ARTICLE

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Small-Scale and Automatable High-Throughput Compositional Analysis of Biomass

Jaclyn D. DeMartini, Michael H. Studer, Charles E. Wyman

Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, 1084 Columbia Avenue, Riverside, California 92507; telephone: 951-781-5703; fax: 951-781-5790; e-mail: charles.wyman@ucr.edu

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ABSTRACT: Conventional wet chemistry methods to determine biomass composition are labor- and time-intensive and require larger amounts of biomass (300 mg) than is often available. To overcome these limitations and to support a high-throughput pretreatment and hydrolysis (HTPH) screening system, this article reports on the development of a downscaled biomass compositional analysis that is based on conventional wet chemistry techniques but is scaled down by a factor of 100 to use significantly less material. The procedure is performed in readily available high-performance liquid chromatography vials and can be automated to reduce operator input and increase throughput. Comparison of the compositional analyses of three biomasses determined by the downscaled approach to those obtained by conventional methods showed that the downscaled method measured statistically identical carbohydrate compositions as standard procedures and also can provide reasonable estimates of lignin and ash contents. These results demonstrate the validity of the downscaled procedure for measuring biomass composition to enable the calculation of sugar yields and determination of trends in sugar release behavior in HTPH screening studies.

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KEYWORDS: biomass; compositional analysis; downscaled; Klason lignin; quantitative saccharification

Introduction

As conventional petroleum reserves dwindle and concerns over associated anthropogenic greenhouse gas emissions grow, alternative renewable energies should be at the forefront of research (Brandt and Farrell, 2007; Lynd et al., 1991). Currently, the only promising resource for large-scale sustainable production of liquid fuels for transportation is lignocellulosic biomass (Farrell et al., 2006; Lynd et al., 1991; Perlack et al., 2005; Ragauskas et al., 2006). However, the primary obstacle to low cost biological production of renewable fuels from lignocellulosic biomass is a plant's recalcitrance or resistance to deconstruction by enzymes or microbes (Lynd et al., 2008; Wyman, 2007). Thus, optimizing pretreatment and enzymatic hydrolysis processes, improvement of enzymes applied, and identification and use of biomass species with reduced recalcitrance are attractive routes to making fuels from lignocellulosic biomass more commercially viable (Lynd et al., 1991, 1999). In pursuing these objectives, accurate and rapid determination of composition, in particular sugar contents, is essential for determining yields accurately and identifying plant-pretreatment–biocatalyst combinations that provide performance advantages.

Conventional wet chemistry techniques employed to determine biomass composition generate accurate and quantitative compositional data through a two-step acid hydrolysis sequence to breakdown structural carbohydrates into components that can be more easily quantified by chromatography and gravimetric methods (Sluiter et al., 2008). Unfortunately, the process is both exacting and labor intensive and also requires a relatively large amount of material (300 mg). These disadvantages are particularly problematic in feedstock studies that aim to identify promising biomass variants for enhanced sugar release in which thousands of samples can be generated for screening and limited amounts of material may be available. As a result, alternative, higher throughput, and smaller scale methods are sorely needed for compositional analyses. Currently, near infrared (NIR) spectroscopy (Hames et al., 2003; Kelley et al., 2004) and pyrolysis molecular beam mass spectrometry (py-MBMS) (Evans and Milne, 1987; Sykes et al., 2008, 2009) coupled with multivariate analytical techniques have been shown to rapidly and accurately determine biomass composition. However, these methods require sophisticated and fairly costly equipment and extensive calibration data from a distinct set of biomasses

Michael H. Studer's present address is Institute of Process Engineering, Swiss Federal Institute of Technology (ETH Zurich), Sonneggstr. 3, CH-8092 Zurich, Switzerland.
Correspondence to: Charles E. Wyman
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that must closely resemble the samples to be tested. Besides the challenges of acquiring biomasses with a sufficient compositional range to support calibration, a large number of calibration samples must be analyzed by the above-mentioned traditional wet chemistry methods to provide a sufficient range of compositions.

