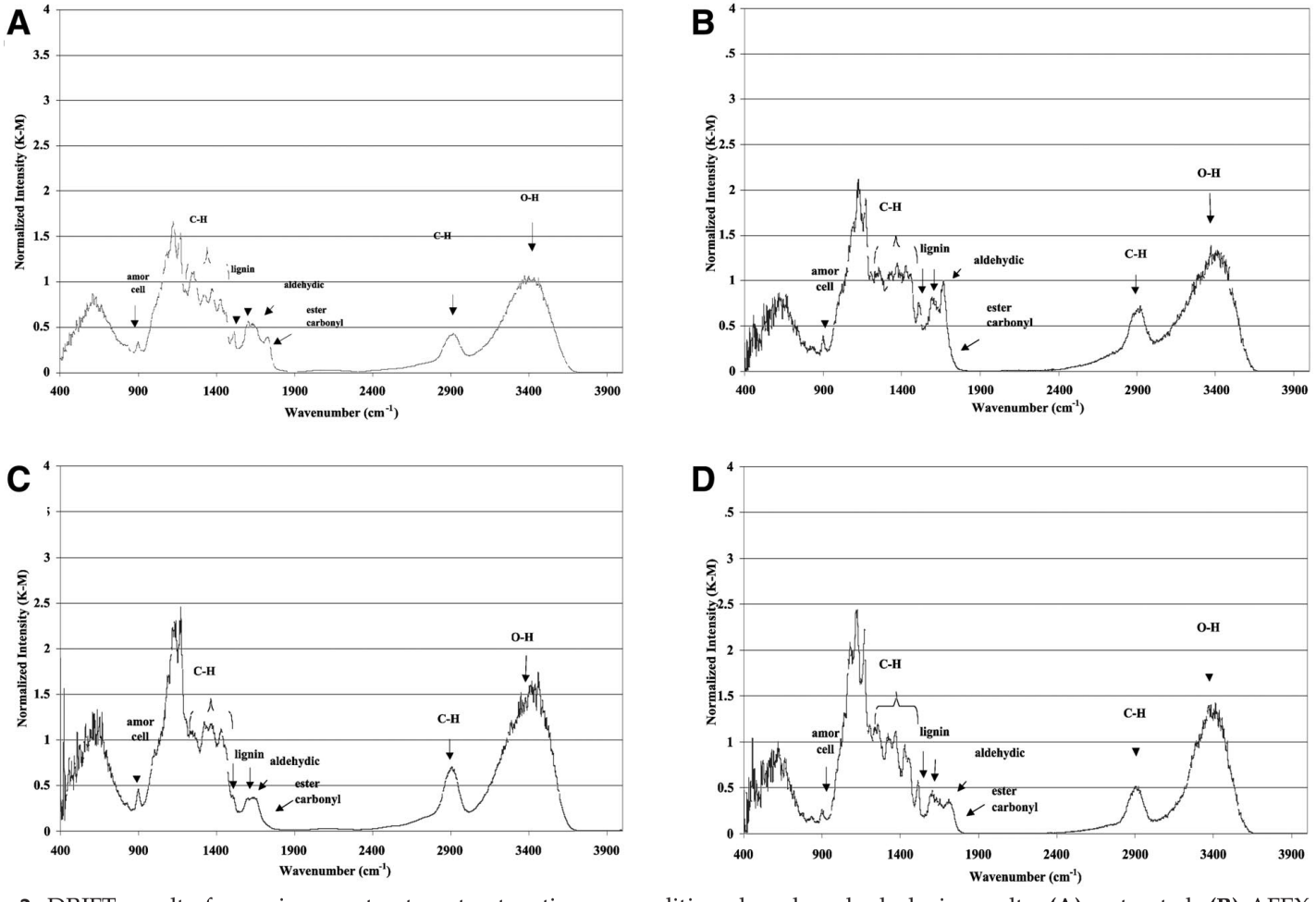

Fig. 2. DRIFT results for various pretreatments at optimum conditions based on hydrolysis results: **(A)** untreated; **(B)** AFEX (60%, 1:1, 90°C); **(C)** ARP (10%NH_3, 170°C, 10 min), **(D)** controlled pH (190°C, 15 min; **(E)** dilute acid (180°C, 1% H_2SO_4, 2 min); **(F)** lime (air, 55°C, 4 months); **(G)** α- cellulose.

