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Water-Soluble Sugars of Pedigreed Sorghum Mutant Stalks and Their Recovery after Pretreatment

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Abstract: Chemical composition of biomass, especially carbohydrate content, is a critical indicator of a biomass source's potential for biofuel applications. This study characterized physico-chemical properties of stalks from 16 representative pedigreed sorghum mutant lines. The objectives of this study were to evaluate the recovery of sucrose and its hydrolysis products, glucose and fructose, during dilute sulfuric acid pretreatment at conditions typically used for lignocellulosic biomass, and to determine the relationship between water-extractive contents and sugar recovery after pretreatment. Dilute acid-pretreated sorghum stalks had enzymatic saccharification of >82.4% glucose yield for all treated samples with more than 82.3% cellulose recovery and 85% hemicellulose removal. A single-step, one-pot process was recommended for sorghum mutant stalks with low water-extractive content (<35%, *w*/*w*) to reduce processing cost and minimize wastewater disposal since the majority of sugars will be recovered after dilute acid pretreatment with minimal degradation products. However, for sorghum mutant stalks with high water-extractive content (>35%, *w*/*w*), a pre-washing step is beneficial to recover the water-soluble sugars before subjecting to the pretreatment process in order to avoid sugar losses during the pretreatment stage. Thus, different processing technologies should be applied to lignocellulosic biomass with various water-extractive contents and water-soluble sugar concentrations.

Keywords: sorghum mutant; biomass; soluble sugars; dilute acid pretreatment; one-pot process

1. Introduction

Bioethanol is currently used as an alternative liquid fuel for transportation in the United States of America (USA) and Brazil. Ethanol with an octane rating of 113, can function as an octane booster to reduce engine knocking and contribute to national energy security by reducing oil imports. Bioethanol is a sustainable alternative fuel, which can be derived from various sustainable feedstocks, including sugar-based crops, starch-based crops, and cellulosic biomass [1–3]. Cellulosic biofuels provide environmental benefits not available from grain- or sugar-based biofuels and are considered as a solid foundation to meet the needs for transportation fuels in a low-carbon economy, albeit with electrified vehicles and other technical advances [4].

Sorghum (*Sorghum bicolor* L. Moench) is a C4 photosynthetic species with high productivity and drought tolerance, ranking the fifth among widely grown cereal crops globally [5]. Unique characteristics of sorghum position the crop as a viable bioenergy source, including high biomass yield and high sugar content, high moisture-use efficiency and strong drought tolerance, a well-established

management system, and breeding potential for genetic improvement [6,7]. Sweet sorghum contains high fermentable sugars such as sucrose, glucose, and fructose in the stalk which can be extracted easily and directly fermented to bioethanol; in contrast, hybrids of grain sorghum provide starch for bioethanol production. Sweet sorghum, previously used as a syrup, is now being researched for use in sugarcane processing facilities to produce ethanol (1.5 generation biofuel), while biomass sorghums contain a significant amount of structural carbohydrates which can be hydrolyzed and fermented to biofuel [7,8]. After pressing sorghum stalks, approximately 50% of water-soluble sugars and 100% water-insoluble structural carbohydrates remain in the bagasse; thus, recovery of all the residual sugars can significantly improve the process economics and increase the overall productivity [9,10].

Lignocellulosic biomass is the most abundant renewable source for biofuel production, mainly consisted of cellulose, hemicellulose, and lignin. Cellulose-based biomass such as sorghum stalk must be pretreated in order to open its complex structure for enzymatic hydrolysis and subsequent fermentation [11–15]. Currently, pretreatment methods mainly include physical methods, chemicals methods, biological methods, and a combination of methods. Numerous pretreatment methods were developed to overcome the recalcitrant structure of sorghum biomass, such as ball milling, steam explosion, liquid hot water, dilute acid, lime, ammonia, organic solvent, and ionic liquid pretreatments [16–19]. Challenges of using the current pretreatment processes include incomplete separation of cellulose and lignin, which results in reduced subsequent enzymatic saccharification efficiency, formation of inhibitors (such as acetic acid, furans, or phenolic compounds) that affect ethanol fermentation, and increased usage of chemicals and energy-intensive processes, as well as high cost of waste disposal [20–22].

A two-step process, consisting of prewashing and a subsequent pretreatment process was recommended to extract considerable amounts of water-soluble sugars to avoid sugar losses or degradation during the pretreatment stage [23]. However, the economic viability of adding an extraction step is questionable due to the increased process cost due to high water usage [24]. Instead, a single pretreatment step without the pre-extraction processing, i.e., a "one-pot" process, was proposed to recover the water-soluble sugars and improve the enzymatic digestibility of cellulosic biomass. The improved digestibility was based on the possibility that sucrose or its immediate hydrolysis products, glucose and fructose, will survive the pretreatment step and will be available to fermentative microorganisms [25].