This article reports on the development of a simple, downscaled biomass compositional analysis that was developed to support a high-throughput pretreatment and enzymatic hydrolysis system used to screen thousands of biomass samples and identify those with reduced recalcitrance (Decker et al., 2009; Studer et al., 2010). Previously, results from these screening studies could only be expressed as the amount of sugar released per amount of total biomass because there was no available method to accurately determine the carbohydrate content of the small amounts of material collected (<50 mg in some cases). Thus, to allow the calculation of glucan and xylan yields, and the estimation of lignin and ash contents, a method was developed that is based on the well-established two-stage acid hydrolysis but is scaled down by a factor of 100 to use significantly less material. Furthermore, downsizing the procedure also lended itself to automation, resulting in far less operator input and higher throughput for large sample sets compared to standard wet chemistry procedures. Results from the use of the high-throughput method were compared to those from application of the conventional method for three different biomasses to verify that the downscaled method produced identical sugar compositions to the standard procedure, and also to evaluate the method's accuracy in measuring lignin and ash contents. Testing was performed with a robotics platform to facilitate all weighing and solid dispensing steps in order to demonstrate that the downscaled method can be automated to reduce operator input and increase throughput. However, this approach can also be applied with simple equipment available in most laboratories that already perform biomass compositional analysis, as shown by performing the downscaled analysis manually for one biomass material. Furthermore, the present study employed readily available high-performance liquid chromatography (HPLC) vials as reaction vessels for downscaled analysis, but other, more elaborate systems, for example, based on well-plate formats, could also be used instead (Studer et al., 2010).

Materials and Methods

Materials

Three biomass materials were tested: *Populus deltoides* (8492 National Institute of Standards and Technology), sugarcane bagasse (8491 National Institute of Standards and Technology), and Alamo switchgrass (BioEnergy Science Center internal standard material). The NIST materials comprised a mesh fraction of −20/+74 ($0.85\,mm > x > 0.19\,mm$), while the switchgrass had a mesh fraction of −20/+80 ($0.85\,mm > x > 0.18\,mm$). All materials were well-mixed, had a moisture content of 5%, and were analyzed as received.

Downscaled Compositional Analysis

The downscaled method reported here is an adaptation of the well-established laboratory analytical procedures "Determination of Structural Carbohydrates and Lignin in Biomass" and "Determination of Ash in Biomass," refined by NREL researchers (Sluiter et al., 2005, 2008, respectively). The first method is based on a two-stage acid hydrolysis, originally developed by NREL in 1996, adopted as the standard method by ASTM, E1758-01, and most recently updated by NREL in 2008. The major differences in the conventional and downscaled methods include (1) the solid–liquid separation step, which is accomplished by centrifugation and decanting in the downscaled version, versus filtration in the conventional method, (2) the lack of mixing in the first hydrolysis step of the downscaled procedure due to the impractical nature of mixing the contents of such small reactors, and (3) the measurement of the entire acid insoluble residue (AcIR) in the downscaled analysis to estimate the lignin content, which unlike the conventional Klason lignin procedure, includes acid insoluble ash.

Carbohydrates and Lignin

For the downscaled system, 1.5 mL high recovery glass HPLC vials that weigh approximately 2,550 mg each (Agilent, Santa Clara, CA) were placed in a muffle furnace (Isotemp Programmable Muffle Furnace, Fisher Scientific, Pittsburg, PA) at 575°C for a minimum of 8 h. After cooling, the vials were weighed and loaded with 3.0 mg (±0.15 mg) of dry *P. deltoides*, bagasse, or switchgrass by a liquid and powder-dispensing robot (Core Module Standard Configuration 2 equipped with Sartorius WZA65-CW balance and ten 25 mL biomass-dispensing hoppers with either 8 or 12 mm openings, Symyx Technologies, Sunnyvale, CA). In regard to the robotics platform, it should be mentioned that the biomass-dispensing hoppers did not require significantly more material than the target amount to be dispensed, and that the Sartorius balance featured a readability of 0.01 mg and a repeatability of 0.03 mg. After vial weighing and biomass addition steps, 30 μL of 72% H_2SO_4 was pipetted manually into all vials (10–100 μL pipetter, Eppendorf, Hamburg, Germany). Then, the vials were held at 30°C (±0.5°C) on the Core Module's platform for 60 min, followed by removing them from the heat source and manually pipetting 840 μL of deionized (DI) water (100–1,000 μL pipetter, Eppendorf). Nine independent replicates were prepared for all three biomasses.

