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Analysis of Biomass Sugars Using a Novel HPLC Method

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Abstract

The precise quantitative analysis of biomass sugars is a very important step in the conversion of biomass feedstocks to fuels and chemicals. However, the most accurate method of biomass sugar analysis is based on the gas chromatography analysis of derivatized sugars either as alditol acetates or trimethylsilanes. The derivatization method is time consuming but the alternative high-performance liquid chromatography (HPLC) method cannot resolve most sugars found in biomass hydrolysates. We have demonstrated for the first time that by careful manipulation of the HPLC mobile phase, biomass monomeric sugars (arabinose, xylose, fructose, glucose, mannose, and galactose) can be analyzed quantitatively and there is excellent baseline resolution of all the sugars. This method was demonstrated for standard sugars, pretreated corn stover liquid and solid fractions. Our method can also be used to analyze dimeric sugars (cellobiose and sucrose).

Index Entries: Biomass hydrolysates; biomass sugars; glucose; HPLC analysis; xylose.

Introduction

The analysis of lignocellulosic biomass has been investigated extensively in the past, but methods vary depending on the feedstocks and the end application. The pulp and paper industry uses the TAPPI standards for hardwood and softwood analysis *(1)*; the forage industry uses the acid and

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neutral detergent methods *(2)*; and the biomass industry uses ASTM standards or combinations thereof *(3,4)*. The ASTM standards were based on the round robin tests conducted in the 1990s on standard NIST biomass reference samples *(5)*. These methods are based on 72% H_2SO_4 hydrolysis and conversion of the sugars to alditol acetates followed by gas chromatography (GC) analysis. The GC method is particularly accurate for low sugar concentrations; however, sample preparation is time consuming.

The alternative sugar analysis method is based on the high-performance liquid chromatography (HPLC) analysis of the acid hydrolysates *(3,6)*. Although this method is relatively fast, it is less sensitive for low sugar concentrations especially when the refractive index detector is used. The pulsed amperiometric detector improves on the detection limits, but suffers from the poor column resolution of the sugar peaks. Another major problem with the HPLC method of sugar analysis is the poor stability of the lead carbohydrate column. Column regeneration is challenging and sample preparation is time consuming. Furthermore, the resolution of sugars such as fructose and mannose and the detection of dimmers are poor. Both the HPLC and GC methods are primary methods.

There has also been extensive research to develop rapid analytical methods such as molecular beam mass spectrometry *(7)*, near-infrared coupled with principal component analysis *(8,9)*, and thermogravimetric analytical method *(10)*. The rapid methods require primary analytical methods to determine values of the sugars for their calibration. Thus, there is a need for the development of a rapid, robust, primary analytical method for the analysis of biomass sugars. In this paper, we present results for the analysis of standard sugars and sugars in pretreated corn stover liquid and solid fractions using a new and improved HPLC method of biomass sugars analysis.

Materials and Methods

The standard sugar samples arabinose, xylose, fructose, mannose, galactose, glucose, sucrose, and cellobiose were acquired from Sigma-Aldrich (St. Louis, MO). Corn stover hydrolysates (liquid fraction), and solid residues were supplied by the National Renewable Energy Laboratory (NREL), Golden, Colorado. The liquid fraction consisted of two groups of samples (P031118CS group and P031209CS group) with different corn stover pretreatment conditions as shown in Tables 1 and 2.

Prevail carbohydrate ES column (250 × 4.6 mm) packed with 5-μm spherical polymer beads coated with proprietary bonding material was used for the HPLC analysis. The precolumn cartridge (7.5 × 4.6 mm) and the analytical column were obtained from Alltech Associates Inc (Deerfield, IL).

HPLC-grade acetonitrile and water mixture was used as mobile phase, and a Shimadzu HPLC 10 AVP instrument (Shimadzu Scientific, Colombia, MD) was used for the analysis. A Shimadzu low-temperature evaporative light-scattering detector (ELSD-LT) and Shmadzu refractive index

Table 1
Sugar Concentrations in Pretreated Corn Stover Liquid Fractions for P031118CS Group of Samples

P031118CS(#)	1	2	3	4	5	6
Component	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)
Arabinose	6.60 ± 0.33	12.46 ± 0.28	10.28 ± 0.11	9.70 ± 0.13	6.83 ± 0.07	7.84 ± 0.27
Xylose	50.56 ± 0.04	65.46 ± 0.11	52.98 ± 0.16	56.96 ± 0.07	49.68 ± 0.09	52.07 ± 0.09
Fructose	5.49 ± 0.05	4.42 ± 0.04	2.35 ± 0.03	4.02 ± 0.08	1.23 ± 0.06	2.68 ± 0.03
Mannose	4.61 ± 0.84	4.28 ± 0.31	3.57 ± 0.44	2.99 ± 0.66	2.77 ± 0.09	3.71 ± 0.30
Galactose	5.21 ± 0.74	7.33 ± 1.12	3.35 ± 0.42	6.27 ± 1.46	3.45 ± 0.44	2.75 ± 0.30
Glucose	18.95 ± 0.12	24.55 ± 0.10	20.21 ± 0.10	20.79 ± 0.06	27.30 ± 0.10	22.97 ± 0.06
Sucrose	0.01 ± 0.00	0.15 ± 0.01	0.14 ± 0.03	0.22 ± 0.01	0.03 ± 0.00	0.05 ± 0.01
Cellobiose	0.03 ± 0.00	0.30 ± 0.01	0.31 ± 0.03	0.44 ± 0.01	0.03 ± 0.00	0.08 ± 0.01
	Pretreatment reaction conditions					
Temperature (°C)	180	180	190	190	200	200
Acid loading (%)	0.045	0.045	0.045	0.045	0.06	0.045
Solids loading (%)	30	35	30	25	25	25
Acid concentration (%)	1.90	2.39	1.91	1.59	1.98	1.49
Severity	4.5	5.6	5.1	4.2	5.8	4.4

The errors are expressed as standard deviations on three replicates.

