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Evaluation and Characterization of Sorghum Biomass as Feedstock for Sugar Production

K. Theerarattananoon, X. Wu, S. Staggenborg, J. Propheter, R. Madl, D. Wang

Abstract. *Conversion of cellulosic biomass, such as agricultural residues, to biofuels offers significant economic, environmental, and strategic benefits. Sorghum is an important energy crops in the U.S. It is a renewable resource and is currently grown on about 10 million acres in the U.S. However, at present, there is a lack of scientific information and knowledge about the use of sorghum biomass for biofuel production. The objective of this research was to evaluate and characterize sorghum biomass as a feedstock for sugar production. Five types of sorghum biomass (brown midrib sorghum, forage sorghum, grain sorghum, photoperiod-sensitive sorghum, and sweet sorghum) were characterized and evaluated for sugar production. Pretreatment with dilute acid was used to increase yield of fermentable sugars. Effects of sulfuric acid concentration, treatment temperature, and residence time on yield of fermentable sugars were studied. Accellerase 1000 was used to hydrolyze cellulose into glucose at 50 °C and pH 4.8 for 96 h. A high percentage of enzymatic conversion of cellulose (ECC) was observed for sorghum biomass that was pretreated under severe pretreatment temperature (85% to 98% ECC for biomass pretreated at 165 °C for 10 min; 65% to 82% ECC for biomass pretreated at 140 °C for 30 min). However, mass recovery and cellulose recovery of the solid fraction after pretreatment decreased under severe pretreatment conditions (70% to 85% cellulose recovery for sorghum biomass pretreated at 140 °C for 30 min; 31% to 58% cellulose recovery for sorghum biomass pretreated at 165 °C for 10 min).*

Keywords. *Cellulose conversion, Enzymatic hydrolysis, Pretreatment, Sorghum biomass.*

As the world population and economy expand, energy demand will increase (USCB, 2008; EIA, 2008). Energy consumption in the U.S. exceeds 100 quadrillion Btu per year, and 85% of this consumption is from fossil fuels (EIA, 2008). Fossil fuel production from the current major energy sources (coal, crude oil, and natural gas) will soon peak (Kharecha and Hansen, 2008), and the solution is to either develop new types of energy sources or produce substitute fuels using available alternative feedstocks. Conversion of lignocellulosic biomass into biofuels is a feasible option for substantial replacement of fossil fuels (Perlack et al., 2005). Lignocellulosic biofuels offer one of the best near-to-midterm alternatives for meeting our nation's transportation energy needs.

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The authors are **Karnnalin Theerarattananoon, ASABE Member Engineer,** Graduate Research Assistant, Department of Biological and Agricultural Engineering; **Xiaorong Wu,** Postdoctoral Research Associate, Department of Biological and Agricultural Engineering; **Scott Staggenborg,** Professor, Department of Agronomy; **Jonathan Propheter,** Graduate Research Assistant, Department of Agronomy; **Ron Madl,** Professor, Department of Grain Science and Industry; and **Donghai Wang, ASABE Member,** Associate Professor, Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, Kansas. **Corresponding author:** Donghai Wang, Department of Biological and Agricultural Engineering, Kansas State University, Manhattan, KS 66506; phone: 785-532-2919; fax: 785-532-5825; e-mail: dwang@ksu.edu.

Production of bioethanol from lignocellulosic biomass through a biological route involves three major steps: pretreatment, enzymatic hydrolysis, and fermentation (Christakopoulos et al., 1993). Pretreatment is a critical step. The purpose of pretreatment is to break up the lignin seal, prehydrolyze the hemicellulose, and disrupt the crystalline structure of the cellulose, thus allowing cellulases better access to cellulose during enzymatic hydrolysis (Corredor et al., 2008; Kadar et al., 2007; Sun and Cheng, 2002). Pretreatment can be achieved through mechanical (size reduction through milling and extrusion processing), physical (steam treatment), thermal-chemical (dilute acid treatment, concentrated acid treatment, alkaline treatment, hydrogen peroxide treatment, hot water treatment, steam explosion, ammonia fiber explosion, and organic solvent treatments), and biological methods (microbial and enzyme degradation) or a combination of these methods (Sun and Cheng, 2002; Zhan et al., 2006; Corredor et al., 2008; Zheng et al., 2008). Ideal pretreatment methods are cost-effective and have as little carbohydrate degradation or loss and formation of inhibitory substances as possible (Sun and Cheng, 2002).

Despite of some limitations on using dilute acid pretreatment, including formation of degradation products, release of potential biomass fermentation inhibitors, washing and neutralization of acid before sugars proceed to fermentation, and the need for corrosion-resistant reactors (Mosier et al., 2005), pretreatment with dilute acid is still considered an effective and relatively inexpensive pretreatment method for several types of biomass, which not only solubilize hemicellulose but also convert solubilized hemicellulose into fermentable sugars. This method also eliminates the use of hemicellulose enzymes during hydrolysis (Zaldivar et al., 2001; Saha et al., 2005).

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The success of using lignocellulosic biomass for bioethanol production greatly depends on the chemical and physical properties of the biomass, pretreatment method, optimization of the process conditions, and efficiency of the hydrolyzing enzymes and fermentation microorganisms (Corredor et al., 2008). Great research efforts have been conducted on pretreatment of corn stover (Wyman et al., 2005; Lau and Dale, 2009), sugarcane bagasse (Dawson and Boopathy, 2007), switchgrass (Dien et al., 2006; Alizadeh et al., 2005), wheat straw (Rosgaard et al., 2007; Kristensen et al., 2008), and hardwood and softwood biomasses (Soderstrom et al., 2002; Sassner and Zacchi, 2008).

In the Midwest region of the U.S., sorghum is considered one of the promising lignocellulosic feedstocks for biofuel production because it is abundant in this region and its production ranks third among cereal crops (Linde et al., 2006; Zhan et al., 2006). Currently, not much work has been conducted on bioconversion of sorghum biomass for biofuels as compared to other types of biomass. Coredor et al. (2009) showed that up to 72% hexose yield and 94% pentose yield from forage sorghum stalk were obtained using modified steam explosion with 2% sulfuric acid at 140°C for 30 min and enzymatic hydrolysis with cellulase (15 FPU g^{-1} cellulose) and β-glucosidase (50 CBU g^{-1} cellulose). Salvi et al. (2010) conducted a study on ethanol production from sorghum fibers by a dilute ammonia pretreatment method and found that theoretical cellulose yield and hemicellulose yield for sorghum fibers pretreated by dilute ammonia and hydrolyzed by enzyme combination of Spezyme Cp (60 FPU g^{-1} glucan) and Novozyme 188 (64 FPU g^{-1} glucan) were 84% and 73%, respectively. The ethanol yield was 25 g per 100 g dry biomass.

The objective of this research was to evaluate and characterize sorghum biomasses from grain sorghum, forage sorghum, sweet sorghum, photoperiod-sensitive sorghum, and brown midrib (BMR) sorghum as feedstocks for sugar production.

Materials and Methods

Materials

Sorghum biomass, including forage sorghum, photoperiod-sensitive sorghum, BMR sorghum, sweet sorghum, and grain sorghum, was harvested from Riley County, Kansas, and air dried in an oven at 70°C to reduce the moisture content for long-term storage. The sorghum biomass was ground into powder with a Retsch cutting mill (Haan, Germany) with a 1.0 mm sieve. Sorghum biomass samples were stored at room temperature for future use. Corn stover grown in the same location was used as a control. All chemicals used for this research were purchased from Sigma Chemical Co. (St. Louis, Mo.). Accellerase 1000 (Danisco US, Inc., Genencor Division, Rochester, N.Y.) enzyme complex was used for hydrolyzing sorghum biomass into sugars. This enzyme complex contains multiple enzyme activities, mainly exoglucanase, endoglucanase (2500 CMC U g^{-1} minimum), hemi-cellulase, and β-glucosidase (400 pNPG U g^{-1} minimum). Exoglucanase activity is reported in carboxymethycellulose (CMC U) activity units (one CMC U unit of activity liberates 1 μmol of reducing sugars in 1 min under specific assay conditions of 50°C and pH 4.8), and β-glucosidase is reported in pNPG units (one pNPG unit denotes 1 μmol of nitrophenol liberated from para-nitrophenyl-B-D-glucopyranoside in 10 min at 50°C and pH 4.8).

Dilute Acid Pretreatment

Pretreatment was carried out in a pressure reactor (Parr Instrument Co., Moline, Ill.) with a 1 L reaction vessel. The ground sorghum biomass and corn stover were mixed with diluted sulfuric acid (2% w/v) to obtained 10% solid content (approximately 53 g in 500 mL diluted sulfuric acid solution). Effects of temperature and reaction time on sugar yield were studied (140°C for 30 min and 165°C for 10 min). Pretreated biomass was washed with hot distilled water and centrifuged four times to remove dissolved sugars and sulfuric acid. The supernatant was collected into a 2 L volumetric flask. A portion of the supernatant was neutralized with $CaCO_3$ and further analyzed for glucose and pentose content by using a high-performance liquid chromatograph (HPLC) with a Rezex RCM column (Phenomenex, Cal.). As hemicellulose is a polymer of hexose and pentose, glucose in the supernatant was considered to be from hydrolysis of both cellulose and hemicellulose, and pentose was counted as sugars released from hydrolysis of hemicellulose. Washed biomass samples were split into two portions. One portion was used for moisture content and chemical composition analyses; the other portion was used for subsequent enzymatic hydrolysis.