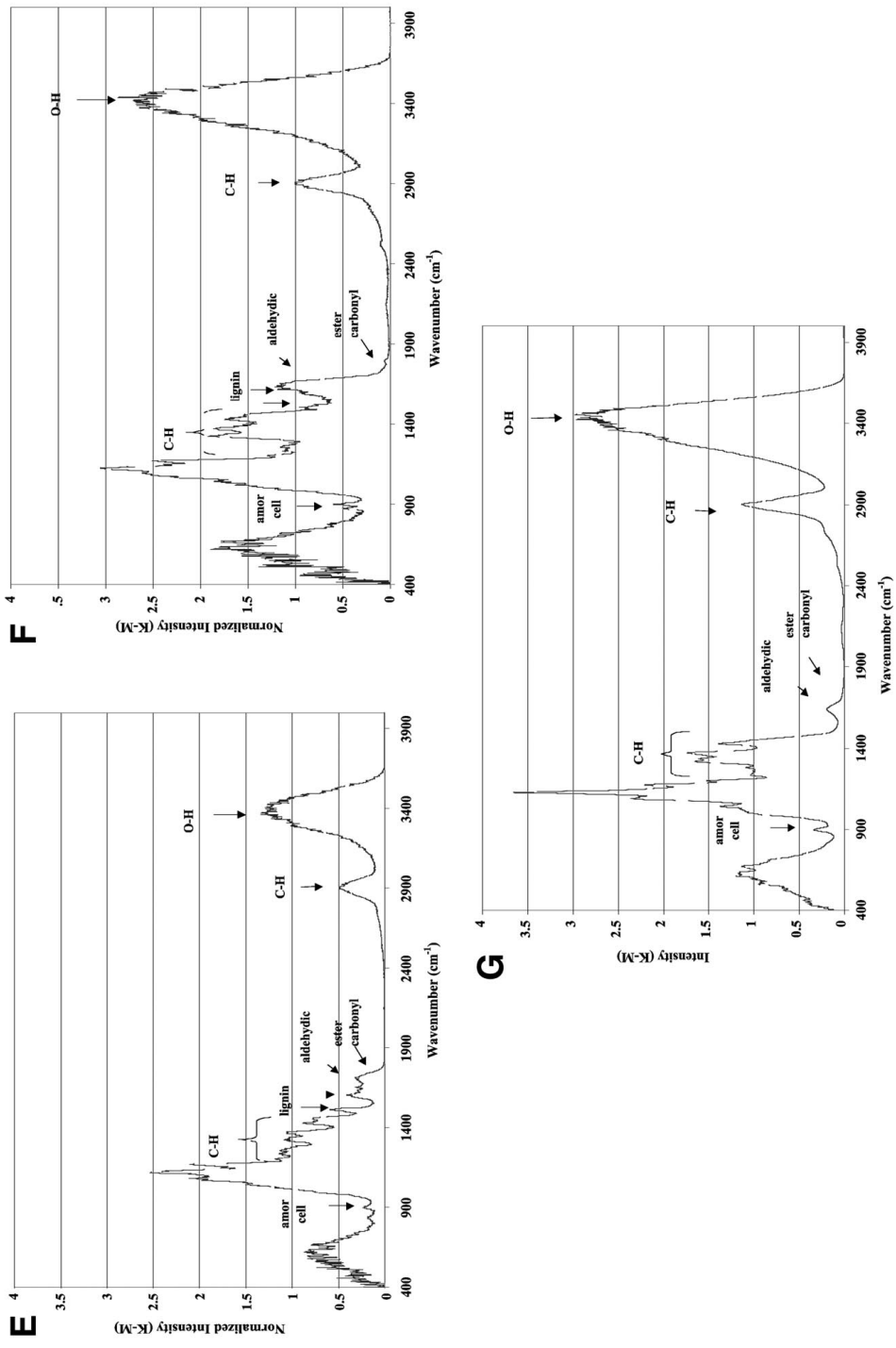

Fig 2. (*Continued*)

