Bioresource Technology 216 (2016) 1077–1082

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Robustness of two-step acid hydrolysis procedure for composition analysis of poplar

Samarthya Bhagia [a,b,c], Angelica Nunez [a,b,1], Charles E. Wyman [a,b,c,*], Rajeev Kumar [b,c]

[a] Department of Chemical and Environmental Engineering, Bourns College of Engineering, University of California Riverside, 900 University Avenue, Riverside, CA 92521, USA
[b] Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, 1084 Columbia Avenue, Riverside, CA 92507, USA
[c] BioEnergy Science Center (BESC), Oak Ridge National Laboratory (ORNL), PO Box 2008 MS6341, Oak Ridge, TN 37831, USA

HIGHLIGHTS

- Compositional analysis was performed in strong acid followed by dilute acid stages.
- Particle size affected only glucan and acid insoluble lignin content.
- Results were unaffected with primary strong acid hydrolysis from 30 to 90 min.
- Secondary hydrolysis of filtered liquor showed results similar to that of slurry.
- Lignin and polysaccharides were completely separated in primary hydrolysis.

ARTICLE INFO

Article history:
Received 11 March 2016
Received in revised form 27 April 2016
Accepted 28 April 2016
Available online 26 May 2016

Keywords:
Compositional analysis
Lignocellulosic biomass
Poplar
Sulfuric acid hydrolysis
Lignin-carbohydrate complex

ABSTRACT

The NREL standard procedure for lignocellulosic biomass composition has two steps: primary hydrolysis in 72% wt sulfuric acid at 30 °C for 1 h followed by secondary hydrolysis of the slurry in 4 wt% acid at 121 °C for 1 h. Although pointed out in the NREL procedure, the impact of particle size on composition has never been shown. In addition, the effects of primary hydrolysis time and separation of solids prior to secondary hydrolysis on composition have never been shown. Using poplar, it was found that particle sizes less than 0.250 mm significantly lowered the glucan content and increased the Klason lignin but did not affect xylan, acetate, or acid soluble lignin contents. Composition was unaffected for primary hydrolysis time between 30 and 90 min. Moreover, separating solids prior to secondary hydrolysis had negligible effect on composition suggesting that lignin and polysaccharides are completely separated in the primary hydrolysis stage.

1. Introduction

The two step acid hydrolysis procedure is the benchmark for measuring the amounts of sugars in the form of cellulose and hemicellulose, and lignin in lignocellulosic biomass feedstocks and accounting for their fate as biomass progresses through pretreatment, enzymatic hydrolysis, fermentation, and other steps of biological processing of biomass (Sluiter et al., 2010; Wyman et al., 2009). Furthermore, because sugar mass balances hinge on accurate compositional analysis, understanding uncertainties inherent in this procedure and factors that affect measured values can be valuable in interpreting the resulting compositional data (Templeton et al., 2010). This procedure has two major steps: 1) strong sulfuric acid hydrolysis at near room temperature followed by 2) a secondary hydrolysis at higher temperature after diluting the primary hydrolysis slurry. The ability of strong sulfuric acid to dissolve crystalline cellulose has been known for over a century. For example, in 1900, Alexander Classen of Aachen, Germany patented a process for sugar recovery in which he added three parts of 55–60° Baumé sulfuric acid (69.65–77.67 wt% H_2SO_4) to one part sawdust by mass, pressed the resulting slurry for half an hour with a hydraulic press, diluted the mixture with four more parts water, and boiled the resulting slurry for another half an hour to release glucose (Classen, 1900). Today, sugar and lignin compositions are determined based on a procedure developed by Professor Johan Peter Klason of Stockholm, Sweden in 1908 in which he added 72% sulfuric acid to biomass isolate what is now

* Corresponding author at: Department of Chemical and Environmental Engineering and Center for Environmental Research and Technology, Bourns College of Engineering, University of California Riverside, 1084 Columbia Ave, Riverside, CA 92507, USA.
E-mail address: cewyman@engr.ucr.edu (C.E. Wyman).
[1] Undergraduate student.

