

Signatures of Biologically Driven Hemicellulose Modification Quantified by Analytical Pyrolysis Coupled with Multidimensional Gas Chromatography Mass Spectrometry

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Supporting Information

ABSTRACT: Biomass storage conditions are a major source of feedstock quality variability that impact downstream preprocessing, feeding, handling, and conversion into biofuels, chemicals and products. Microbial activity in the stored biomass can result in heating that can modify or degrade the cell walls of the biomass, changing its characteristics. Analytical pyrolysis has been used to characterize biomass, but at temperatures typically used (~600 °C), the differentiation of samples having different storage histories is subtle or nonexistent. In this study, lower-temperature (400 °C) pyrolysis was used to show large differences in corn stover samples that had experienced different biological heating histories, indicated by pyrolysis products that were identified and, in several cases, quantified using two-dimensional gas chromatography/mass spectrometry. Pyrolysis of the samples originating from biomass that had experienced biological heating during storage generated small oxygenates such as furfural, 5-methyl furfural, and 2-(5H)-furanone with efficiencies that were as much as ten times greater than those measured for samples that were not significantly heated. Most of the pyrolysis products with enhanced efficiencies were C5 oxygenates, suggesting formation from hemicellulosic precursor polymers in the corn stover. The findings suggest that biological heating disrupts the cell wall structure, fragmenting the hemicellulose or cellulose chains and generating more polymer termini that have a higher efficiency in the generation of oxygenates at lower temperatures. Further, analytical pyrolysis conducted at lower temperatures may be a beneficial strategy for improved biomass cell wall characterization and the provision of insights to understand and manage the feedstock variability and inform harvest and storage best management practices.

KEYWORDS: Biomass feedstock, Variability, Biological heating, Two-dimensional gas chromatography, GC×GC, Internal standard method, Hemicellulose degradation



INTRODUCTION

The conversion of biomass to fuels or to value-added chemicals is resource intensive and frequently cannot compete economically with alternative forms of energy or chemical production methods. One contributing factor to this lack of economic competitiveness is biomass feedstock variability. Feedstock variability in chemical, physical, and mechanical properties arises from environmental and genetic factors, agronomic practices, and supply chain operations such as growth condition, harvest, collection, biomass aging during storage, and preprocessing.^{1,2} There is a lack of understanding of the source, magnitude, range, and distribution of feedstock variability and its impact on downstream feeding, handling, preprocessing, and conversion processes. A fundamental understanding of the sources of feedstock variability could

lead to mitigation strategies that could improve conversion efficiency and cost competitiveness.

As part of the biomass supply chain, in-field bale storage unit operation is one of the major sources of herbaceous biomass feedstock variability. The seasonal biomass supply requires a storage process to ensure the year-round availability. However, depending on moisture content, bale storage could present favorable conditions for microbial growth, biological heating,³ and biomass degradation, which results in feedstock loss, quality changes,⁴ and risks to downstream conversion processes and worker health and safety. Field studies have observed that the aged biomass generated by storage can

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reduce bale integrity.^{5,6} Biological heating can alter composition and cell wall structures that are manifest in macro-scale mechanical properties. Many studies have established that the combination of living cell respiration, biological degradation, and thermo-chemical oxidative reactions bring about significant changes to critical biomass feedstock attributes and process efficiency during storage.^{7,8} Some of these alterations may be potentially advantageous for conversion, reduction of biomass recalcitrance, lowering of energy demand, processing time, and production of value-added coproducts. However, the means for evaluating the effects of microbial action have not been developed to demonstrate and understand the fundamental mechanism of biological degradation and subsequent heating and how that impacts cell wall breakdown.

The characterization of biomass and, more significantly, changes in biomass that occur during storage or as a result of preprocessing is not a trivial undertaking. Biomass consists of high molecular weight polymers and cross-linked cell wall structures that include lignin, cellulose, and hemicellulose, together with a number of lower molecular weight extractable compounds and inorganic species.^{9–11} Analytical characterization methods that operate on a real-time or fast turnaround basis are most readily targeted toward low molecular weight compounds and require tedious biomass digestion and preparation process and accordingly, are not compatible with the characterization of biomass cell wall structures.

One approach that has seen extensive use for biomass characterization is analytical pyrolysis, and there are numerous reports in the literature over the past 25 years describing this application.^{11–17} Pyrolysis involves rapid heating of the polymers in a nonoxidizing atmosphere, which breaks covalent bonds, liberating small molecules that can be identified and correlated back to the composition and structure of the original polymer in the plant cell wall. Typically, pyrolysis would be conducted by rapidly ramping sample temperatures to 600 °C, which is sufficient to break many chemical bonds and form complex mixtures of compounds.

However, the complexity of the mixtures generated by pyrolysis further adds to the challenges of characterization. Normally, the analysis of the complex mixtures involves the chromatographical separation of the mixture components, which enables spectroscopic identification and quantification. However, even the use of this strategy can be difficult because biomass-derived samples can contain hundreds of compounds that can chromatographically coelute. This makes spectroscopic identification and quantification difficult or impossible.

A way to improve chromatographic separation is to utilize multidimensional chromatography, and there are a number of examples of this in the literature, including studies by Tassarolo, Fullana and Sfetsas, and others that have shown the utility of two-dimensional gas chromatography/mass spectrometry (GC×GC/MS) to identify individual compounds in biooils generated from the pyrolysis of biomass.^{18–22} The distinct advantage of the GC×GC/MS approach is that it provides a more complete separation of the pyrolysis compounds, which enables the acquisition of more pure mass spectra, providing a better chance at compound identification by library matching.

