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Porosity and Its Effect on the Digestibility of Dilute Sulfuric Acid Pretreated Corn Stover

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Enzyme accessibility has been proposed as a limiting factor in the enzymatic conversion of the cellulose in biomass to glucose. Prior work has shown a strong correlation between porosity, measured as the change in the volume of pores accessible to a cellulase-sized molecule, and the initial digestibility of biomass pretreated by various methods. The goal of this work was to determine if porosity was one of the factors governing the overall enzymatic digestibility of the cellulose in dilute acid pretreated biomass. The porosity of wet pretreated corn stover was determined using the methods of solute exclusion and ^{1}H nuclear magnetic resonance (NMR) thermoporometry. The solute exclusion method identified differences in the accessible pore volume of the pretreated samples compared to untreated corn stover; however, only very small differences in porosity were observed among samples pretreated with a range of severities, giving ethanol yields from 70 to 96%. No correlation was found between the volume accessible to an enzyme-sized molecule (diameter estimated to be 51 Å) and the digestibility of the cellulose in dilute acid pretreated corn stover. ^{1}H NMR thermoporometry was used to measure the amount of water in pores ranging from 20 to 200 Å. As was the case for the solute exclusion method, a difference was observed in the pore volume of untreated and acid pretreated corn stover, but no significant differences in pore volume were measured for the different pretreated samples.

KEYWORDS: Corn stover; pretreatment; porosity; solute exclusion; thermoporometry; NMR; digestibility

INTRODUCTION

Lignocellulosic materials can be a renewable source of fuels and energy. However, the complex structure of the plant cell wall and the close interaction of its constituents result in a material resistant to degradation, processing, and transformation. A thermal, chemical, mechanical, and/or biological treatment (pretreatment) is required to disrupt the lignin−hemicellulose−cellulose interaction to increase the susceptibility of cell wall cellulose to the action of cellulolytic enzymes.

There are two established processes for liberating glucose from the cellulose in lignocellulosic biomass: (a) acid hydrolysis and (b) enzymatic hydrolysis. Method a requires high temperatures and pressures, strong acids, and hence expensive reactor materials and results in less than quantitative yields because of significant sugar degradation that occurs during the process. Use of method b, in a biomass to sugars process, is viewed as being more likely to generate products that can compete with petroleum-derived products on a cost basis, because yields of sugars are higher and the use of corrosive chemicals and expensive equipment is avoided. In addition, the cost of the biomass to sugars process using method b can be reduced when biomass is pretreated to increase the rate and extent of cellulose hydrolysis and/or decrease the loading of expensive cellulase catalyst (*1*).

Pretreatment conditions are selected on the basis of their ability to modify the structural and chemical characteristics of biomass that limit the accessibility of enzymes to cellulose in cell wall microfibrils or to increase the susceptibility of their action. The increase in cellulase reactivity attributed to pretreating lignocellulosics has usually been related to the creation of surface openings or internal slits, voids, or spaces, by the removal of cell wall components (*2*, *3*), enhancing the direct physical contact between the enzymes and the substrate (*4*). Therefore, the characterization of the cell wall pores (i.e., size and size distribution) has been proposed as a means to predict the reactivity of the substrate to enzymatic hydrolysis (*4*−*6*).

Different general methods for estimating porosity in materials have been developed; however, most of these methods, that is, electron microscopy, gas adsorption, and mercury porosimetry, require a dry sample. Water removal from nonrigid porous materials, such as biomass, often produces the collapse, partial or total, of the internal structure (*7*). For this reason, the best techniques to measure pores in pretreated biomass samples are those directly applicable to wet materials. Two methods, solute

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Table 1. Properties of ToyoPearl HW Resins (from the Manufacturer)

ToyoPearl	particle size (μm)	pore size (Å)	M_w separation limits (dextrans)
HW-40S	20–40	50	100–7000
HW-40C	50–100	50	100–7000
HW-50F	30–60	125	500–20000
HW-55S	20–40	500	1000–200000

exclusion and ^{1}H NMR thermoporometry, can determine the porosity of materials without removing the original moisture and altering the native structure.