http://dx.doi.org/10.1016/j.biortech.2016.04.138
0960-8524/

known as Klason lignin (Klason, 1908). The National Renewable Energy Laboratory (NREL) in Golden, Colorado, has documented the resulting standard procedure as "Determination of Structural Carbohydrates and Lignin in Biomass" (Sluiter et al., 2008b). The first primary hydrolysis step specifies mixing 0.3 g of biomass with 3 ml of 72 wt% sulfuric acid (4.9 g by weight) at 30 °C for 60 min, followed by dilution with water to reduce the acid concentration to 4%. Next, a secondary hydrolysis step holds the diluted 4% acid slurry at 121 °C for another 60 min, at the end of which, the slurry is filtered through a ceramic crucible. The filtered liquor is neutralized for HPLC analysis for sugars and acetic acid from which the weight fractions of cellulose and hemicellulose in biomass can be calculated. The generally small amount of lignin soluble in the acid solution can be quantified by UV–vis spectrophotometry and is termed acid soluble lignin (ASL). Gravimetric analysis of the solid residue left in the crucible is applied to determine the acid insoluble residue (AIR) and ash content, with the acid insoluble lignin (AIL) calculated as the difference between the mass of the acid insoluble residue and ash.

Although this procedure has been a workhorse for biomass analysis for decades, it is important to understand the method's consistency in measuring the makeup of structural components in lignocellulosic biomass in different sized particles from a single biomass source. It is also important to know how robust this technique is to variations in the primary hydrolysis time from the recommended time of 60 min. Another concern was whether lignin carbohydrate complexes still present after the primary hydrolysis would not be accounted for in the final HPLC measurements, thereby misleading the final results.

2. Materials and methods

The National Renewable Energy Laboratory (NREL), Golden, Colorado, graciously provided BESC (Bioenergy Science Center, Oak Ridge National Laboratory, Oak Ridge TN) standard poplar (BESC STD) chips for this study. These chips were knife milled through a 1 mm screen (Model 4 Wiley Mill Thomas Scientific, Swedesboro NJ) at University of California at Riverside. The moisture content of BESC STD was 7% determined by a halogen moisture analyzer (HB43-S; Mettler Toledo, Columbus OH). No extraction was applied to the poplar prior to analysis because extractives levels in poplar are low and the purpose of this study was to identify how deviations in analysis conditions impacted compositional results for the same material.

Biomass samples were sieved through ASTM E 11 standard sieves (USA Standard Testing Sieve) of mesh sizes 20, 40, 60, 80, and a bottom collection pan that were stacked in that order and shaken by a Ro-Tap® sieve shaker (W.S. Tyler, Model# RX-29) for 30 min. The depth of the sample placed evenly on the number 20 sieve before shaking was less than 7 cm. The sieves prior to loading and after shaking were weighed to calculate weight fraction of biomass collected on each sieve after shaking.

Fig. 1 outlines the standard procedure as well as the experimental strategy employed in this study. To determine the effect of varying particle sizes, the compositions of the biomass collected on each sieve after shaking (+20, −20/+40, −40/+60 and −60/+80), along with the fines (−80 fraction) collected in the bottom pan were individually analyzed. Here symbol "+" represents the fraction retained by a sieve and "−" represents the fraction that passed through a sieve. For example, −20/+40 represents the sample that passed through mesh 20 sieve but retained on mesh 40 sieve. The other conditions were the same as standard NREL procedure. In brief, about 0.3 g biomass was weighed on Fisherbrand® weighing paper (Cat. No. 09-898-12B) on analytical balance (Mettler-Toledo AB54-S, Columbus OH) and transferred into glass conical graduates (Kimble® Kimax® No. 60345, Fisher Scientific, Thermo Fisher Scientific Inc., Waltham MA). Moisture content was 7% for all sieved fractions. The conical graduates were kept in water bath (Cole-Parmer StableTemp Water Bath 20L Item# EW-14575-16, Vernon Hills IL) at 30 °C and stirring rods were put in them and allowed to equilibrate for 10 min. Then, 3 ml of 72% sulfuric acid (lot # R8191600-4A, Ricca Chemical Company through Fisher Scientific, Thermo Fisher Scientific Inc., Waltham MA) was accurately pipetted into the conical graduates. Time was started when sulfuric acid was added to the last conical graduate. Mixing with stirring rods was done every 5 min. At the end of 60 min, the conical graduates were quickly removed from the water bath and the contents diluted with 14 ml water to stop the strong acid primary hydrolysis. This slurry was transferred to 125 ml serum bottles (Wheaton® Cat# 06-406K), and another 70 ml of water was added to the conical graduates and transferred to serum bottles to bring the total to 84 ml. The serum bottles were then sealed and autoclaved (Model HA-300MII, Hirayama Manufacturing Corp., Tokyo, Japan) at 121 °C for 60 min. After the autoclave cooled down to 40 °C, bottles were removed and filtered through crucibles of known weight. Samples of the filtered liquor were collected for HPLC analysis after which more DI water was added to each bottle to recover solids left in the bottles. The crucibles were dried at 105 °C for 24 h and then allowed to cool down in a desiccator prior to being weighed. Next, these crucibles were then placed in a muffle furnace (Thermo Fisher Scientific Inc., Isotemp® Programmable Muffle Furnace, Model 750) and ashed using the ramp-up program mentioned in the NREL procedure (Sluiter et al., 2008b). The filtered liquor without neutralization was analyzed for sugars and acetic acid on Waters® e2695 Separations Module with detection on Waters® 2414 RI detector (Waters Corp., Milford MA). Bio-Rad® Aminex® HPX-87H column conditioned at 65 °C was used for all separations using 5 mM sulfuric acid mobile phase at a flow rate of 0.6 ml/min (Sluiter et al., 2008a). Calibration of cellobiose, glucose, xylose and acetic acid was done according to the concentrations prescribed in the standard NREL procedure. The fractions of glucose and xylose left after secondary hydrolysis of sugar recovery standards were 0.95 and 0.9, respectively. Neutralization of secondary hydrolysis liquor was unnecessary in that the Aminex HPX-87H column uses a 5 mM sulfuric acid mobile phase and poplar contains negligible amount of galactose that could otherwise interfere with the xylose retention time.