Enzymatic Hydrolysis

Pretreated biomass samples were enzymatically hydrolyzed in solution with sodium acetate buffer (50 mM, pH 4.8) and 0.02% (w/v) sodium azide to prevent microbial growth during hydrolysis. The dry mass content of the hydrolysis slurries was 5% (w/v). Enzymatic hydrolysis was carried out in 125 mL flasks with 50 mL of slurry in a 50°C water bath shaker agitating at 140 rpm for 96 h. The enzyme loading (Accellerase 1000, Danisco US, Inc., Genencor Division, Rochester, N.Y.) was 1 mL g^{-1} of cellulose. During enzymatic hydrolysis, the hydrolysis slurries were sampled periodically up to 96 h after the addition of enzyme by withdrawing 0.1 mL of slurry from each flask. Sample slurries were then mixed with 0.9 mL double-distilled water in 1.5 mL vials, and the vials were placed to boil in a water bath for 15 min to deactivate the enzyme. After enzyme inactivation, samples were centrifuged at 13,500 rpm for 15 min. The supernatants then were further diluted and filtered into 1.5 mL autosampler vials through 0.2 μm hydrophilic PTFE syringe filters (Millipore, Billerica, Mass.). Filtered samples were kept at 4°C before HPLC analysis.

The conversion efficiency of cellulose was expressed in terms of the percentage of cellulose enzymatically converted to glucose, i.e., enzymatic conversion of cellulose (ECC). ECC was calculated by comparing the glucose yield (g) after enzymatic hydrolysis with the initial glucose content (1.11 times the initial cellulose content) in the untreated biomass (Varga et al., 2004). The following formula was used to calculate ECC:

$$ECC = \frac{c \cdot V}{1.11 \cdot m} \cdot 100\% \quad (1)$$

where c is the concentration (g L^{-1}) of D-glucose in the sampled hydrolysate determined by HPLC analysis, V is the total volume (L), and m is the weight of cellulose before enzymatic hydrolysis (g). The factor 1.11 is the cellulose to glucose conversion factor.

Crystalline Structure Analysis Using X-Ray Diffraction

The crystalline structure of the sorghum biomass samples before and after pretreatment was analyzed by wide-angle x-ray diffraction (XRD) with a Bruker AXS D-8 diffractometer (AXS GmbH, Karlsruhe, Germany) operating at 40 kW, 40 mA. The radiation was copper Kα (λ = 1.54 Å), and grade range was between 5° and 40° with a step size of 0.03°. Aperture, scatter, and detector slits each were 1°. The scan speed was set at 5° min^{-1}. The presence of crystallinity in a sample can be detected by absorption peaks. The crystallinity index (CrI) was calculated using the method of Segal et al. (1959) as follows:

$$\mathrm{CrI} = \frac{I_{002} - I_{amorphous}}{I_{002}} \cdot 100 \qquad (2)$$

where I_{002} is the intensity of the crystalline portion of biomass at about 2θ = 22.5°, and $I_{amorphous}$ is the peak for the amorphous portion at about 2θ = 16°. In this study, the second highest peak after 2θ = 22.5° was at 2θ = 16° and was assumed to correspond to the amorphous region. However, the amorphous peak is reported to be around 2θ = 18.7° in the literature. The diffractogram was smoothed using a smooth function in MATLAB (see the Appendix).

Chemical Structure Analysis Using FTIR

Fourier transform infrared (FTIR) spectra are frequently used for investigating the structure of constitutes and chemical changes in lignocellulosic biomass. Cellulose decrystallization is usually associated with reduced crystallinity. This suggests that crystallinity can be used to analyze sorghum biomass before and after acid pretreatment and enzymatic hydrolysis. FTIR measurements were performed using a Nexus 670 FT-IR spectrophotometer (Thermo-Nicolet Corp., Madison, Wisc.) equipped with a Smart Collector. Reagent KBr and samples were dried for 24 h at 50°C and then prepared by mixing 2 mg of sample with 200 mg of spectroscopy-grade KBr. All spectra were recorded in the absorbance mode in the wave number range of 400-4000 cm^{-1} with a detection resolution of 4 cm^{-1} and 32 scans per sample. OMNIC 6.1a software (Thermo-Nicolet Corp., Madison, Wisc.) was used to determine peak positions and intensities.

Morphological Structure Analysis Using Scanning Electron Microscopy

Scanning electron microscopy (SEM) was used to measure the surface properties and microstructure of sorghum biomass before and after treatment. A Hitachi S-3500M SEM with an S-6542 absorbed-electron detector (Hitachinaka, lbaraki, Japan) was used to exam the microstructure of sorghum biomass before and after treatment from 1.5K to 3K. Specimens were mounted on conductive adhesive tape, sputter coated with 4 nm of a 60% gold and 40% palladium mixture, and observed using a voltage of 15 to 20 kV.

Analytical Methods

Moisture content of ground sorghum biomass and corn stover was determined by drying about 2 g of each sample in a forced-air oven at 105°C for 4 h (Sluiter et al., 2008b). Moisture content of pretreated wet samples was determined by drying approximately 2.5 g of sample in a forced-air oven at 49°C overnight and further drying at 105°C for a minimum of 4 h.

Extractives in dry, untreated biomass and chemical composition of untreated and pretreated biomass were determined by following NREL laboratory analytical procedures (Sluiter et al., 2005; Sluiter et al., 2008a). Structural carbohydrates in biomass were reported as percentages of glucan and xylan. Glucan is basically cellulose, and xylan is the major hemicellulose constituent. Lignin, the major noncarbohydrate component, is the sum of acid-insoluble and acid-soluble lignin.

Glucose, xylose, mannose, and arabinose in acid-hydrolyzed samples were determined by analyzing the supernatant from pretreated samples with an HPLC (Shimadzu, Kyoto, Japan) equipped with an RCM-monosaccharide column (300 × 7.8 mm; Phenomenex, Torrance, Cal.) and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). The mobile phase was 0.6 mL min^{-1} of double-distilled water, and the oven temperature was 80°C. The supernatants of pretreated samples were neutralized with $CaCO_3$ to pH 6 before being filtered through 0.2 μm hydrophilic PTFE syringe filters (Millipore, Billerica, Mass.).

The experiment for each biomass sample was replicated twice. Analysis of variance (ANOVA) and least-significant difference (LSD) at the 0.05 level were performed using SAS (2005 ver., SAS Institute, Inc., Cary, N.C.).

Results and Discussion

Effect of Pretreatment Conditions on Yield of Fermentable Sugars

The effect of sulfuric acid concentration (1.0%, 1.5%, and 2.0%) on grain sorghum biomass conversion efficiency was studied at constant temperature and residence time (140°C for 30 min). Glucose yield increased as sulfuric acid concentration increased. Pretreatment with 2% sulfuric acid yielded the highest conversion efficiency of glucose (82% ECC at the 70th h of hydrolysis time) compared with lower concentrations of sulfuric acid (fig. 1). Therefore, 2% sulfuric acid was considered optimum and used in subsequent experiments.

When the untreated biomass is used as a reference point, glucan content in biomass increased significantly after dilute acid pretreatment, especially for pretreatment at mild temperature (table 1). There was a significant increase in lignin content of biomass after dilute acid pretreatment. This phenomenon was more pronounced at higher pretreatment temperature, except for the case of grain sorghum and sweet sorghum. Most xylan (mainly hemicellulose) was hydrolyzed during dilute acid pretreatment, as seen from the significant decrease in xylan content of pretreated solid residues (table 1).

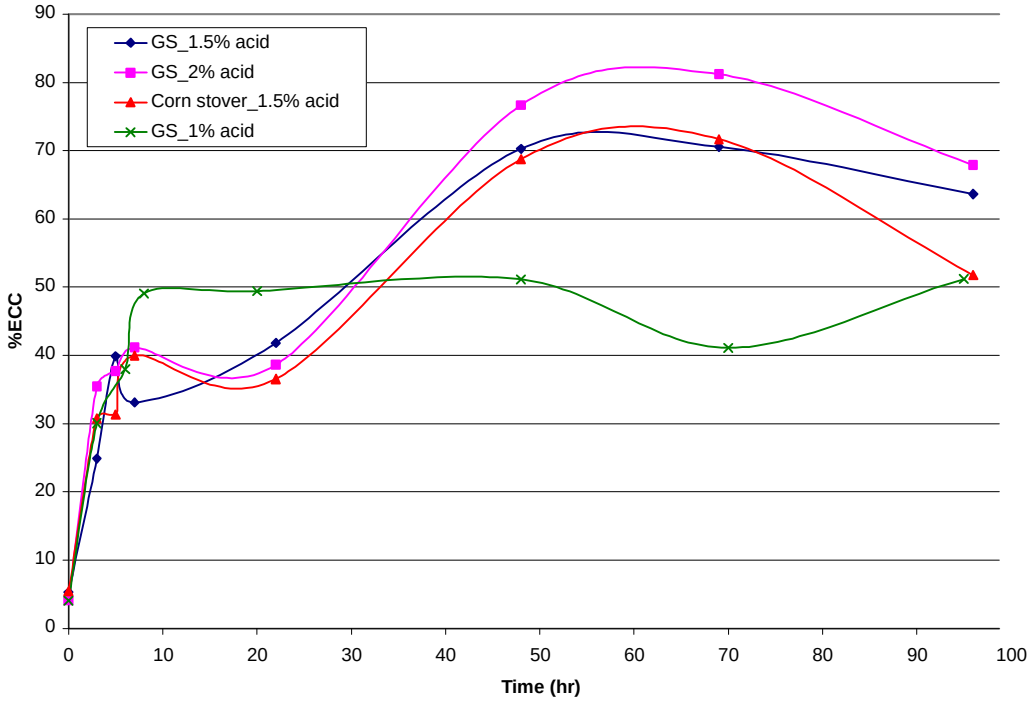

Figure 1. Effect of sulfuric acid concentration on glucose yield.

Table 1. Components in solid fractions of pretreated biomass.