Here, GC×GC/MS is used to characterize compounds generated by 400 °C micro-pyrolysis of corn stover samples subjected to moderate- and severe-biological heating during storage; these results are compared with those of samples that underwent mild heating. The approach follows that employed

previously:²³ biomass samples are rapidly heated in the pyrolyzer and swept directly into the injector of the GC×GC/MS. Pyrolytically generated compounds are identified on the basis of mass spectral library matching and, in selected cases, by retention time matching with standard compounds. For those compounds that have standards, the production efficiency is quantitatively measured using an internal standard method. In the present study, pronounced differences in three types of corn stover samples were observed by the use of a lower maximum pyrolysis temperature, namely, 400 instead of 600 °C. These observations suggest that a better understanding of the structural breakdown of corn stover cell wall components resulting from feedstock storage can be achieved, which can provide an improved understanding of variability that arises prior to processing. The higher level of detail regarding feedstock variability may have implications for downstream preprocessing and conversion processes and inform possible storage best management practices.

■ EXPERIMENTAL SECTION

Samples. Corn stover bales were obtained from Story County, Iowa (harvest date 10/27/2017) and selected for evidence of degradation. A biologically degraded bale was identified for this study in order to evaluate the variability in structural polymeric attributes due to aging and degradation that occur during field-side storage. It was visually observed that some portions of the bale were moderately biologically heated (medium brown coloration) or severely biologically heated (dark brown to almost black) while some of portions of the bale, typically near the edges of the bale, underwent only mild- or negligible biological heating (tan to light brown). Biological heating profiles are a result of the combined effects of intrabale moisture and oxygen-rich or depleted microenvironments throughout the bale, and thus location in the field-side storage stack will likely influence degradation as will the microbial communities native to the soil and biomass itself. Flakes of the bale were deconstructed to collect samples representing variable profiles of biologically induced heating and degradation (Figure 1). Corn stover



Figure 1. Dissection of bale demonstrating a range of biologically degraded regions. Grab samples were collected from mildly heated (control), moderately heated (browned), and severely heated (almost black) sections of the bale.

samples visually identified as severely-, moderately-, and mildly biologically heated were directly sampled from the bale and placed into bags without drying for further processing. Samples from corn stover dissections were milled first through a 2 mm screen using a Thomas Model 4 Wiley mill (Thomas Scientific, Swedesboro, NJ, U.S.A.) followed by milling to pass a 0.2 mm screen using a Retsch ZM200 (Haan, Germany).

Sample Preparation. Samples were prepared for pyrolysis-GC×GC/MS analysis by weighing approximately 300 μg into a 38 mm pyrolysis tube fitted with a 19 mm (short) spacer (CDS Analytical, Oxford, PA) and a small glass wool plug. Once the biomass was in place on top of the plug, it was capped with a second glass wool plug and then spiked with 3 nanomoles of 9-(9H)-fluorenone, which served as an internal standard for the analysis. The fluorenone was injected into the plug as 1 μL of a 3 mM solution in acetonitrile.

Pyrolysis. Pyrolysis was conducted using a CDS Analytical 5250 pyrolyzer unit, which is equipped with a 36-sample carousel that enables analysis of batches of sample tubes. The samples were dropped into the pyrolysis chamber, initially subjected to a 2 s drying time at 100 °C, and then held for an additional second at 100 °C. The temperature was then ramped at 50 °C/second to the maximum pyrolysis temperature (T_{\max}) and then held at T_{\max} for 5 s. The sample tube was then ejected, and the chamber was cleaned by being heated to 1200 °C for 10 s.

GC×GC Separation. Two-dimensional gas chromatography was conducted using an Agilent (Santa Clara, CA) 7890 gas chromatograph modified for GC×GC by a four jet modulator and a secondary oven, both located within the primary oven. The first chromatographic dimension used a 28 m 0.25 mm i.d. column with a 0.5 μm Rxi-5 ms (Restec, Bellafonte, PA) stationary phase, which is 5% diphenyl/95% dimethyl polysiloxane. The second chromatographic dimension used a 1 m, 0.1 mm i.d. column with a 0.1 μm Rxi-17 (Restec, Bellafonte, PA) stationary phase, which is 50% diphenyl/50% dimethyl polysiloxane. An additional 21 cm of the Rxi-17 column served as the transfer capillary between the secondary oven and the mass spectrometer. The Rxi-5 ms column primarily separates compounds on the basis of boiling point, while the Rxi-17 column separates on the basis of compound polarity.

The carrier gas flow was controlled at 1 mL/min throughout the analysis. The pyrolysis compounds were split in the injector using a split ratio of 20:1. The injector was maintained at 300 °C. Upon initiation of the analysis, the primary column was held at 50 °C for 0.5 min, then ramped at 7.5 °C/min to a target temperature of 260 °C, and finally held at this temperature for an additional 3 min. The transfer capillary was maintained at 280 °C.

The secondary oven and 4-jet modulator were maintained at 5 and 15 °C (respectively) above the temperature of primary oven. A 3.00 s modulation period was used, and hot and cool pulse times were varied to efficiently trap and desorb light compounds early in the run and heavier compounds later in the run. Before a retention time of 394 s, the hot pulse and cool times were 0.50 and 1.00 s, respectively. Note that this is repeated twice per modulation cycle by the 4-jet modulator. After 394 s, the hot pulse and cool times were 1.00 and 0.50 s, respectively.

MS Detection. Mass spectrometry detection and analysis were conducted using a Leco Pegasus 4D instrument (St. Joseph, MI), which integrates a time-of-flight mass spectrometer with the Agilent 7890 GC. An acquisition delay of 220 s was employed to allow very light compounds to pass through the MS before analysis was initiated. The instrument was scanned from m/z 43 to 300 at a rate of 200 spectra/second. The fast scan rate enables the deconvolution of closely eluting compounds. The electron impact ion source was operated at 250 °C, with an ionization energy of 70 V.

