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Fast Hemicellulose Quantification Via a Simple One-Step Acid Hydrolysis

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ABSTRACT: As the second most common polysaccharides in nature, hemicellulose has received much attention in recent years for its importance in biomass conversion in terms of producing high yields of fermentable sugars and value-added products, as well as its role in reducing biomass recalcitrance. Therefore, a time and labor efficient method that specifically analyzes hemicellulose content would be valuable to facilitate the screening of biomass feedstocks. In this study, a one-step acid hydrolysis method was developed, which applied 4 wt% sulfuric acid at 121°C for 1 h to rapidly quantify XGM (xylan + galactan + mannan) contents in various types of lignocellulosic biomass and model hemicelluloses. This method gave statistically identical results in XGM contents compared to results from conventional two-step acid hydrolysis while significantly shortening analysis time.

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KEYWORDS: hemicellulose; acid hydrolysis; quantification; one-step; biomass

Introduction

Lignocellulosic biomass, including agricultural and forestry residues and herbaceous and woody crops (Wyman et al., 2005), provides the only sustainable resource for large-scale and low-cost production of liquid fuels and organic chemicals that are currently produced from dwindling and nonrenewable fossil resources (Dale, 2005; Farrell et al., 2006; Ragauskas et al., 2006; Wyman et al., 2005). However, the plant's recalcitrance to deconstruction by enzymes or microbes is the primary obstacle to low cost biological production of renewable fuels from lignocellulosic biomass (Himmel et al., 2007; Wyman, 2007). Therefore, versatile approaches are applied to make the conversion from biomass to fuels or chemicals more commercially viable. On one hand, optimization or improvement of key operations, including developing effective pretreatment technologies and improving the enzymes and microbes applied, can play an important role in reducing recalcitrance (Lynd et al., 1999; Yang and Wyman, 2008). On the other hand, attention to selection of biomass species with reduced recalcitrance and genetic modification of biomass for less recalcitrance also needs to be addressed (Studer et al., 2011; Xin et al., 2011). In pursuing these objectives, accurate and rapid determination of composition, particularly sugar content, is essential for identifying plant-pretreatment-biocatalyst combinations with performance advantages (DeMartini et al., 2011).

Lignocellulosic biomass is a complex material that is composed of three major structural polymers: cellulose, hemicellulose, and lignin. Cellulose is a linear polysaccharide of β-1,4-glucose units, which are linked by intra- and intermolecular hydrogen bonds to form a crystalline or amorphous structure (Lynd et al., 2002). Lignin is a cross-linked and three-dimensional phenolic polymer (Himmel, 2008). Hemicellulose is the second most common polysaccharides in nature and makes up about 20–35 wt% of lignocellulosic biomass (Wyman, 1996). Unlike cellulose, hemicellulose is not chemically homogeneous, and the chemical nature depends on the source. In general, the dominant component of hemicellulose from hardwoods and agricultural plants is xylan, while mannan is prevalent in softwoods. The xylan backbone is a major ingredient that is composed of 1,4-linked β-xylose units(Saha, 2003). Xylan

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substitutions varies from species to species, typically with arabinose sugar acids as well as acetyl groups (Aspinall, 1980). Hemicellulose is amorphous and hydrophilic and therefore more easily removed from cell walls than the cellulose polysaccharide.

Utilization of hemicellulose has received much attention in recent years for its importance in efficient and low-cost conversion of lignocellulosic biomass to fuel ethanol and other value-added products, with both biological and chemical strategies applied for hemicellulose conversion (Saha, 2003). Xylose and xylooligomers are often the major products from pretreatment and enzymatic hydrolysis of hemicellulose. Application of chemicals, such as sulfuric acid, at mild conditions is also capable of generating xylose monomer and oligomers from hemicellulose. Although traditional microorganisms, such as *Saccharomyces cerevisiae* and *Zymomona mobilis* ferment glucose to ethanol rapidly and efficiently, they cannot ferment other pentose, such as xylose. However, several recombinant strains have been developed to successfully ferment pentoses to ethanol under both laboratory and industrial conditions (Hahn-Hagerdal et al., 2007). Value-added products, or reactive intermediates (RIs) such as xylitol, furfural, and levulinic acid for the production of chemicals and polymers can also be generated from hemicellulose polysaccharides via appropriate catalytic approaches (Aden et al., 2004; Alonso et al., 2010).

Some studies suggested that the content as well as composition of hemicellulose also affects cell wall bioconversion (York and O'Neill, 2008). The interactions between hemicellulose and cellulose microfibrils as well as lignin-carbohydrate linkages (LCCs) are believed to sterically hinder enzyme attack (Chundawat et al., 2011; Hsu, 1996). Transgenic *Arabidopsis* with less methyl groups on glucuronoxylan side chains released more xylose than the wild type control at lower severity conditions (Urbanowicz et al., 2012). Reduced glucuronoxylan content in genetically modified poplar was also reported to result in increased cellulose digestibility (Lee et al., 2009).