Table 2
Distribution of Sugar Components in Pretreated Corn Stover Liquid Fraction for P031209CS Group of Samples

P031209CS (#) series	1	2	3	4	5	6	7
Component	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)	Conc (mg/mL)
Arabinose	7.052 ± 0.34	6.46 ± 0.13	8.66 ± 0.43	8.99 ± 0.25	6.48 ± 0.29	8.63 ± 0.26	6.90 ± 0.35
Xylose	39.71 ± 0.34	45.24 ± 0.04	49.39 ± 0.23	58.82 ± 0.03	48.75 ± 0.10	58.00 ± 0.21	28.16 ± 0.14
Fructose	0.50 ± 0.03	1.09 ± 0.01	5.12 ± 0.05	3.59 ± 0.02	3.24 ± 0.05	2.89 ± 0.03	8.16 ± 0.02
Mannose	2.61 ± 0.20	2.55 ± 0.23	3.29 ± 0.10	4.32 ± 0.58	2.60 ± 0.19	5.23 ± 0.78	3.59 ± 0.28
Galactose	2.64 ± 0.29	3.02 ± 0.12	4.60 ± 0.21	5.49 ± 0.69	3.78 ± 0.60	6.12 ± 0.93	2.87 ± 0.17
Glucose	38.31 ± 0.04	26.77 ± 0.09	20.60 ± 0.07	24.23 ± 0.10	18.53 ± 0.03	23.91 ± 0.19	12.68 ± 0.07
Sucrose	0.05 ± 0.00	0.01 ± 0.00	0.04 ± 0.01	0.11 ± 0.02	0.04 ± 0.00	0.14 ± 0.02	0.04 ± 0.00
Cellobiose	0.04 ± 0.00	0.02 ± 0.00	0.07 ± 0.00	0.37 ± 0.02	0.04 ± 0.00	0.43 ± 0.02	0.06 ± 0.01
Pretreatment reaction conditions							
Temperature (°C)	200	200	200	190	190	180	180
Acid loading (%)	0.06	0.045	0.03	0.045	0.048	0.06	0.03
Solids loading (%)	35	30	30	30	25	30	25
Acid concentration (%)	3.16	1.92	1.28	1.91	1.59	2.53	0.99
Severity	9.3	5.7	3.8	5.1	4.2	6.0	2.3

The errors are expressed as standard deviations on three replicates.

detector (RID) were used for compound detection. The ELSD-LT was operated at 40°C, 250 kPa and air was used as the nebulizing gas. The compounds in the unknown mixture were identified by comparing their retention times with those of authentic standards analyzed under similar conditions. The samples were also spiked to further confirm the identity of the compounds. The sample data were acquired and quantified using Shimadzu CLASS-VP 7 chromatographic analysis software.

Standard Sugars Analyses and Calibration Curve

A four-point calibration curve was developed for each of the following sugars: arabinose, xylose, glucose, mannose, galactose, sucrose, and cellobiose. The external calibration curves were developed by analyzing concentrations of 1.5 mg/mL, 2.5, 5 mg/mL, 10 mg/mL, and 20 mg/mL of standard sugar solutions of arabinose, xylose, fructose, mannose, galactose, and glucose. For the dimeric sugars, sucrose and cellobiose, the concentrations were 0.0625, 0.125, 0.25, 0.5, and 1 mg/mL.

About 2 mL of standard sugar solutions were filtered through a 0.25-µm syringe filter into auto-sampling vials and loaded into the Shimadzu SIL-10AXL auto-injector. The mobile phase consisted of 100% deionized and degassed water (pump A) and 100% degassed acetonitrile (pump B). For the monomeric sugars, the samples were analyzed in the isocratic mode while for mixtures of monomers and dimmers the gradient mode was more suitable.

In the isocratic mode, the acetonitrile (85%) and water (15%) were pumped at a total flow rate of 1.0 mL/min. The sample injection volume was 10 µL, the run time was 30 min, the oven temperature was 30°C, the ELSD-LT detector temperature was 40°C, and the pressure was 250 kPa.

For the simultaneous analysis of the monomeric and dimeric sugars, the analysis time was extended to 45 min. In this case, the flow rate was constant at 1 mL/min for 25 min and the mobile phase composition was kept constant at 85/15 ratio (acetonitrile/water ratio). At the 25th minute, the mobile phase flow was changed to the gradient mode. That is, the water content was increased from 15% to 35% from 25 min to 35 min. After that, the water content was decreased from 35% to 15% over 10 min. Thus, total analysis time was 45 min. Calibration curves were obtained by plotting log area vs log concentration.

Analysis of Pretreated Corn Stover Liquid Fraction

The NREL pretreated corn stover liquid fractions (liquid fraction) had pH 2.0. About 2 mL of the liquid fractions were measured into a 2.5-mL microcentrifuge tubes, centrifuged at 10,000*g* for 10 min, and then decanted. The decanted samples were filtered through 0.25-µm syringe filter into 2-mL auto-sampling vials. The samples (5 µL) were injected unto the column using the Shimadzu SIL-10AXL auto-injector. Note that the pHs of the NREL liquid samples were not adjusted with any base before the analysis.