The solute exclusion method of Stone and Scallan (*7*) measures the pore volume of water-swollen materials using a series of molecules of known molecular size (molecular probes). These molecules function as "filler gauges". When a solution of known concentration of molecular probes is mixed with a porous material saturated with water, a dilution will be observed if the solute is small enough to penetrate the pores; that is, the water contained in the pores is accessible and contributes to the total volume of the system. As progressively larger molecules are used, some of the smaller pores and, finally, all of the pores become inaccessible to the probe molecules and unavailable for dilution of the solution. Using this approach, the accessible pore volume of biomass can be determined by measuring the change in concentration of a relevant molecular probe solution.

Thermoporometric methods generally use the change in physical properties of a substance when it is confined within small spaces, that is, melting point depression. Two techniques have been widely used for these measurements: differential scanning calorimetry (DSC) (*8*−*12*) and nuclear magnetic resonance (NMR) (*13*−*16*). DSC thermoporometry measures heat transfer during the phase change of a substance in the pores, whereas NMR thermoporometry records the fraction of unfrozen liquid as a function of temperature. The amount of unfrozen liquid at a specific temperature is directly related to the pore volume of a defined size.

Overall, our goal is to understand the relationship between pretreatment conditions and the chemical and structural changes they produce in biomass. In this work, solute exclusion and ^{1}H NMR thermoporometry were used to measure the porosity of corn stover after dilute sulfuric acid pretreatment. The pretreated samples that were studied gave high ethanol yields (70−96%) after 7 days of simultaneous saccharification and fermentation (SSF). Our research tested if biomass porosity was a critical factor in determining the overall enzymatic digestibility of pretreated corn stover in these highly digestible, process relevant, substrates. We anticipated that the results of this work could help to identify research directions that would lead to even more digestible substrates.

MATERIALS AND METHODS

Substrates. Four ToyoPearl HW chromatographic resins of different particle sizes and exclusion limits were obtained from Tosoh Bioscience LLC. These resins are hydrophilic, semirigid, spherical beads, synthesized by copolymerization of ethylene glycol and methacrylate type polymers. The properties and exclusion limits of these resins are summarized in **Table 1**.

Pretreated samples were produced from corn stover (Pioneer 34M95) harvested in Colorado in 2002. The corn stover was subjected to dilute acid pretreatment in a pilot-scale vertical reactor using a fixed residence time of approximately 1 min at temperatures ranging from 180 to 200 °C, solid loadings between 25 and 35% (w/w), and acid loadings of 0.03−0.06 g of acid/g of dry biomass using procedures described in Schell et al. (*17*). Following pretreatment, the samples were stored at 4 °C and pH ∼1. Prior to use, the samples were thoroughly washed with distilled water until the wash water was colorless and neutral in pH. After the last washing, the samples were filtered into a press cake of approximately 20% (w/w) solids.

Table 2. Molecular Masses and Diameters of Dextrans and PEGs

solute	mol mass[a] (Da)	mol diameter[b] (Å)
glucose	180	8
Dextran FP1	1060	18
Dextran 4	4900	32
Dextran 8	11800	47
Dextran 15	18100	56
Dextran 40	36021	89
Dextran 60	65100	110
Dextran 100	115000	140
Dextran 2000	2000000	560
PEG 1	1240	27
PEG 3	3710	50
PEG 8	8570	84
PEG 10	10360	98
PEG 20	19710	130
PEG 35	30040	240

[a] From the manufacturer. [b] From Stone and Scallan (*7*).

Chemical Analysis and Enzymatic Digestibility. The composition and cellulose digestibilities of these samples were measured using the methods detailed in Schell et al. (*17*). The composition was measured according to the National Renewable Energy Laboratory (NREL) method for the determination of structural carbohydrates and lignin in biomass (*17*). The enzymatic digestibility of cellulose was determined via an SSF process using Genencor's Spezyme at a loading of 20 mg of protein/g of cellulose (*17*).