For experiments to study effect of primary hydrolysis time, the same batch of biomass that had been knife-milled through 1 mm screen was used. However, each of the particle size ranges (+20, −20/+40, −40/+60 and −60/+80, and −80) were mixed in equal parts, i.e., the contribution of each of the sizes to our "mixed" poplar was one-fifth of the total weight. Primary hydrolysis of mixed poplar was performed for 30, 45, 60, 75 and 90 min at 30 °C in exactly the same way as for the varying particle size experiments.

The third leg of this study used the same mixed poplar for varying primary hydrolysis times. Primary hydrolysis was carried out at three different times: 30, 60, and 90 min. After primary hydrolysis, the liquor was diluted quickly similar to the other experiments. After dilution to 4% acid concentration, approximately 10 ml of liquor was filtered through crucible. A 0.5 ml aliquot was withdrawn from each of the filtered samples and kept for HPLC analysis. The rest of the 10 ml filtered liquor was autoclaved at 121 °C for 60 min. The liquors after autoclaving were centrifuged (Eppendorf® Microcentrifuge Model 5424, Eppendorf North America, Hauppauge, NY) at 15000 rpm for 5 min, followed by HPLC analysis similar to the other experiments.

Acid soluble lignin content was determined in the liquors after secondary hydrolysis in 96 well plate (Corning® UV-Transparent Microplate 3635) in triplicates for each sample with 0.3 ml in each