This was because we determined previously that the results for neutralized and nontreated samples were similar and therefore this step was eliminated, and thus simplifying the analysis. Further, the Prevail carbohydrate ES column can be operated using a mobile phase pH range of 2.0 to 13.0.

Because the liquid fraction samples contained both monomers and dimmers, the analysis was conducted as that described above for the standard mixture of monomers and dimmers. To compensate for any variations in the HPLC column and detector conditions during the analysis, standard mixtures containing seven sugars at three concentration levels were analyzed simultaneously with the unknown samples. All samples were analyzed in triplicate.

Analysis of Pretreated Corn Stover Solid Fraction

The hydrolysis of the NREL pretreated corn stover solid fraction (solid fraction) was carried out according to ASTM E1721-01 *(11)*. The filtrate obtained from this process was filtered again through 0.2-μm syringe filter into autosampling vials. The same protocol for the HPLC analysis of the pretreated corn stover liquid fraction was used for the analyses of these samples. The samples were injected directly unto the column without any pH adjustment.

Results and Discussion

The Analysis of Standard Sugars

Typical chromatograms of the standard sugar mixtures with various retention times are shown in Fig 1. All monomeric sugars had baseline resolution, but the dimeric sugars areas showed a hump because of the change in mobile phase composition. All the calibration curves were plotted as $\log_{10}$ area vs. $\log_{10}$ concentration because the ELSD is nonlinear for area vs concentration plot. The $\log_{10}$ area vs $\log_{10}$ concentration were linear for all sugars with r^2 values greater than 0.97. The worse r^2 value (0.97) was obtained for the cellobiose sample, because of the change in phase composition, which gave rise to a hump in the baseline.

The analysis of the standard sugars on the Prevail carbohydrate ES column showed strong dependence on the composition of the mobile phase. For good baseline resolution, a mobile phase composed of 15% water and 85% acetonitrile analyzed in the isocratic mode resolved all the biomass monomeric sugars such as arabinose, xylose, fructose, mannose, galactose, and glucose, as shown in the chromatogram (Fig. 1). The manufacturer recommended mobile phase composition of 25% water and 72% acetonitrile was not suitable for the biomass sugars.

In the gradient mode, the best results were obtained when the initial mobile phase composition was 15% water and 85% acetonitrile and gradually increasing the composition to 25% water. The major advantage of the gradient system was shorter analysis time (15 min), but the peaks were not well resolved compared to the isocratic mode (data not shown).