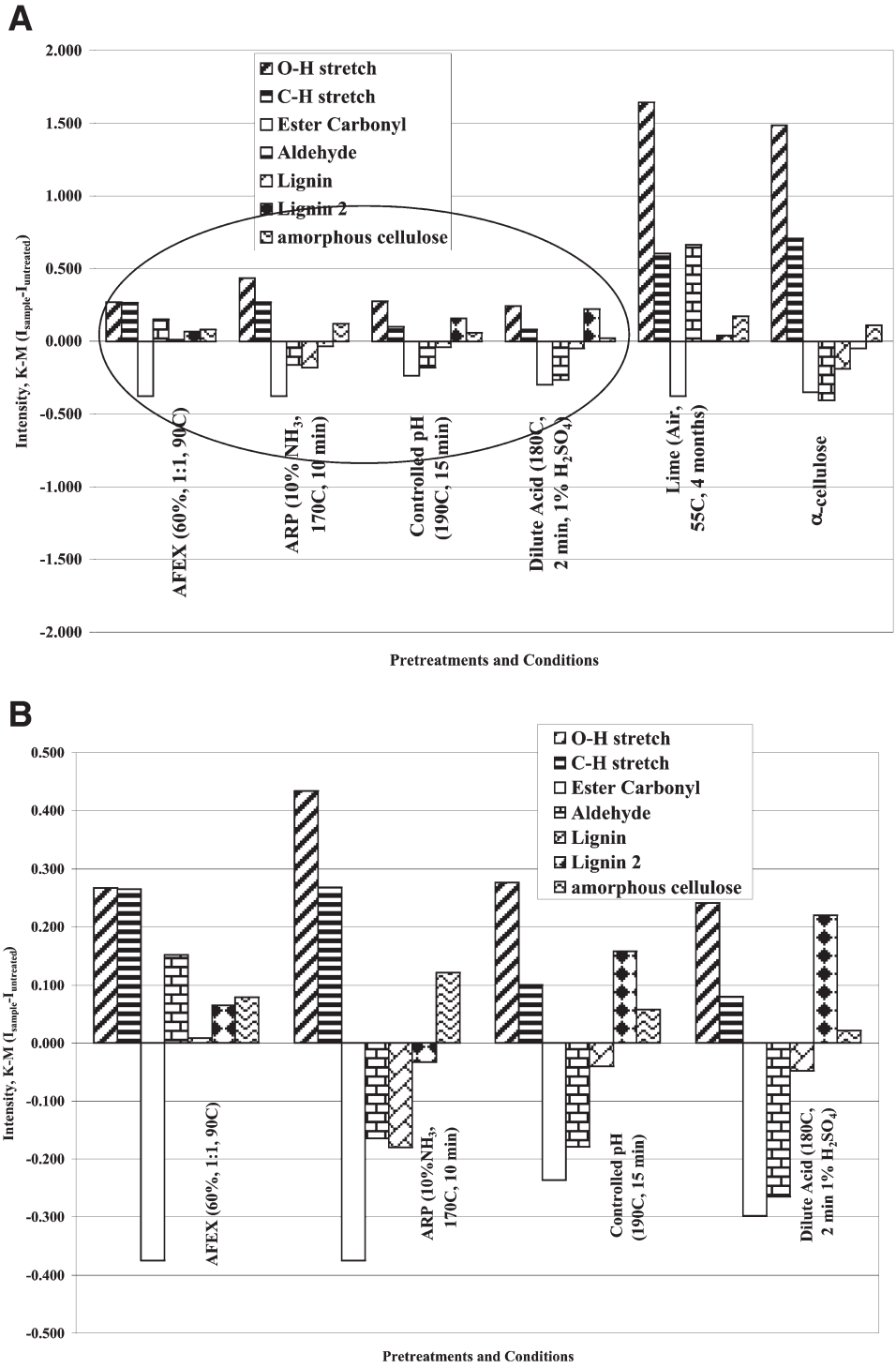

Fig. 3. Intensity difference between untreated and treated corn stover using various pretreatments: **(A)** all the pretreatments; **(B)** magnification of the area selected.