Data Processing, Compound Identification, and Quantification. Mass spectral data pertinent to a given compound were deconvoluted using the Leco ChromaTOF software, using automatic smoothing and a baseline offset value of 1.0, which correlates to just above the spectrometer noise level. Deconvoluted mass spectra were searched against the NIST and Wiley mass spectral libraries, and identification was based on forward and reverse similarity indices, probability,²⁴ and the judgment of the analyst.

The severely biologically heated samples could be differentiated from those that were mildly heated on the basis of differences in the abundances of small oxygenate compounds. Accordingly, calibration curves were generated for 11 oxygenates by the direct injection of a series of serially diluted standards containing all 11 compounds and the fluorenone internal standard (Table 1). These analyses utilized

Table 1. Small Oxygenate Compounds Used for Generating Calibration Curves for the Pyrolysis-GC×GC/MS Analyses

Compound	First dimension retention time, sec	m/z for quantitation
Acetic acid	301	45 + 60
2,3-Butanedione	310	86
2-Furfural	562	96 + 95 + 67
2-Cyclopenten-1-one	565	82 + 54
Acetoxyacetone	592	116 + 86 + 73 + 43
2(5H)-Furanone	667	84 + 55
5-Methyl furfural	733	110 + 109 + 81 + 53
2-Hydroxy-γ-butyrolactone	763	58 + 57 + 44
5-Deoxy-L-arabino-1,4-lactone	994	73 + 70 + 69 + 60 + 55 + 45
5-(Hydroxymethyl) furfural	1054	126 + 97 + 69 + 53 + 51 + 50
2-Deoxy-D-ribose-1,4-lactone	1246	104 + 101 + 83 + 61 + 57 + 55 + 44
9-(9H)-Fluorenone	1612	180 + 152 + 151 + 150 + 76 + 63

the rail sampler and not the pyrolyzer. Extracted ion profiles were generated for m/z values in the mass spectra of the oxygenates that were compound-diagnostic and unique in the GC×GC/MS analyses.

Calibration equations were generated by performing regression analyses of plots of the ratio of the peak areas for the reference oxygenates to the peak area of the internal standard times the ratio of the quantity of internal standard to the quantities of the reference oxygenate compounds (see Appendix 2 in Supporting Information). The equations could be used to convert the areas of the diagnostic ions for the oxygenates from the pyrolysis analyses to absolute quantities generated. Normalization for the sample masses resulted in pyrolysis production efficiency, expressed here in nanomol/μg.

Compositional Analyses. Structural and extracted sugars were measured according to National Renewable Energy Laboratory analytical procedures.²⁵ Samples dissected from one bale were ground to pass a 2 mm sieve. Extractable sugars were measured prior to an acid hydrolysis step (generating values for monomeric sugars) and after an acid hydrolysis step (generating monomeric + oligomeric sugars).

RESULTS AND DISCUSSION

Qualitative Sample Differentiation with 400 °C Pyrolysis. Pyrolysis of the corn stover samples conducted at 600 °C generated a large number of compounds that are categorized as small organic oxygenates, anhydrosugars, or lignols, consistent with what has been reported in the literature previously. Differences between the mildly heated, moderately heated, and severely heated samples, pyrolyzed at 600 °C, were not obvious (Figure S1,S2). However, the pyrolysis products generated and their abundances were greatly affected by the maximum pyrolysis temperature (Figure S3).²³ A qualitative comparison of the mildly heated and severely heated samples using pyrolysis-GC×GC/MS analyses conducted at 400 °C samples showed significantly greater intensities of the small oxygenates in the severely heated samples. These phenomena are displayed in a comparison of the 3D chromatograms generated from a 260 μg, mildly heated sample compared with those of a 380 μg, severely heated sample (Figure 2). In the comparison depicted in Figure 2, the color scale (which is the z-axis) of the mildly heated sample was increased by a factor of 292/200 relative to the scale of the analysis of the severely heated sample. This scales the response of the mildly heated analysis to match that of the severely heated analysis, in a

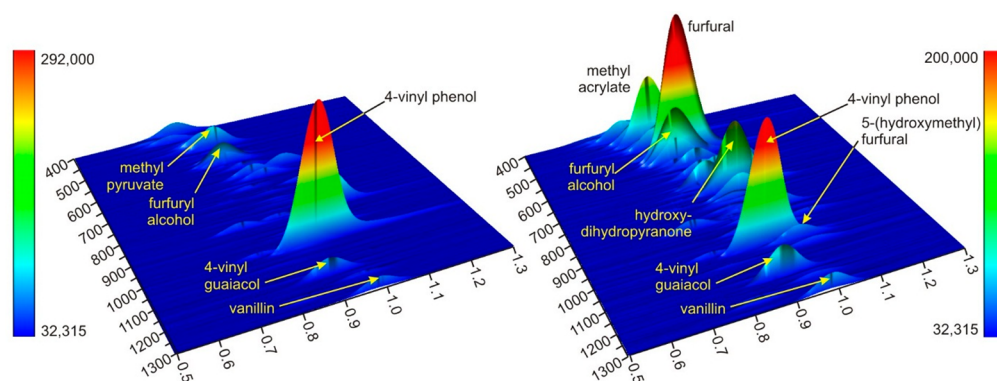


Figure 2. Comparison of 3D chromatograms of a mildly heated, 260 μg corn stover sample (left), with a 380 μg severely heated sample (right) pyrolyzed at 400 $^{\circ}\text{C}$. The z axis color scale for the 260 μg sample was multiplied by the ratio of the sample masses (380/260), normalizing its GC \times GC/MS response to that of the 380 μg sample so as to account for the difference in sample masses.

fashion that accounts for the difference in sample masses (260 vs 380 μg , respectively) and thus enables a more valid visual comparison. Most notably, furfural is greatly enhanced as are methyl acrylate, furfuryl alcohol, and 4-hydroxy-5,6-dihydro-(2H)-pyran-2-one, which are all C5 oxygenates. Two C6 oxygenates, 5-methyl furfural, and 5-(hydroxymethyl)-furfural are formed in abundance in the 400 $^{\circ}\text{C}$ pyrolysis of the severely heated sample, but are barely formed in the mildly heated sample. By way of contrast, the intensity of 4-vinyl phenol was the same for both sample types.