The objective of this research was to evaluate the recovery of sucrose and its hydrolysis products, glucose and fructose, during dilute sulfuric acid pretreatment process at conditions typically used to pretreat lignocellulosic biomass. Sixteen representative stalk samples (sorghum stalks without panicle and leaves) from pedigreed mutant sorghum lines with various water-extractive contents (18 to 52%) were selected from a pedigreed sorghum mutant library consisting of 6000 individually mutagenized M_4 seed pools for the proposed one-pot process test and determination of the relationship between water extractive contents and sugar recovery after pretreatment.

2. Materials and Methods

2.1. Materials

Stalk samples from 16 pedigreed sorghum mutant lines, including wild-type sorghum used as control, were selected from a large sample pool provided by the Plant Stress and Germplasm Development Unit of the US Department of Agriculture Agricultural Research Services (Lubbock, TX, USA). Sorghum stalks were manually harvested. Sorghum stalks were ground (<1 mm) using a cutting mill (SM 2000, Retsch Inc., Newton, PA, USA) and placed into a plastic bag kept at room temperature. The moisture content was measured according to the convection oven drying method described in NREL LAP Determination of Total Solids in Biomass [26]. One gram of sorghum biomass was placed in a pre-weighed aluminum weighing dish and dried to a constant weight at 105 °C. Total solid percentage was defined as dry weight of sample per sample weight as received [27].

All chemicals, such as 72% (*w*/*w*) sulfuric acid, pure ethanol 200 proof, and HPLC grade water, used for this research were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Compositional Analysis

2.2.1. Extractives

Water and ethanol extraction was used to determine extractive content of the sorghum samples. Five grams of sorghum biomass samples were put into a Soxhlet thimble and washed using distilled water for 8 h according to the NREL Laboratory Analytical Procedure (LAP) Determination of Extractives in Biomass [26]. Then, ethanol was added to continue extraction for another 16 h. Extractive percentage was defined as dry weight of sample per sample weight as received. Water-soluble extractives contain inorganic materials, non-structural sugars, and nitrogenous materials [27].

2.2.2. Structural Carbohydrates, Lignin, and Ash

The chemical composition of samples was determined according to the National Renewable Energy Laboratory (NREL) procedure [26]. Extraction-free samples were placed into pressure tubes and through a two-step acid hydrolysis with 72% (*w*/*w*) sulfuric acid at 30 °C for 60 min, followed by 4% (*w*/*w*) dilute sulfuric acid at 121 °C for another 60 min. Carbohydrates such as cellulose and hemicellulose were converted to monosaccharides glucose, fructose, xylose, and arabinose, which were determined by high-performance liquid chromatography (HPLC) equipped with an RCM monosaccharide column (300 × 7.8 mm) (Phenomenex, Torrance, CA, USA) and a refractive index detector, under a mobile phase of 0.6 mL·min^{-1} water and a column temperature of 80 °C. Acid-soluble lignin in the hydrolysis liquor was determined using an ultraviolet (UV)–visible spectrophotometer at 320 nm wavelength blanked against distilled water [26]. Acid-insoluble lignin was weighed from the residue solids after oven heating overnight at 105 °C and then at 575 °C using a muffle furnace for at least 6 h to measure the ash content [27].

2.3. Dilute Acid Pretreatment

For dilute acid pretreatment, sulfuric acid (2%, *w*/*v*) was applied as a reaction medium, and reaction temperature was kept at 120 °C for 60 min (Figure 1). Once the treatment was complete, the reactor (Parr Instrument Co., Moline, IL, USA) was removed from the electric heater and placed into tap water to cool down within 50 °C in 5 min. Then, the slurry was vacuum-filtered using Whatman Paper (No. 4). Treated biomass was washed thoroughly with water and collected for composition analysis and enzymatic hydrolysis to evaluate the effect pretreatment on sugar yields and other hydrolytes.

$$Mass\ recovery\ (\%) = \frac{M_{tre}}{M_{ori}} \times 100\%, \quad (1)$$

$$Cellulose\ recovery\ (\%) = \frac{M_{tre} \times C_{tre}}{M_{ori} \times C_{ori}} \times 100\%, \quad (2)$$

$$Hemicellulose\ recovery\ (\%) = \left(1 - \frac{M_{tre} \times H_{tre}}{M_{ori} \times H_{ori}}\right) \times 100\%, \quad (3)$$

where M_{tre} is dry mass weight after pretreatment, M_{ori} is original dry mass weight, C_{tre} is the cellulose percentage of solid biomass after pretreatment, C_{ori} is the cellulose percentage in raw material, H_{tre} is the hemicellulose percentage of solid biomass after pretreatment, and H_{ori} is the hemicellulose percentage in raw material.