Sample	Pretreatment Conditions	Component in Solid Fractions (%)[a]				Mass Recovery (%)	Cellulose Recovery (%)
		Lignin	Glucan	Xylan	Ash		
Forage sorghum	Unpretreated	20.29 a	37.90 a	29.79 a	10.94 a		
	140°C/30 min	26.82 b	47.91 b	3.62 b	10.47 b	67.08	84.79
	165°C/10 min	31.55 c	42.16 c	2.54 c	12.43 c	51.45	57.23
Photoperiod-sensitive sorghum	Unpretreated	19.22 a	44.02 a	27.40 a	7.49 a		
	140°C/30 min	28.17 b	55.37 b	4.26 b	6.01 b	61.41	77.24
	165°C/10 min	47.90 c	44.76 a	2.42 c	7.54 a	30.85	31.36
BMR sorghum	Unpretreated	15.48 a	40.48 a	26.16 a	8.71 a		
	140°C/30 min	22.67 b	55.24 b	4.22 b	8.03 b	56.81	70.56
	165°C/10 min	28.20 c	42.29 a	2.94 b	9.38 c	45.02	42.81
Sweet sorghum	Unpretreated	18.03 a	34.24 a	26.81 a	4.27 a		
	140°C/30 min	33.82 b	54.15 b	2.64 b	4.58 b	46.38	73.35
	165°C/10 min	32.30 b	36.17 a	2.41 b	5.43 c	44.65	47.17
Grain sorghum	Unpretreated	18.11 a	37.84 a	24.60 a	10.87 a		
	140°C/30 min	28.21 b	44.49 b	4.34 b	12.06 ab	59.07	74.57
	165°C/10 min	28.02 b	41.44 c	2.58 c	12.43 b	51.71	56.62
Corn stover	Unpretreated	18.10 a	37.30 a	24.61 a	5.88 a		
	140°C/30 min	25.18 b	55.01 b	3.99 b	5.45 b	66.12	97.51
	165°C/10 min	30.05 c	46.54 c	2.63 b	5.23 c	47.41	59.16

[a] Means in the same biomass followed by different letters are significantly different at $p < 0.05$.

Mass recovery and cellulose recovery for certain pretreatment conditions varied among biomass types. Under the pretreatment condition of 140°C for 30 min, cellulose recovery of various types of biomass ranged from 70% to 85%, whereas cellulose recovery biomass pretreated at 165°C for 10 min ranged from 31% to 58%. Mass recovery and cellulose recovery from the solid fraction after pretreatment decreased under more severe pretreatment conditions (165°C, 10 min; table 1).

Content of hexose sugar (glucose) in the filtrate fraction of samples after pretreatment increased with the increase in pretreatment temperature (table 2). Pentose content (xylose and arabinose) in the filtrate fraction decreased as pretreatment temperature increased. These results indicate that more glucose and less pentose were present in the liquid fraction after pretreatment at higher temperature. The decrease in pentose sugar was probably due to degradation of pentose at higher temperature.

Enzymatic Hydrolysis

The maximum ECC of pretreated cellulose was between 65% and 82% for biomass pretreated at 140°C for 30 min (fig. 2) and between 85% and 98% for biomass pretreated at 165°C for 10 min (fig. 3). For biomass pretreated at 140°C for

Table 2. Sugar yield in filtrate after dilute acid pretreatment.

Samples	Pretreatment Conditions	Components in Filtrate Fractions[a]		
		Xylose	Arabinose	Glucose
Forage sorghum	140°C/30 min	14.82 a	2.75 a	18.28 a
	165°C/10 min	9.50 b	1.02 b	20.85 b
Photoperiod-sensitive sorghum	140°C/30 min	15.44 a	2.69 a	14.14 a
	165°C/10 min	8.10 b	2.07 b	21.42 b
BMR sorghum	140°C/30 min	16.68 a	3.22 a	15.77 a
	165°C/10 min	9.72 b	1.01 b	20.92 a
Sweet sorghum	140°C/30 min	17.86 a	2.41 a	21.20 a
	165°C/10 min	5.67 b	1.68 b	24.78 b
Grain sorghum	140°C/30 min	15.29 a	3.00 a	15.49 a
	165°C/10 min	9.94 b	2.47 a	18.06 b
Corn stover	140°C/30 min	17.54 a	3.30 a	12.90 a
	165°C/10 min	9.37 b	1.69 b	22.68 b

[a] g per 100 g of dry, untreated biomass. Means in the same biomass followed by different letters are significantly different at $p < 0.05$.

30 min, corn stover yielded the highest ECC, followed by BMR sorghum, forage sorghum, photoperiod-sensitive sorghum, sweet sorghum, and grain sorghum. The ECC of corn stover (82.3%) was not much higher than that for BMR sorghum (80.5%). For biomass pretreated at 165°C for 10 min, BMR sorghum yielded the highest ECC, followed by sweet sorghum, corn stover, photoperiod-sensitive sorghum, forage sorghum, and grain sorghum. In this case, the ECC of corn stover (95%) was a bit lower than that of sweet sorghum (97%). The ECC of sorghum biomass was not much different from that of corn stover, regardless of pretreatment conditions, which indicates that there is some potential for using sorghum stover in biofuel applications. Among different types of sorghum biomass, BMR sorghum yielded the highest ECC during enzymatic hydrolysis. This probably is because BMR sorghum has less lignin content than other types of sorghum, even less than corn, and a high ratio of cellulose to lignin. In general, biomass with less lignin is more digestible.

Table 3. Crystallinity index values for different types of biomass.[a]

Sample	Untreated	140Prt	165Prt	140EH	165EH
BMR	37.04	46.51	45.39	37.00	32.90
Corn stover	47.82	63.32	60.68	45.88	25.83
Forage sorghum	40.99	56.47	52.91	35.72	38.30
Grain sorghum	39.53	49.49	49.63	45.95	34.19
P-S sorghum[b]	45.52	55.00	59.19	49.13	29.01
Sweet sorghum	32.58	56.06	33.92	36.57	38.55

[a] 140Prt = pretreated at 140°C for 30 min, 165Prt = pretreated at 165°C for 10 min, 140EH = enzymatic hydrolysis of biomass pretreated at 140°C for 30 min, and 165EH = enzymatic hydrolysis of biomass pretreated at 165°C for 10 min.

[b] Photoperiod-sensitive sorghum.

X-Ray Diffraction

The crystallinity patterns of each biomass sample after pretreatment and after enzymatic hydrolysis look similar to their patterns before treatment (fig. 4). However, the intensity of crystallinity and amorphous peaks varied with treatment conditions as well as biomass types. Among different types of sorghum, photoperiod-sensitive sorghum had the highest intensity of both the crystalline peak and amorphous peak. Lower crystallinity has been associated with cellulose decrystallization as well as a high value of amorphous material. As shown in table 3, the CrI values for corn were higher than those for any type of sorghum. The increase of CrI values along with the significant increase of glucan content and the significant decrease of xylan content in pretreated solid residues (table 1) confirmed that dilute acid pretreatment was an efficient method for hydrolyzing the amorphous portion (hemicellulose) and disrupting the crystalline structure of the biomass. For most biomass samples, enzymatic hydrolysis resulted in a decrease of CrI values compared with untreated samples. However, the relationship between the crystallinity index of hydrolyzed biomass and its corresponding glucose conversion efficiency is not well defined. A high crystallinity index of biomass after hydrolysis does not mean that the biomass is difficult to

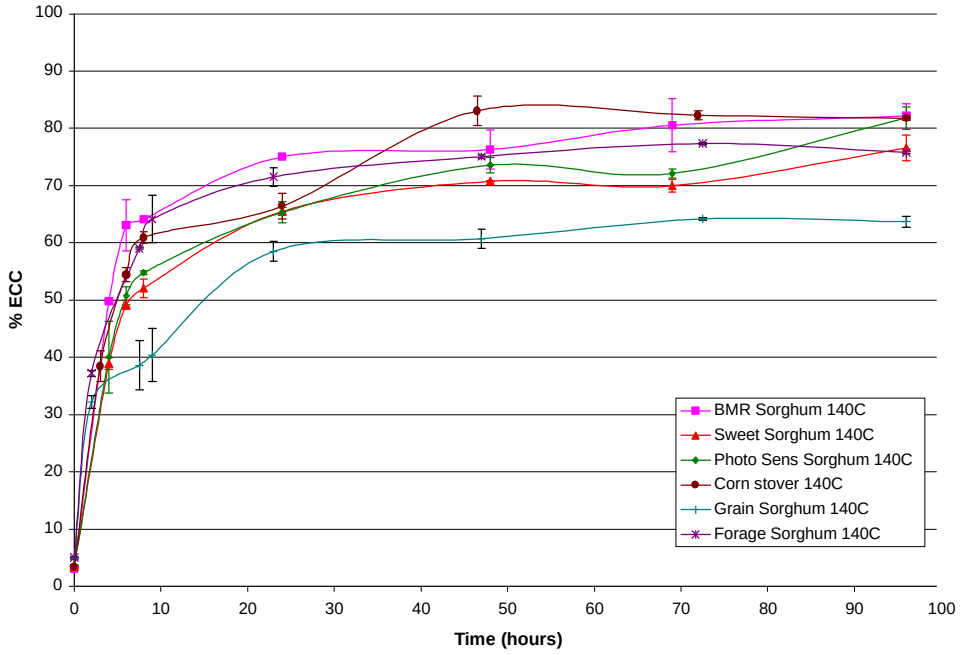

Figure 2. Percentage of enzymatic conversion of cellulose for corn stover and different types of sorghum biomass pretreated with 2% w/v sulfuric acid at 140°C for 30 min.