A set of glucose and xylose sugar recovery standards (SRS) was also prepared to support the correction for losses due to sugar degradation during the second hydrolysis stage

(Sluiter et al., 2008). The glucose and xylose concentrations of the three standards ranged from 1.8 to 7.6 and 1.0 to 4.5 g/L, respectively. For each, 300 μL of standard, 540 μL of DI water, and 30 μL of 72% H_2SO_4 were manually pipetted into 1.5 mL high recovery vials.

The vials containing biomass and those containing the SRS were placed in an aluminum reactor block (Symyx Technologies) that could house forty-eight 1.5–2 mL glass vials. The block was then clamped between two aluminum plates with a flat Silicone gasket (thickness 1.5875 mm, durometer hardness A40), and the resulting sandwich was clamped together using four ¼ inch-20 threaded bolts (6.35 mm-20) placed in each corner of the plate, thereby sealing all of the vials. The technique was used to save time as compared to closing each vial individually and has been successfully applied in similar cases that involve much higher absolute and differential pressures between the inside of the reaction vessel and the surrounding environment (Studer et al., 2010). The reactor block was then placed in an autoclave (HA-MII Hirayama, Westbury, NY) and held at 121°C for 60 min.

After allowing the vials to cool to room temperature, the reactor block was opened. A sealing tape (Nunc, Rochester, NY) was secured to the top of all vials, and the entire reactor block was centrifuged (CS-6R Centrifuge, Beckman, Fullerton, CA) for 10 min at a force of 4,200*g* using a 96-well plate carrier adaptor (Microplate carriers SX4750, VWR International, West Chester, PA). In contrast to the conventional technique, the downscaled method uses centrifugation and decanting because the filtration and weighing of such small amounts of residue are not practical. After spinning down, the sealing tape was removed, and up to 840 μL of the SRS as well as of the hydrolyzate supernatant was transferred to 2 mL polypropylene (PP) centrifuge tubes (Safe-Lock 2.0 mL test tubes, Eppendorf), while the acid insoluble residues were left in the glass HPLC vials. The liquid hydrolyzates were neutralized in the centrifuge tubes by adding $CaCO_3$ in steps until the pH was between 5 and 6 (using approximately 50 mg of $CaCO_3$ in total), as monitored by pH indicator strips (EMD Chemicals, Gibbstown, NJ) and as described in more detail by Sluiter et al. (2008). Tubes were then centrifuged (5415D, Eppendorf) for 5 min at 18,200*g*, and 300 μL supernatant of the neutralized hydrolyzate was transferred to a 0.5 mL PP 96-well plate (Agilent) for HPLC analysis.

The acid insoluble residues remaining in the glass vials were washed two times by centrifugation and re-suspension with 1 mL of DI water for each wash, with centrifugation at 4,200*g* for 10 min. Afterwards, the reactor block containing vials with washed residues was placed in an oven set to 105°C for at least 36 h. Then, the vials were removed from the oven, allowed to cool in a desiccator, and weighed by the Core Module to determine the final acid insoluble residue (AcIR) content.

Additionally, the entire procedure described above was also performed manually for *P. deltoides* without the use of a robotics platform but instead employed a laboratory microbalance for weighing (MX5, Mettler Toledo, Columbus, OH). The MX5 balance featured a readability of 0.001 mg, and at a load of 0–2 g, a repeatability of 0.0008 mg. To facilitate manual weighing of 3 mg of biomass, a specially made aluminum weighing beaker that held a volume corresponding to the target mass was used to scoop biomass into vials (Studer et al., 2010).