Table 2
Crystallinity Indices for Different Pretreatments

Pretreatment and conditions	Crystallinity index (%)
60%, 1.3:1, 60°C	25.95
60%, 1.3:1, 70°C	29.82
60%, 1.3:1, 80°C	26.50
60%, 1.3:1, 90°C	26.98
60%, 1:1, 60°C	27.00
60%, 1:1, 70°C	23.15
60%, 1:1, 80°C	22.96
60%, 1:1, 90°C	***36.29***
60%, 0.7:1, 60°C	20.09
60%, 0.7:1, 70°C	24.00
60%, 0.7:1, 80°C	22.81
60%, 0.7:1, 90°C	31.94
40%, 1.3:1, 60°C	12.40
40%, 1.3:1, 70°C	19.25
40%, 1.3:1,80°C	19.25
40%, 1.3:1, 90°C	22.30
40%, 1:1, 60°C	23.45
40%, 1:1, 70°C	25.09
40%, 1:1, 80°C	13.71
40%, 1:1, 90°C	23.48
20%, 0.7:1, 60°C	20.21
20%, 0.7:1, 70°C	23.07
20%, 0.7:1, 80°C	23.61
20%, 0.7:1, 90°C	16.77
Dilute Acid (180°C, 2 min, 1% H_2SO_4)	52.51
Controlled pH (190°C, 15 min)	44.52
Lime (Air, 55°C, 4 months)	56.17
ARP (10% NH3, 170°C, 10 min)	25.98
Untreated	***50.30***
α-Cellulose	***66.53***

Table 2 shows the values of the crystallinity index for AFEX-treated samples. In general, lower moisture content in AFEX tends to produce less crystalline samples. Table 2 also presents the crystallinity index values for the different pretreatments at their optimal conditions (highest sugar yield in enzymatic hydrolysis). AFEX and ARP are effective in decrystallizing cellulose. Controlled pH also shows a decreased CrI and lime and dilute acid gives an apparent increase in CrI.

Figure 4 is a plot of glucan conversion vs crystallinity index for AFEX-treated corn stover. For a particular crystallinity index, different conversions can be obtained. It seems that after a certain degree of decrystallization is obtained, cellulose crystallinity is then not an important factor to achieve complete conversion. These results confirm that crystallinity is not the only factor that affects the enzymatic hydrolysis of corn stover.

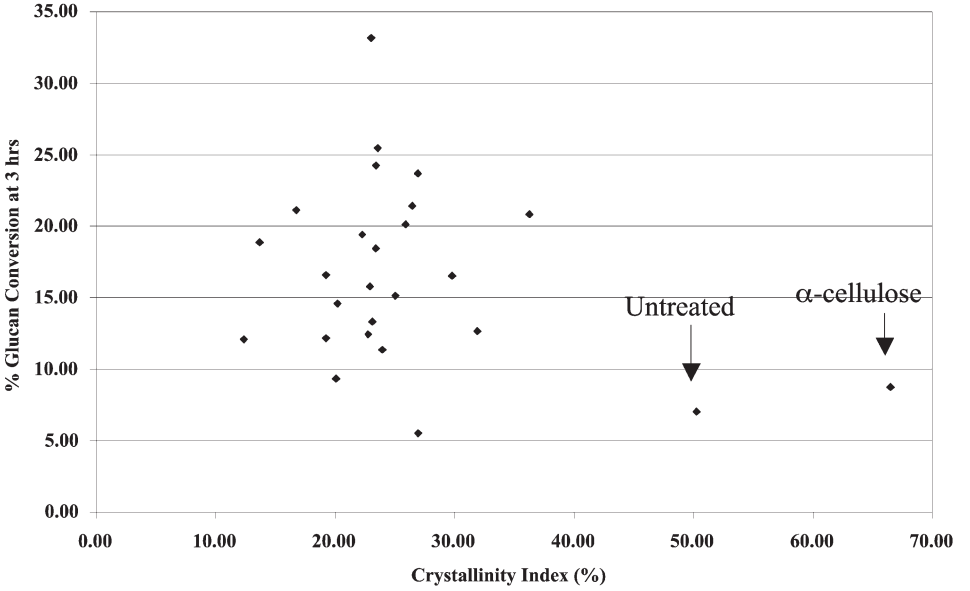

Fig. 4. Effect of CrI on glucan conversion at 3 h for AFEX-treated corn stover.