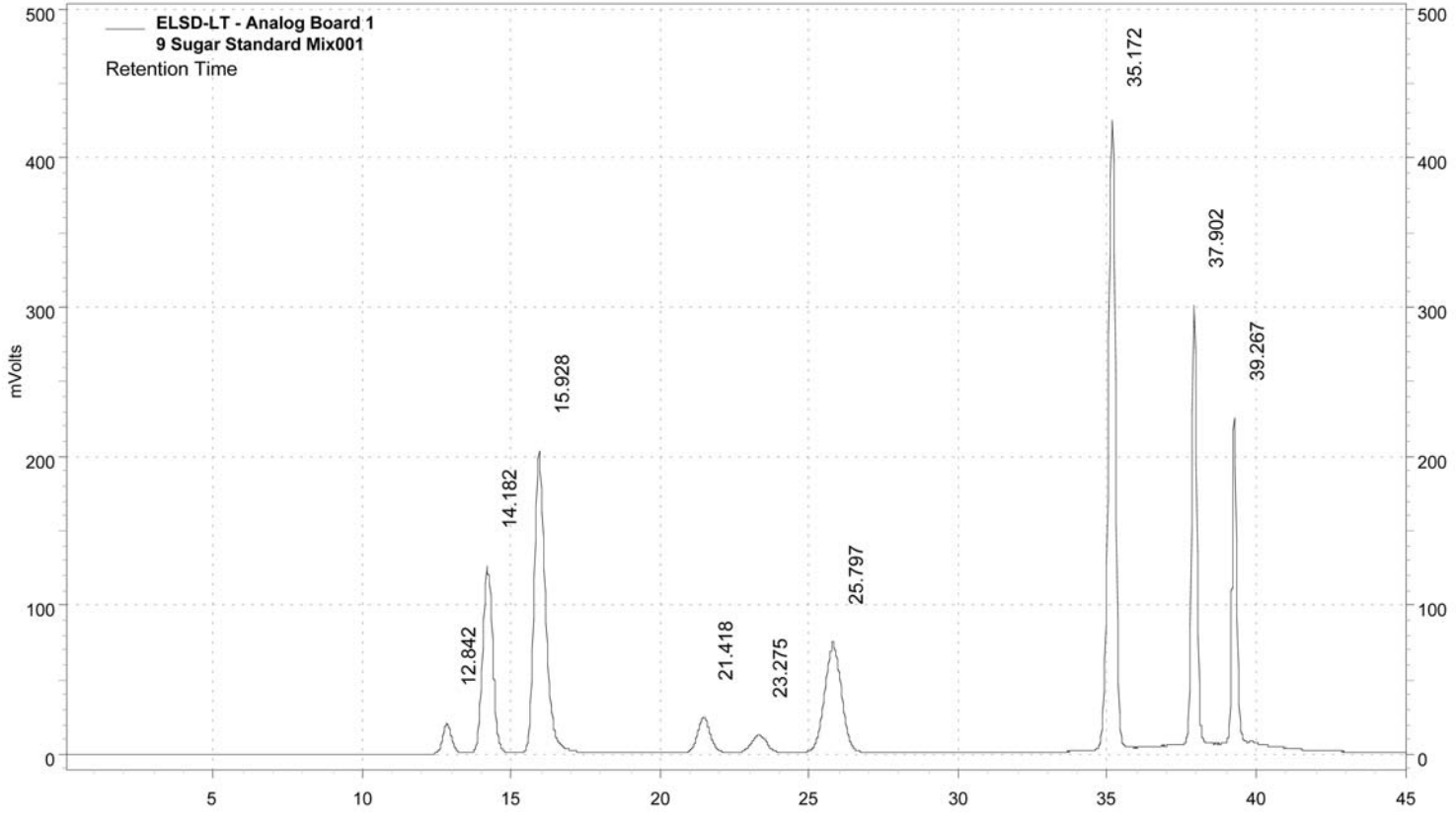

Fig 1. Chromatogram of seven standard sugar mixtures and internal standard showing their retention times (RT) in minutes. RT 12.84, arabinose; RT 14.12, xylose; RT 15.92, fructose; RT 21.41, mannose; RT 23.27, galactose; RT 25.79, glucose; RT 35.17, inositol (internal standard); RT 37.95, sucrose; and RT 39.59, cellobiose.

The resolution of the sugars obtained using the Prevail carbohydrate ES column was superior to those obtained using either Pb^{2+}, Ca^{2+}, H^{+} carbohydrate columns, or silica-based amino columns *(3,12,13)*. In Pb^{2+} carbohydrate column, xylose and glucose are not baseline resolved, and furthermore, mannose, galactose, and fructose are not resolved.

In addition to the monomeric sugars, the column could also resolve dimeric sugars such as sucrose, cellobiose and maltose. The resolution of these sugars also depended very strongly on the composition of the mobile phase. Although the isocratic regime could resolve the monomeric sugars, it was impossible to resolve dimeric sugars. The dimmers were retained on the column, but when the mobile phase was made more polar by increasing the water content; the dimmers eluted and were resolved. The cellobiose and sucrose peaks were well resolved (Fig 1), but the baseline was not flat because of the change in the mobile phase composition.

The detection limits for fructose, glucose, and galactose using the ELSD-LT detector were 24, 50, and 85 ng. Mannose and galactose were less sensitive than the other sugars. Because the detection mode of this instrument is based on the light scattering, the dimmers showed stronger response than the monomeric sugars.

Analysis of NREL-Pretreated Corn Stover Liquid Fraction

The chromatograms of the liquid fraction from the two groups of samples (P031118CS group and P031209CS group) were similar and typical chromatograms are shown in Fig. 2A,B. Each chromatogram contained three groups of peaks: unknown group of compounds (retention time 0 to 10 min); monomeric sugars (retention time 11 to 30 min); and dimmers and oligomeric sugars (retention time 30 to 45 min).

We believe that the unknown compounds were not sugars because after conversion of the liquid fraction into alditol acetates, this group of compounds was not derivatized (data not shown). In addition, they showed strong absorption at 254 nm and 280 nm, indicating that they may be aromatic or lignin-derived. Further, the absorption at 254 nm and 280 nm were similar to those that we observed in corn stover hydrolysates analyzed on C18 column and by ^{13}C-NMR *(14)*. The ^{13}C-NMR spectra showed that most of the compounds were aromatic and were potential microbial inhibitors. Although the our liquid fractions were analyzed on a carbohydrate column, because of the strong absorbance at the same wavelengths, this suggested that these compounds were not sugars, but may have similar structures as the microbial inhibitors reported by Agblevor et al. *(14)*.

P031118CS Group of Pretreated Corn Stover Liquid Fraction

The HPLC method was very effective in the analysis of this group of samples. The peaks were well resolved and the reproducibility of the analysis was very good (*see* Table 1). All the samples had very high xylose and glucose contents and low arabinose, fructose, galactose, mannose, and