Determination of Ash Content

To determine the ash content, 15 ± 0.75 mg of biomass was weighed out by the Symyx Core Module into previously dried (at 575°C) 1.5 mL glass HPLC vials. Next the vials were placed in a muffle furnace and heated to 575°C following the Furnace Temperature Ramp Program described elsewhere (Sluiter et al., 2008). Then, the vials were removed, allowed to cool in a desiccator, and weighed by the Core Module. The ash content was calculated as the difference in weight between the vial plus ash, minus the weight of the empty vial, all divided by the original dry weight of the sample and multiplied by 100. The downscaled ash procedure was also performed manually for *P. deltoides* using a laboratory microbalance for weighing (MX5, Mettler Toledo).

Conventional Procedures

The conventional procedure to determine sugar, AcIR, and Klason lignin content was performed following the NREL Laboratory Analytical Procedure "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter et al., 2008) using 300 mg material per test, while the NREL Laboratory Analytical Procedure "Determination of Ash in Biomass" (Sluiter et al., 2005) was used to determine the ash content with 500–2,000 mg material per test.

Sugar Analysis

Sugar concentrations were measured by HPLC (Agilent 1200 Series RI detector) using an Aminex HPX-87H column (BioRad, Hercules, CA) heated to 65°C and using 0.005 M sulfuric acid as the eluent. However, since sample hydrolyzates were neutralized, an Aminex HPX-87P column could also have been used for the measurement of additional sugars.

Statistical Analysis

The carbohydrate and lignin/AcIR analysis of each biomass was performed using nine independent replicates for both the conventional and downscaled methods, while the ash analysis was based on six independent replicates. To test whether the two methods produced carbohydrate compositional data that were statistically the same, an equivalence test was performed to compare glucan and xylan results from the conventional and downscaled analyses. Irrelevant

difference criteria were defined to be ±10% of the mean value for glucan and xylan, which was defined based on the composition uncertainties for *P. deltoides* and bagasse reported by NIST at a 95% confidence level. The reported errors of these materials ranged from ±10% for glucan to ±15% for xylan, and were determined by gathering compositional data performed on the NIST materials in a 2001 inter-laboratory study (National Institute of Standards and Technology, 2001). For the current study, a non-paired *t*-test was performed on the measured glucan and xylan data for all three biomasses to determine the corresponding 95% confidence interval (CI), which had to fall within the defined irrelevance range to confirm that the compositional data from the downscaled method were statistically identical to that of the conventional analysis. The glucan and xylan composition data from the downscaled and conventional methods were also compared to reference values for *P. deltoides* and bagasse by testing whether the measured values fell within the NIST-reported 95% CI calculated from the standard deviation of the mean (SDOM) for these standard materials. No error analysis was available for the BESC switchgrass used.

To evaluate AcIR measurements, values determined by the downscaled and conventional methods were compared to one another. To further gage whether AcIR measurements were within the correct range, Klason lignin measurements from the conventional method were evaluated against the reference lignin values. For ash contents, measurements taken by the downscaled and conventional methods were compared to one another and also evaluated against the reference values to assess their ability in estimating the ash content.

Results and Discussion

As demonstrated in Figure 1, we achieved our primary goal of developing a downscaled method that measures virtually identical carbohydrate compositions as standard wet chemistry procedures. The carbohydrate measurements from the downscaled and conventional methods were in good agreement with each other and produced comparable standard deviations. Furthermore, there was no discernible trend to suggest that the downscaled method consistently resulted in over- or under-estimation of the composition as compared to the conventional method. To compare the results produced by both methods, relative differences will be reported in percent with the conventional measurements serving as the reference. Accordingly, the average glucan content determined by the downscaled method was slightly lower than the value determined by the conventional method for bagasse and *P. deltoides*, producing a 0.8% and 3.6% lower glucan content, respectively. However, for switchgrass, the glucan content measured by the downscaled method was 3.8% higher than the conventional result. For xylan, the values resulting from the downscaled method were slightly higher than what was determined by the