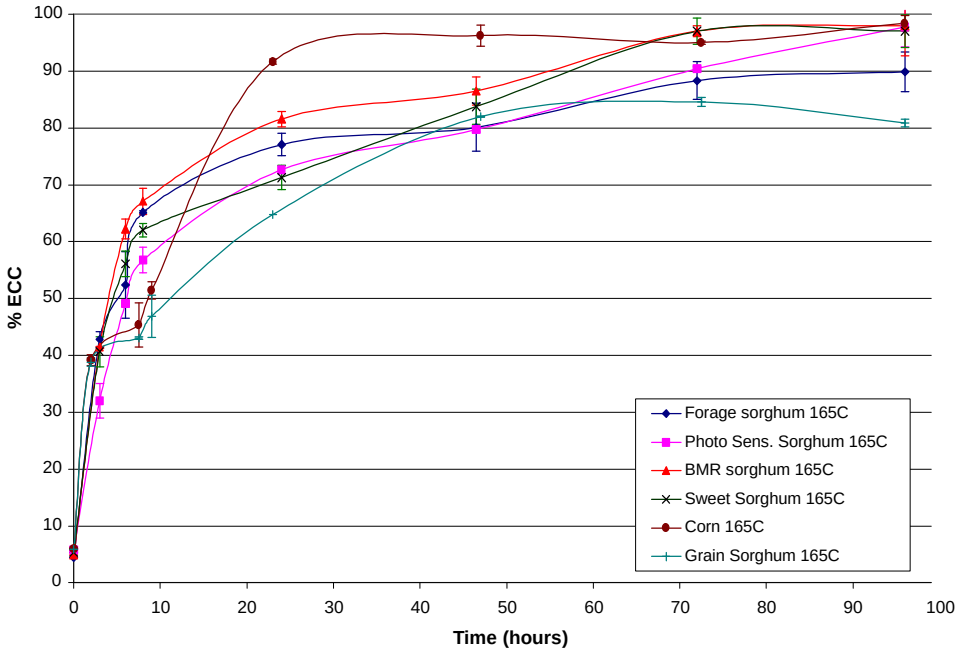

Figure 3. Percentage of enzymatic conversion of cellulose of corn stover and different types of sorghum biomass pretreated with 2% w/v sulfuric acid at 165°C for 10 min.

enzymatically hydrolyze. For example, the CrI value of enzymatically hydrolyzed corn stover pretreated at 140°C was 45.88, which was relatively higher than the CrI of many sorghum samples, but its cellulose conversion after hydrolysis was the highest (82.3%) of all samples.

Chemical Structure

The FTIR spectra of sorghum biomass and corn stover show several absorption bands that can be assigned to major structural components: hemicellulose, lignin, and cellulose. The assignment of FTIR absorption bands for sorghum biomass and corn stover is summarized in table 4. As shown in figure 5, the 4000-1800 cm^{-1} region of the absorbance spectra has only a few bands, which are attributed to the O-H group (at around 3340 cm^{-1}) and the C-H group (at around 2927 cm^{-1}). These bands are pure, whereas other bands in the fingerprint region (1800-900 cm^{-1}) are complex; this is a result of various vibration modes in carbohydrates and lignin (Gilbert et al., 1993; Pandey, 1999). Therefore, this investigation focused on the fingerprint region (figs. 6 to 10). In the fingerprint region, C-H bending modes appear at 1435-1431 cm^{-1} (asymmetric) and 1381-1373 (symmetric) cm^{-1}, respectively, and the C-O of guaiacyl ring lies at 1273-1271 cm^{-1}. Although all biomass spectra were similar, slight changes were observed from spectrum to spectrum. For example, there were no peaks at 1714, 1660, 1273, 1207, and 1088 cm^{-1} for the untreated biomass. The hemicellulose band appeared at 1738 cm^{-1} for all original samples (Guo et al., 2008; Kumar et al., 2009; Pandey, 1999; Sun and Tomkinson, 2004). No hemicellulose band was observed after treatment and hydrolysis, indicating that hemicellulose was greatly hydrolyzed during the pretreatment process. The data in table 1, indicating that pretreated samples contained only about 2% to 4% xylose, further supported this claim. The chemical composition analysis of biomass (table 2) supports the FTIR observations that the hemicellulose (xylan) content of biomass significantly decreases after pretreatment.

Lignin-related bands in the FTIR spectra were seen around 1273, 1518, 1610, and 1715 cm^{-1} (Kumar et al., 2009; Pandey, 1999; Sun et al., 1998). The band at 1518-1514 cm^{-1}, attributed to the C=C of lignin, was observed for all untreated biomass and was strong in intensity for photoperiod-sensitive sorghum and sweet sorghum. This spectrum remained after pretreatment and was still seen after enzymatic hydrolysis. Detection of an absorption band at 1715 cm^{-1}, due to the C=O stretching of the phenyl ester side chains of the lignin structure, in pretreated solid residues showed that the phenyl ester linkages between lignin and a few hemicelluloses had not been cleaved by dilute acid pretreatment. This finding supports the previous result for chemical composition analysis of biomass samples; dilute acid pretreatment can remove most of the xylan (hemicellulose) from biomass, while most of the glucan (cellulose) and lignin remain in the solid residues. The band at 1606-1610 cm^{-1} is associated with the α-β double bond of the propanoid side group in lignin-like structures (Corredor et al., 2009; Kumar et al., 2009; Pandey, 1999). For all samples, this band was defined after pretreatment but became weaker after enzymatic hydrolysis. Sorghum biomass and corn stover have two types of lignin: guaiacyl and syringyl rings. The band at 1514-1518 cm^{-1} is associated with the guaiacyl ring in lignin (Corredor et al., 2009; Pandey, 1999; Sun et al., 1998). This band was observed in all untreated biomass samples and remained after pretreatment and enzymatic hydrolysis. The band around 1435 cm^{-1} is due to absorption of syringyl rings in lignin (Corredor et al., 2009; Gastaldi et al., 1998; Pandey, 1999). This band was observed in all untreated and treated samples. Among various types of sorghum after the pretreatment process, BMR sorghum, which had the lowest ratio of syringyl to guaiacyl rings in its lignin structure, yielded the highest ECC.

Cellulose-related bands in the FTIR spectra were seen around 904, 1381, 1435, 2927, and 3340 cm^{-1} (Gastaldi et al., 1998; Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999;

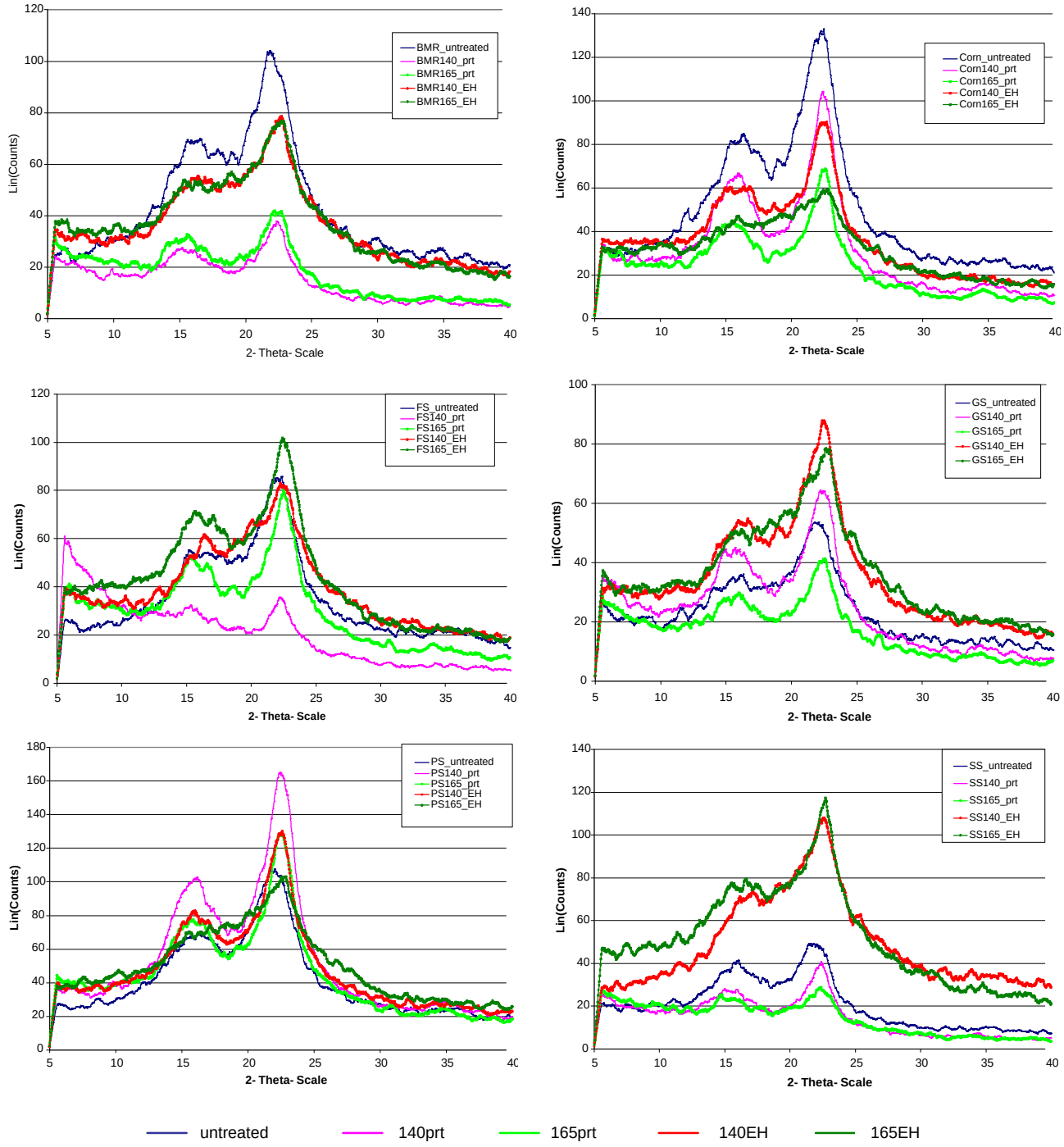

Figure 4. X-ray diffraction of untreated and treated sorghum stalks and corn stover: (a) BMR sorghum, (b) corn stover, (c) forage sorghum, (d) grain sorghum biomass, (e) photoperiod-sensitive sorghum, and (e) sweet sorghum bagasse.

Sun et al., 1998). The band at 1381-1373 cm^{-1} is due to C-H deformation (symmetric) of cellulose (Gastaldi et al., 1998; Gilbert et al., 1993; Pandey, 1999). This band was observed in all original samples at 1381 cm^{-1}. After pretreatment, the band shifted to 1373 cm^{-1} and decreased in intensity. The decrease in peak intensity was more pronounced after enzymatic hydrolysis. This decrease implies that cellulose is decrystallized because of the applied pretreatment and further hydrolyzed after enzymatic hydrolysis.