Solute Exclusion Method. A series of dextrans (obtained from Serva Electrophoresis GmbH), polyethylene glycols (PEGs from Sigma-Aldrich and J. T. Baker), and analytical grade glucose were used as molecular probes. The molecular masses and corresponding solution diameters of the above dextrans, PEGs, and glucose are shown in **Table 2**.

Three replicates for each dextran, PEG, and glucose solution (total of 15 probes) were prepared with distilled water. Approximately 1.0 g of ToyoPearl beads or 0.5 g of corn stover was weighed into tared plastic snap cap centrifuge tubes to which 1 mL of 1.0% (w/v) solution was added. The tubes were periodically hand shaken for about 30 s during 2−3 h. After this time, the samples were centrifuged, and the supernatant was separated using a syringe. The solution was filtered using a 0.45 μm nylon filter and then transferred and sealed into HPLC vials for analysis. The remaining solids were washed with distilled water and dried to constant weight at 105 °C to determine the dry solid weights.

The concentration of the molecular probes in the filtered liquids was measured using an Agilent 1100 HPLC equipped with a refractive index detector. The HPLC column was replaced with a union fitting between the injector and the detector. The eluent was nanopure water delivered at a flow rate of 0.4 mL/min. The injection volume was 10 μL. Analyses were repeated four or five times for each vial. Distilled water blanks were prepared for each set of untreated or pretreated corn stover samples. The refractive index of the blank was used to correct for the response caused by extractives and other soluble materials.

The inaccessible volume (d_i) for a solute of diameter i, in mL of water per g of dry substrate, can be calculated from the initial and final concentration of the probe solution using the expression (*18*)

$$d_i = \left(\frac{W+q}{p}\right) - \left(\frac{W}{p}\cdot\frac{C_i}{C_f}\right) \quad (1)$$

where W is the mass of solution, q is the mass of water in the sample, p is the dry mass of the sample, C_i is the initial solute concentration, and C_f is the final solute concentration.

The largest probe molecule used, Dextran 2000, had an estimated diameter of 560 Å and was considered to be completely excluded from the pores; it was thus used as a measure of the total excluded volume in the system. Knowing the total volume and the inaccessible volume for each probe, the accessible pore volume for each probe was calculated using

$$A_i = d_{560} - d_i \quad (2)$$

where d_{560} was the water in the pores that was inaccessible to the 560 Å size probe molecule.

^{1}H NMR Thermoporometry. The ^{1}H NMR experiments were conducted with a Varian Unity 300 MHz (7.0 T) spectrometer. The spectra were collected using a $\pi/3$ pulse and a 5 s recycle delay. For each experiment, approximately 0.8−1.2 g of hydrated corn stover was introduced into a 10 mm NMR tube. The sample was cooled to 230 K, and then the NMR signal of the unfrozen water was recorded at intervals of 10 K until 285 K was reached. Samples were allowed to equilibrate for 5−7 min at each temperature.

The intensity of the NMR signal at 285 K is directly proportional to the total amount of water in the sample (calculated from the sample weight and moisture content), and at temperature T (below 273 K), the NMR signal represents the fraction of unfrozen water in the sample. The pore volume expressed in g of water per g of biomass can be derived using

$$\text{pore volume (g of water/g of dry biomass)} = \frac{I_T}{100}\cdot\frac{w}{m} \quad (3)$$

where I_T is the intensity of the signal at temperature T, w is the total weight of water in the sample, and m is the dry weight of the sample.

The melting point depression of water is related to pore size through the Gibbs−Thompson equation (*10*)

$$\Delta T_m = T_m - T_m(x) = -\frac{4\sigma_{sl}T_m}{x\Delta H_f\rho} \quad (4)$$

where T_m is the melting point of bulk water, $T_m(x)$ is the melting point of water in pores of diameter x, σ_{sl} is the surface energy of the solid−liquid interface, ΔH_f is the enthalpy of fusion of bulk water, and ρ is the density of the solid. In a simplified form, the equation is usually rewritten as

$$\Delta T = \frac{K(x)}{x} \quad (5)$$

where ΔT is the melting point depression of the liquid, $K(x)$ is a constant depending on the characteristics of the material, and x is the pore diameter.