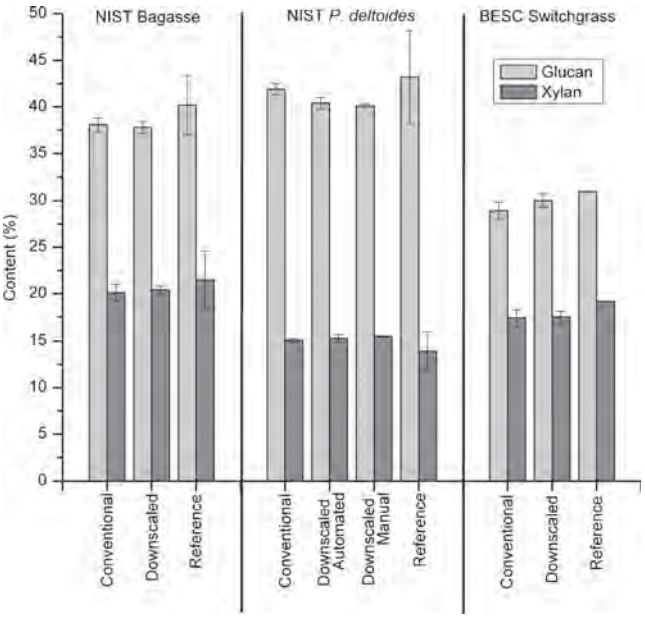

Figure 1. Glucan and xylan contents of bagasse, *P. deltoides*, and switchgrass, determined by the downscaled and the conventional methods, along with the reference values. The compositions are displayed as mass percent, and the error bars represent the uncertainty at the 95% confidence level as reported by NIST for the reference materials and calculated from the SDOM of nine independent measurements for the downscaled and conventional analyses.

conventional method for all three biomasses. For *P. deltoides*, the xylan content measured by the downscaled method was 1.3% higher than that measured by the conventional method, while it was 1.4% and 0.6% higher for bagasse and switchgrass, respectively.

The carbohydrate data produced by the two methods was further tested for statistical equivalence by comparing a 95% CI of a heteroscedastic *t*-test with the above-defined levels of scientific indifference. For data to be statistically equivalent, the calculated 95% CI had to be smaller and fall within the defined indifference interval. Based on this criterion, the glucan and xylan measurements of all biomass materials tested were statistically identical for the downscaled and conventional analysis methods, as shown in Table I. These results demonstrate that we were able to quantify the carbohydrate contents of lignocellulosic biomasses using the newly introduced downscaled method to obtain identical results as conventional methods.

We also sought to demonstrate that the downscaled procedure can be performed manually. Thus, the composition of *P. deltoides* was also analyzed by performing all steps, including weighing and biomass dispensing, manually. The glucan content measured by the manual downscaled version was 4.3% lower than that measured by the conventional method and was 0.9% lower than the automated downscaled measurement. The xylan content that was measured by the manual downscaled process was 2.7% higher than the conventional measurement and was 1.2% higher than that

Table I. To compare whether glucan and xylan contents measured by the downscaled and conventional methods are the same, the level of scientific indifference calculated from the conventional measurements (outer bracket) and the 95% confidence intervals for a heteroscedastic *t*-test for biomass compositions analyzed by the downscaled method (inner bracket) is shown. Results are considered identical if the 95% confidence interval falls within the defined indifference interval of ±10% of the mean determined by the conventional analysis. Downscaled results include the automated procedure for all biomasses, in addition to the manual procedure for *P. deltoides*.