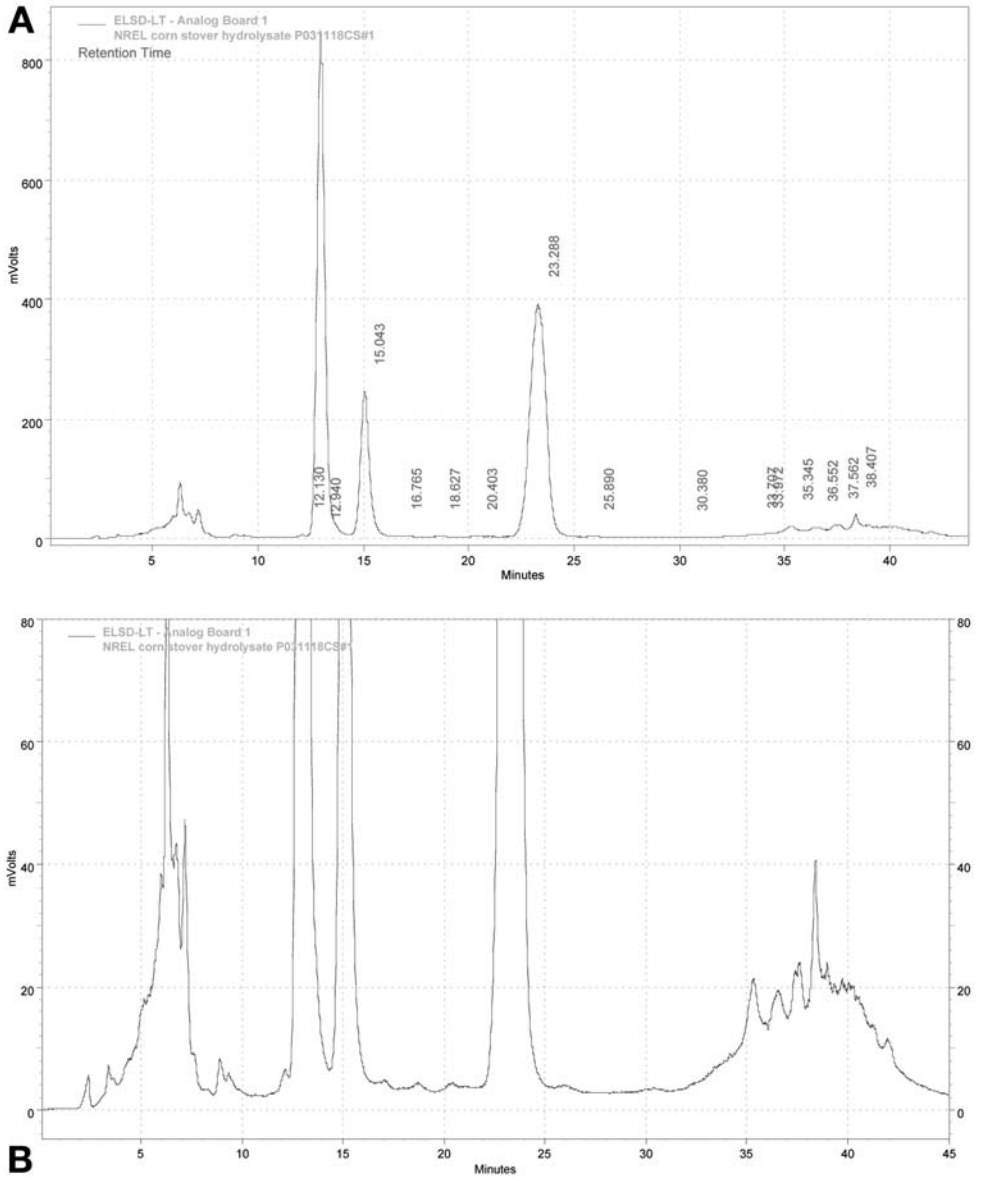

Fig. 2. **(A)** Chromatogram of pretreated corn stover liquid fraction showing three regions of unknown compounds (0–10 min), monomeric sugars (10–30 min), and oligomeric sugars (30–45 min). **(B)** Expanded version of **A** showing the three major groups of compounds.

cellobiose contents. Because of the different pretreatment conditions used for each corn stover sample (Table 1), the composition of the liquid fractions varied widely.

The severity of pretreatment varied from 4.2 to 5.8, and temperatures ranged from 180 to 200°C (Table 1). To compare the effect of severity on the

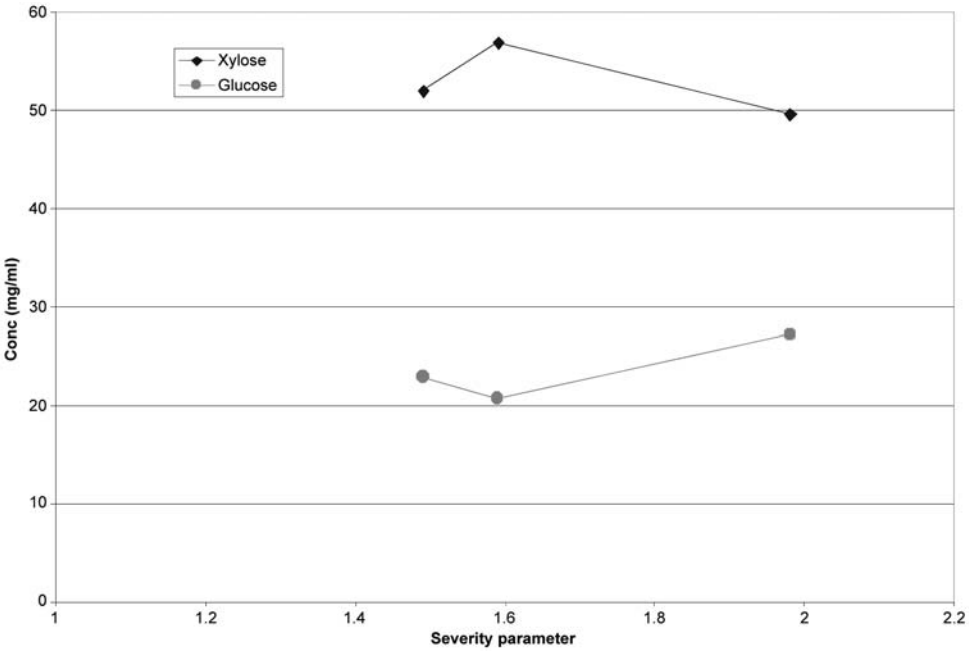

Fig 3. The effect of severity parameter on the concentration of xylose and glucose in the pretreated corn stover liquid fraction (P031118CS group of samples).

concentration of sugars in the liquid fraction, we assumed similar solids loading, because the solids loading affects the concentration of sugars in the solution but it is independent of the severity parameter. It is clear from Table 1 and Fig. 3 that the severity parameters affected glucose and xylose concentrations differently. Xylose concentrations in the liquid fraction were highest at low severity parameters and low at high severity parameters. The trends in fructose, mannose, arabinose, and galactose concentrations were similar to that of xylose (Table 1) (Fig 4).

The trend in the glucose concentration in the liquid fraction was the exact opposite of that observed for the xylose for similar severity parameters (Fig 3). The glucose concentration was highest at the highest severity and *vice versa*.

The highest xylose concentration (65.46 mg/mL) was achieved at the highest solids loading (35%). However, the highest solids loading did not result in the highest glucose concentration because glucose release was strongly influenced by the severity parameter. At the highest solids loading, although the acid concentration was high, the reaction temperature was relatively low (180°C) and therefore glucose release was not very high. The highest glucose concentration (27.30 mg/mL) corresponded to the highest severity parameter for this group of samples (Table 1).