Due to the importance of hemicellulose in biomass conversion in terms of producing high yields of fermentable sugars and value-added products, as well as its role in reducing biomass recalcitrance, a time and labor efficient method that specifically analyzes hemicellulose content would be valuable. Methods based on acid hydrolysis of biomass to determine structural carbohydrate and lignin content have been used for decades (Sluiter et al., 2008b, 2010). Strong acid such as sulfuric acid is most commonly used. Other acid such as trifluoroacetic acid (TFA) was used as a substitute for strong acid for analysis of cell wall hemicellulose (Albersheim et al., 1967; Foster et al., 2010). Unlike strong acid, which require neutralization before sugar analysis, TFA can be easily removed by evaporation after hydrolysis. Nowadays, a well-established and widely-used procedure from the National Renewable Energy Laboratory (NREL) employs two-step acid hydrolysis to breakdown structural carbohydrates into components that can be quantified by chromatography and gravimetric methods to accurately determine total sugar content in cellulosic biomass (Sluiter et al., 2008b). This method uses 72 wt% sulfuric acid in a first step followed by 4 wt% sulfuric acid in a second step to hydrolyze both cellulose and hemicellulose to sugars that can then be quantified via HPLC or other methods.

In this work, a one-step acid hydrolysis method was developed and was found to be effective for fast quantification of the hemicellulose sugars in biomass. The method applied 4 wt% sulfuric acid to hydrolyze biomass samples at 121°C for 1 h. Then the sugars released were measured to determine the hemicellulose sugar content. The method was applied to compounds enriched in hemicellulose and to agricultural, herbaceous, and woody biomasses, and the results were compared with those from a conventional two-step acid hydrolysis.

Materials and Methods

Materials

Five hemicellulose compounds, four biomass standards, three typical biomass materials, pure cellulose with different crystallinities, and starch were used in this study. Beechwood xylan (Lot BCBS8393V) was purchased from Sigma Chemicals (St. Louis, MO). Glucomannan was a dietary fiber derived from the root of the konjac plant (Konjac foods, Sunnyvale, CA). Carob galactomannan (Lot 10501b), wheat arabinoxylan (Lot 20301b), and xyloglucan (Lot 100402) were all purchased from Megazyme International (Wicklow, Ireland).

Sugarcane bagasse (NIST 8491), Eastern cottonwood (NIST 8492), Monterey pine (NIST 8493), and Wheat straw (NIST 8494) standard biomass materials of known composition as established by the National Institute of Standards and Technology (NIST) were obtained from NIST. The particle size range of the NIST standards was 20–74 mesh (0.19–0.85 mm). All biomass materials were well mixed and moisture content was measured as less than 10% before analysis.

The three typical biomass materials were tested: corn stover, switchgrass, and poplar wood. Corn stover was obtained from Michigan State University Farms (East Lansing, MI). This stover was harvested in September, 2008 from corn hybrid NK 49-E3 (Syngenta, Basel, Switzerland), a typical CS hybrid grown in the Great Lakes Region. The switchgrass, *Panicum virgatum*, and poplar wood, *Populus trichocarpa*, were provided by the BioEnergy Science Center (BESC). The poplar was debarked, split, and chipped (Yard Machine 10HP, MTD Products, Inc., Cleveland, OH). The corn stover, switchgrass, and poplar wood chips were further knife milled (Model 4, Wiley Mill, Thomas Scientific, Swedesboro, NJ) through a 1 mm screen. After that, all materials were air dried for approximately 1-month followed by sieving to collect fractions with a particle size between 20- ($<$0.85 mm) and 80-mesh ($>$0.180 mm) (RX-29, W.S. Tyler, Mentor, OH). Particles larger than 20-mesh

were collected and sieved again, and the resulting 20–80 mesh fraction was mixed with the previously obtained 20–80 mesh fraction.

Microcrystalline and amorphous cellulose as well as starch were also tested by conventional two-step acid hydrolysis and the new one-step approach. Pure cellulose (Avicel® PH101, Cat No. 11365, Lot 1094627) was purchased from FMC Corporation, Philadelphia, PA. Regenerated amorphous cellulose (RAC) was prepared from phosphoric acid decrystallization of Avicel PH 101, according to a method reported by Zhang et al. (2006). Starch powder (Batch #076K0181) from potato was from Sigma Chemicals. Seventy-two weight percent sulfuric acid (Lot.19093268) used for acid hydrolysis was from Ricca Chemicals (Arlington, TX; Sigma).

Methods

The major components of all the materials were determined by the well-established laboratory analytical procedures (LAPs) "Determination of structural carbohydrates and lignin in biomass" refined by NREL researchers (Sluiter et al., 2008b). The method is based on two-step acid hydrolysis, in which 0.3 ± 0.01 g biomass is first hydrolyzed by 3 mL 72 wt% sulfuric acid at 30°C for 1 h, followed by its dilution to 4 wt% acid for additional hydrolysis at 121°C for 1 h. A set of sugar recovery standards (SRS), summarized in Table I, were prepared to correct for losses due to sugar degradation during the second acid hydrolysis. After hydrolysis, about 700 μL of liquid was drawn, centrifuged to separate solids from the liquid in a microcentrifuge (Model 5424, Eppendorf North America, Hauppauge, NY) at 14,600 rpm for 5 min, pipetted into 500 μL polyethylene HPLC vials (Grace, Deerfield, IL), and then stored at 4°C until analysis for sugar content.

Hemicellulose Analysis by One-Step Acid Hydrolysis

The hemicellulose content of all materials was also determined by the fast one-step acid hydrolysis method developed here and compared with values determined by conventional NREL method (Sluiter et al., 2008b). The fast hemicellulose quantification used 0.3 ± 0.01 g biomass (dry weight) that was hydrolyzed in 87 mL of 4 wt% sulfuric acid hydrolysis at 121°C for 1 h. Then the liquid was drawn, centrifuged, and stored at 4°C until analysis for sugar content.