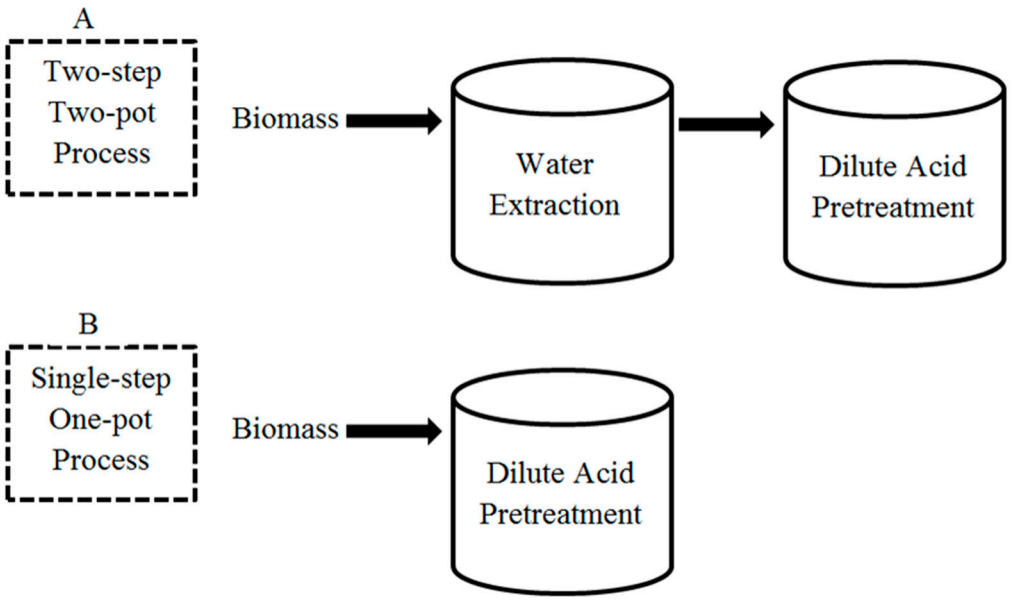

Figure 1. Flowchart of process designs: (**A**) two-step, two-pot process; (**B**): single-step, one-pot process.

The National Renewable Energy Laboratory (NREL) method for the determination of degradation products in pretreatment hydrolysate was applied to measure the furfural and hydromethylfurfural (HMF) concentration in biomass hydrolysates using HPLC. The injection volume was 20 µL; the solvent was water containing 0.005 M sulfuric acid at a flow rate of 0.6 mL/min. The column and refractive index detector (RID) temperatures were set at 60 and 45 °C.

2.4. Enzymatic Saccharification

After pretreatment, biomass (4%, *w*/*v*) was added to flasks to perform enzymatic hydrolysis using Accellerase 1500, generously provided by Genencor Dupont, at the recommended dosage (0.5 mL/g cellulose). Flasks were incubated at 50 °C in a rotary shaker (Model I2400, New Brunswick Scientific Inc., Edison, NJ, USA) with a speed of 150 rpm. After 72 h of enzymatic hydrolysis, supernatants were extracted by filtration and stored at 4 °C for further experiments. Glucose concentration was measured by HPLC. All reactions were performed in duplicate. Glucose yield was calculated as follows:

$$Glucose\ yield\ (\%) = \frac{Released\ sugar\ amount}{Theoretical\ sugar\ amount\ in\ raw\ material} \times 100\%. \quad (4)$$

2.5. Elemental Analysis

The elemental composition of sorghum stalks was determined using a CHNS/O Elemental Analyzer (PerkinElmer 2400 Series II, PerkinElmer Inc., Waltham, MA, USA). Approximately 2 mg of ground sorghum stalk packed with foil was burned under a pure oxygen atmosphere. The gases including CO_2, N_2, SO_2, and H_2O were burned and separated in a quartz column containing copper wires detected by a thermo-conductometer detector and reported as a percentage of initial dry weight (*w*/*w*, db) [27].

2.6. Heating Value

The energy content of pedigreed sorghum mutant stalks was determined using a calorimeter (IKA-Calorimeter C 200, IKA-Werke GmbH and Co. KG, Staufen, Germany) with a benzoic acid standard. A crucible with approximately 1.00 g of biomass sample was placed into an adiabatic bomb. After sealing the bomb, it was filled with oxygen and placed into the calorimetric equipment filled

with water. The sample was ignited electrically through the cotton line. Powder sorghum stalks were pressed into pellets in order to reduce experimental error caused by incomplete combustion resulting from loose samples blown away during burning. The resultant water temperature increase allows the calculation of high heating value of the sample [27]. The heat capacity of the calorimeter was measured using benzoic acid as a reference standard.