Morphological Structure

Untreated samples seemed to have deposits on the outer surface (fig. 11). This surface layer can include waxes, hemicellulose, lignin, and other binding materials. The internal plant structure consists of vascular bundles and holes in the cellulose wall that are used for ventilation and metabolism. After dilute acid pretreatment, the surfaces were clean and smooth (figs. 12 and 13), a result of the removal of the outer surface layer by acid. Some annular rings and

Table 4. Assignment of FTIR absorption bands for sorghum biomass and corn stover.

Wavenumbers (cm^{-1})	Pattern in[a]	Assignment	Reference
3340	All	O-H stretching (indicates rupture of cellulose hydrogen bonds)	Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999
2927	All	C-H stretching (indicates rupture of methyl/methylene group of cellulose)	Gilbert et al., 1993; Kumar et al., 2009; Pandey, 1999
1738	Untreated	C=O ester; strong carbonyl groups in branched hemicellulose	Guo et al., 2008; Kumar et al., 2009; Pandey, 1999; Sun and Tomkinson., 2004
1714-1713	140Prt, 165Prt, and 165EH	C=O stretching (carboxylic acids/ester groups) from lignin	Kumar et al., 2009; Pandey, 1999
1660-1637	Untreated 140EH	Absorbed H_2O, C=O with intramolecular hydrogen bond	Gilbert et al., 1993; Guo et al., 2008
1610-1606	All	Aromatic skeletal vibration + C=O stretching (related to lignin removal)	Kumar et al., 2009; Pandey, 1999
1518-1514	All	C=C (related to lignin removal) guaiacyl ring of lignin	Corredor et al., 2009; Pandey, 1999; Sun et al., 1998
1435-1431	All	C-H deformation (asymmetric) of cellulose; syringyl absorption of hardwood	Corredor et al., 2009; Gastaldi et al., 1998; Pandey, 1999
1381-1373	All	C-H deformation (symmetric) of cellulose	Gastaldi et al., 1998; Gilbert et al., 1993; Pandey, 1999
1340-1335	All	O-H in-plane deformation	Pandey, 1999
1273-1271	140Prt, 165Prt, 140EH, and 165EH	C-O of guaiacyl ring and C-O stretching	Pandey, 1999
1238-1236	140Prt and 165Prt	O-H in-plane deformation	Gilbert et al., 1993
1207-1203	140Prt and 165Prt	O-H in-plane deformation	Gilbert et al., 1993; Pandey, 1999
1136-1126	All	Antisymmetric C-O-C beta-1,4 glycosyl linkage of cellulose	Gilbert et al., 1993
1088	140EH	C-O of secondary alcohols	Pandey, 1999
904-901	All	Glucose ring stretch, C-H deformation (removal of amorphous cellulose)	Kumar et al., 2009; Pandey, 1999

[a] 140Prt = pretreated at 140°C for 30 min, 165Prt = pretreated at 165°C for 10 min, 140EH = enzymatic hydrolysis of biomass pretreated at 140°C for 30 min, and 165EH = enzymatic hydrolysis of biomass pretreated at 165°C for 10 min.

macrofibrils were also observed. The diameter of individual cellulose microfibers was about 7 to 9 microns. Pretreatment at higher temperature disrupted microfibrils much more and had a greater impact on particle size reduction than the lower temperature pretreatment condition. SEM images of pretreated biomass also revealed formation of some holes on the biomass surface and disruption of the biomass network consistent with hemicellulose removal during pretreatment. The compact outer layer was removed after enzymatic hydrolysis (figs. 14 and 15), revealing the holes as part of the internal structure of cellulose. The microfibers are about 4 to 7 micron in width. Thus, enzymatic hydrolysis reduced and degraded cellulose, leaving a small, final solid that might require further degradation.

CONCLUSIONS

Forage sorghum, photoperiod-sensitive sorghum, BMR sorghum, sweet sorghum, and grain sorghum biomasses were evaluated as potential feedstocks for biofuel production. FTIR, SEM, and XRD were used to characterize the chemical structure, morphological structure, and crystallinity of the sorghum biomasses. At pretreatment conditions of 165°C for 10 min with dilute sulfuric acid solutions, the enzymatic conversion of cellulose ranged from 85% to 98%. BMR sorghum yielded the highest cellulose conversion rate (98%), followed by sweet sorghum, photoperiod-sensitive sorghum, forage sorghum, and grain sorghum. At pretreatment conditions of 140°C for 30 min, cellulose conversion rate ranged from 65% to 82%. BMR sorghum yielded the highest cellulose conversion rate (81%), followed by forage sorghum, photoperiod-sensitive sorghum, sweet sorghum, and grain sorghum. Pretreatment conditions had a significant effect on solid mass recovery and cellulose fraction recovery. Under pretreatment conditions of 140°C for 30 min and 165°C for 10 min, cellulose recoveries of sorghum biomass ranged from 70% to 85% and from 31% to 58%, respectively. Considering both sugar recovery and energy consumption, pretreatment of biomass at mild temperature is more favorable than pretreatment at high temperature. Structural analysis results showed that a low ratio of syringyl to guaiacyl rings in the lignin structure makes BMR sorghum easy to hydrolyze enzymatically.

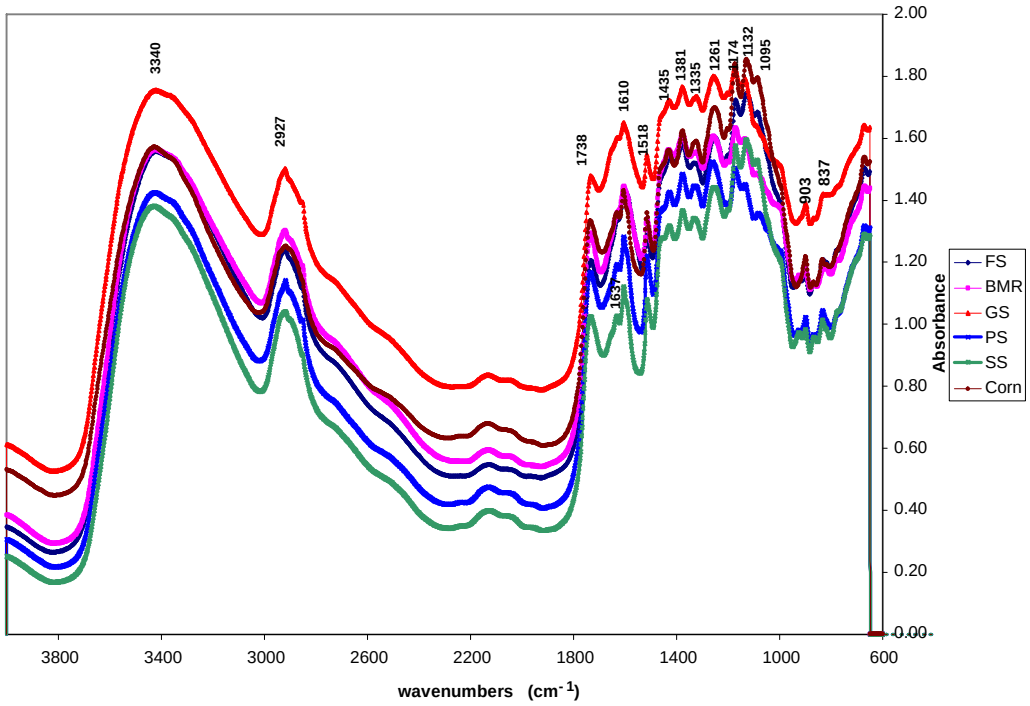

Figure 5. FTIR spectra of untreated sorghum biomass and corn stover.

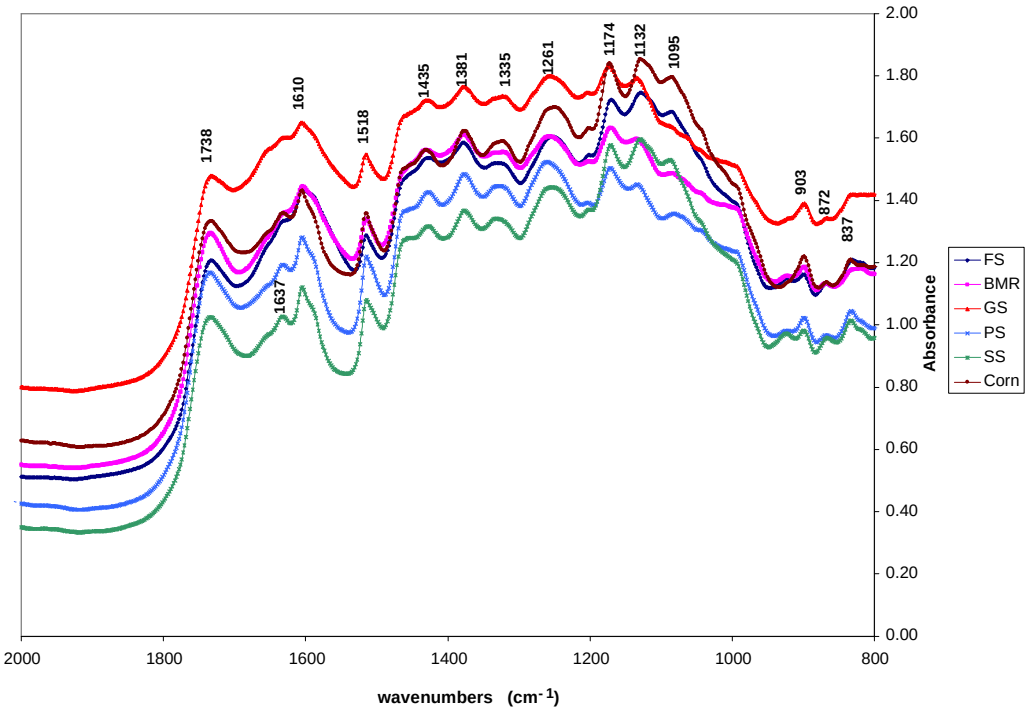

Figure 6. FTIR spectra of untreated sorghum biomass and corn stover in the fingerprint region (900-1800 cm^{-1}).