Component Removal by One-Step Acid Hydrolysis

After one-step acid hydrolysis of corn stover, switchgrass, and poplar wood, the solid residues were collected by filtration, dried in a 105°C oven overnight, and weighed to calculate the solid yield. Then, the composition of the solid residues was determined by NREL LAPs (Sluiter et al., 2008b) for component removal calculation.

Sugar Analysis

For analysis, samples along with appropriate calibration standards were run on a Waters Alliance HPLC system (Model e-2695, Waters Corporation, Milford, MA) employing an Aminex HPX-87H column (Bio-Rad Laboratories, Life Science Research, Hercules, CA). Samples were processed at an eluent of 5 mM sulfuric acid with flow rate of 0.60 mL/min using a refractive index (RI) detector (Model 2414, Waters Corporation). The chromatograms were recorded and processed with Empower® 2 software (Waters Corporation).

Calculation of Sugar Content, Solid Yield, and Component Removal

The glucan, xylan, and arabinan contents were calculated as:

$$\text{Glucan content}\,(\%) = 100 \times \frac{(\text{GH}\,(\text{g}) + \text{CB}\,(\text{g}) \times 1.053)/1.111}{\text{Sample}\,(\text{g})}$$

$$\text{Xylan content}\,(\%) = 100 \times \frac{\text{XH}\,(\text{g})/1.136}{\text{Sample}\,(\text{g})}$$

$$\text{Arabinanx content}\,(\%) = 100 \times \frac{\text{AH}\,(\text{g})/1.136}{\text{Sample}\,(\text{g})}$$

Galactose and mannose have similar retention times and responses as xylose in the Aminex HPX-87H column. However, when the three sugars did not coexist, the content of each was determined as:

$$\text{Glucan content}\,(\%) = 100 \times \frac{\text{GaH}\,(\text{g})/1.111}{\text{Sample}\,(\text{g})}$$

Table I. Sugar recovery standards and their concentrations range used in the methods applied.

Name	Vendor	Lot number	Concentration (mg/mL)
D(+)-glucose	Sigma–Aldrich, St. Louis, MO	089K00601	1–4
D(+)-xylose	Acros Organic, Morris Plains, NJ	A0308408	1–4
L(+)-arabinose	Alfa Aesar, Ward Hill, MA	10162224	1–4
D(+)-mannose	Acros Organic, Morris Plains, NJ	A0308014	1–4
D(+)-galactose	Acros Organic, Morris Plains, NJ	A0244833	1–4

$$\text{Mannan content}\,(\%) = 100 \times \frac{\text{MH}\,(\text{g})/1.111}{\text{Sample}\,(\text{g})}$$

Otherwise, the content was reported as XGM (xylan + galactan + mannan), which was calculated by the following equation:

$$\text{XGM content}\,(\%) = 100 \times \frac{\text{XH}\,(\text{g})/1.136}{\text{Sample}\,(\text{g})}$$

in which GH, CB, XH, AH, GaH, and MH represent glucose, cellobiose (if any), xylose, arabinose, galactose, and mannose released during acid hydrolysis, and 1.053, 1.111, and 1.136 are the mass conversion factors for cellobiose to glucose, glucose (mannose or galactose) to cellulose (mannan or galactan), and xylose (arabinose) to xylan (arabinan), respectively. The mass of sample used here was on a dry weight basis.

The solid yield of samples after one-step acid hydrolysis was defined as:

$$\text{Solid yield}\,(\%) = 100 \times \frac{\text{Total dry solid after acid hydrolysis}\,(\text{g})}{\text{Total dry solid before acid hydrolysis}\,(\text{g})}$$

The components (glucan, xylan, and lignin) removed in one-step acid hydrolysis were calculated as:

$$\text{Glucan removal}\,(\%) = 1 - \frac{\%\,\text{Glucan in solid after one step acid hydrolysis}}{\%\,\text{Glucan in raw biomass}} \times \text{Solid yield}$$

$$\text{Xylan removal}\,(\%) = 1 - \frac{\%\,\text{Xylan in solid after one step acid hydrolysis}}{\%\,\text{Xylan in raw biomass}} \times \text{Solid yield}$$

$$\text{Lignin removal}\,(\%) = 1 - \frac{\%\,\text{Lignin in solid after one step acid hydrolysis}}{\%\,\text{Lignin in raw biomass}} \times \text{Solid yield}$$

Statistical Analysis

The xylan content (XGM) in corn stover, switchgrass, poplar wood, and the four NIST standards was determined by the conventional two-step acid hydrolysis (NREL LAP) and the new one-step acid hydrolysis using quadruplicate measurements. To test whether the xylan contents from the two methods were statistically the same, an equivalence test was performed. First, a two-tailed *F*-test was performed to check if the variances of two sample populations were the same:

$$F_{\text{calculate}} = \frac{S^2_{\text{the larger value}}}{S^2_{\text{the smaller value}}}$$

where S is the sample standard deviation, n is the number of samples, and the degree of freedom is $n-1$ for both the numerator and the denominator. $F_{\text{calculate}}$ was compared with the critical value at the 5% significance level ($\alpha = 0.05$). If the $F_{\text{calculate}} \le F_{\text{critical}}$, then the variances of the two sample populations were accepted as equal. If the $F_{\text{calculate}} > F_{\text{critical}}$, then the variances of the two sample populations were considered to be different.