2.7. Statistics

Analysis of variance and pairwise comparisons for the means using the Tukey adjustment at the confidence level of 95% were performed with SAS (SAS Institute, Inc., Cary, NC, USA). Mean values and standard deviations from the duplicated experiments are reported.

3. Results and Discussion

3.1. Physical and Chemical Properties of Pedigreed Sorghum Mutant Stalks

Sixteen sorghum mutant stalks with evenly distributed water-extractive contents ranging from the lowest, 18%, to the highest, 52%, were selected for this study (Table 1). The carbon and oxygen contents of selected samples ranged from 37.4% to 41.4% and 49.2% to 55.3%, respectively. The hydrogen content was approximately 5% to 6%, and nitrogen and sulfur contents were typically less than 2%. The wild-type sorghum samples (E25a and E25b, referred to as the control) contained relatively low water-extractive content of 25.8% with an average of 39.4% carbon, 52.5% oxygen, 6.4% hydrogen, 0.8% nitrogen, and 1.3% sulfur contents (Table 1). The selected sorghum mutants with higher water-extractive contents were expected to contain significantly larger portions of non-structural carbohydrates, including sucrose, glucose, and fructose, as compared to the control wild-type sorghum biomass (Table 2).

Table 1. Physico-chemical characteristics of sorghum stalks.

Sample Identity	Water-Soluble Extractive (%)	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Sulfur (%)	Oxygen (%)	Heating Value (J/g)
E18A	17.6 ± 0.6a [1]	40.3 ± 0.8a	6.4 ± 0.2a	0.96 ± 0.3a	1.3 ± 0.3a	51.1 ± 0.6a	16,419.5 ± 21a
E18B	18.7 ± 0.8a	41.4 ± 0.9a	6.5 ± 0.3a	0.91 ± 0.2a	1.3 ± 0.2a	49.9 ± 0.7a	17,047.4 ± 31a
E20A	21.2 ± 0.9b	38.9 ± 1.3b	5.6 ± 0.2b	0.64 ± 0.3b	1.2 ± 0.2a	53.6 ± 0.8a	15,935.1 ± 26b
E20B	21.0 ± 0.8b	37.4 ± 0.7b	5.5 ± 0.4b	0.73 ± 0.1b	1.1 ± 0.1a	55.3 ± 0.4b	14,748.6 ± 19b
E25A	25.4 ± 1.3b	39.4 ± 1.6a	6.4 ± 0.1a	0.77 ± 0.2b	1.2 ± 0.1a	52.2 ± 0.5a	16,039.3 ± 21b
E25B	26.3 ± 1.6b	39.4 ± 0.7a	6.4 ± 0.2a	0.73 ± 0.3b	1.3 ± 0.2a	52.2 ± 0.6a	15,953.4 ± 18b
E30A	30.3 ± 1.8c	39.5 ± 0.6a	6.4 ± 0.3a	1.10 ± 0.2c	1.5 ± 0.2b	51.5 ± 0.7a	16,651.4 ± 25a
E30B	30.3 ± 1.6c	41.1 ± 1.5a	6.5 ± 0.4a	1.83 ± 0.3c	1.5 ± 0.2b	49.2 ± 0.6c	17,327.4 ± 24a
E35A	35.3 ± 2.2d	38.9 ± 1.8b	5.8 ± 0.5b	0.72 ± 0.1b	1.2 ± 0.1a	53.4 ± 0.8a	16,071.9 ± 29b
E35B	35.3 ± 2.1d	39.9 ± 0.9a	5.2 ± 0.2b	0.92 ± 0.2a	0.9 ± 0.1c	53.0 ± 0.9a	16,510.8 ± 28a
E40A	40.3 ± 2.3d	38.6 ± 0.8a	5.6 ± 0.3b	1.07 ± 0.1a	1.1 ± 0.2a	53.6 ± 0.6a	16,085.9 ± 28b
E40B	40.8 ± 1.9d	38.3 ± 0.7a	5.9 ± 0.5a	0.68 ± 0.1b	1.2 ± 0.1a	53.9 ± 1.1d	16,144.3 ± 22b
E45A	45.1 ± 2.3e	37.6 ± 0.5a	5.8 ± 0.4b	1.01 ± 0.2a	1.2 ± 0.2a	54.4 ± 1.2d	16,035.2 ± 17b
E45B	46.4 ± 2.1e	39.0 ± 0.6a	5.9 ± 0.6a	1.00 ± 0.2a	1.2 ± 0.1a	52.9 ± 0.8a	16,461.8 ± 26a
E52A	52.7 ± 2.5e	39.7 ± 0.8b	5.5 ± 0.3b	0.78 ± 0.1b	1.1 ± 0.1a	52.9 ± 0.5a	16,594.9 ± 25a
E52B	53.4 ± 2.6e	40.0 ± 1.3b	5.8 ± 0.4b	0.70 ± 0.1b	1.8 ± 0.2d	51.8 ± 0.7a	16,436.9 ± 21a

Note: Samples E25A and E25B are wild-type sorghum used as control; the remaining samples are pedigreed sorghum mutant stalks. [1] Column means with different letters than the control are significantly different at the 0.05 level.