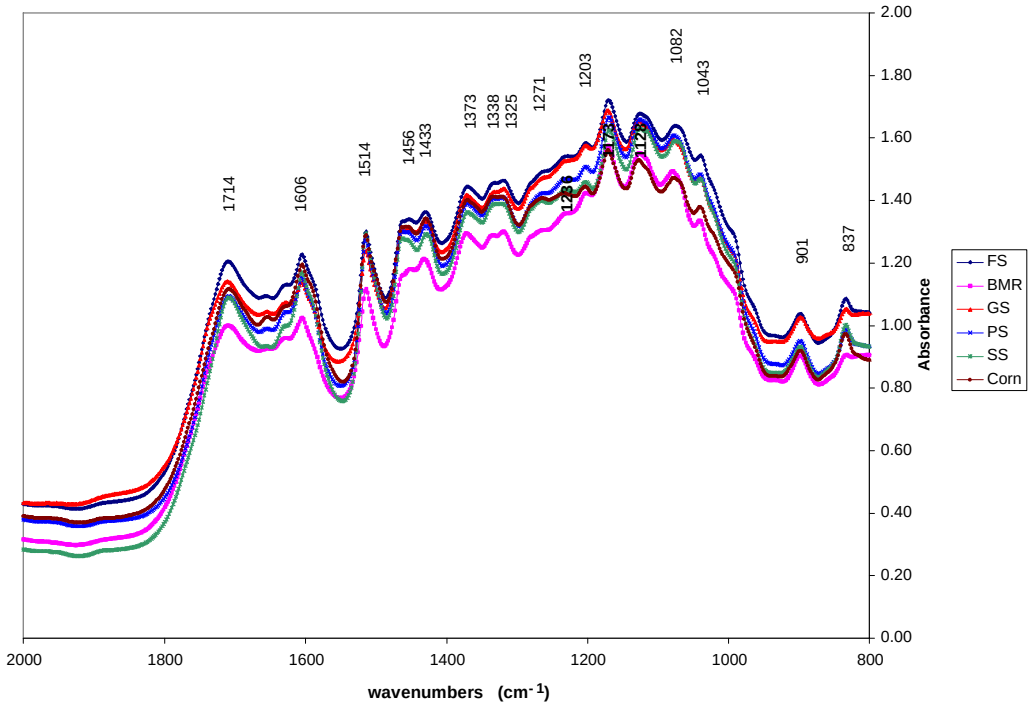

Figure 7. FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm^{-1}) after dilute acid pretreatment at 140°C for 30 min with 2% w/v acid.

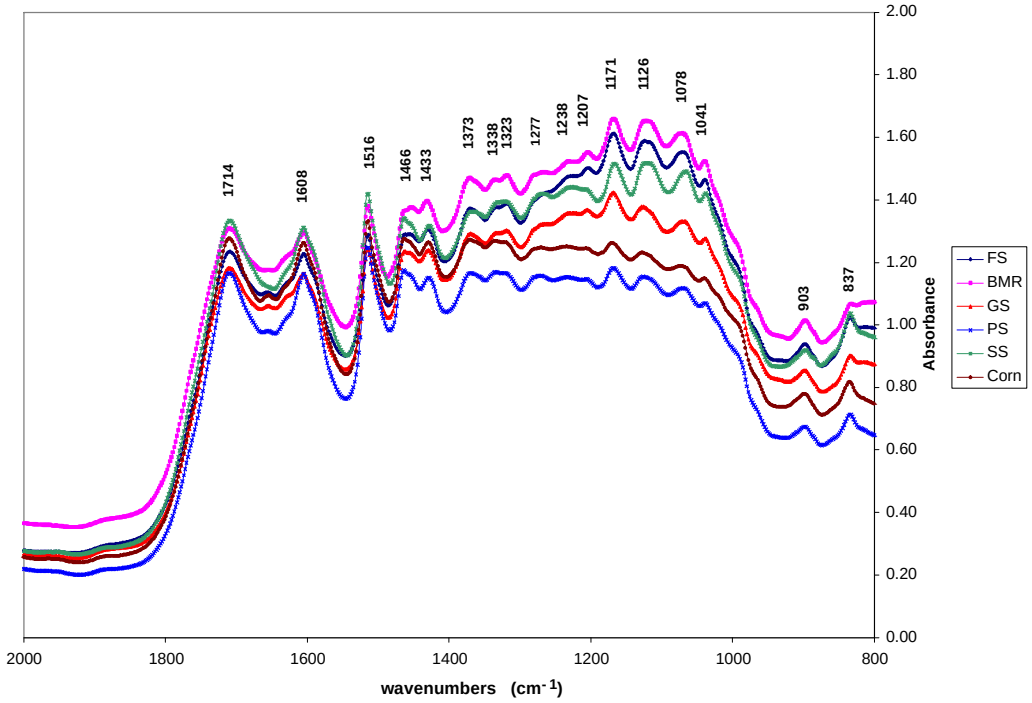

Figure 8. FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm^{-1}) after dilute acid pretreatment at 165°C for 10 min with 2% w/v acid.

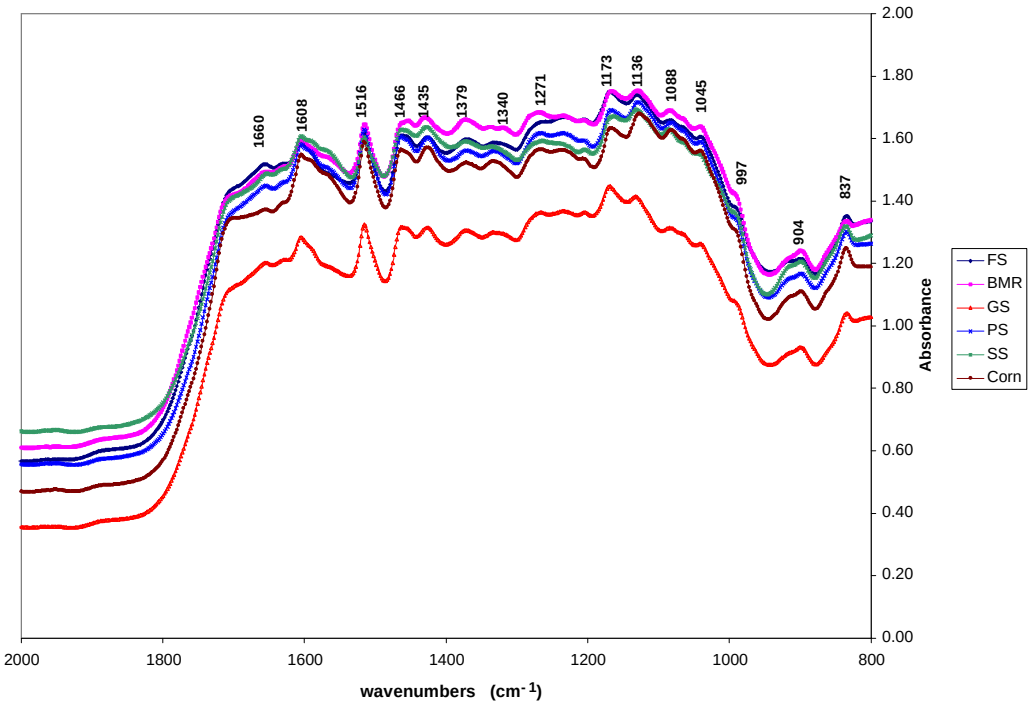

Figure 9. FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm^{-1}) after dilute acid pretreatment at 140°C for 30 min with 2% w/v acid and enzymatic hydrolysis.

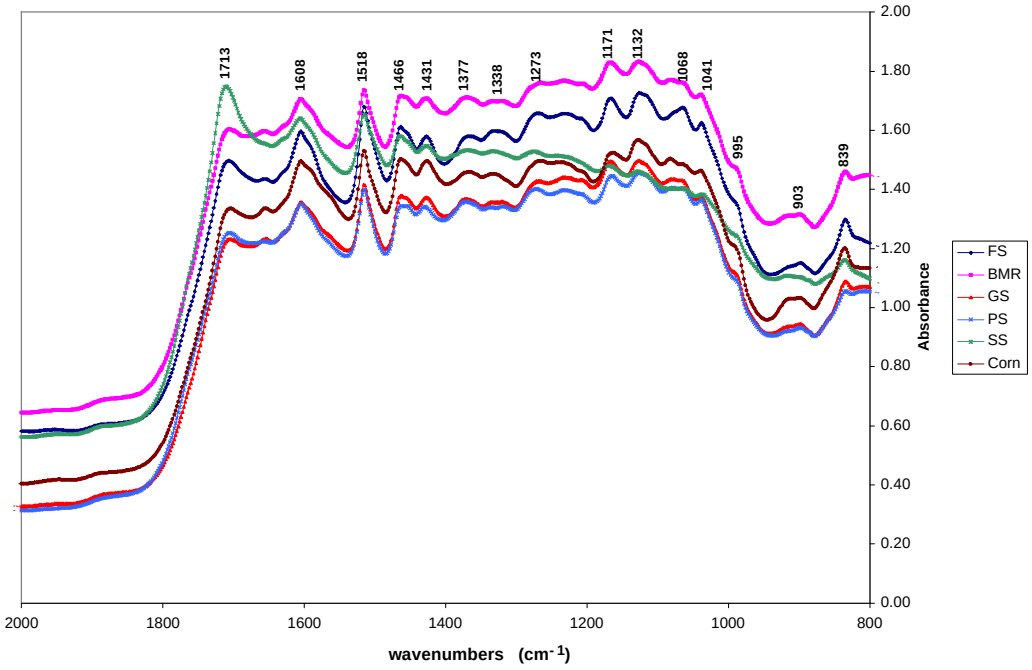

Figure 10. FTIR spectra of sorghum biomass and corn stover in the fingerprint region (900-1800 cm^{-1}) after dilute acid pretreatment at 165°C for 10 min with 2% w/v acid and enzymatic hydrolysis.