In addition, the two-tailed *t*-test was used to test if the mean values of the two sample populations were the same. As for the *F*-test, if the variances of the two populations were determined to be equal, then t was calculated as:

$$t_{\text{calculate}} = \frac{\bar{X}_1 - \bar{X}_2}{((S_p^2/n_1) + (S_p^2/n_2))^{1/2}}$$

in which $S_p = (((n_1-1)S_1^2 + (n_2-1)S_2^2)/(n_1+n_2-2))$, the degree of freedom is $\text{df} = n_1 + n_2 - 2$. When the variances of the two populations were not equal, then t was calculated as:

$$t_{\text{calculate}} = \frac{\bar{X}_1 - \bar{X}_2}{((S_1^2/n_1) + (S_2^2/n_2))^{1/2}}$$

with the degree of freedom calculated by the following equation to the closest integer larger then the calculated value:

$$\text{df} = \frac{((S_1^2/n_1) + (S_2^2/n_2))^2}{((S_1^2/n_1)^2/n_1 + (S_2^2/n_2)^2/n_2)}$$

In this equation, $\bar{X}_1$ and $\bar{X}_2$ are average values of the samples, S_1^2 and S_2^2 are variances of the samples, and n_1 and n_2 are the number of tests in a sample.

The value of $t_{\text{calculate}}$ was compared to the critical value at the 5% ($\alpha = 0.05$) significance level. If $t_{\text{calculate}} \le t_{\text{critical}}$, then the one- and two-step acid hydrolysis methods were considered to give statistically the same xylan content. Otherwise, the xylan contents determined by the one- and two-step acid hydrolysis were considered to not be statistically equal.

Results and Discussion

Selection of Conditions for One-Step Method

The one-step acid hydrolysis applied acid loadings of 2, 4, and 6 wt% at 121°C for 0.5, 1, and 1.5 h on beechwood xylan,

corn stover, switchgrass, and poplar wood and the resulting xylan plus galactan plus mannan (XGM) were compared with the values obtained from conventional two-step hydrolysis. Among all the tested conditions, 4 wt% acid loading at 121°C for 1 h gave comparable XGM results as the two-step acid hydrolysis. NREL method for analysis of soluble oligosaccharides in hydrolyzate applied the same condition (Sluiter et al., 2008a). For other combinations of acid loading and reaction time in one-step hydrolysis, the XGM value was much lower than that determined by the two-step acid hydrolysis (not shown). Moreover, the sugar recovery standards run with 6 wt% showed very severe degradation of xylose (~30%), which suggested that 6 wt% acid loading was not a proper choice (Sluiter et al., 2008a). As a result, this condition of 4 wt% acid loading at 121°C for 1 h was chosen for the following tests.

Composition of Hemicellulose Compounds

The one-step acid hydrolysis was first applied to the five compounds rich in hemicellulose, and the results were compared to compositional data from the conventional two-step acid hydrolysis (NREL–LAP). As shown in Figure 1, one-step hydrolysis at 121°C with 4 wt% sulfuric acid for 1 h gave virtually identical sugar contents as conventional two-step acid hydrolysis for all five hemicellulose compounds, in that the sugar contents measured by one-step acid hydrolysis were in agreement with the conventional method and the standard deviations were also comparable. Overall, there was no discernible trend that suggested over- or under-estimation of the sugar compositions by the one-step acid hydrolysis method. Regardless of the type of hemicellulose-rich compound, the one-step method was capable of complete deconstruction of the hemicellulose to monomeric sugars for quantitative analysis.

Xylan and Arabinan Content of NIST Standards and Lignocellulosic Biomass Samples

Next, the xylan and arabinan contents of the four NIST standards were measured by one-step acid hydrolysis, and the results were compared with the reference value provided by NIST (Wise and Watters, 2011a,b,c,d) as well as the results from application of the conventional NREL methods in our laboratory. As shown in Figure 2, both the xylan plus galactan plus mannan (XGM) and arabinan contents determined by one-step acid hydrolysis were comparable to results from the conventional method and the reference value.

The XGM contents measured by one-step and conventional two-step acid hydrolysis were further tested for statistical equivalence at a 5% significance level ($\alpha = 0.05$) by applying the widely-used two-tailed *t*-test. However, the *F*-test at the 5% significance level ($\alpha = 0.05$) was applied first to test the equivalence of variances from the two methods as shown in Table II. The critical value of F at $\alpha = 0.05$ with df = 3 for both sample populations is 15.44 according to an *F*-table. Based on the results from *F*-test, the variances of XGM content from one-and two-step acid hydrolysis were not equal for sugarcane bagasse, cottonwood, wheat straw, and poplar wood, but equal for Monterey pine, corn stover, and switchgrass. Then the *t*-test was employed because the sample size was small (<30), with the distribution of the population assumed to be normal or approximately normal. There are