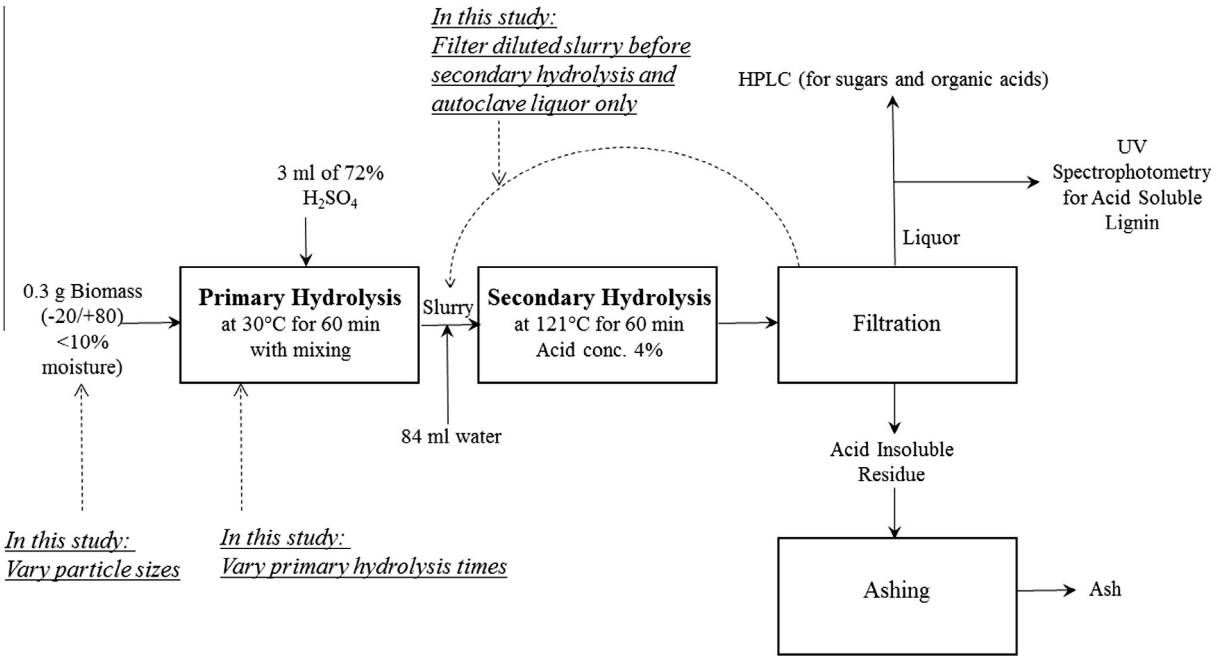

Fig. 1. Experimental design.

well through Spectramax® M2e Plate Reader (Molecular Devices, Sunnyvale, CA, USA) equipped with SoftMax® Pro data acquisition software. Concentration of acid soluble lignin was calculated by the Beer-Lambert-Bougeur's Law based on absorption coefficient of 25 L/g cm at 240 nm (Sluiter et al., 2008b).

Three replicates were kept for each condition and all calculations were done following the standard NREL procedure (Sluiter et al., 2008b). Standard deviations were calculated using in-built functions in Microsoft® Office® Excel® Professional 2013. The overall average value represents arithmetic mean of values found for each condition. The standard deviation associated with overall average was calculated through square root of summation of variances for each of the values.

3. Results and discussion

3.1. Effect of particle size

The mass distribution of particle sizes after milling through 1 mm screen were as follows: 4.16% between 1 and 0.850 mm (+20), 53.09% between 0.850 and 0.425 mm (−20/+40), 29.99% between 0.425 and 0.250 mm (−40/+60), 4.81% between 0.250 and 0.180 mm (−60/+80), and 7.96% less than 0.180 mm (−80). Fig. 2A and B show the composition of each of these fractions as determined by the standard compositional analysis procedure, while Table 1 reports the values along with standard deviations for the data presented in these graphs. Fig. 2A shows only about a 1% deviation in measured glucan content (as weight percent of biomass on a dry basis) from an average value of 45.2% for particle sizes ranging from 1 mm to 0.250 mm (1 mm to +60 However, the average glucan content dropped significantly to 39% for a particle size less than 0.250 (−60 and less). On the other hand, acid insoluble lignin (AIL) measurements rose continuously from 21.83% to 25.90% with decreasing particle size. Because the ash content in this poplar was too low to be measured accurately, the contents of acid soluble lignin and acid soluble residue were pretty much the same.

The high acid insoluble lignin content in the −60/+80 and −80 sizes can be due to formation of humic substances through glucose degradation as glucan% was lower in these two sizes (Hu et al., 2012). As shown in Table 1, the overall average AIL was 24% with 1.34% standard deviation. The data in Table 1 and Fig. 2 indicates no meaningful trend in xylan content with variation in particle size. Although a xylan content of 14% was measured for the +20 fraction, xylan levels increased to about 15% in the −20/+60 range, dropped back to 14% in −60/+80 fraction, and increased to 15% in the −80 fines. Thus, the difference between the lowest and largest values for xylan content was only 1.5% while the overall average was 14.5% with 0.8% standard deviation (Table 1). The deviations in xylan content seem to be due to experimental error rather than particle size and was also lower than the deviations measured in glucan content. Fig. 2B shows that neither the acid soluble lignin (ASL) nor acetate content were affected by varying the particle size, with an overall average of 3.87% with only a 0.1% deviation for ASL and 3.4% with a 0.35% standard deviation for acetate. In summary, this data indicates if a substantial portion of biomass has particle sizes below 0.250 mm, measurements of glucan and acid insoluble lignin may not truly representative of the overall biomass composition of poplar. Furthermore, it could prove valuable to check the particle size distribution if sugar mass balances cannot be closed or lignin removal seems lower than expected. Also, the strong sulfuric acid concentration could be reduced to determine if this change affects measurements of glucan concentrations.