	NIST bagasse	NIST *P. deltoides*	BESC switchgrass
Glucan			
Automated	[−3.81 [−0.68, 1.41] +3.81]	[−4.19 [−2.39, 0.21] +4.19]	[−2.89 [−2.38, 0.20] +2.89]
Manual		[−4.19 [−2.81, −0.82] +4.19]	
Xylan			
Automated	[−2.01 [−1.44, 0.90] +2.01]	[−1.51 [−0.70, 0.26] +1.51]	[−1.74 [−1.27, 1.10] +1.74]
Manual		[−1.51 [0.17, 0.65] +1.51]	

Results are expressed as follows: [−indifference interval [upper 95% CI, lower 95% CI] +indifference interval].

measured by the automated downscaled method. Thus, the downscaled method can be performed manually to measure statistically identical glucan and xylan contents as the conventional wet chemistry method, which is important for labs that do not have robotics systems available.

To ensure that our measurements were reasonable, we also compared the values measured by conventional and downscaled methods to reference values reported by NIST. Direct comparison between the measured and reference values should not be used as a strict guideline because the latter were obtained using extractive-free material, while values measured in this study were obtained from raw biomass. We did not use extractive-free materials because the small amounts of material that will be available in future applications of the downscaled analysis will not suffice to accomplish an extraction step. Figure 1 shows that the measured carbohydrate values were in agreement with the reference values, as demonstrated by all measurements falling within the 95% CIs of the NIST standards *P. deltoides* and bagasse. For switchgrass, although there was no error analysis available for comparison, both the conventional and downscaled measured values differed from the reference values by <10%, which is in line with the 10–15% uncertainties reported for the NIST *P. deltoides* and bagasse.

After confirming that the downscaled method produced statistically identical carbohydrate measurements to the conventional method, we evaluated the feasibility of applying it to lignin and ash measurements. The lignin content determined by the Klason lignin procedure is a lumped measure that includes all acid insoluble non-sugar and non-ash components. The downscaled analysis, however, does not allow determination of the ash content of the Klason lignin acid insoluble residue because 3 mg of starting material is not sufficient to accurately quantify ash content (as discussed later). Therefore, the acid insoluble residue (AcIR) contents were quantified by both the downscaled and conventional methods, and the resulting values were compared in Figure 2 and Table II. While AcIR contents measured by the downscaled method were 1.1% and 3.5% lower than that measured by the conventional method for switchgrass and *P. deltoides*, respectively, the downscaled measurement for bagasse was 19.8% higher than that from the conventional method. When performed manually, the AcIR content of *P. deltoides* measured by the downscaled procedure was 11.1% higher than that measured by the conventional method. All downscaled AcIR measurements were within a 20% relative error range as compared to conventional AcIR measurements, which is only slightly higher than the errors reported for the lignin values of NIST bagasse (14.9%) and *P. deltoides* (19.5%).

To help gauge whether AcIR contents measured by the downscaled and conventional approaches were within the correct range, we next compared conventionally measured Klason lignin values with reference lignin values reported by NIST and found that all Klason lignin measurements were statistically identical to reference values (Fig. 2 and Table II). Because of this good agreement between reference and