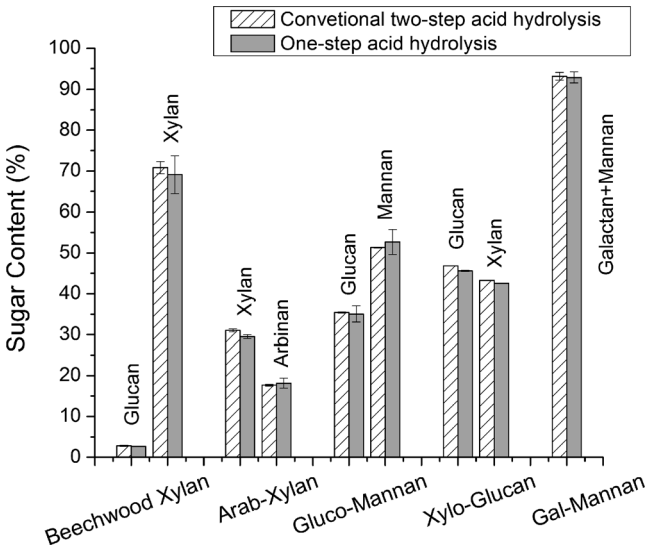

Figure 1. Sugar contents of Beechwood xylan, arabinoxylan, glucomannan, xyloglucan, and galactomannan as determined by one-step and conventional two-step acid hydrolysis methods. The compositions are displayed as mass percent, and the error bars represent standard deviation from four independent measurements.

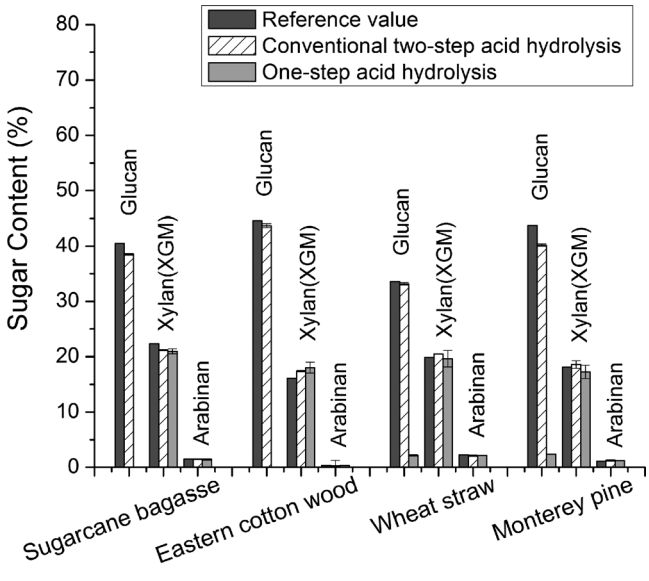

Figure 2. Glucan, xylan plus galactan and mannan (XGM), and arabinan contents of the NIST standards determined by the one-step and conventional two-step acid hydrolysis methods, and their reference values. The compositions are displayed as mass percent, and the error bars represent standard deviation from four independent measurements.

Table II. Application of *F*-test at 10% significance level to determine the variance of XGM content from one- and two-step acid hydrolysis methods were statistically the same.

	Conventional two-step acid hydrolysis			One-step acid hydrolysis					
	$\overline{X_1}$	S_1	n_1	$\overline{X_2}$	S_2	n_2	$F_{calculate}$	$F_{critical}$	$S_1^2 = S_2^2$
Corn stover	23.1	0.84	4	22.4	0.32	4	6.79	15.44	Yes
Switchgrass	22.0	1.04		21.4	0.57		3.35		Yes
Poplar wood	20.7	2.51		21.4	0.60		17.54		No
Sugarcane bagasse	21.1	0.21		20.9	0.43		4.19		Yes
Eastern cottonwood	17.4	0.17		18.0	0.98		34.69		No
Monterey pine	18.6	0.70		17.3	1.21		2.97		Yes
Wheat straw	20.4	0.06		19.6	1.44		641.1		No

$\overline{X}$, average of four independent measurements; S, standard deviation of four independent measurements; n, number of samples.
$F_{critical}$ is at 5% significance level ($\alpha = 0.05$).

two equations to calculate the *t*-value as explained in the Methods section, with one generally used when the variances of the two populations are assumed equal and the other when the variances are not equal. In the following, the *t*-test at significance level of 5% ($\alpha = 0.05$) was applied to calculate the *t*-value $|t_{calculate}|$ which was then compared to the critical value from a *t*-distribution table. As shown in Table III, $|t_{calculate}|$ values of all samples were smaller than $t_{critical}$, suggesting that the XGM contents of all the NIST standards measured by the rapid one-step acid hydrolysis and the conventional two-step acid hydrolysis were statistically equivalent.

Corn stover, switchgrass, and poplar wood are feedstocks that hold great potential to support large-scale fuel production. The XGM and arabinan contents for these three species were evaluated by both the one-step acid hydrolysis and the conventional method, with the results shown in Figure 3. The XGM and arabinan contents from one-step acid hydrolysis were in good agreement with those determined by conventional method. In addition, Tables II and III show that the *F*-test and *t*-test, respectively, proved that these two analysis methods produced statistically equivalent XGM contents. According to NREL procedure for a complete biomass composition analysis (Sluiter et al., 2008b), water and ethanol extraction is applied before acid hydrolysis. However, as an easy and fast method to determine hemicellulose content, one step acid hydrolysis was directly applied on raw material. In this study, negligible effect was observed in determining the hemicellulose content without extraction. But, it should be kept in mind that if significant amount of free sugar exist in biomass feedstock, such as fresh sugarcane bagasse, an extraction or washing step should be performed before apply the method.