The differences between the severely heated and mildly heated samples were further illustrated by subtracting the 3D, un-normalized chromatogram of a mildly heated sample from that of a 260 μg severely heated sample (Figure 3). This showed pronounced enhancements of formic and acetic acids, methyl acrylate, the furfural derivatives, and 4-hydroxy-5,6-dihydropyran-2-one in the severely heated sample. Note that

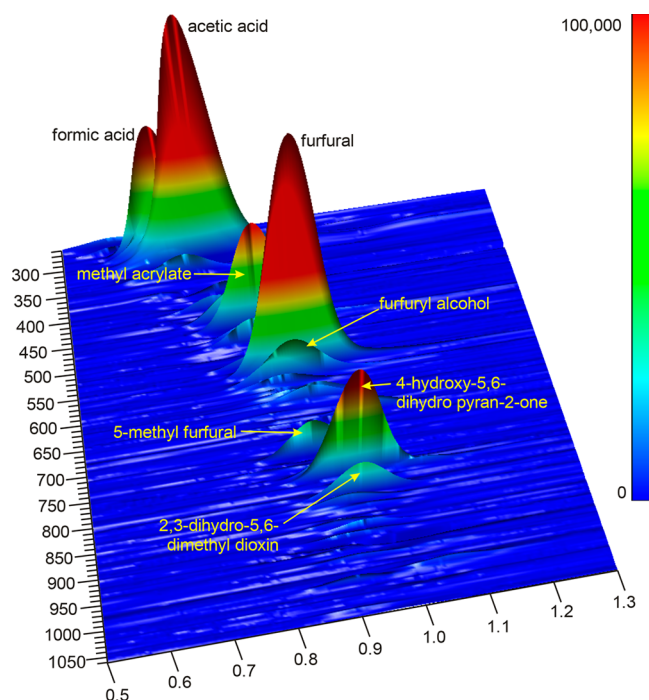


Figure 3. 3D chromatogram of a mildly heated sample subtracted from that of a severely heated sample. Three dimensional total ion chromatograms were generated from pyrolysis at 400 $^{\circ}\text{C}$.

not all of the small oxygenates generated in the pyrolysis analyses were enhanced in the microbially heated samples. Acetol, for example, was actually present in higher abundance in the mildly heated sample.

A more detailed examination of 2D extracted ion chromatograms reveals a number of other oxygenates whose production is markedly enhanced by biological heating. Examples of the enhancement are provided by the 2D plots of 5-(hydroxymethyl) furfural (HMF, m/z 126) and 1,5-anhydro arabinofuranose (m/z 57) (Figure 4). HMF is more abundant

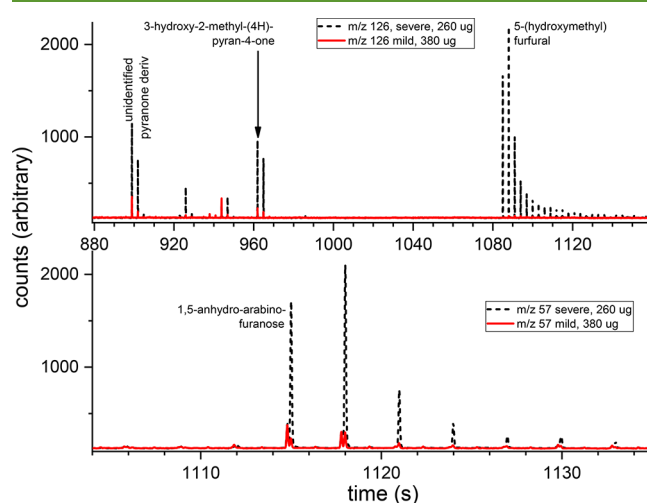


Figure 4. Top, extracted ion chromatograms for m/z 126, which is the diagnostic ion for 5-(hydroxymethyl) furfural (HMF) and 3-hydroxy-2-methyl-(4H)-pyran-4-one. Bottom, extracted ion chromatograms for m/z 57, the diagnostic ion for 1,5-anhydro arabinofuranose. The analyses of the mildly heated sample are represented by the red, solid lines and are superimposed on the analyses of the severely heated sample (dashed black lines). Note that the peaks are split into multiple slices, which is a consequence of the modulator used in the GC \times GC analyses.

in the severely heated sample by at least 2 orders of magnitude, while the arabinofuranose derivative is more abundant by more than a factor of 10. The abundances of both formic and acetic acids are also much greater in the severely heated sample (Figure S4), with peak heights for formic acid increasing from $<1 \times 10^4$ counts to $\sim 6 \times 10^4$. The situation for acetic acid is similar; this compound is difficult to quantify because its peak splits as a result of column overloading; nevertheless, it is clear

that significantly more acetic acid is produced in the moderately- and severely heated samples. The four carbon oxygenates, furan, tetrahydrofuran-3-one, 2,3-butanedione, and methyl acrylate are also enhanced in the moderately- and severely heated samples (Figure S5) as are the two furanone derivatives, although the effect of biological heating on the production of these compounds is not as pronounced. Figure S6 shows the enhancement of furfural (2D extracted ion chromatogram for m/z 96) and three pyranone derivatives (m/z 112, 114).