3.2. Effect of primary hydrolysis time

Since 83% of the milled biomass had particle sizes in the range of 0.850–0.250 mm, the effect of particle sizes outside this range might not be apparent over the primary hydrolysis time. For example, particles sizes less than 0.250 mm (−60) might appear to have lower sugar contents due to greater degradation than larger sizes when subjected to long primary hydrolysis times. Therefore, the composition was measured of a mixture of equal portions by mass of each of the size ranges (1 mm to +20, −20/+40, −40/+60, −60/+80, and less than −80) for primary hydrolysis times ranging from 30 to 90 min. Secondary hydrolysis after primary hydrolysis was done according to the conventional NREL method. Surprisingly, Fig. 2C and D and Table 2 show no significant differences in the amounts of glucan, xylan, AIL, ASL, and acetate measured. This result shows that this procedure is robust with respect to primary

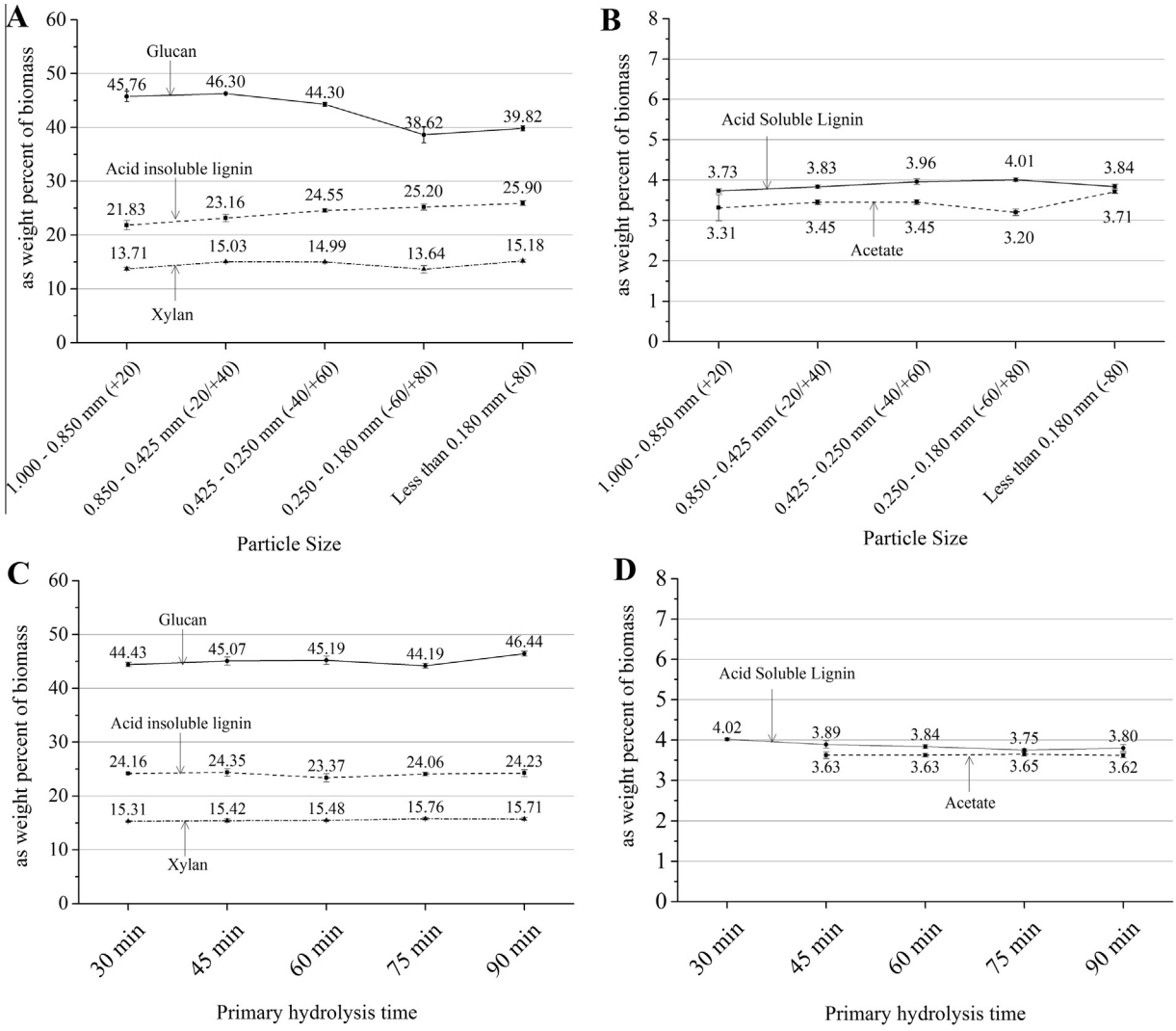

Fig. 2. Effect of particle size and primary hydrolysis time on composition. (A) Shows glucan, xylan and AIL contents whereas (B) shows acetate and ASL contents with variation in particle size. (C) Shows glucan, xylan and AIL contents whereas (D) shows acetate and ASL contents with variation in primary hydrolysis time. Error bars represent standard deviation from three replicates.