Overall, the one-step acid hydrolysis method gave statistically identical XGM and arabinan contents as the conventional two-step approach for different biomass species, including agricultural residues (corn stover, sugarcane bagasse, and wheat straw), grasses (switchgrass), softwood (Monterey pine), and hardwood (poplar wood and eastern cottonwood). Thus, the one-step acid hydrolysis provides a viable method for rapid evaluation of the hemicellulose content in many different types of biomass. Compared with the conventional method for structural sugar measurement, the one-step acid hydrolysis reduces the analysis time by approximately half, because only a 1 h hydrolysis at 121°C is needed, instead of 1 h at 30°C followed by 1 h at 121°C. In addition, the one-step method employs only a 4 wt% sulfuric acid solution and not concentrated sulfuric acid, reducing the hazard of dealing with concentrated sulfuric acid. Moreover, the one-step acid hydrolysis method can also be integrated with the high throughput small-scale compositional analysis system, which employs

Table III. The results of *t*-test performed to compare the equivalence of XGM contents from one- and two-step acid hydrolysis methods.

Sample	df	$\lvert t_{calculate}\rvert$	$t_{critical,0.1}$	Equivalence
Corn stover	6	1.449	2.447	Yes
Switchgrass	6	1.009	2.447	Yes
Poplar wood	5 (4.46)	0.544	2.571	Yes
Sugarcane bagasse	6	2.470	2.571	Yes
Eastern cottonwood	5 (4.23)	1.262	2.571	Yes
Monterey pine	6	1.922	2.447	Yes
Wheat straw	5 (4.01)	1.133	2.571	Yes

df, degree of freedom, the value of df was calculated as introduced in Methods section.
The numbers between brackets are the calculated value of df when variance of two sample sets were unequal and the closest integer larger than the calculated value was used as df.
$t_{critical}$ at 5% significance level.

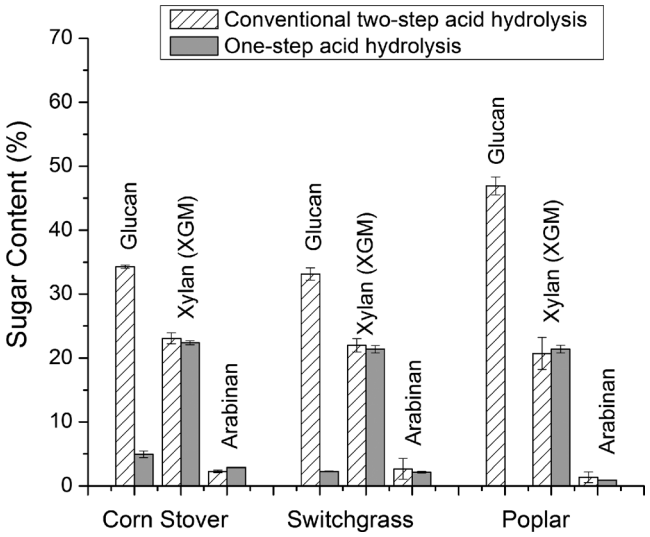

Figure 3. Glucan, xylan plus galactan and mannan (XGM), and arabinan contents in corn stover, switchgrass, and poplar wood determined by the one-step and conventional two-step acid hydrolysis methods. The compositions are displayed as mass percent, and the error bars represent standard deviation from four independent measurements.

only 3.0 mg samples in 1.5 mL glass vials for measurement (DeMartini et al., 2011). Thus, the one step approach can be an appealing method in terms of labor and time efficiency, especially when dealing with large numbers of samples, for example, transgenic plants with modified hemicellulose structure and/or content.

One-Step Acid Hydrolysis on Crystalline, Amorphous Cellulose, and Starch

Figures 2 and 3 show that other than XGM and arabinan, minor amounts (<5%) of glucan were also released in one-step acid hydrolysis for some biomasses. The most possible source of the glucan release was from β-glucan and hemicellulose side chains. In addition, starch in biomass would also be a possible source of glucose release in one-step acid hydrolysis. Starch is a storage component in most biomass, and its content can vary with part of the plant and harvest season and time of day (Decker et al., 2012). The content of starch is typically low in mature wood or field dried herbaceous crops but varies from 2 to 8 wt% in green material of switchgrass, as reported by Decker et al. (2012). Crystalline cellulose, however, is very unlikely to be a large source of glucan release from 4 wt% acid hydrolysis at 121°C for 1 h due to its high recalcitrance at these conditions (Bobleter, 1994; Lynd et al., 2002).

To gain an understanding of how cellulose and starch behave in one-step acid hydrolysis, glucose release from crystalline cellulose (Avicel) and regenerated amorphous cellulose (RAC) as well as starch from potato was measured in terms of glucan equivalents, as shown in Table IV. At the given conditions, starch was completely hydrolyzed into glucose. In contrast, as expected, negligible amounts of glucan were released from crystalline cellulose (Avicel). The solids recovery data in Table IV also suggest that one-step acid hydrolysis hydrolyzed starch completely but not crystalline cellulose.

RAC was also subjected to both one-step and conventional two-step acid hydrolysis. The glucan content in RAC was first determined by conventional two-step acid hydrolysis as 75.2 ± 0.6%. One-step acid hydrolysis partially released glucose from RAC, and not all the RAC was recovered after one-step hydrolysis. However, it should be kept in mind that the structure of RAC regenerated from phosphoric acid swollen cellulose disrupted hydrogen bonds, so they were different from the amorphous region in natural cellulose in biomass (Zhang et al., 2006). Considering the tightly packed structure of crystalline cellulose, we anticipate that very minor amount of glucan that released during one-step acid hydrolysis may be from the amorphous region of cellulose in biomass.