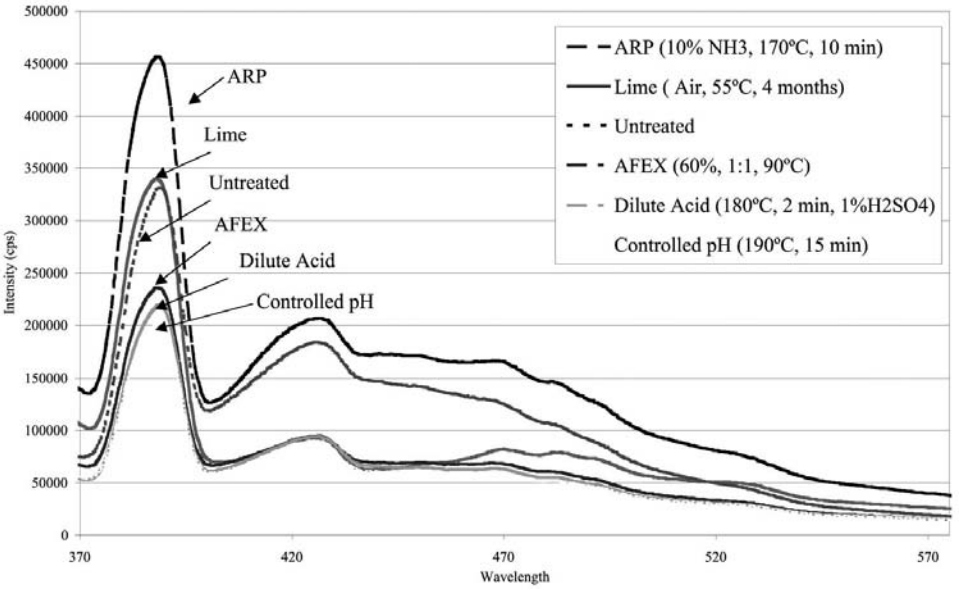

Fig. 5. Fluorescence results for different pretreatments.

Figure 5 presents the results of the fluorescence analysis for the different pretreatments. When the powdered sample is excited at 350 nm, a peak at 425 nm related to lignin will appear. All the pretreatments except ARP show a decrease in this peak as compared to that for untreated corn stover. However, it is well known that ARP is a delignification process, so this result for ARP may not be particularly noteworthy. It is believed that

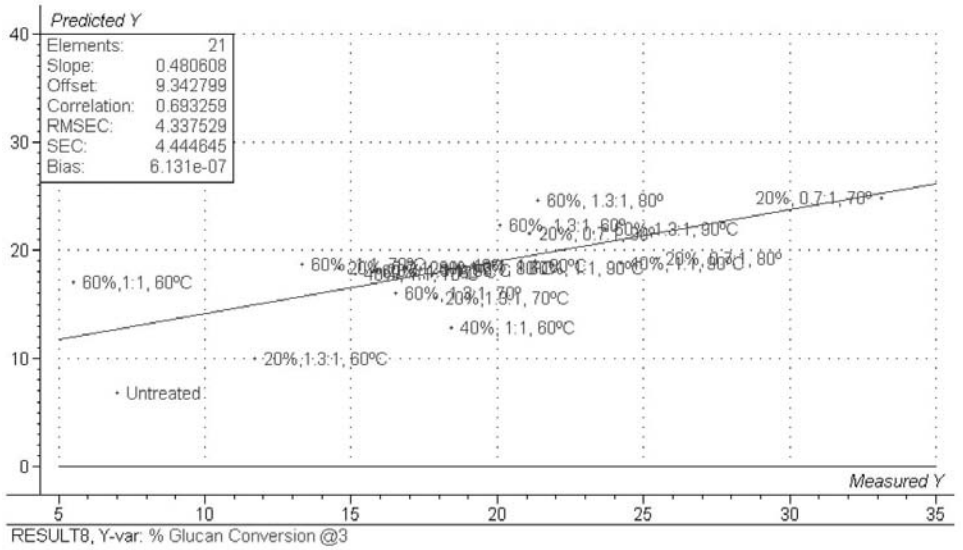

Fig. 6. MLR model for the initial hydrolysis rate of AFEX-treated corn stover. Points indicate the data used to create the model and the line indicates the model.

Table 3
MLR Model B Coefficients for Hydrolysis (3 and 72 h)

Variables	*B* (X-vars + interactions) for 3 h	*B* (X-vars + interactions) for 72 h
Bo	10.69	1.204
CrI	−0.224	1.277
OH	0.509	10.49
CH	3.570	28.47
Ester carbonyl	−12.09	−60.99
Aldehyde	−9.952	−19.71
Lignin	3.918	−4.022
Lignin2	0.392	−9.020
Amorphous cellulose	19.44	36.71

since ARP extracts lignin some lignin might be re-deposited on the surface. Fluorescence, a surface-analysis method, might thereby show a higher lignin content.