Table 2. Water-soluble sugars in the raw biomass and recovery of sugars after pretreatment.

Sample Identity	Raw Biomass			Pretreatment Hydrolysate						Glucose Recovery (%)	Fructose Recovery (%)
	Water-Soluble Sugars (g/L)			Water-Soluble Sugars (g/L)				Sugar Degradation (g/L)			
	Sucrose	Glucose	Fructose	Glucose	Fructose	Xylose	Arabinose	HMF	Furfural		
E18A	0.13a [1]	0.1a	0.08a	0.16a	0.14a	4.3a	0.56a	0a	0.07a	97.4a	97.0a
E18B	0.16a	0.16a	0.07a	0.23a	0.14a	4.42a	0.51a	0.04a	0.06a	96.2a	93.9a
E20A	0.12a	0.08a	0.07a	0.14a	0.12a	4.28a	0.59a	0.02a	0.06a	96.9a	92.8a
E20B	0.26a	0.11a	0.09a	0.23a	0.20a	3.9a	0.52a	0.03a	0.05a	96.4a	91.5a
E25A	0.27a	0.15a	0.16a	0.28a	0.28a	4.07a	0.55a	0.04a	0.05a	98.8a	95.4a
E25B	0.28a	0.17a	0.19a	0.3a	0.31a	3.99a	0.81a	0.07a	0.06a	97.3a	94.4a
E30A	0.57b	0.49a	0.47a	0.77b	0.69b	3.52b	0.66a	0.24a	0.06a	99.8a	91.8a
E30B	0.77b	0.4a	0.42a	0.73b	0.68b	4.12a	0.75a	0.23a	0.06a	93.5a	84.9b
E35A	1.42b	0.78b	0.89b	1.39b	1.33b	3.51b	0.53a	0.39b	0.05a	93.8a	83.5b
E35B	0.82b	0.83b	1.07b	1.21b	1.29b	3.49b	0.54a	0.38b	0.05a	97.9a	87.4b
E40A	1.78b	0.78b	0.95b	1.48b	1.51b	3.17b	0.53a	0.45b	0.04a	89.1b	82.5b
E40B	1.63b	1.23b	1.44b	1.82b	1.52b	3.11b	0.44b	0.48b	0.04a	89.4b	67.7b
E45A	3.36b	1.32b	1.29b	2.55b	1.68b	2.66b	0.49b	0.55b	0.05a	85.5b	56.9b
E45B	3.05b	1.24b	1.40b	2.39b	1.78b	2.52b	0.40b	0.66b	0.04a	87.0b	61.2b
E52A	5.20b	1.15b	1.10b	3.20b	2.06b	2.28b	0.37b	0.79b	0.03b	86.0b	56.1b
E52B	4.75b	1.17b	1.08b	2.85b	2.06b	2.39b	0.34b	0.72b	0.04a	81.0b	60.1b

Note: Samples E25A and E25B are wild-type sorghum used as control; the remaining samples are pedigreed sorghum mutant stalks. [1] Column means with different letters than the control are significantly different at the 0.05 level.

Biomass samples with high carbon content are generally predicted to exhibit high caloric energy. The control samples had a mean heating value of 15,996.4 J/g, relatively lower than other selected sorghum mutant samples, especially those with higher water-extractive content. The heating value of selected sorghum mutant samples was positively correlated to the carbon content (R^2 = 0.66), while negatively correlated with the oxygen content (R^2 = 0.69). The heating value is critical for biomass pyrolysis evaluation and provided as comprehensive information for alternative use.

3.2. Sugar Recovery after Dilute Acid Pretreatment

The control sample contained minimal amounts of water-soluble sugars with approximately 0.28 g/L sucrose, 0.16 g/L glucose, and 0.18 g/L fructose (totally <3%, *w*/*w* of dry biomass) (Table 2). The content of water-soluble sugars in sorghum stalks increased as water-extractive content increased. Significantly larger amounts of water-soluble sugars were detected in sorghum mutant stalks with higher water-extractive content, as high as 4.98 g/L sucrose, 1.16 g/L glucose, and 1.09 g/L fructose (approximately 36%, *w*/*w* of dry biomass).