Components Removal by One-Step Acid Hydrolysis

The composition of the starting raw material and the material recovered from one-step acid hydrolysis as well as the solid yield are summarized in Table V. These results show that 48.0%, 54.9%, and 68.4% of the corn stover, switchgrass, and poplar wood were recovered from hydrolysis with 4 wt% acid at 121°C for 1 h. Although, it is known that acid treatment of biomass mainly removes hemicellulose from biomass, one-step acid hydrolysis also partially removed glucan and lignin, as shown in Table V. And as discussed before, the glucan may have come from the hemicellulose, β-glucan, and/or starch in biomass. The method almost completely removed xylan, with only negligible amounts left in biomass: 0.9% for corn stover,

Table IV. Summary of glucan release and solid yields from one- and two-step acid hydrolysis of Avicel, RAC, and starch.

	Avicel	RAC	Starch
Conventional two-step acid hydrolysis			
Solid yield	0	0	N/A
Glucan (%)	98.4 ± 0.4	75.2 ± 0.6	N/A
One-step acid hydrolysis			
Solid yield	97.0 ± 1.0	56.7 ± 1.7	0
Glucan (%)	2.7 ± 0.2	14.6 ± 2.2	97.0 ± 1.4

N/A, not analyzed.

Table V. Summary of the raw biomass and residual solids compositions, and components removal and solid yields of various feedstocks after one-step acid hydrolysis.

	Composition	Corn stover	Switchgrass	Poplar
Raw	Solid (g)	100	100	100
	Glucan (%)	34.3 ± 0.3	33.1 ± 1.0	46.9 ± 1.4
	XGM (%)	23.1 ± 0.8	22.0 ± 1.0	20.7 ± 2.5
	K-lignin (%)	17.2 ± 1.0	18.8 ± 1.2	23.4 ± 1.0
Residual solids after one-step acid hydrolysis	Solid (g)	48.0 ± 0.4	54.9 ± 1.2	68.4 ± 0.1
	Glucan (%)	63.2 ± 0.2	59.0 ± 0.8	65.7 ± 0.3
	XGM (%)	0.9 ± 0.07	0.9 ± 0.4	1.0 ± 0.05
	K-lignin (%)	30.0 ± 1.3	29.0 ± 1.2	29.0 ± 1.2
Removal by one-step hydrolysis	Solid (g)	52	45.1	31.6
	Glucan (%)	10.7 ± 0.9	2.9 ± 1.4	4.15 ± 0.6
	XGM (%)	98.0 ± 0.1	97.8 ± 1.1	96.7±0.2
	K-lignin (%)	20.1 ± 2.2	18.1 ± 2.45	15.9 ± 2.3

0.9% for switchgrass, and 1.0% for poplar wood. Therefore, one-step acid hydrolysis is a promising method for removal of hemicellulose from the solids so its content can be measured in terms of dissolved sugars.

Conclusions

A one-step acid hydrolysis method was developed for rapid quantification of XGM content in various types of lignocellulosic biomass. By hydrolysis with 4 wt% sulfuric acid at 121°C for 1 h, the hemicellulose was almost totally released from various types of biomass as sugar monomer that could then be quantified by HPLC. This method gave statistically identical results in XGM contents compared to results from conventional two-step acid hydrolysis while significantly shortening analysis time. Thus, the one-step acid hydrolysis method provides a rapid and simple approach for xylan (and other) hemicelluloses quantification.

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References

Aden ABJ, Holladay J, White J, Manheim A, Elliot D, Lasure L, Jones S, Gerber M, Ibsen K, Lumberg L, Kelley S, Werpy J, Petersen G. 2004. Top value added chemicals from biomass, volume I—results of screening for potential candidates from sugars and synthesis gas. Oak Ridge: U.S. Department of Energy. Available at www.eere.energy.gov/biomass/pdfs/35523.pdf

Albersheim P, Nevins DJ, English PD, Karr A. 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohydr Res 5:340–345.

Alonso DM, Bond JQ, Dumesic JA. 2010. Catalytic conversion of biomass to biofuels. Green Chem 12(9):1493–1513.

Aspinall G. 1980. Chemistry of cell wall polysaccharides. In: Preiss J, editor. The biochemistry of plants (a comprehensive treatise), vol 3. Carbohydrates: Structure and function. New York: Academic. p 473–500.

Bobleter O. 1994. Hydrothermal degradation of polymers derived from plants Prog Polym Sci 19(5):797–841.

Chundawat SPS, Beckham GT, Himmel ME, Dale BE. 2011. Deconstruction of lignocellulosic biomass to fuels and chemicals. In: Prausnitz JM, editor. Annual review of chemical and biomolecular engineering, vol 2. Palo Alto, CA. p 121–145.

Dale BE. 2005. Special issue: Coordinated development of leading biomass pretreatment technologies. Bioresour Technol 96(18):1959–2032.

Decker SRC M, Selig MJ, Doeppke C, Davis M, Sykes R, Turner G, Ziebell A. 2012. Reducing the effect of variable starch levels in biomass recalcitrance screening. In: Himmel ME, editor. Biomass conversion. Methods in molecular biology. New York: Humana Press. p 181–195.

DeMartini JD, Studer MH, Wyman CE. 2011. Small-scale and automatable high-throughput compositional analysis of biomass. Biotechnol Bioeng 108(2):306–312.

Farrell AE, Plevin RJ, Turner BT, Jones AD, O'Hare M, Kammen DM. 2006. Ethanol can contribute to energy and environmental goals. Science 311(5760):506–508.