A multilinear regression (MLR) was performed relating the seven prominent peaks in the DRIFT spectra, the crystallinity index, and the total amount of lignin in the biomass as determined by acid hydrolysis to the initial rate of the enzymatic hydrolysis (3 h glucan yield) and the digestibility at 72 h. Figure 6 shows the correlation for AFEX data for 21 samples. The solid line indicates the hydrolysis initial rate model obtained from the data. The ANOVA gives a correlation of $R^2 = 0.481$. The

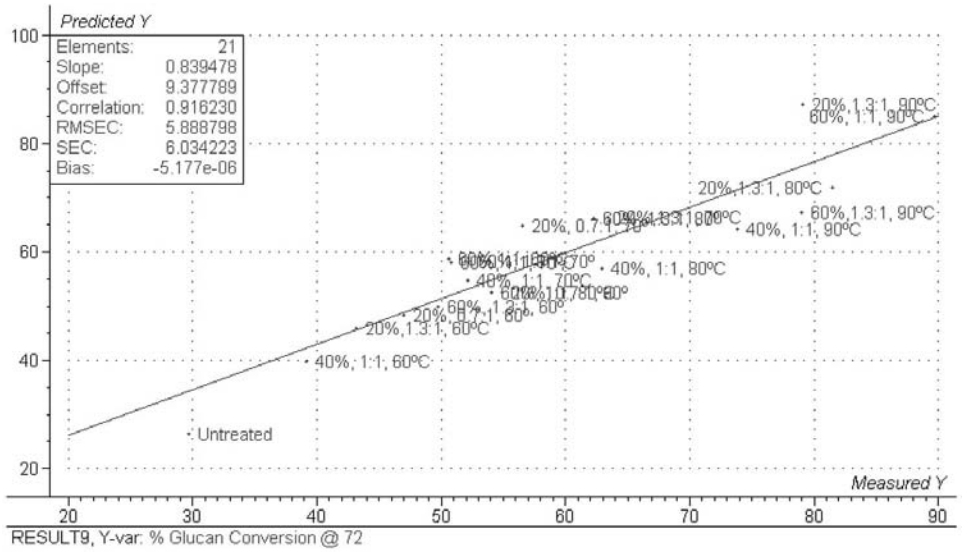

Fig. 7. MLR model for 72 h hydrolysis of AFEX-treated corn stover. Points indicate the data used to create the model and line indicates the model.

regression coefficients for each of the variables used in the model are presented in Table 3. The absolute values presented for each coefficient represent the weight or importance of each parameter to the model. The parameters that most influence the model are the amorphous cellulose peak, followed by ester carbonyl and aldehyde bonds.

Figure 7 shows the correlation for AFEX data for 21 samples. The solid line indicates the 72 h digestibility model obtained from the data. The ANOVA gives a correlation of $R^2 = 0.839$. The regression coefficients for each of the variables used in the model are presented in Table 3. In this model the parameter with the most weight in the determination of the 72 h hydrolysis yields is ester carbonyl bonds and crystallinity, followed by C–H bonds.

The absolute value of the coefficient indicates the weight of the different parameters in the model. According to the MLR model, the parameter that most affects the glucan conversion initial rate is the cellulose crystallinity of the sample, while at 72 h the parameter that most influences the conversion is the ester carbonyl bonds that are directly related to the hydrolysis of hemicellulose. This agrees with the CrI data presented before. After a certain amount of decrystallization, the crystallinity of cellulose in the biomass is not the limiting parameter in hydrolysis. The acetyl content then starts to play an important role in the glucan conversion.

The initial rate prediction using AFEX is presented in Table 4. It also shows the results of glucan conversion prediction using AFEX for 72 h hydrolysis. There is a wide range in the accuracy of the predictions obtained. It would seem that the predictive value of the initial rate is not

accurate. However, it should be noted that in this study only crystallinity, lignin, and selected chemical bonds were used as input to the model. Previous studies have shown that there are other parameters that affect the hydrolysis initial rate, including pore volume and size and surface area accessibility.

The MLR model was then used to predict the glucan conversion values for different pretreatments. These results are presented in Table 4. However, the accuracy of these predictions cannot be confirmed because complete enzymatic hydrolysis information is not yet available from our collaborators. When these data become available for the various pretreated samples, the accuracy of this prediction can be confirmed. Additional information will not only test the accuracy of the model but will also help us understand how the model applies to other pretreatments. Further analysis and experimentation are underway and these results will be presented in a subsequent paper. It is important to mention that one important goal in this study is to find the simplest model using spectroscopic data that will give a reasonable prediction of hydrolysis results.