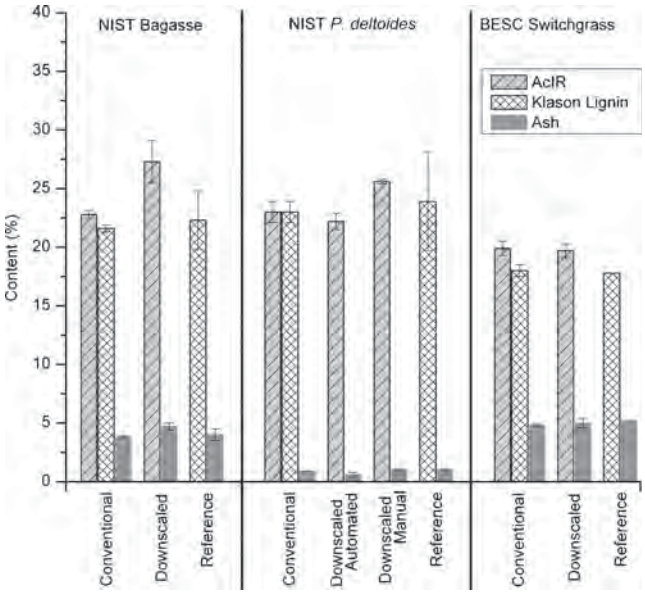

Figure 2. For bagasse, *P. deltoides*, and switchgrass, Klason lignin, acid insoluble residue (AcIR), and ash contents determined by the conventional analysis, AcIR, and ash contents determined by the downscaled analysis, and reference Klason lignin and ash contents are reported. The compositions are displayed as mass percent, and the error bars represent the uncertainty at the 95% confidence level as reported by NIST for the reference materials and calculated from the SDOM of nine independent measurements for the downscaled and conventional AcIR and Klason lignin analyses and six independent measurements for the ash analyses.

Table II. For bagasse, *P. deltoides*, and switchgrass, mean acid insoluble residue (AcIR) contents determined by the conventional and downscaled methods, as well as Klason-lignin (K-lig) contents determined by the conventional method. Values are compared to the reference K-lig values. All data include corresponding 95% confidence intervals as reported by NIST for the reference materials and calculated from the SDOM of nine independent measurements for the downscaled and conventional analyses. Also shown is the mean acid insoluble residue content and corresponding 95% confidence interval for *P. deltoides* as measured by the downscaled method performed manually. Downscaled results include the automated procedure for all biomasses, in addition to the manual procedure for *P. deltoides*.

NIST bagasse				NIST *P. deltoides*					BESC switchgrass			
Downscaled AcIR	Conv. AcIR	Conv. K-lig	Ref. K-lig	Downscaled AcIR		Conv. AcIR	Conv. K-lig	Ref. K-lig	Downscaled AcIR	Conv. AcIR	Conv. K-lig	Ref. K-lig
				Automated	Manual							
27.3 ± 1.8	22.8 ± 0.3	21.6 ± 0.3	22.3 ± 2.5	22.2 ± 0.7	25.6 ± 0.2	23.0 ± 0.9	23.0 ± 0.9	23.9 ± 4.2	19.7 ± 1.6	19.9 ± 0.6	18.0 ± 0.5	17.8

conventionally measured lignin values, we can assume that the conventional measurements of AcIR were an accurate reflection of the biomass' acid insoluble residue content. Furthermore, since we previously determined that conventional and downscaled AcIR measurements were comparable to one another, we can then deduce that the AcIR contents measured by the downscaled analysis were also reasonable. Thus, even though the Klason lignin content cannot be directly measured by the downscaled method, we have shown that this approach can determine the AcIR content of biomass, which can provide meaningful information about lignin content since Klason lignin is just the AcIR minus the acid insoluble ash content, which is a relatively low percentage of biomasses (<10%). For materials that are particularly low in ash, such as woody materials, this AcIR measurement can provide an estimate of the Klason lignin content, while for materials that are high in ash content, such as herbaceous plants, the AcIR measurement will significantly overestimate the Klason lignin content. However, it is also important to keep in mind that the primary purpose of this method is to support a high-throughput screening system from which sample outliers will be selected for further study to undergo more thorough testing, including a detailed analysis of lignin content and composition.

In the process of developing the downscaled Klason lignin method, it was determined that while 3 mg of raw biomass was sufficient to determine carbohydrate and acid insoluble residue contents, this amount was insufficient to determine an accurate ash content for all biomass materials tested (Table III). Reasonable ash values were determined for bagasse and switchgrass using 3 mg of material, but due to the low ash content of *P. deltoides*, this same amount produced erratic ash contents. If only 3 mg of material were used to measure the ash content of a biomass containing 1% ash (such as *P. deltoides*), the balance would have to be capable of accurately weighing 0.03 mg. Although this weight is above the detection limit of the Sartorius balance used in the automated downscaled experiments, it is already at the limit of the balance's repeatability. As a result, to develop a single method that could be used to more accurately estimate the ash content of all biomasses tested, it was established that a minimum mass of 15 mg had to be used, which for a biomass containing 1% ash, now results in an ash weight (0.15 mg) that is 15 times greater than the balance's detection limit and 5 times greater than its repeatability. All values measured by the conventional and downscaled procedures were in the same range as one another, as well as with reference values (Fig. 2 and Table III). The results suggest that 15 mg of material used in the downscaled method is sufficient to estimate the ash content of a diverse set of materials.