RESULTS

High levels of xylan and/or lignin removal from lignocellulosics have been used as indicators of the effectiveness of pretreatment. In general, a direct relationship can be observed between the removal of either or both of these two components and the conversion of cellulose to ethanol during enzymatic hydrolysis and saccharification (*19*−*23*). Within this set of 35 samples of dilute sulfuric acid pretreated corn stover, a similar trend was also observed between xylan removal and cellulose digestibility (**Figure 1**). As the removal of xylan increased, the digestibility of the cellulose increased. Several groups have indicated that this effect is related to the creation of pores by the removal of the cell wall components (*2*, *3*).

The changes in pore volume of corn stover due to dilute acid pretreatment were measured by using the solute exclusion method and NMR thermoporometry. Prior to using the solute exclusion method on pretreated biomass samples, ToyoPearl chromatographic resins were employed to validate the technique. These resins were selected as standards because (i) they are

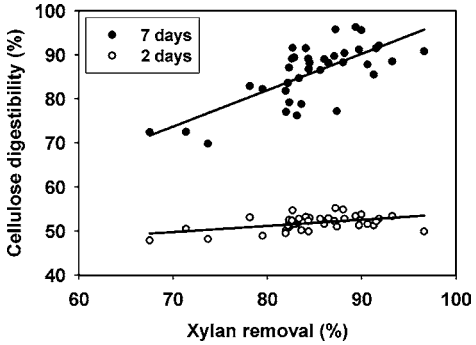

Figure 1. Cellulose digestibilities after 2 and 7 days versus xylan removal for pretreated corn stover.

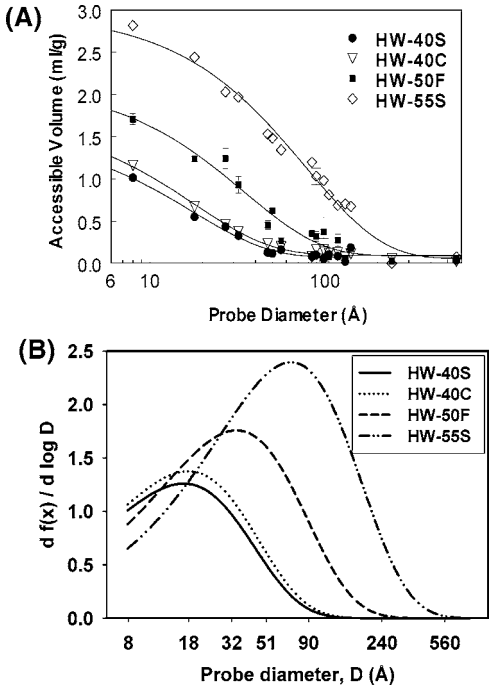

Figure 2. Accessible pore volume obtained by solute exclusion for Toyopearl resins. (**A**) Symbols indicate the experimentally determined accessible pore volumes for probes described in **Table 2**. Lines are the fit of the accessible pore volume using eq 6. (**B**) Lines represent the first derivative of the fitted curves, d $f(x)$/d log D, which are the pore size distribution curves.

available in a variety of pore and particle sizes, which allowed us to test the independence of the porosity technique (in a range relevant to enzymatic hydrolysis, 20−200 Å) from variation in particle size; and (ii) they are homogeneous materials, which also allowed us to test the reproducibility of the porosity measurement system without the complicating factor of the biomass's inherent heterogeneity.

In **Figure 2**, the accessible pore volume and the pore size distribution curves for the ToyoPearl resins are shown. The error bars indicate the magnitude of one standard deviation in the measured accessible volume for each probe, based on three measurements in each case. The pore size distribution curves were obtained from the first derivative of the fitted function of the accessible pore volume distributions using the equation

$$V_i = a_0 + a_1 \exp\left(-\frac{D}{a_2}\right) \quad (6)$$

where V_i is the accessible pore volume, D is the probe diameter, and a_0, a_1, and a_2 are fitting parameters.