The striking increases in formic acid, acetic acid, furfural, and HMF in the moderately- and severely heated samples are suggestive of structural modifications occurring as a result of biological heating. Acetic acid is likely generated from the cleavage of acetyl groups present in the hemicellulose of biomass, while the furfural is often derived from hemicellulose or other pentose rich polysaccharides such as xylan, xyloglucans, and β -glucans. HMF is generally synthesized by the loss of water molecules from hexose units which comprise cellulose but also can be produced from C6 carbohydrate moieties present in hemicellulose. Furfural and HMF, once formed from the dehydration of hemicellulose and cellulose, could be further degraded to form organic acids such as formic and acetic acids. Using the same analytical approach, the mildly heated samples did not produce significant quantities of these compounds, indicating that the biological degradation/heating process occurring during storage caused the breakdown of corn stover cell wall structure mainly involving the degradation of hemicellulose but possibly degrading cellulose as well.

This conclusion draws support from the wet chemical analysis of the carbohydrate materials in the three samples (Table 2). The structural xylan concentration decreases from

Table 2. Chemical Composition (Weight Percentages) Measured for Corn Stover Samples That Experienced Mild-, Moderate-, and Severe-Biological Heating^a

component (wt %)	mildly heated	moderately heated	severely heated
Extractable Monomeric Glucose	0.30 (0.00)	0.67 (0.01)	0.75 (0.01)
Extractable Monomeric +Oligomeric Glucose	0.68 (0.02)	2.21 (0.02)	2.19 (0.05)
Extractable Monomeric Xylose	0.00 (0.00)	0.19 (0.01)	0.19 (0.02)
Extractable Monomeric +Oligomeric Xylose	0.26 (0.06)	4.52 (0.01)	4.58 (0.13)
Glucan	33.39 (1.35)	32.23 (0.33)	30.23 (1.79)
Xylan	18.19 (0.43)	13.08 (0.20)	10.51 (1.05)

^aMean values are listed, with the difference between analytical duplicates in parentheses.

18.2% in the mildly heated samples to 10.5% in the severely heated samples, which, at first glance, would seem to be contrary to the observation that the severely heated samples are producing so much more of the C5 oxygenates in the pyrolysis analyses. However, more extractable xylose (both oligomeric and monomeric) is produced in the moderately- and severely heated samples. The self-consistent explanation that reconciles the data is that hemicellulose is being broken down by the biological heating, thus disrupting cross-linking, generating oligomers and thus more polymer termini, and producing extractable xylan. These processes would be expected to produce a material that has an increased ability

to release C5 oxygenates upon pyrolysis. The same trend can be seen in the analytical results for glucan, only the magnitudes of the changes are more muted: structural glucan decreases from 33% in the mildly heated sample to 30% in the severely heated sample, while extractable glucan (monomeric + oligomeric) increases from 0.68% to 2.2%.

These phenomena are consistent with the different structures of cellulose and hemicellulose. The linear and crystalline structure of cellulose, which has uniform β -1,4 glycosidic linkages, is more recalcitrant to degradation, which requires effective and specific cellulase biocatalysts. Hemicellulose, with various monosaccharide units, heterolinkages, and different branch lengths, is susceptible to attack from the natural microbial community (i.e., bacterial, yeast, fungi) in the storage environment. Biomass dry matter, which is composed mainly of cellulose, hemicellulose, lignin, and ash, may undergo selective degradation of the cellulose and hemicellulose fractions. These valuable structural carbohydrates largely define the quality of feedstock, which effects downstream preprocessing, conversion performance, and yields. For the in-field corn stover bales experiencing biological degradation and heating during storage, the dry matter and convertible sugar loss will result in a significant mass basis reduction of total convertible sugars postdegradation and will increase feedstock cost for downstream conversion. Concurrently, the biomass becomes enriched with nonconvertible components such as ash or nonreadily convertible lignin.⁴ The consequences of higher ash content are increased equipment wear during preprocessing²⁶ and conversion operations, and deactivation of enzyme and microbial activity via hydrolysis and fermentation. Further, increased lignin content will require more aggressive pretreatment processing with accompanying increased energy consumption and operation cost. The utilization of large quantities of corn stover for biofuels and or conversion to biochemicals on an industrial scale requires a long-term and reliable storage method in order to maintain the quality, reduce and manage the variability, and supply biorefineries continuously year-round. The control of carbohydrate loss during storage is a key driver motivating the storage of industrial biomass in a controlled environment to ensure a sustainable feedstock supply.

Quantitative Evaluation of Biologically-Heated Samples. The internal standard method provides an approach for the quantitative comparison of the pyrolysis production efficiencies of those oxygenates for which standards were available, i.e., could be commercially procured. Responses of different product compounds during the pyrolysis event are variable, depending on sample mass, sample tube morphology, and many other factors, not all of which are understood. However, it is likely that the response of the product compounds will track that of the internal standard to some degree, which provides a measure of normalization to the responses, and provides quantitative meaning to the mass spectrometer response. 9-(9H)-fluorenone was selected as an internal standard because our experiments showed that it does not undergo significant degradation during the pyrolysis temperatures used in the current analyses.²³

Seven of the oxygenate products (Figure 5) were quantitated using the internal standard method, enabling measurement of pyrolysis efficiency. The most dramatic differences are seen in the furfural: at 300 °C, the efficiency of production of furfural during analytical pyrolysis is on the order of 0.004 nanomol/ μ g in the severely heated samples, which is about 40 \times greater than