In addition to the methods and materials presented here, which are capable of analyzing up to 64 different biomasses in triplicate per run, there are also important opportunities to further simplify and improve it. For example, while our current robotics platform houses ten 25 mL biomass-dispensing hoppers, other Symyx robotics platforms can house up to one hundred forty-four 10 mL hoppers on deck at a time, with an option for additional off-deck hoppers. In this article, we have demonstrated the ability to scale down and automate the compositional analysis procedure, but the

Table III. Mean ash values with corresponding 95% confidence intervals calculated from the SDOM, as determined by the downscaled (3 and 15 mg biomass) and conventional methods (300 mg biomass) compared to reference values. Data from performing the downscaled analysis manually with 15 mg of *P. deltoides* are also listed. Downscaled results include the automated procedure for all biomasses, in addition to the manual procedure for *P. deltoides*.

NIST bagasse				NIST *P. deltoides*					BESC Switchgrass			
Downscaled		Conv.	Ref.	Downscaled			Conv.	Ref.	Downscaled		Conv.	Ref.
				Automated		Manual						
3 mg	15 mg			3 mg	15 mg	15 mg			3 mg	15 mg		
3.9 ± 0.4	4.7 ± 0.3	3.8 ± 0.1	4.0 ± 0.5	−2.0 ± 0.4	0.6 ± 0.2	1.0 ± 0.1	0.9 ± 0.0	1.0 ± 0.1	7.4 ± 1.8	5.0 ± 0.4	4.8 ± 0.1	5.2

degree of automation will depend on the specific robotics platform used. Additionally, the solid and liquid dispensing robot can also be applied to dispense water and acid, as well as decant and transfer liquids. Neutralization with $CaCO_3$ or other bases can also be accomplished with a pH probe that is installed on many laboratory automation systems. Thus, only the autoclaving, centrifugation, and ashing steps need to be performed manually. Furthermore, although we neutralize samples prior to HPLC analysis to provide flexibility in column usage, samples can be directly injected on an Aminex HPX-87H column to eliminate the neutralization step and significantly speed analysis. Conversely, we also demonstrated that the downscaled method works equally well in determining accurate glucan and xylan contents when it is performed manually without the use of a robotics platform. Other possible equipment simplifications include the use of crimp caps instead of the top and bottom plates with a flat gasket, which were also found to seal glass vials during high-temperature hydrolysis (data not shown). Furthermore, to spin down solids in the HPLC vials, these vials could alternatively be inserted into larger centrifuge tubes for centrifugation instead of employing the reactor block and well plate carrier adaptor as reported here.

Besides these variations, the method can also be extended to measure additional biomass components. Once plant material has been broken down in the two-stage hydrolysis into components that can be more easily quantified, the measurement of a wide range of sugars is only limited by the selectivity and resolution of the chromatography column. Finally, the ability to process very small amounts of biomass is not only applicable in determining the composition of raw materials, it can also be very beneficial in following the progress of laboratory-scale hydrolyses or fermentations with solid substrates, for which the small samples available are inadequate for conventional methods.

Conclusions

In support of a high-throughput pretreatment and enzymatic hydrolysis system, a rapid and simple method was developed that can determine statistically identical carbohydrate contents of very small amounts of biomass as conventional wet chemistry procedures and enables the subsequent calculation of sugar yields in screening studies. Furthermore, the method can also measure acid insoluble residue and ash contents to help identify compositional outliers that warrant further study and also identify correlated trends in sugar release data. The result of the modified procedure presented here is a new method that can process a much higher number of samples per time than is possible with the conventional method, and can also dramatically reduce the amount of material that must be sacrificed for compositional analysis.

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