Table 1
The composition of different particle size fractions produced by milling and screening poplar.

Particle size range	Glucan%		Xylan%		Acetate%		ASL%		AIL%	
	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.
1.000–0.850 mm (+20)	45.48	0.96	13.71	0.29	3.31	0.32	3.73	0.05	21.83	0.87
0.850–0.425 mm (−20/+40)	46.03	0.18	15.03	0.09	3.45	0.06	3.83	0.04	23.16	0.67
0.425–0.250 mm (−40/+60)	44.01	0.34	14.99	0.07	3.45	0.06	3.96	0.07	24.55	0.26
0.250–0.180 mm (−60/+80)	38.34	1.48	13.64	0.68	3.20	0.08	4.01	0.05	25.20	0.56
Less than 0.180 mm (−80)	39.54	0.46	15.18	0.26	3.71	0.04	3.84	0.05	25.90	0.45
Overall average	42.68	1.86	14.51	0.79	3.42	0.35	3.87	0.12	24.13	1.34

hydrolysis over times that were well below and well above the recommended 60 min. Table 2 shows that overall compositional averages were similar for all components measured over the range of primary hydrolysis times applied to those for the varying particle size experiments other than for the glucan content measured for particle sizes less than 0.250 mm.

3.3. Filtration of slurry before secondary hydrolysis

In the third leg of this study, a 4% primary hydrolysis slurry produced at times of 30, 60, and 90 min was filtered before subjecting it to secondary hydrolysis. However, unlike the brittle solids produced by secondary hydrolysis that are easy to separate in accordance with the conventional procedure, filtering the 4% slurry from primary hydrolysis was so slow due to formation of lignin sludge on the crucible that only 10 ml of 4% primary hydrolysis slurry was filtered. Thus, it was seen that secondary hydrolysis significantly changed the physical nature of lignin such that the AIL content could not be measured. Before subjecting the filtered liquor to secondary hydrolysis, samples taken in aliquots were analyzed by HPLC and UV–vis spectrophotometry to determine how the fraction of monomeric versus oligomeric sugars after dilution

Table 2
The composition of poplar as measured after application of primary hydrolysis times from 30 to 90 min followed by secondary hydrolysis according to standard protocols. The standard primary hydrolysis time is 60 min.

Primary hydrolysis time	Glucan%		Xylan%		Acetate%		ASL%		AIL%	
	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.
30 min	44.15	0.39	15.31	0.08	N.D.[a]	N.D.	4.02	0.03	24.16	0.19
45 min	44.79	0.73	15.42	0.32	3.63	0.08	3.89	0.09	24.35	0.71
60 min	44.91	0.77	15.48	0.15	3.63	0.04	3.84	0.05	23.37	0.74
75 min	43.91	0.47	15.76	0.23	3.65	0.05	3.75	0.03	24.06	0.33
90 min	46.16	0.46	15.71	0.28	3.62	0.05	3.80	0.09	24.23	0.65
Overall average	44.78	1.31	15.54	0.52	3.63	0.12	3.86	0.14	24.03	1.27

[a] N.D. Not Detemined.