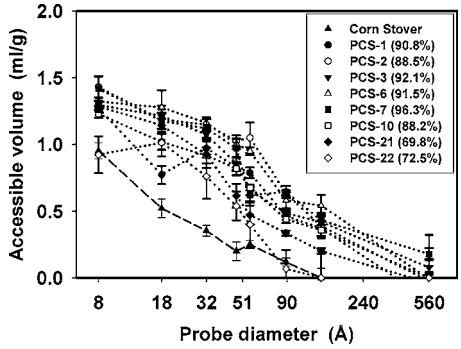

Figure 3. Accessible pore volume of untreated corn stover and pretreated corn stover measured by solute exclusion. Cellulose digestibilities after 7 days are indicated in parentheses. Error bars represent the standard deviation of three replicates.

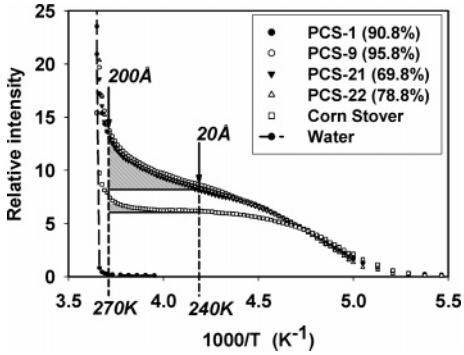

Figure 5. Normalized NMR signal intensity versus inverse of temperature (IT) curves for untreated and pretreated corn stover. The shaded area under the curve represents the total pore volume in the 20–200 Å range. Cellulose digestibilities after 7 days are indicated in parentheses.

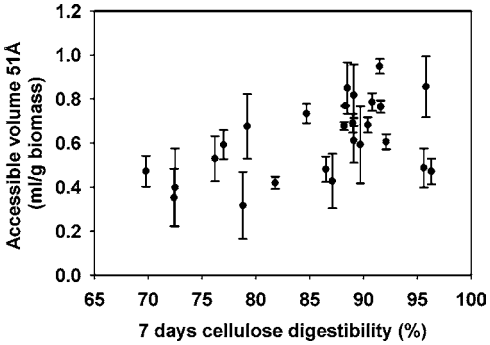

Figure 4. Accessible pore volume for a 51 Å probe obtained by solute exclusion for pretreated corn stover versus 7 days cellulose digestibility. Error bars represent the standard deviation of three replicates.

The solute exclusion method was readily able to distinguish between the ToyoPearl resins on the basis of their different average pore sizes. The pore sizes given in **Table 1** were determined by the manufacturer by finding the smallest molecule excluded from a resin when used as a chromatographic packing material. Examination of the curves in **Figure 2** indicates that the pore size limits for the ToyoPearl resins are in agreement with the product description. Two resins with the same pore size distribution, but different particle sizes, give almost identical results, showing that this method is unaffected by different particle sizes. This result is essential for measuring the porosity in heterogeneous natural materials, such as pretreated biomass, that are expected to have a broad range of particle sizes.

After testing and verifying the solute exclusion method with the ToyoPearl resins, the same conditions were then used to determine the porosity of pretreated corn stover (**Figure 3**). The results show that there is significantly more accessible pore volume in the acid pretreated corn stover samples compared to that in the untreated corn stover, particularly in the range from 10 to 100 Å. Dilute acid pretreatment increased the accessible pore volume of corn stover; however, differences within the selected samples could not be clearly observed because of the variability in the results. Only a very poor correlation was found between the pore volume accessible to a 51 Å molecule, determined by solute exclusion, and 7-day cellulose digestibility (**Figure 4**).

^{1}H NMR thermoporometry represented an alternative method for measuring the pore volume in wet biomass. The melting (or freezing) temperature of the trapped water can be related to pore size (radius or diameter) using the Gibbs−Thompson equation (eq 4). In the NMR spectrum, only the unfrozen water contributes to the signal intensity, because the signal for the frozen water is broad and can no longer be observed. The intensity of the NMR signal decreases with temperature as water freezes in the larger pores or increases with temperature as water melts in the smaller pores. The change of intensity reflects the pore size distribution in the samples.