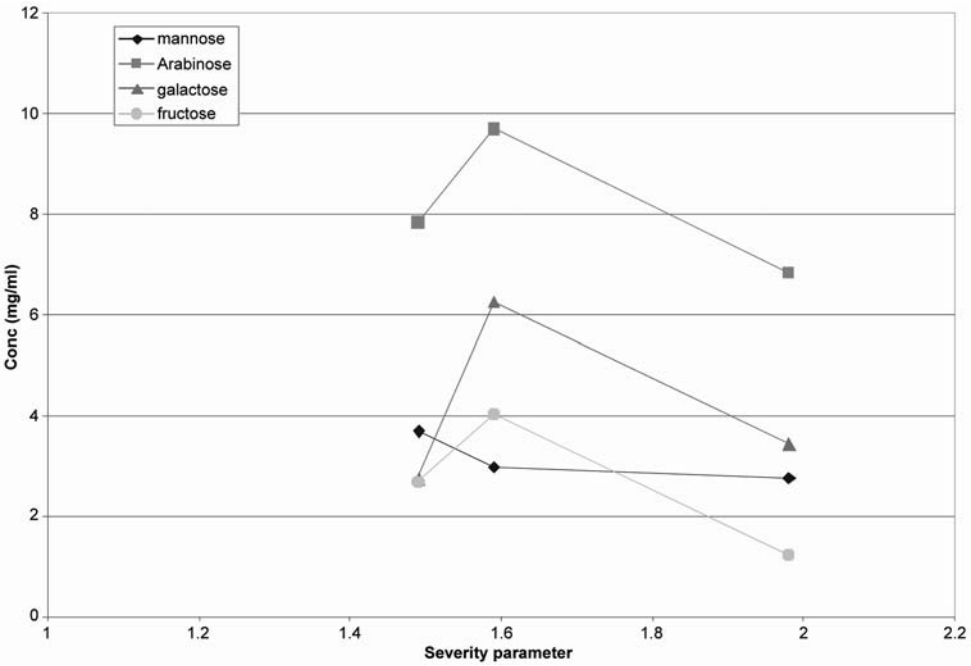

Fig 4. The effect of severity parameter on the concentration of monomeric sugars (mannose, arabinose, galactose, and fructose) for group P031118CS samples.

P031209CS Group of Pretreated Corn Stover Liquid Fraction

The HPLC method was effective in the analysis of this group of samples. There was baseline peak resolution and good reproducibility of the data (Table 2).

Similar to the P031118CS group of samples, the P031209CS group of samples was treated at different severities, solids loadings, acid concentrations, and acid loadings. The severity of pretreatment varied from 3.8 to 9.3 and temperatures varied from 180 to 200 °C (Table 2). Because of the wide range of treatment conditions, the sugar concentrations also varied widely.

In order to assess the effect of severity parameter on the concentration of various sugars released into the solution, samples with similar solids loading (30%) were compared. It can be seen from Fig. 5 that for a constant solid loading (30%), the xylose concentration did not correlate with the severity parameter probably because of xylose degradation. The xylose concentration (45.24 mg/mL) at severity of 5.7 was lower than the xylose concentration (58.82 mg/mL) at severity of 5.1 (*see* Table 1). Furthermore, the xylose concentration at severity of 6 was similar to the xylose concentration at severity 5.1 and both were higher than that at severity of 5.7. This suggests that temperature has a stronger influence on xylose production than the acid concentration, which is in agreement with the data reported by Buhner and Agblevor *(15)* on the dilute acid hydrolysis of corn fiber.

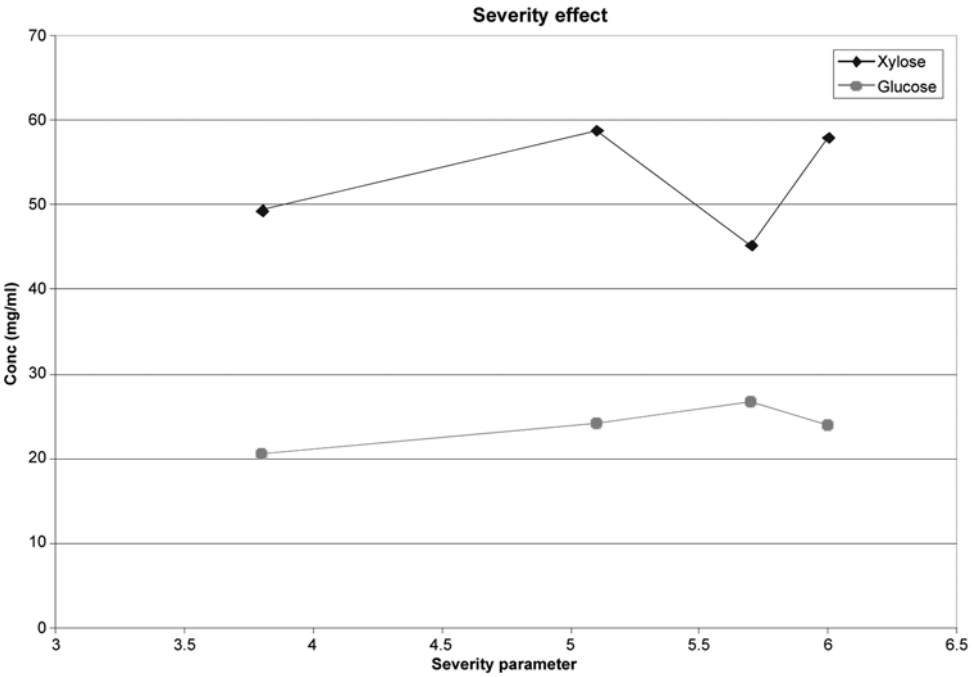

Fig 5. The effect of severity parameter on the concentration of xylose and glucose in the pretreated corn stover liquid fraction (P031209CS group of samples).