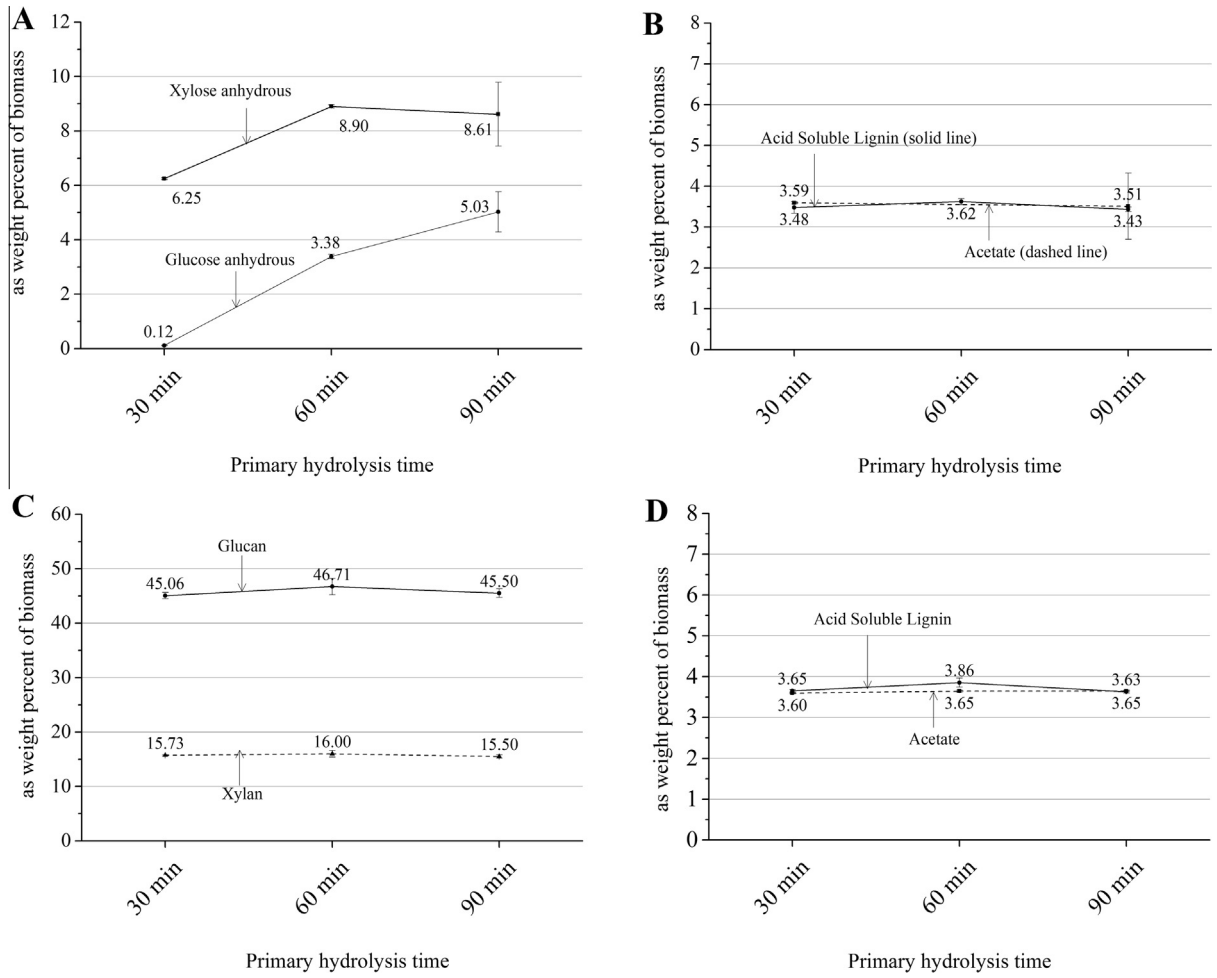

Fig. 3. Effect of filtration of diluted primary hydrolysis slurry before secondary hydrolysis on composition. (A) Shows glucan and xylan contents in monomeric form, and (B) shows acetate and ASL contents in diluted primary hydrolysis filtered liquor without secondary hydrolysis for three primary hydrolysis times. (C) Shows glucan and xylan, whereas (D) shows acetate and ASL for the same filtered liquors but after secondary hydrolysis for three primary hydrolysis times. Error bars represent standard deviation from three replicates.

of primary hydrolysis liquor was affected by different primary hydrolysis times. The results in Fig. 3A (glucan and xylan) and 3B (acetate and ASL), as well as Table 3, show that the amount of monomeric glucan was negligible at the end of 30 min, rose to 3% after 60 min, and increased further to 5% by 90 min. However, 6% of the 16% total xylan content was monomeric at the primary hydrolysis time of 30 min but increased to about 9% in 60 and 90 min. Thus, monomeric xylan plateaued after 60 min, while monomeric glucan kept increasing over the entire time period considered. At the primary hydrolysis time of 60 min specified in the standard procedure, about 9% of xylan and only 3% of glucan was monomeric with the rest oligomers. Because the acetate content stayed at the same value of about 3.5% without secondary hydrolysis, as shown in Fig. 3B, the first 30 min of primary hydrolysis is sufficient to de-acetylate all of the hemicellulose. The figure also shows that acid soluble lignin (ASL) was slightly lower (0.2%) when analyzed after primary hydrolysis than after secondary hydrolysis, likely due to formation of sugar dehydration products in secondary hydrolysis that absorb the same wavelength range of ASL compounds.