Sucrose present in sorghum stalks was completely hydrolyzed during the dilute acid pretreatment since no sucrose remained after treatment. Sucrose is a non-reducing disaccharide composed of β-D-fructose and α-D-glucose. In commercial industrial production, sucrose is hydrolyzed to monomers under typically low temperature (<120 °C) to minimize fructose and glucose degradation [24]. Both glucose and fructose are usually degraded when temperatures are higher than 106 °C and pH is less than 2.0, which is similar to the conditions present during dilute acid pretreatment of lignocellulosic biomass. The primary degradation pathway is dehydration of the sugars, glucose and fructose, to 5-hydroxymethylfurfural (5-HMF), which hydrolyzes and further degrades to levulinic and formic acid. Appreciable concentrations of sucrose hydrolysis products, glucose and fructose, were detected after dilute acid pretreatment (Table 2). Glucose and fructose levels were as high as 3.2 g/L and 2.06 g/L, respectively, in sample E52A, which were significantly higher than the control E25A and E25B. However, higher amounts of the sugar degradation product (approximately 0.79 g/L), 5-HMF, were detected in the pretreatment hydrolysate. A significant concentration of the hemicellulose degradation product, xylose, was also detected in the pretreatment hydrolysate, ranging from 2.28 to 4.42 g/L. Relatively low amounts of xylose degradation product, furfural, were detected in all the selected sorghum samples (Table 2).

For wild-type sorghum, approximately 98% and 95% recovery was achieved for glucose and fructose, respectively. However, as the water-soluble sugar content in the sorghum stalks increased, significant amounts of sugars were lost during the dilute acid pretreatment, especially for fructose. When the water-extractive content was greater than 35% (*w*/*w*), fructose recovery was usually less than 60% and 5-HMF concentration was significantly higher (>0.7 g/L). In contrast, the glucose recoveries of all the selected sorghum samples were higher than 80%, which further proved that fructose was easier to degrade than glucose, as found in other studies [24,25].

For sorghum mutants with high water-extractive content (>35%, *w*/*w*), a pre-washing step was beneficial to recover the present water-soluble sugars before performing the pretreatment process in order to avoid sugar losses or degradation during the pretreatment stage [23,28]. However, for those sorghum mutants with less water-extractive content, a single-step, one-pot process was recommended to reduce processing cost and minimize wastewater disposal since a majority of sugars can be recovered after dilute acid pretreatment with minimal amount of degradation products.

For high water-extractive biomass, an integrated bioprocess of combined advanced solid-state fermentation technology (ASSF) and alkaline pretreatment can be used to convert all the present sugars, including sucrose and structural carbohydrate. This process allows soluble sugars in the biomass (e.g., sweet sorghum stalks) to be converted into ethanol by ASSF using crushed stalks directly, and then ethanol distillation combined with alkaline pretreatment is simultaneously performed using a single distillation reactor. This integrated one-pot process can significantly reduce the production cost and minimize the wastewater pollution [29].

3.3. Chemical Composition of Pretreated Solid Biomass

For raw biomass, the maximum cellulose content of the selected 16 sorghum mutants was 31.3%, whereas the minimum was only 18.1%. The average cellulose content was 24.6% with a standard deviation of 4.8% (Table 3). Large variations of hemicellulose content were also observed from 14.7% to 26.6%, with an average of 20.4% and standard deviation of 4.20%. Relatively low amounts of lignin content were observed in this study, ranging from 6.6% to 13.9% with a mean value of 10.4% and standard deviation of 2.3%, as compared to corn stover and herbaceous grass [3,12].

Significant amounts of hemicellulose were hydrolyzed into xylose and arabinose after the dilute acid pretreatment. Hemicellulose content after the dilute acid pretreatment was reduced to approximately 6%, which resulted in significantly higher cellulose and lignin content. The majority of hemicellulose (>85%) was hydrolyzed during the pretreatment, which could increase the pore size and provide greater access for enzymes to digest cellulose [7,23]. Lignin relocation and removal were reported to enhance the enzymatic hydrolysis of treated sweet sorghum bagasse [30,31]. Kim and Day reported that sweet sorghum bagasse contained 45% cellulose, 27% hemicellulose, and 21% lignin [32]. The maximum cellulose content after dilute acid pretreatment was 55.8%, whereas the minimum was still as high as 47.0% with an average value of 50.7% and standard deviation of 2.2% (Table 3), which were higher than the cellulose content of acid-treated sweet sorghum bagasse reported by Yu et al. [33]. The increased cellulose content contributed to higher fermentable sugar concentration after enzymatic hydrolysis as compared to the untreated biomass [34,35]. A relatively high proportion of solid biomass was lost after dilute acid pretreatment, especially for those samples with high water-soluble sugar percentages. For pretreated pedigreed sorghum mutant stalks, the maximum solid recovery was 53.7%, and the minimum solid recovery was only 29.3% (E52A) (Table 3). However, it was more important to preserve as much of the cellulose present in the pretreated biomass. Cellulose recovery was defined as the cellulose ratio before and after pretreatment multiplied by the mass recovery. All the sorghum samples obtained greater than 82.3% cellulose recovery with a maximum recovery of 92.5%.