Initial experiments were carried out by collecting spectra at 1° intervals. The sample was rapidly cooled to 180 K and then slowly ramped to 285 K. In **Figure 5**, the NMR signal intensity versus inverse of temperature (IT) curves for untreated, pretreated corn stover samples, and water are shown. The IT curves can be used to estimate the pore size distribution by fitting the curve to the function shown in eq 7 (*2*, *3*)

$$I(X) = \sum_{i=1}^{N} \frac{I_{0i}}{\sqrt{\pi}} \int_{-\infty}^{(X - X_{ci})/\sqrt{2\Delta_i}} \exp(-u^2)\, du \qquad (7)$$

where $I(X)$ represents the amount of mobile pore water at inverse temperature X (=1000/T), I_{0i} is the amount of confined pore water, X_{ci} represents the phase transition temperature (i.e., the temperature at which water freezes in a pore), and Δ_i is the width of the transition curve. The analytical solution of eq 7 is to fit the IT curve with a series of Gaussian functions centered at X_{ci} with a half-width of Δ_i, where the number of Gaussian functions is equal to the number of phase transitions (i.e., number of uniquely different pore sizes). Broad pore size distributions will have a larger Δ_i, and narrow distributions will have a Δ_i close to zero. A more complete description of curve fitting of the freezing behavior can be found in previous papers (*13*−*15*). However, the IT curves for the pretreated materials could not be easily fit to eq 7 because of the very broad nature of the transitions observed in the pretreated samples, especially for larger pores with water thawing at temperatures near 273 K. The number of pores within a specified size range was then estimated from the changes in the integrated intensity within a temperature range corresponding to a range of pore sizes determined from the constant, K.

Several values for K have been reported for water melting in porous solids (*16*, *24*). The $K(r)$ values are in the range of 500−700 KÅ for water in materials with spherical pores and around 300−400 KÅ for cylindrical pores (*25*, *26*). The plant cell wall is described as a multilamellar structure, and as components are removed from the cell walls by pretreatment, the developing porosity is probably found in slitlike spaces between these lamellae (*2*, *3*). According to this, a $K(r)$ value of 323.3 KÅ (*25*) that corresponds to water melting in cylindrical pores was

Table 3. Melting Temperatures and Calculated Pore Diameters $K(r) = 323.3$ KÅ

temperature (K)	ΔT	pore diameter (Å)
270	–3	216
265	–8	81
260	–13	50
250	–23	28
240	–33	20
230	–43	15
220	–53	12

chosen as this seemed to be the most suitable given the likely structure of pores in pretreated corn stover. The pore diameter corresponding to various temperatures using a $K(r)$ value of 323.3 KÅ are listed in **Table 3**. Choosing this value of K may underestimate the diameter of more spherical pores, but it allows a relative comparison of the distribution of pores in pretreated biomass.

The cumulative pore volume for a range of diameters can be determined by the difference in the NMR signal intensity measured at the temperatures corresponding to pores sizes in a range from ~20 to ~200 Å (from 200 to 270 K, respectively). Using this method, obtaining the complete IT curves was not necessary. Recording intensities at intervals of 5−10 K was sufficient to provide the information required to determine the pore volume changes in the specified range. The results of this analysis are shown in **Table 4**. Pretreated corn stover showed a larger porous structure in the 20−200 Å range than the starting corn stover, but little or no significant difference was found between the pretreated corn stover samples (shaded area in **Figure 5**). Most of the pretreated samples analyzed appeared to have the same total pore volume; therefore, no strong correlation with cellulose digestibility could be established with pore volume (**Figure 6**).

Although all of the pretreated samples had similar intensity differences between 270 and 240 K, the intensity measured at the lowest temperature (240 K) varied among the samples. A relationship was identified between the water that remained unfrozen at 240 K and the remaining xylan content for the series of pretreated corn stover samples (**Figure 7**). This result indicates that pore volume in the smallest pores (<20 Å) decreases as xylan is increasingly removed.