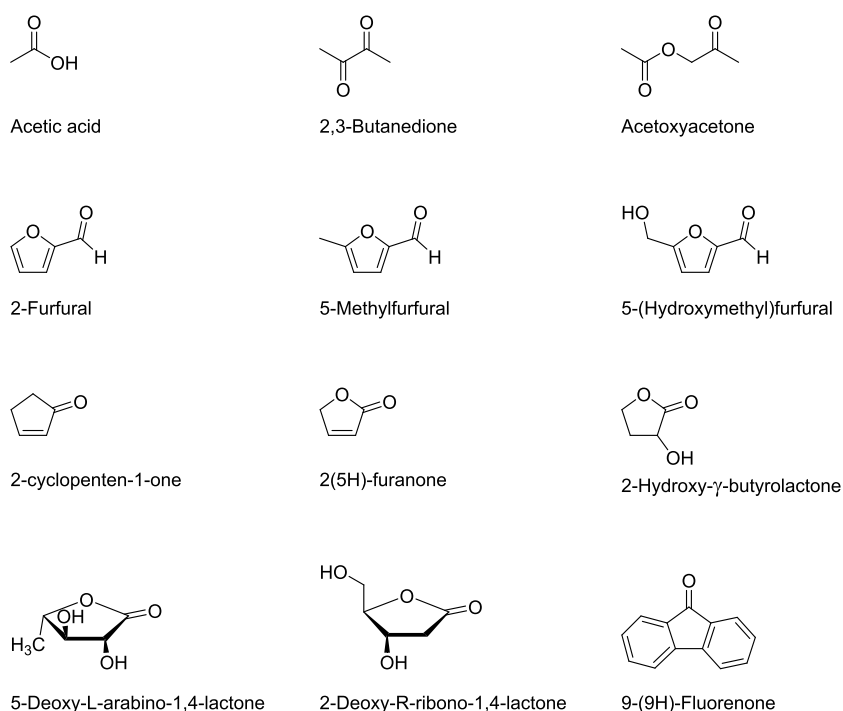


Figure 5. Structures of small oxygenates calibrated for quantitative analysis and 9-(9H)-fluorenone, which is the internal standard. The pyrolysis efficiencies were quantitatively measured for acetic acid, 2-furfural, 5-methyl furfural, 5-(hydroxymethyl)furfural, 2-cyclopenten-1-one, 2(5H)-furanone, and 2-hydroxy- γ -butyrolactone. The other compounds were not generated in abundance by the pyrolysis event.

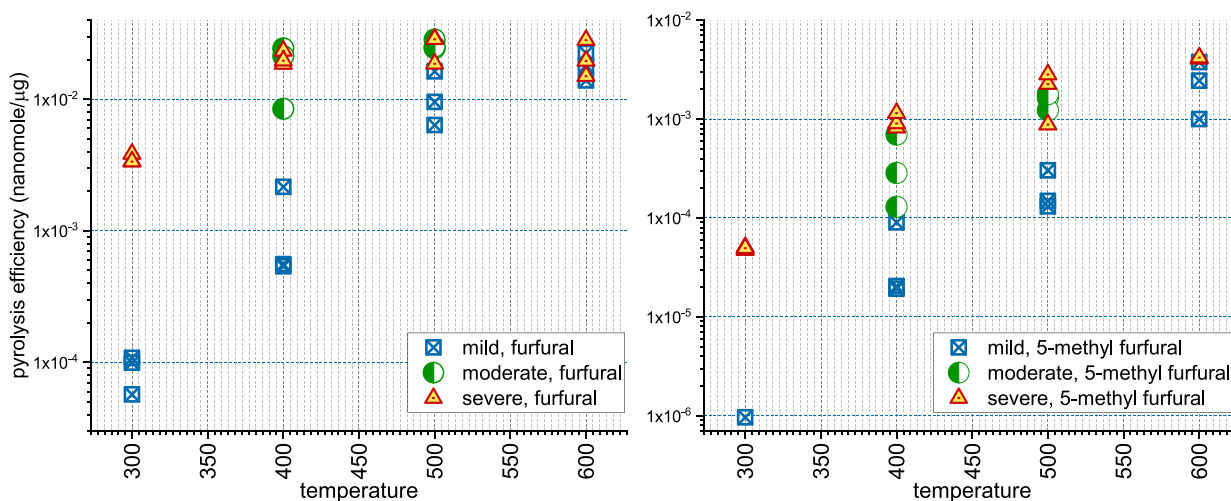


Figure 6. Comparison of the mildly, moderately-, and severely-heated samples in terms of pyrolysis efficiency for production of furfural (left) and 5-methyl furfural (right).

that for the mildly heated samples (Figure 6). At 400 °C, efficiency rises to about 0.02 nanomol/ μ g for the severely heated samples, again more than an order of magnitude greater than the mildly heated samples. At 500 and 600 °C, efficiency plateaus for both the moderately heated and severely heated samples, and values are approached by the mildly heated samples. This is probably the clearest example of the higher pyrolysis temperatures washing out differences between the different sample types. The behavior of 5-methyl furfural is similar (Figure 6 right); note that at 300 °C, it is not detected at all in the mildly heated samples, while at 400 °C, there is an order of magnitude greater efficiency measured for the severely heated samples compared to that for the mildly heated samples. Higher production efficiency is also seen for the

moderately heated and severely heated samples in the 500 and 600 °C pyrolysis experiments, although the differences between these samples and the mildly heated samples are not as dramatic as in the case of the lower temperature experiments.