Table 3. Chemical composition of sorghum stalks before and after pretreatment.

Sample Identity	Raw Sorghum Stalk			Pretreated Sorghum Stalk			Mass Recovery (%)	Cellulose Recovery (%)	Hemicellulose Removal (%)
	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)			
E18A	31.3 ± 0.2a [1]	26.5 ± 0.6a	13.8 ± 0.2a	53.4 ± 0.3a	6.4 ± 0.1a	29.1 ± 0.6a	53.7a	91.6a	87.0a
E18B	29.3 ± 0.3b	26.6 ± 0.7a	13.9 ± 0.3a	52.0 ± 0.2a	7.6 ± 0.2a	29.6 ± 0.5a	52.1a	92.5a	85.2b
E20A	28.0 ± 0.1b	24.6 ± 0.9b	12.8 ± 0.2a	50.0 ± 0.4a	5.9 ± 0.3a	28.3 ± 0.4a	50.8b	90.9a	87.9a
E20B	29.8 ± 0.2b	22.6 ± 1.2b	12.2 ± 0.1a	52.0 ± 0.9a	5.8 ± 0.2a	25.7 ± 0.6b	53.0a	92.3a	86.3a
E25A	30.2 ± 0.5b	23.9 ± 0.6b	11.9 ± 0.1b	55.8 ± 1.4b	6.9 ± 0.4a	27.2 ± 1.1c	48.8c	90.4a	85.9b
E25B	28.0 ± 0.7c	24.0 ± 0.5b	11.8 ± 0.2b	50.3 ± 0.6a	5.8 ± 0.2a	27.4 ± 0.8c	48.5c	86.9b	88.3a
E30A	27.7 ± 0.8c	21.0 ± 0.3c	11.0 ± 0.5b	52.2 ± 0.7a	5.0 ± 0.3b	27.5 ± 0.9c	43.6d	82.3c	89.6a
E30B	25.7 ± 0.2d	23.2 ± 0.2b	11.6 ± 0.6b	50.9 ± 0.5a	6.9 ± 0.6a	30.6 ± 0.8a	42.4d	84.0c	87.4a
E35A	24.2 ± 0.4e	19.3 ± 0.5d	9.6 ± 0.6c	49.6 ± 0.5a	5.4 ± 0.2b	26.9 ± 0.6d	42.5d	87.3b	88.0a
E35B	24.3 ± 0.3e	19.5 ± 0.1d	10.0 ± 0.5c	49.5 ± 0.6a	4.8 ± 0.3c	29.1 ± 0.7a	40.7f	83.1c	90.0a
E40A	19.8 ± 0.2f	18.0 ± 0.2d	9.5 ± 0.4c	47.0 ± 0.8c	5.2 ± 0.2b	29.5 ± 0.5a	36.8e	87.4b	89.3a
E40B	21.2 ± 0.5f	18.1 ± 0.3d	8.9 ± 0.3c	50.3 ± 0.3a	5.3 ± 0.2b	27.0 ± 0.6c	36.9e	87.5b	89.2a
E45A	18.6 ± 0.8f	15.2 ± 0.5e	8.5 ± 0.2c	47.1 ± 0.2c	5.3 ± 0.5b	28.8 ± 0.5a	35.5e	89.7a	87.6a
E45B	19.2 ± 0.6f	14.9 ± 0.4e	8.1 ± 0.4d	48.5 ± 0.4c	4.3 ± 0.3c	28.9 ± 0.8a	32.9g	83.1c	90.5a
E52A	18.3 ± 0.4f	14.9 ± 0.3e	6.6 ± 0.2d	51.5 ± 0.5a	5.5 ± 0.4b	27.2 ± 0.3c	29.3h	82.3c	89.2a
E52B	18.1 ± 0.5f	14.7 ± 0.2e	6.7 ± 0.3d	51.3 ± 0.7a	5.3 ± 0.2b	28.1 ± 0.4c	29.9h	84.5c	89.3a

Note: Samples E25A and E25B are wild-type sorghum and used as control; the remaining samples are pedigreed sorghum mutant stalks. [1] Column means with different letters than the control are significantly different at the 0.05 level.