The effect of the pretreatment severity parameter on the trends in concentrations of galactose, mannose, and arabinose were similar to that of xylose (*see* Figs. 5 and 6) and did not correlate with the severity parameter. The glucose concentration was only partly correlated with the severity parameter. In this case, the temperature appeared to have a stronger effect than the acid concentration. The glucose concentration in the solution (26.77 mg/mL) at severity 5.7 was higher than that at severity 6.0 (23.91 mg/mL; *see* Table 2). Unlike the xylose, it appeared glucose degradation was not a major factor. The temperature at severity 5.7 was 200°C compared to 180°C for the severity at 6.0 whereas the acid concentrations were the reverse. Thus, unlike the xylose, the higher reaction temperature resulted in higher glucose release compared to the lower temperature.

The above observations on the effect of severity parameter on the release of sugars from corn stover during pretreatment are similar to those observed for steam explosion pretreatments of cotton gin waste and sugar cane bagasse *(6,16)*. These trends are contrary to those observed for the pretreatment of woody biomass *(17)* which showed correlation between severity of pretreatment and release of sugars. Because cotton gin waste, sugar cane bagasse, and corn stover are all herbaceous biomass (agricultural residues), it appears that these materials behave differently under pretreatment conditions compared to woody biomass and therefore their

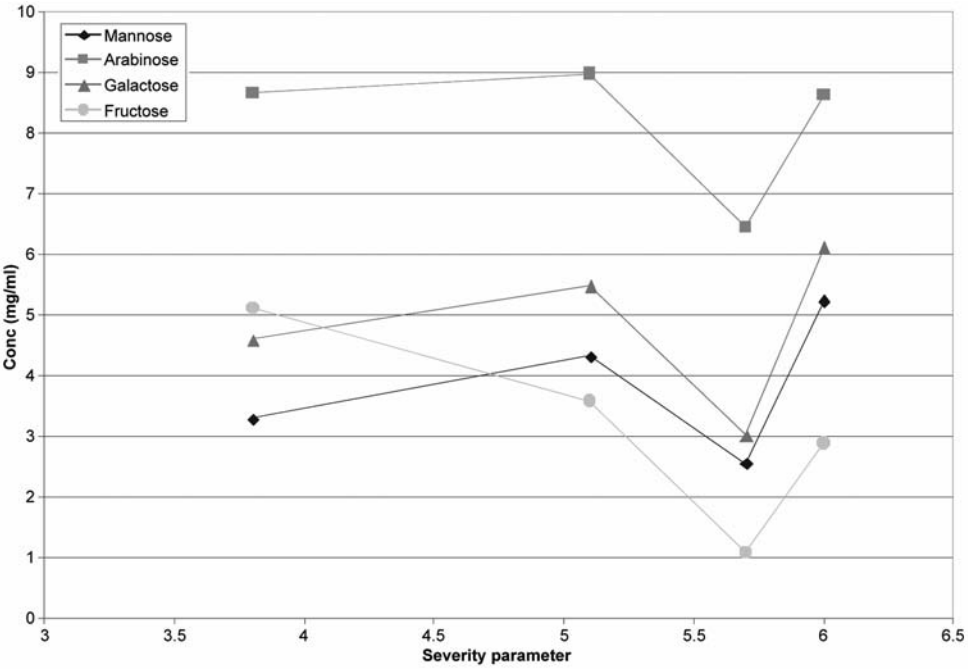

Fig 6. The effect of severity parameters on the concentration of minor sugars (arabinose, fructose, galactose, and mannose) in pretreated corn stover liquid fraction for P031209 group of samples.

pretreatments cannot be modeled using the severity parameter. In these agricultural residues, the temperature appeared to have a much stronger effect than the other parameters. In the case of steam explosion pretreatment *(16)*, the residence time had less influence than the temperature.

It is interesting to note that although dilute sulfuric acid was used in the corn stover pretreatment, which is a much stronger acid than the organic acids generated during steam explosion pretreatment in the absence of additives, the trend in the sugar release were similar for both processes *(16)*. The apparent contradiction of severity parameter model in the case of agricultural residues could be attributed to the physico-chemical properties of these materials. The ability of the reactants to penetrate the substrate and the faster reaction rates at higher temperatures may be key factors. These suggest that the activation energy of the reaction may be the key parameter in modeling these reactions.

One can assess the effect of solids loading on product distribution by comparing P031209CS#1 to P031118CS#2. Although both samples had similar solids loading (35%), the severity factor appeared to play a very important role in the products distribution. For P031118CS#2, the severity was 5.6 compared to 9.3 for P031209CS#1. In the case of P031118CS #2, the xylose concentration (65.46 mg/mL) was two and a half times higher than

the glucose concentration (24.55 mg/mL). On the contrary, for P031209CS#1, which had a higher severity, the xylose concentration (39.71 mg/mL) was almost the same as the glucose concentration (38.31 mg/mL). Thus, the higher severity was detrimental to the xylose but favorable for the glucose production.