DISCUSSION

Enhancement in the lignocellulose-to-ethanol conversion of pretreated materials has been related to the creation of a more accessible and digestible cellulose substrate (*2*, *3*). Stone and co-workers (*4*) used the solute exclusion method as a means to estimate the accessibility of cellulases to cellulose. In their work, they found a good correlation between the accessible pore volume to a molecule of 30 Å in phosphoric acid swollen cotton and spruce sulfite pulps and the initial rate of dissolved cellulose per hour. They also suggested that an interesting extension of their study would be to use this method for substrates containing lignin and hemicelluloses, because the solute exclusion method should not be influenced by chemical composition. Accessible pore volume could then be correlated with the reactivity of the substrate. Several researchers have since used this method (*6*, *18*, *27*−*31*) and observed good correlations between the surface area estimated to be available to a 51 Å molecule and glucose yields after 2 h of saccharification (*5*).

In this work, the solute exclusion method was used to determine the pore volume of a series of dilute sulfuric acid pretreated corn stover samples pretreated at different severities. All of the pretreated samples studied showed higher pore volume, especially in the larger pore size range (>30 Å), when compared to untreated corn stover. However, the differences within the pretreated corn stover samples were unclear, partly because of the variability in the results. The likely cause of the variability in the measurement of pore volumes in the pretreated corn stover is the heterogeneity of the samples. Visual inspection clearly shows a lack of homogeneity in the substrates, especially in those treated at low severity, which still appear to be quite fibrous. The more severely treated samples seem to be more homogeneous, having a mudlike consistency.

The results reported here do not agree with some published correlations of pore volume with cellulose digestibility (*6*). This may be due, in part, to the fact that few authors have attempted to compare results between such highly digestible substrates as studied here. One of the motivations of our work is characterize cellulosic substrates that are highly digestible so that we can lower the cost of producing ethanol from biomass to make the process competitive with gasoline production from fossil fuels. Our research is attempting to discern which physical and chemical factors control the digestibility of cellulose at higher levels of conversion. Our research indicates that porosity may distinguish lignocellulosic substrates of low digestibility from those of high digestibility as was seen in this work comparing the pretreated corn stover to the untreated corn stover. However, our results indicate that porosity may be less of a limiting factor for more highly digestible materials and the overall yields of ethanol under process-relevant conditions.

^{1}H NMR thermoporometry was also used in this study as an alternative method to measure total pore volume in the range of 20−200 Å, being a relatively rapid, reproducible, and accurate technique. The results described above clearly showed that the pretreated samples have a more porous structure than the corn stover starting material. However, the thermoporometry method also showed that most of the pretreated samples analyzed appeared to have the same total pore volume, and thus no correlation of cellulose digestibility with pore volume could be established.

When the measurement of pore sizes in the plant cell wall is discussed, it must be pointed out that different techniques can give considerably different results. For example, Suurnäkki and co-workers (*32*) found that the median pore diameter of pine kraft fibers measured by NMR was significantly greater than the diameter estimated by the solute exclusion technique, i.e., about 130 Å compared to 30 Å, respectively. This result was due primarily to basic differences between the two methods. The NMR technique determines the surface to volume ratio of water within the plant fiber wall where the surface is defined as all surfaces that are available for water interaction. Therefore, the NMR technique is not affected by changes in pore shape, the form of pore openings, and the presence of restricted pore openings (ink-bottle-shaped pores), whereas the solute exclusion measurement depends on the physical penetration of probe molecules into the pores. In contrast, in this work, higher values of pore size were obtained by the solute exclusion method compared to NMR thermoporometry. This result possibly indicates the absence of restricted pore openings and the presence of ripples or slit-type pores as observed and described by several authors in lignocellulosics (*2*, *3*).