Remarks and Future Work

As mentioned above pretreatment of lignocellulosic biomass is necessary to obtain high sugar yields by enzyme catalysis. However, the fundamental characteristics of biomass that limit its enzymatic conversion are not clearly understood. A better fundamental understanding of these factors would help improve pretreatment/hydrolysis systems.

It is in the best interest of the biomass industry to understand biomass structure and the effects of pretreatments on structure in order to minimize the cost of both enzyme and pretreatment. The creation of a model may provide an easy, fast, and inexpensive way to predict ethanol/sugar yield based on structural changes in biomass (corn stover) as a result of pretreatment. In addition, modeling will broaden the knowledge of biomass structure and the effect that pretreatment has on such structures. This knowledge will help identify parameters that can be tailored in order to obtain an opti mum sugar conversion. In addition, this model will give information as to what parameters most affect the initial hydrolysis rate.

AFEX has been widely studied using different feedstocks and ample literature is available. The study of corn stover during this research has shown the effectiveness of AFEX. AFEX is the basis for the model because of our expertise in this process. It has been shown that the changes produced by AFEX in chemical bonds can be easily identified by DRIFT and the decrystallinization of cellulose by X-ray diffraction.

The analytical methods used here are easy to use, fast, and inexpensive as compared to enzyme hydrolysis. DRIFT has been identified as a reliable method for monitoring structural changes in biomass. X-ray diffraction has also been used in the field for decades to determine the

Table 4
Hydolysis (Glucan Conversion at 3 and 72 hr) Prediction Based on MLR Model

Sample	Predicted` conversion at 3h (%)	Measured conversion at 3h† (%)	Percentage difference at 3 h ‡	Predicted conversion at 72 h (%)	Measured conversion (%) at 72 hr†	percentage difference at 72 h‡
60%, 0.7:1, 60°C	14.5	9.29	44.12	32.7	30.7	6.40
60%, 0.7:1, 70°C	17.0	11.34	40.13	37.0	36.1	2.60
60%, 0.7:1, 80°C	22.9	12.42	59.41	35.6	35.2	1.20
60%, 0.7:1, 90°C	17.3	12.65	31.10	45.5	17.1	91.0
40%, 1.3:1, 60°C	18.9	12.07	44.09	25.3	38.0	40.2
40%, 1.3:1, 70°C	22.5	12.15	59.58	31.6	59.7	61.6
40%, 1.3:1,80°C	17.6	16.55	6.03	31.8	30.4	4.40
40%, 1.3:1, 90°C	20.0	19.37	3.04	35.2	65.8	60.8
Dilute acid (140°C, 40 min, 1% H_2SO_4)	13.2	N/A	—	74.8	N/A	—
Dilute acid (160°C, 10 min, 1% H_2SO_4)	16.9	N/A	—	97.5	N/A	—
Dilute acid (180°C, 1 min, 1% H_2SO_4)	8.48	N/A	—	74.3	N/A	—
Dilute acid (180°C, 2 min, 1% H_2SO_4)	13.0	N/A	—	102	N/A	—
Controlled pH (190°C, 15 min)	7.37	N/A	—	68.2	N/A	—
Lime (02)	16.1	N/A	—	163	N/A	—
ARP (M-1)	41.9	N/A	—	179	N/A	—

†The measured conversion was obtained by enzymatic hydrolysis.
‡Predicted vs. measured.
N/A Not available.

biomass crystallinity. The determination of total lignin is performed using acid hydrolysis in a standard procedure developed for biomass. However, the data obtained from these methods have not been investigated to complement each other in the analysis of biomass depolymerization.

A statistical analysis will correlate mathematically these spectroscopic data to experimental data (e.g., hydrolysis initial rate and yield). Multivariate analysis is widely used in spectral analyses. A model is made using MLR multivariate technique. This model will predict a sugar/ethanol yield closest to the one determined by wet chemistry. Based on the coefficients of the model the parameter that most affects the hydrolysis can be determined. In this case the initial rate is limited by the cellulose crystallinity and by the lignin content at 72 h of hydrolysis.

In this research the techniques, analysis, and data handling have been widely studied previously in various biomass areas. However, this is the first time that all of these factors were combined for corn stover and for the breadth of pretreatments studied. We believe this combination of analytical methods, enzymatic hydrolysis, modeling, and very different pretreatments will give us much greater insight into this important technology area.

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