Fig. 3C and D show results from subjecting the liquor that was filtered from the solids after primary hydrolysis of the 4% slurry to

Table 3
Composition of filtered and diluted primary hydrolysis liquors before and after application of the standard secondary hydrolysis procedure.

	Primary hydrolysis time	Glucan%		Xylan%		Acetate%		ASL%	
		Value	Std. dev.	Value	Std. dev.	Value	Std. dev.	Value	Std. dev.
Before secondary hydrolysis	30 min	0.12	0.01	6.25	0.03	3.59	0.03	3.48	0.14
	60 min	3.38	0.08	8.90	0.06	–	–	3.62	0.07
	90 min	5.03	0.74	8.61	1.18	3.51	0.81	3.43	0.05
After secondary hydrolysis	30 min	44.78	0.57	15.73	0.11	3.60	0.01	3.65	0.03
	60 min	46.44	1.50	16.00	0.62	3.65	0.02	3.86	0.11
	90 min	45.22	0.80	15.50	0.33	3.65	0.01	3.63	0.04

secondary hydrolysis, while Table 3 presents the values and standard deviations for these results. No significant difference was found in glucan, xylan, acid soluble lignin, and acetate contents whether filtration was done before or after secondary hydrolysis. The overall averages of glucan, xylan, ASL, and acetate contents listed in Table 3 are similar to the measurements for the different particle sizes and 3 primary hydrolysis times. Thus, the primary concentrated sulfuric acid hydrolysis step can completely break cellulose and hemicellulose away from the lignin in poplar even for times as low as 30 min.

4. Conclusions

Compositional analysis of poplar particle sizes smaller than 0.250 mm showed significantly lower glucan content and higher acid insoluble lignin content than larger particle sized samples while xylan, acetate, and acid soluble lignin measurements were unaffected. Varying the primary hydrolysis time from 30 to 90 min had little effect on composition values of mixed poplar. Compositional results for mixed poplar were similar when only diluted primary hydrolysis filtered liquor was subjected to secondary hydrolysis instead of diluted primary hydrolysis slurry. Furthermore, primary hydrolysis completely separated polysaccharides from lignin, while secondary hydrolysis depolymerized sugar oligomers to monomers.

Acknowledgements

This work was supported by the Office of Biological and Environmental Research in the Department of Energy (DOE) Office of Science through the BioEnergy Science Center (BESC) at Oak Ridge National Laboratory (Contract DE-PS02-06ER64304). Stipend for undergraduate research was awarded by UCR Hispanic Serving Institutions (HSI) Undergraduate Research Program through the U. S. Department of Education. We thank Daniel Lee, Department of Chemical and Environmental Engineering at UCR for assistance in milling of poplar used in this study. We also acknowledge the Center for Environmental Research and Technology (CE-CERT) of the Bourns College of Engineering for providing the facilities and the Ford Motor Company for funding the Chair in Environmental Engineering that facilitates projects such as this one.

References

Classen, A., 1900. Process of Converting Cellulose into Sugar, US654518.
Hu, F., Jung, S., Ragauskas, A., 2012. Pseudo-lignin formation and its impact on enzymatic hydrolysis. Bioresour. Technol. 117, 7–12.
Klason, P., 1908. Chemical composition of deal (Fir wood). Ark. Kemi. Mineral. Geol. 3, 1–10.
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 Laboratory Analytical Procedure, NREL/TP-510-42623.
Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008b. Determination of Structural Carbohydrates and Lignin in Biomass Laboratory Analytical Procedure, NREL/TP-510−42618.
Sluiter, J.B., Ruiz, R.O., Scarlata, C.J., Sluiter, A.D., Templeton, D.W., 2010. Compositional analysis of lignocellulosic feedstocks. 1. Review and description of methods. J. Agric. Food Chem. 58 (16), 9043–9053.
Templeton, D.W., Scarlata, C.J., Sluiter, J.B., Wolfrum, E.J., 2010. Compositional analysis of lignocellulosic feedstocks. 2. Method uncertainties. J. Agric. Food Chem. 58 (16), 9054–9062.
Wyman, C.E., Dale, B.E., Elander, R.T., Holtzapple, M., Ladisch, M.R., Lee, Y.Y., Mitchinson, C., Saddler, J.N., 2009. Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. Biotechnol. Prog. 25 (2), 333–339.