A direct and reliable correlation between a physical property (i.e., porosity) and the biological response (digestibility) of the highly digestible substrates was not found in this work. These results support the view (*33*, *34*) that porosity may not be the

Table 4. NMR Signal Intensities Measured at Different Temperatures in Untreated and Pretreated Corn Stover

sample	7-day cellulose digestibility (%)	xylan content (%)	NMR signal intensities[a] (g of water/g of dry biomass)					Δintensity (270 K– 240 K)
			240 K	250 K	260 K	265 K	270 K	
untreated	17.7[b]	24.1	0.353	0.363	0.377	nd[c]	0.481	0.128
PCS0-1	90.8	2.1	0.210	0.237	0.273	0.310	0.367	0.157
PCS-2	88.5	3.7	0.226	0.251	0.290	0.329	0.413	0.187
PCS-3	92.1	4.2	0.231	0.252	0.293	0.329	0.412	0.181
PCS-4	87.8	4.3	0.214	0.235	0.277	0.309	0.393	0.179
PCS-5	85.5	4.6	0.210	0.236	0.276	0.307	0.389	0.179
PCS-6	91.5	4.7	0.208	0.229	0.267	0.300	0.383	0.175
PCS-7	96.3	5.0	0.229	0.257	0.299	0.335	0.412	0.183
PCS-8	95.6	5.2	0.230	0.256	0.298	0.335	0.415	0.185
PCS-9	95.8	5.9	0.247	0.273	0.315	0.351	0.437	0.190
PCS-10	88.2	7.0	0.232	0.264	0.308	0.343	0.423	0.191
PCS-11	86.5	7.2	0.232	0.257	0.304	0.339	0.421	0.189
PCS-13	81.8	7.5	0.236	0.259	0.298	0.328	0.404	0.168
PCS-14	78.8	7.6	0.231	0.256	0.303	0.336	0.416	0.185
PCS-15	77.0	7.6	0.255	0.285	0.325	0.363	0.454	0.199
PCS-16	91.6	7.6	0.248	0.277	0.325	0.361	0.447	0.199
PCS-17	89.4	8.3	0.267	0.286	0.325	0.360	0.450	0.183
PCS-18	87.1	8.9	0.270	0.293	0.338	0.375	0.471	0.201
PCS-19	82.2	9.5	0.264	0.285	0.329	0.369	0.460	0.196
PCS-20	82.9	10.3	0.268	0.290	0.336	0.376	0.461	0.193
PCS-21	69.8	10.4	0.288	0.313	0.358	0.392	0.480	0.192
PCS-23	72.4	14.1	0.310	0.330	0.374	0.430	0.501	0.191

[a] Standard deviation of at least three replicates (0.002–0.015). [b] Measured with a single cellulase Cel7A. [c] Not determined.

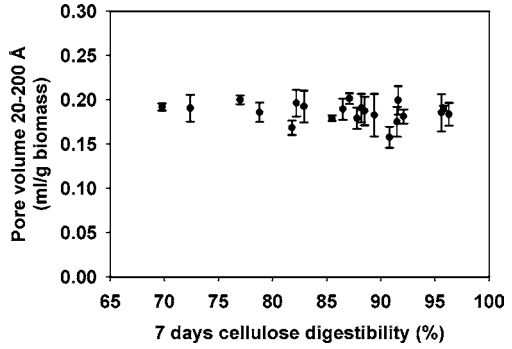

Figure 6. Pore volume in the 20–200 Å range measured by NMR thermoporometry for pretreated corn stover versus cellulose digestibility after 7 days. Error bars represent the standard deviation of at least three replicates.

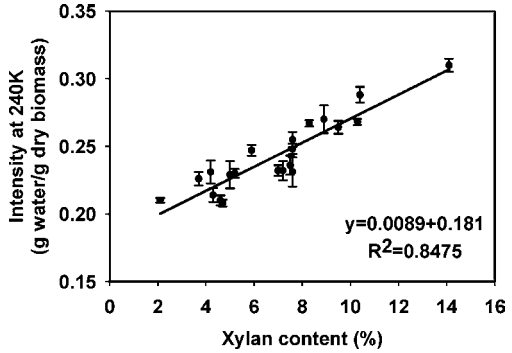

Figure 7. NMR signal intensity at 240 K versus xylan content for pretreated corn stover. Error bars represent the standard deviation of at least three replicates.

only factor limiting cellulose digestibility and that other factors, that is, chemical composition, crystallinity, and cellulose accessibility, also play a role in the cellulase−cellulose interaction and, consequently, in the digestibility of substrates.

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