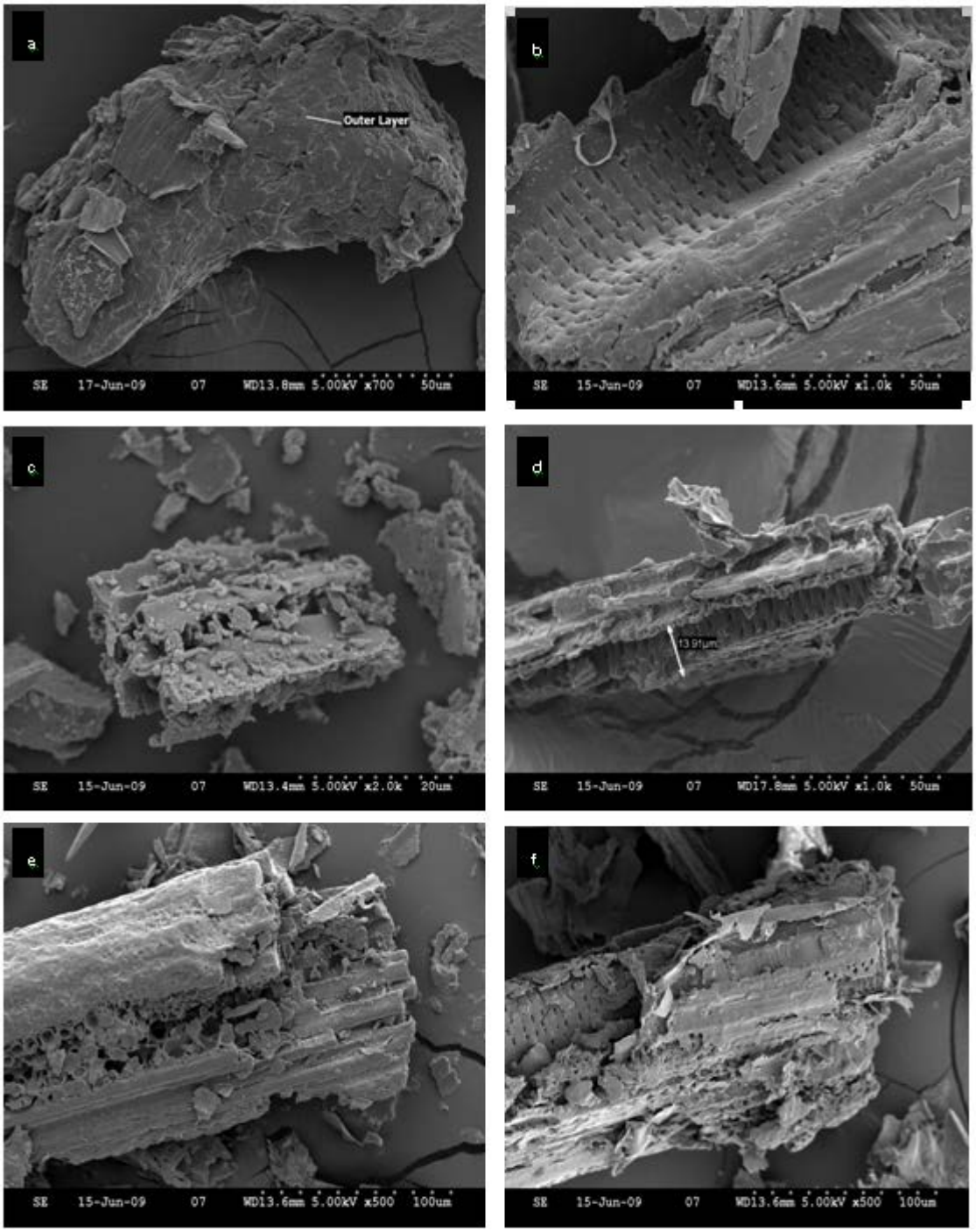

Figure 11. SEM images of untreated samples: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.

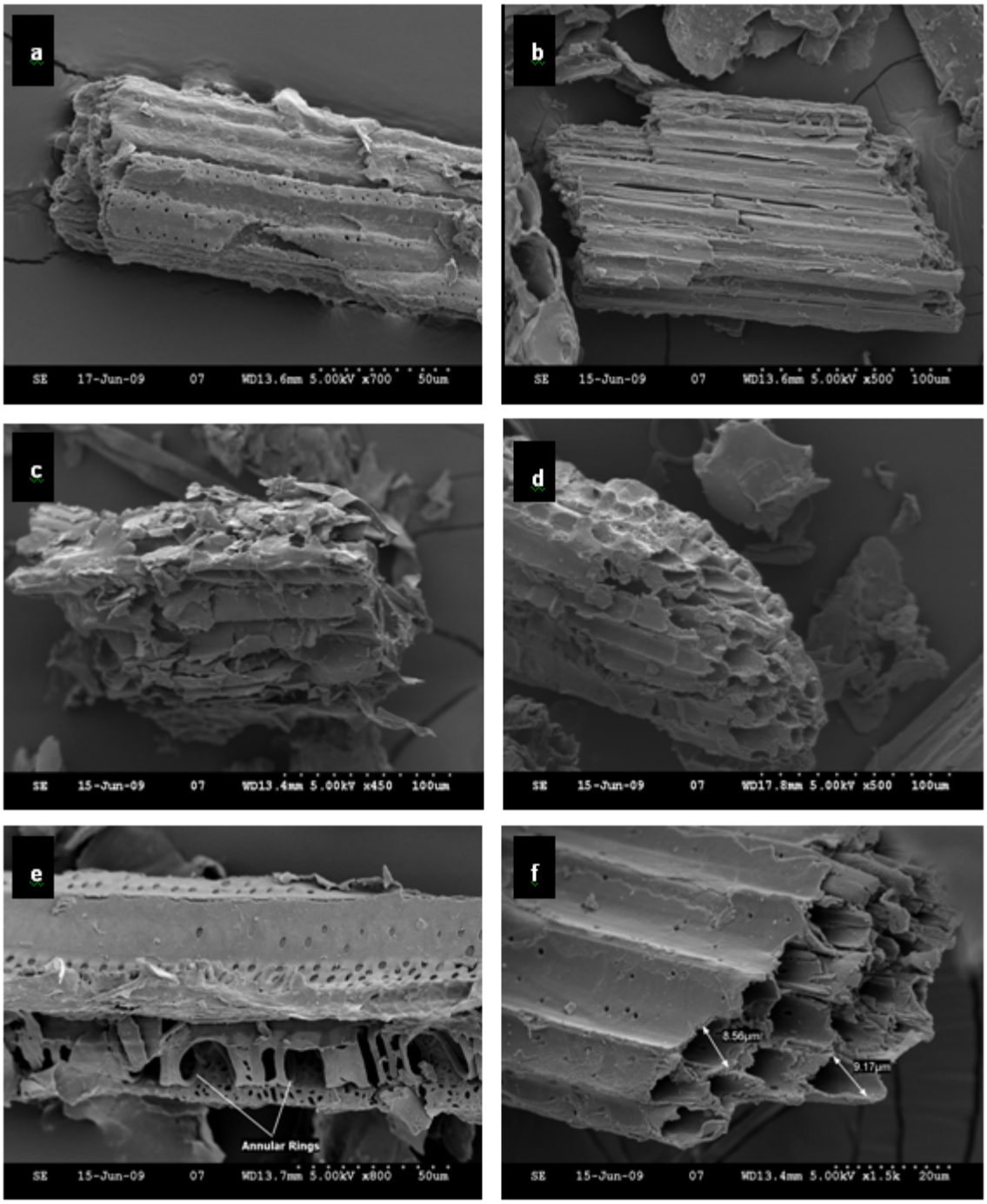

Figure 12. SEM images of samples pretreated at 140°C for 30 min with 2% sulfuric acid: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.

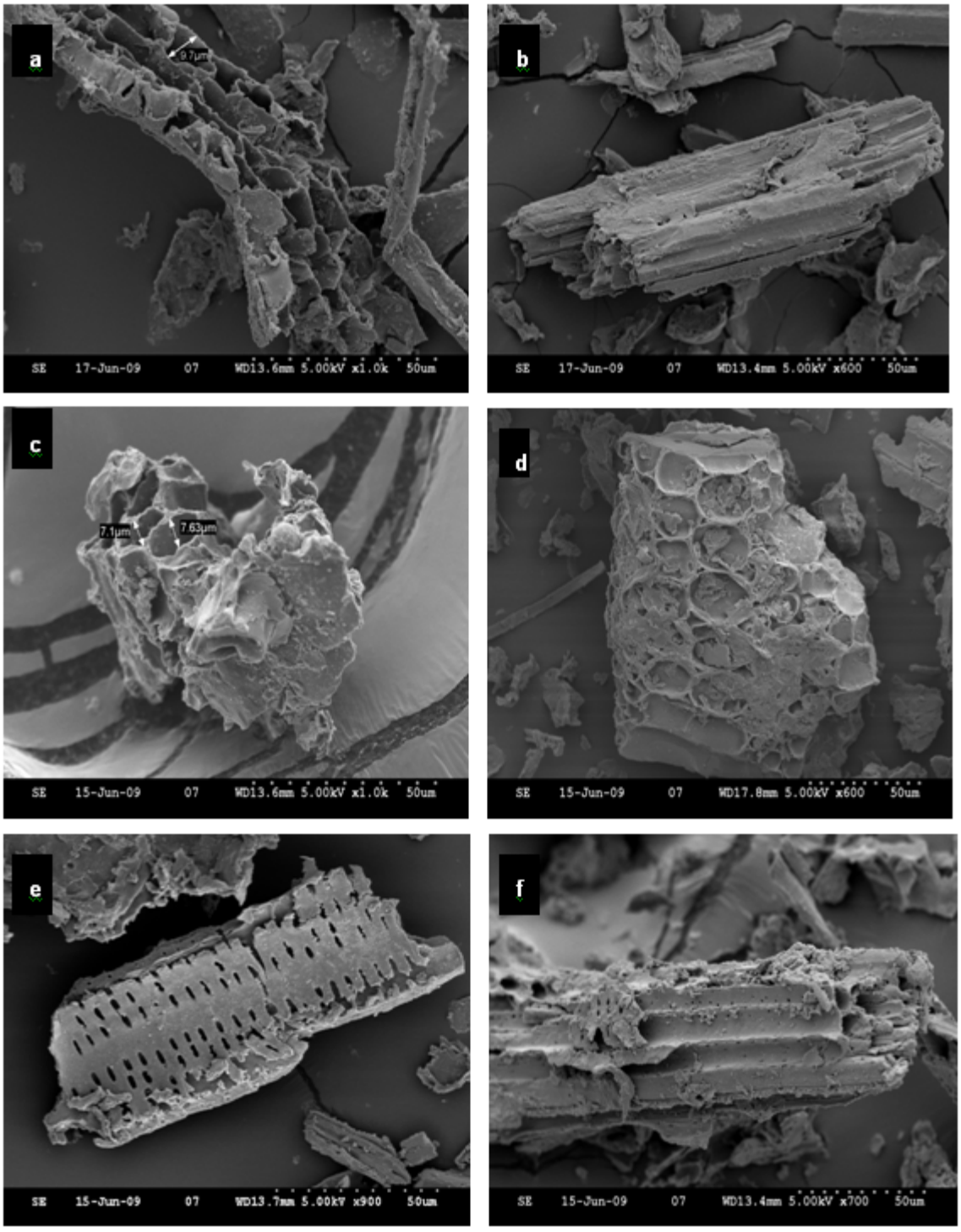

Figure 13. SEM images of samples pretreated at 165°C for 10 min with 2% sulfuric acid: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.