3.4. Glucose Yield from Enzymatic Hydrolysis of Dilute Acid-Treated Solid Biomass

The reaction temperature in dilute acid pretreatment ranged from 120 to 160 °C, while sulfuric acid levels were in the range of 0.5 to 2% (*w*/*w*) [12,35]. The yield of reducing sugars through enzymatic hydrolysis was mainly influenced by acid concentration, reaction temperature, and reaction time. Acid hydrolysis releases oligosaccharides and monosaccharides but also results in the formation of degradation products such as aldehydes. In order to reduce the decomposition of the sugars, a hydrolysis time of 60 min was used in this study. Dilute acid-pretreated sorghum stalks demonstrated good enzymatic digestibility with more than 82.4% glucose yield for all the treated samples (Figure 2), which showed that the dilute acid pretreatment condition of reaction temperature 120 °C for 60 min with 2% (*w*/*v*) sulfuric acid was sufficient to disrupt the rigid cell-wall structure of sorghum biomass and significantly improve the enzymatic hydrolysis of treated sorghum biomass. Half of the selected sorghum mutants achieved greater than 90% glucose yields with a maximum glucose yield of 98.2% for sample E40B.

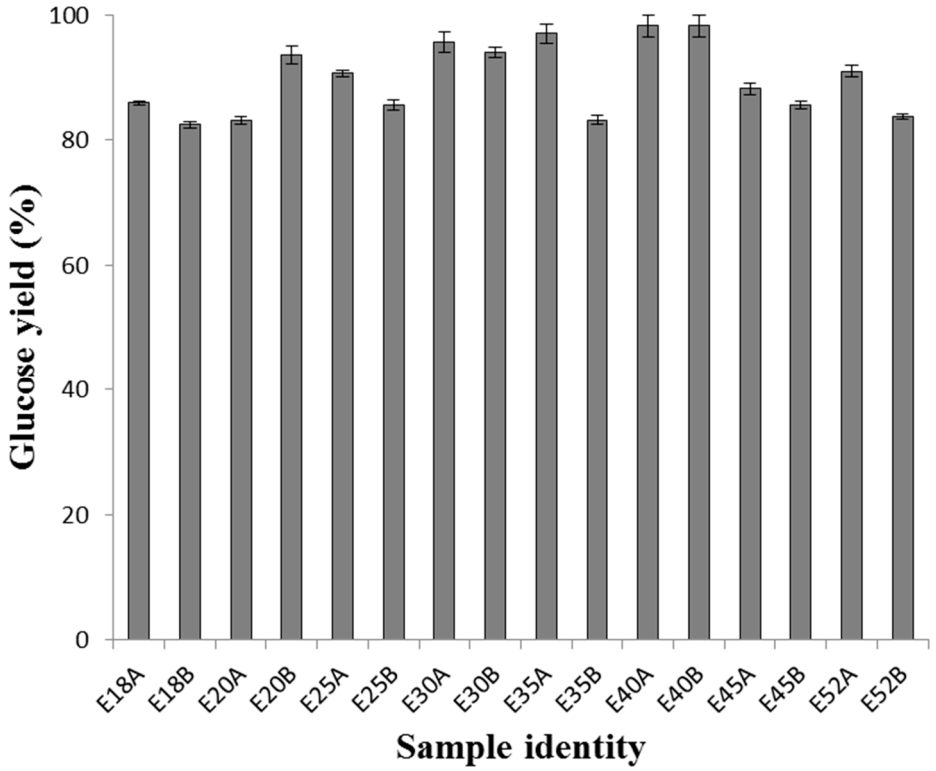

Figure 2. Glucose yield of dilute acid-pretreated sorghum stalks.

4. Conclusions

The content of water-soluble sugars in pedigreed sorghum mutant stalks increased as water-extractive content increased. The recovery of sugars including sucrose, glucose, and fructose during dilute sulfuric acid pretreatment also increased as water-extractive content increased. More than 82.4% of enzymatic hydrolysis yield and more than 82.3% cellulose recovery and 85% hemicellulose removal were achieved for all pretreated sorghum samples. To consider the effect of water-soluble content on the bioconversion process, a single-step, one-pot process is recommended for sorghum mutants with water-extractive content of equal or less than 35% (*w*/*w*), which can reduce the production cost and minimize wastewater generation, since the majority of sugars can be recovered after pretreatment with a minimum amount of degradation products. However, for sorghum mutants with higher water-extractive content (>35%, *w*/*w*), a pre-washing step is recommended to extract the exiting water-soluble sugars before subjecting to the pretreatment process in order to avoid sugar

losses or degradation during the pretreatment stage. Thus, different processing strategies should be considered for the lignocellulosic biomass with various water-extractive contents and various water-soluble sugar levels.

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