Another interesting observation was the effect of acid concentration on the distribution of fructose in the liquid fraction. In general, fructose concentration in the liquid fraction decreased with increasing acid concentration, except in the case of sample P031209CS#2 where there appeared to be a severe temperature effect on the fructose concentration. The highest fructose concentration (8.16 mg/mL) was obtained for the lowest solids loading and the lowest pretreatment temperature. On the contrary, the lowest fructose concentration (0.45 mg/mL) was obtained for the highest solids loading at the highest severity parameter (highest acid concentration and reaction temperature). Thus, it appears both temperature and acid loading have strong influence on the fructose concentration.

Analysis of Corn Stover Pretreatment Solids Fraction

The acid-insoluble material (AIM) content of the pretreated corn stover solid ranged from 29.8% to 32 wt% for the P031118CS group of samples (Table 3). The HPLC analysis of the 72% H_2SO_4 hydrolysates of the solid residues showed high levels of glucose and trace amounts of xylose that were not quantifiable. The chromatogram shows only one strong baseline resolved peak identified as glucose.

The glucose content ranged from 60 wt% to 70.7 wt% (Table 3) and showed more variation than the AIM content. The sum of glucose and AIM content was between 91 and 101%. The variation in the mass closure showed that other compounds may be present in these materials that could not be analyzed using the above methods.

In the case of the P031209CS group of pretreated corn stover solid residue samples, there was more variation in the AIM content than those obtained for the P031118CS group of samples. The AIM content ranged from 23 wt% to 39.8 wt% (Table 4). The highest AIM content (39.8 wt%) corresponded to the highest severity parameter (9.3) and the lowest AIM content (23 wt%) corresponded to the lowest severity parameter (2.3). The AIM content appeared to increase with increasing severity of treatment. However, the temperature appeared to have a strong influence on the AIM content as discussed above for the liquid fractions. The high AIM content at high severity also suggests that several other components such as protein and Maillard reaction products may have condensed with the lignin. At lower severities, the condensation of these compounds appeared to be less and hence the lower AIM content.

The glucose content in the pretreated corn stover residues ranged from 56 wt% to 72 wt%, which is much wider than those obtained for the P031118CS group of samples. In this case, the lowest severity corresponded to the lowest glucose content, but the highest severity combined with the

Table 3
Composition of Pretreated Corn Stover Solid Fraction for P031118CS Group of Samples

P031118CS(#)	1	2	3	4	5	6
Acid insoluble material (%)	31.07 ± 0.59	32.04 ± 1.16	30.53 ± 0.60	29.77 ± 0.99	30.24 ± 0.95	32.17 ± 0.67
Glucose (%)	60.20	62.23	70.72	67.82	68.45	67.82
Pretreatment reaction conditions						
Temperature (°C)	180	180	190	190	200	200
Acid loading (%)	0.045	0.045	0.045	0.045	0.06	0.045
Solids loading (%)	30	35	30	25	25	25
Acid concentration (%)	1.90	2.39	1.91	1.59	1.98	1.49
Severity	4.5	5.6	5.1	4.2	5.8	4.4

The errors are expressed as standard deviations on three replicates.

highest solids loading did not correspond to the highest glucose content. It appeared the highest severity resulted in the hydrolysis of the cellulose as shown by the high glucose concentration in the corresponding liquid fraction of this sample. The highest glucose content (72.2 wt%) was obtained for severity of 5.7. As the severity increased above 5.7, there was a corresponding decrease in the glucose content of the residues and increase in the AIM contents.

Total mass closure on this group of samples ranged from 79 % to 104%. The sample with lowest severity had the worst mass closure, probably because of the formation of oligomeric products that could not be accounted for in any of the methods used in this analysis.

Conclusions

The Prevail carbohydrate ES column was used successfully in analyzing the standard sugars, liquid fraction, and solid residue of pretreated corn stover feedstock. The concentration of sugars in the liquid fraction was affected by several factors including the severity parameter. However, temperature appeared to have a much stronger influence on the product concentration than other factors. The severity parameter is probably not a good predictor of the monomeric sugars that were hydrolyzed during the pretreatment.

Xylose, arabinose, mannose, and galactose concentrations decreased when the severity increased above 1.6 for the P031118CS group of samples. The trend for these sugars was quite different for the P031209CS group of samples. However, for both group of samples, the fructose decreased with increase in severity. In general, for both group of samples, the glucose concentration in the liquid fraction increased with increase in severity of treatment.

Table 4
Composition of Pretreated Corn Stover Solid Residue
of P031209CS Group of Samples

P031209CS (#)	1	2	3	4	5	6	7
Acid insoluble material (%)	39.78 ± 0.23	32.27 ± 0.47	27.99 ± 0.71	27.38 ± 0.09	26.81 ± 0.20	20.40 ± 2.00	23.17 ± 0.26
Glucose (%)	60.90	7.23	60.14	65.04	65.17	64.74	56.78
	Pretreatment reaction conditions						
Temperature (°C)	200	200	200	190	190	180	180
Acid loading (%)	0.06	0.045	0.03	0.045	0.048	0.06	0.03
Solids loading (%)	35	30	30	30	25	30	25
Acid concentration (%)	3.16	1.92	1.28	1.91	1.59	2.53	0.99
Severity	9.3	5.7	3.8	5.1	4.2	6.0	2.3

The errors are expressed as standard deviations on three replicates.

The pretreated corn stover solid residue appeared to be composed of only cellulose and acid insoluble material. There was hardly any other sugar present in any of these samples. The glucose content of these samples ranged from 56 wt% to 72 wt%. Thus, the pretreatment was very effective in removing the hemicellulose and sucrose components from the corn stover.

The treatment conditions had different effects on various samples. Thus, using the HPLC method, the samples can be screened and the most suitable conditions selected for pretreatment and subsequent product development.

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