Similar trends were observed for HMF: pyrolysis efficiencies for the severely heated samples were 2 orders of magnitude higher compared to those of the mildly heated samples at 400 °C, with the values for the moderately heated samples being intermediate. At 500 °C, severely heated and moderately heated samples are still more than 10 \times more efficient than the mildly heated samples, while at 600 °C, differences in pyrolysis efficiencies are less pronounced.

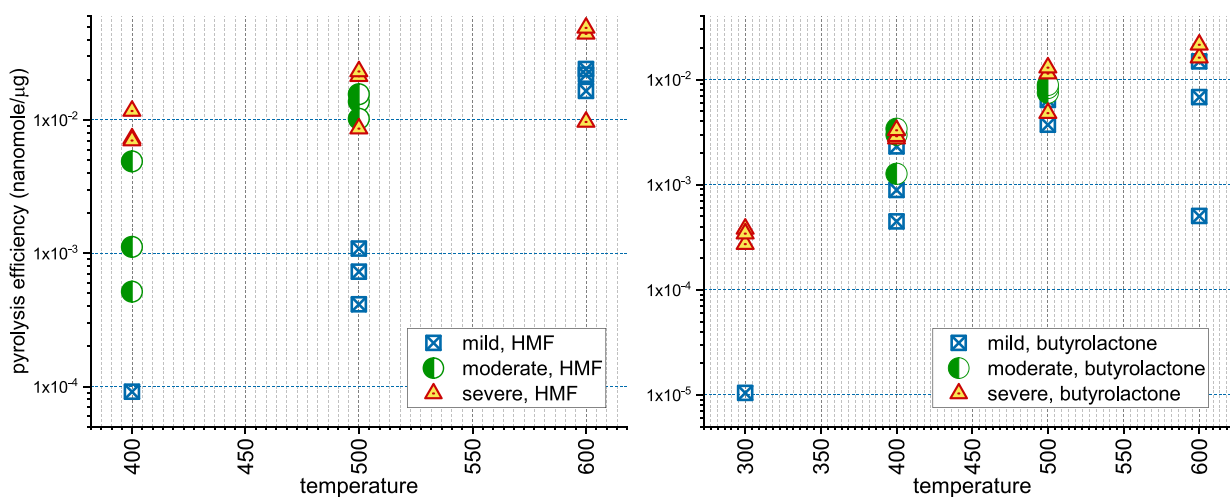


Figure 7. Comparison of the mildly-, moderately-, and severely-heated samples in terms of pyrolysis efficiency for production of 5-(hydroxymethyl) furfural (HMF, left) and 2-hydroxy- γ -butyrolactone (HBL, right).

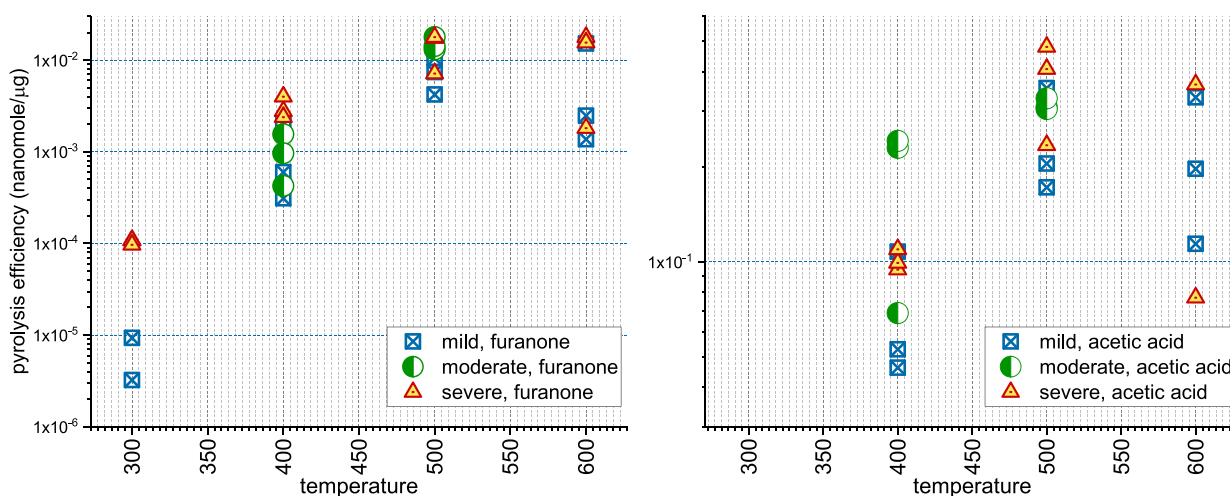


Figure 8. Comparison of the mildly-, moderately-, and severely-heated samples in terms of pyrolysis efficiency for production of 2-(5H)-furanone (left) and acetic acid (right).

2-Hydroxy- γ -butyrolactone (HBL) is another compound that has notably higher pyrolysis efficiency in the severely heated and moderately heated samples when pyrolyzed at 400 °C, with values that are on the order of 5–10 \times greater than those for the mildly heated samples (Figure 7, right). At 500 °C, the differences in the three samples are less noticeable, but when the pyrolysis temperature is increased to 600 °C, the HBL pyrolysis efficiency from the mildly heated sample drops off again, perhaps signaling secondary reactions involving this compound that are not occurring in the moderately heated and severely heated samples. This behavior is similar to that seen for 2-(5H)-furanone, which shows sharp differences in comparing the mildly heated samples with the severely heated samples pyrolyzed at 400 °C, with pyrolysis efficiencies being 5–10 \times higher in the latter (Figure 8, left). Values for the moderately heated samples were intermediate at this temperature. An increase in the temperature to 500 °C resulted in an increased production efficiency of the furanone but afforded less differentiation between the three sample types. At 600 °C, efficiency plateaus with some scatter in the data; however, the severely heated samples are on average an order of magnitude higher than the mildly heated samples, which suggests that

secondary reactions consume the compound in the latter, a process that perhaps does not occur in the severely heated samples.