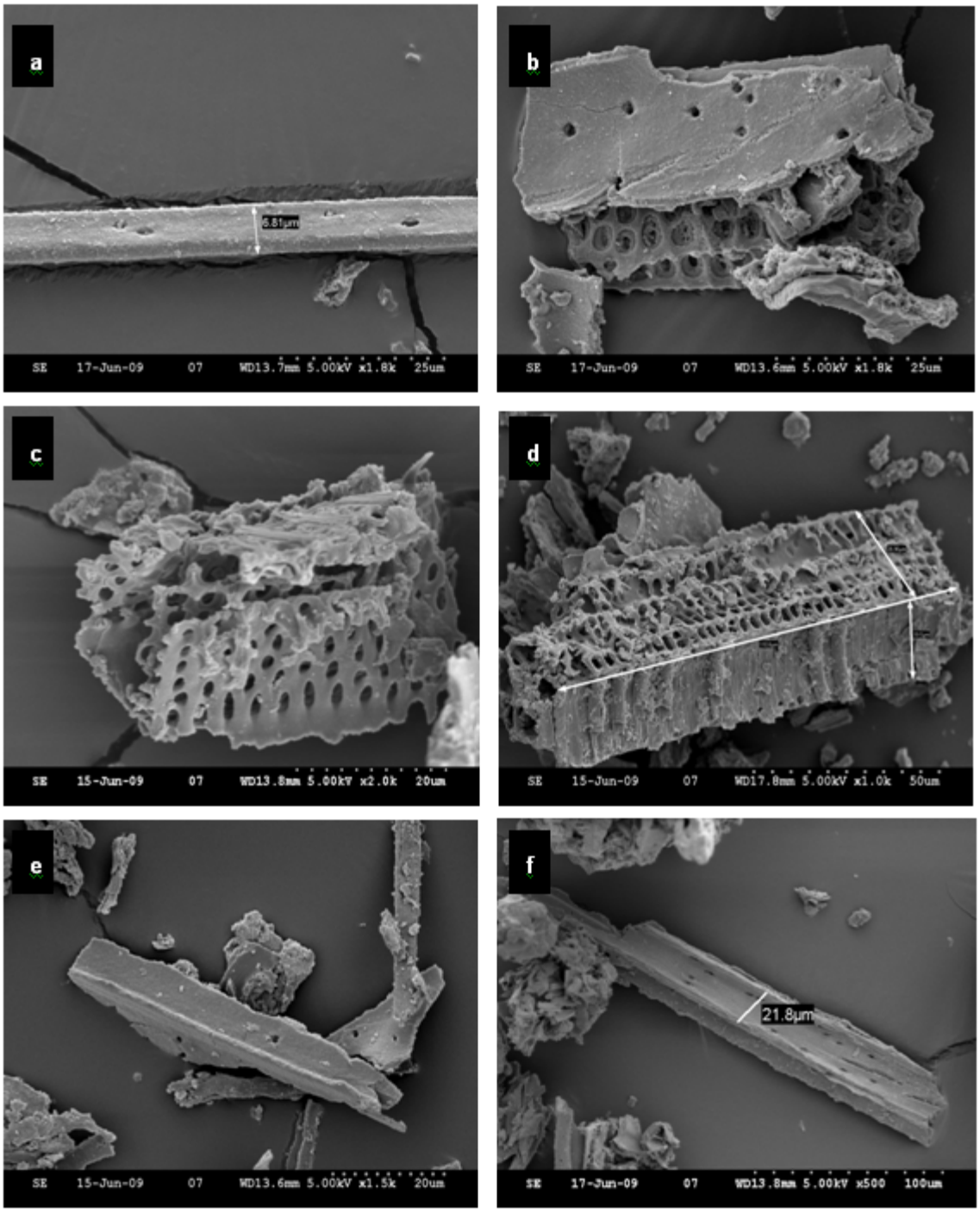

Figure 14. SEM images of samples after pretreatment at 140°C for 30 min with 2% sulfuric acid and enzymatic hydrolysis: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.

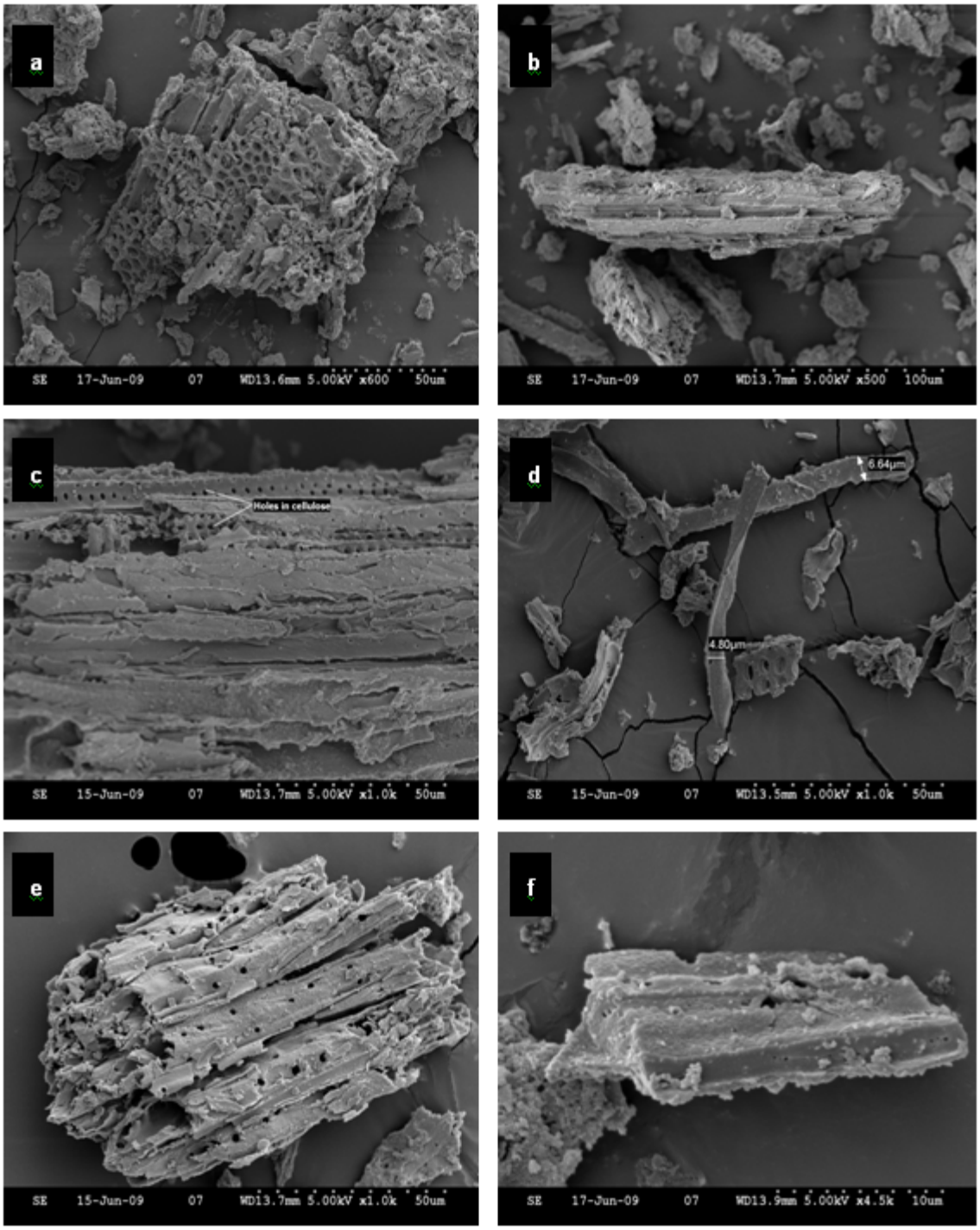

Figure 15. SEM images of samples after pretreatment at 165°C for 10 min with 2% sulfuric acid and enzymatic hydrolysis: (a) BMR sorghum (b) corn, (c) forage sorghum, (d) grain sorghum, (e) photoperiod-sensitive sorghum, and (f) sweet sorghum.

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APPENDIX

MATLAB CODE FOR SMOOTHING OF X-RAY DIFFRACTION SPECTRA

```
clc
clear
A = [imported y-values from Excel file];
B = [imported x-values from Excel file];
windowsize = 20;
b = ones(1,windowsize)/windowsize;
A1 = filter(b,1,A)
//copy the values of A1 (data after smoothing)
into a column of Excel file
figure(1)
subplot(2,1,1)
plot(B,A)
subplot(2,1,2)
plot(B,A1)
```