Foster CE, Martin TM, Pauly M. 2010. Comprehensive compositional analysis of plant cell walls (lignocellulosic biomass) part II: Carbohydrates. J Vis Exp Mar 12(37):e1837.

Hahn-Hagerdal B, Karhumaa K, Fonseca C, Spencer-Martins I, Gorwa-Grauslund MF. 2007. Towards industrial pentose-fermenting yeast strains. Appl Microbiol Biotechnol 74(5):937–953.

Himmel M. 2008. Biomass recalcitrance: Deconstructing the plant cell wall for bioenergy. Oxford, UK: Wiley-Blackwell. 528 p.

Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, Brady JW, Foust TD. 2007. Biomass recalcitrance: Engineering plants and enzymes for biofuels production. Science 315(5813):804–807.

Hsu TA. 1996. Pretreatment of biomass. In: Wyman CE, editor. Handbook on bioethonal production and utilization. Washing, DC: Taylor & Francis.

Lee C, Teng Q, Huang W, Zhong R, Ye Z-H. 2009. Down-regulation of PoGT47C expression in poplar results in a reduced glucuronoxylan content and an increased wood digestibility by cellulase. Plant Cell Physiol 50(6):1075–1089.

Lynd LR, Wyman CE, Gerngross TU. 1999. Biocommodity engineering. Biotechnol Prog 15(5):777–793.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: Fundamentals and biotechnology. Microbiol Mol Biol Rev 66(3):506–739.

Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, Eckert CA, Frederick WJ, Hallett JP, Leak DJ, Liotta CL, Mielenz JR, Murphy R,

Templer R, Tschaplinski T. 2006. The path forward for biofuels and biomaterials. Science 311(5760):484–489.

Saha BC. 2003. Hemicellulose bioconversion. J Ind Microbiol Biotechnol 30(5):279–291.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D. 2008a. Determination of sugars, byproducts, and degradation products in liquid fraction process samples. NREL Laboratory Analytical Procedure NREL/TP-510-42623. Golden, CO: National Renewable Energy Laboratory.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. 2008b. Determination of structural carbohydrates and lignin in biomass. NREL Laboratory Analytical Procedure NREL/TP-510-42618. Golden, CO: National Renewable Energy Laboratory.

Sluiter JB, Ruiz RO, Scarlata CJ, Sluiter AD, Templeton DW. 2010. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J Agric Food Chem 58(16):9043–9053.

Studer MH, DeMartini JD, Davis MF, Sykes RW, Davison B, Keller M, Tuskan GA, Wyman CE. 2011. Lignin content in natural Populus variants affects sugar release. Proc Natl Acad Sci U S A 108(15):6300–6305.

Urbanowicz BR, Pena MJ, Ratnaparkhe S, Avci U, Backe J, Steet HF, Foston M, Li H, O'Neill MA, Ragauskas AJ, Darvill AG, Wyman CE, Gilber HJ, York WS. 2012. 4-O-methylation of glucuronic acid in Arabidopsis glucuronoxylan is catalyzed by a domain of unknown function family 579 protein. Proc Natl Acad Sci U S A 109(35):14253–14258.

Wise SA, Watters RJ. 2011a. Report of investigation reference material 8491, sugarcane bagasse whole biomass feedstock. Gaithersburg, MD. Available at: https://www-s.nist.gov/srmors/view_report.cfm?srm=8491

Wise SA, Watters RJ. 2011b. Report of investigation reference material 8492, eastern cotton wood whole biomass feedstock. Gaithersburg, MD. Available at: https://www-s.nist.gov/srmors/view_report.cfm?srm=8492

Wise SA, Watters RJ. 2011c. Report of investigation reference material 8493, monterey pine whole biomass feedstock. Gaithersburg, MD. Available at: https://www-s.nist.gov/srmors/view_report.cfm?srm=8493

Wise SA, Watters RJ. 2011d. Report of investigation reference material 8494, wheat staw whole biomass feedstock. Gaithersburg, MD. Available at: https://www-s.nist.gov/srmors/view_report.cfm?srm=8494

Wyman C. 1996. Handbook on bioethanol: Production and utilization. Taylor&Francis, Washington, DC.

Wyman CE. 2007. What is (and is not) vital to advancing cellulosic ethanol. Trends Biotechnol 25(4):153–157.

Wyman CE, Dale BE, Elander RT, Holtzapple M, Ladisch MR, Lee YY. 2005. Coordinated development of leading biomass pretreatment technologies. Bioresour Technol 96(18):1959–1966.

Xin Z, Watanabe N, Lam E. 2011. Improving efficiency of cellulosic fermentation via genetic engineering to create "smart plants" for biofuel production. In: Buckeridge MS Goldmann GH editors. Routes to Cellulosic Ethanol. New York: Springer p 181–197.

Yang B, Wyman CE. 2008. Pretreatment: The key to unlocking low-cost cellulosic ethanol. Biofuels Bioprod Biorefining 2(1):26–40.

York WS, O'Neill MA. 2008. Biochemical control of xylan biosynthesis—which end is up? Curr Opin Plant Biol 11(3):258–265.

Zhang YHP, Cui JB, Lynd LR, Kuang LR. 2006. A transition from cellulose swelling to cellulose dissolution by o-phosphoric acid: Evidence from enzymatic hydrolysis and supramolecular structure. Biomacromolecules 7(2):644–648.