The production of acetic acid is only observed in small quantities in the pyrolysis experiments conducted at 300 °C (Figure S3, Figure 8, right). However, in the 400 °C experiments, significant production efficiency is seen, with values of ~ 0.1 nanomol/ μg for the severely heated samples. The efficiency for the moderately heated samples is even higher, although there is scatter in the data. Acetic acid integration problems, noted above, no doubt contribute to scatter in pyrolysis efficiency data. Nevertheless, the 400 °C experiments indicate that acetic acid pyrolysis efficiencies in the mildly heated samples are about 5 \times lower than those in the severely heated samples. At 500 °C, the severely heated and moderately heated samples have greater efficiencies, on average, than the mildly heated samples; however, there is significant overlap and scatter in the data. The same may be said for the 600 °C data; however, the pyrolysis efficiencies are notably decreased, suggesting that acetic acid is being consumed by secondary reactions.

It is worth noting that not all the small oxygenates show differences in pyrolysis efficiencies in the comparisons of the three samples. 2-Cyclopentenone is not produced at all at when the samples are pyrolyzed at 300 °C, but values around 0.0001 nanomol/ μg (0.1 picomol/ μg) are reproducibly measured at 400 °C (Figure S6); this was true for both the mildly heated and severely heated samples. The compound almost exactly coelutes with furfural; however, a small difference in the second chromatographic dimension enables unambiguous differentiation. Pyrolysis efficiencies increase by a factor of 10 upon increase of the maximum pyrolysis temperature to 500 °C and by another factor of 10 for 600 °C. However, there are no notable differences in the pyrolysis efficiencies measured for the three samples.

CONCLUSIONS

Corn stover biomass subjected to biological heating undergoes cell wall structure modification resulting from hemicellulose and cellulose breakdown that is observable in enhanced pyrolysis efficiency for the production of small oxygenates such as furfural-, furanone-, and pyranone-derivatives. The pyrolysis efficiency differences between the mildly heated, moderately heated, and severely heated samples are readily observable when the pyrolysis analyses are conducted at 400 °C but are not obvious at higher pyrolysis temperatures. Most of the compounds that are enhanced in the analyses of the moderately heated and severely heated samples are C5 oxygenates, suggesting that the pyrolysis products may be formed from hemicellulosic precursor polymers in the corn stover, consistent with the wet chemical analysis that showed degradation of structural xylan. Cellulosic precursors are likely also involved, although probably not to the same extent as the hemicellulose. The fact that pyrolysis efficiency in the moderately heated and severely heated samples is so high may indicate that the microbial heating fragments the hemicellulose and cellulose chains, generating more polymer terminal groups that are responsible for higher pyrolysis efficiency at lower temperature. This result would be consistent with the findings of Krumm, who noted intrachain scission in cellulose beginning at 467 °C.²⁷

It is worth pointing out that the conclusions of the present study are based on the behaviors of a relatively small number of compounds that displayed enhanced pyrolysis efficiency for the biologically heated samples: seven compounds were quantitated, and perhaps twice as many were qualitatively identified. In reality, hundreds of pyrolysis products are formed, most at lower abundances compared to those emphasized in this report but nevertheless likely to contain significant information regarding polymer of origin and storage or preprocessing history. It is likely that further insight into the molecular changes occurring upon biological heating during storage will be uncovered by a more detailed study of the temperature-dependent pyrolysis behavior of an expanded set of product compounds, focusing on replicate sets of samples that have been subjected to varying storage histories.

The results also suggest that pyrolysis, in particular those conducted at lower temperatures, combined with GC \times GC/MS, can furnish improved biomass cell wall characterization, providing insights to understand and manage feedstock variability as well as informing harvest and storage best management practices. The results also suggest that additional studies to correlate pyrolysis temperature dependence with

polymer alteration will likely provide improved characterization and augmented control over product distribution.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acssuschemeng.9b06524>.

Chromatographic Figures, 2D color maps of pyrolysis-GC \times GC/MS analyses, 3D profiles of pyrolysis-GC \times GC/MS analyses at different temperatures, 2D extracted ion chromatograms, pyrolysis efficiency plot for 2-cyclopenten-1-one, quantitative analysis using 9-(9H)-fluorenone internal standard, quantitative data for cyclopentenone, and calibration plot for cyclopentenone (PDF)

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Notes

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REFERENCES

- (1) Li, C.; Aston, J. E.; Lacey, J. A.; Thompson, V. S.; Thompson, D. N. Impact of feedstock quality and variation on biochemical and thermochemical conversion. *Renewable Sustainable Energy Rev.* **2016**, *65*, 525–536.
- (2) Williams, C. L.; Westover, T. L.; Emerson, R. M.; Tumuluru, J. S.; Li, C. Sources of Biomass Feedstock Variability and the Potential Impact on Biofuels Production. *BioEnergy Res.* **2016**, *9* (1), 1–14.
- (3) Tian, X.; Zhang, H.; Sheng, C. Self-Heating of Agricultural Residues During Storage and Its Impact on Fuel Properties. *Energy Fuels* **2018**, *32* (4), 4227–4236.
- (4) Wendt, L.; Murphy, J. A.; Smith, W.; Robb, T.; Reed, D.; Ray, A.; Liang, L.; He, Q.; Sun, N.; Hoover, A.; Nguyen, Q. Compatibility of high-moisture storage for biochemical conversion of corn stover: storage performance at laboratory and field scales. *Front. Bioeng. Biotechnol.* **2018**, *6*, 1–13.
- (5) Smith, W. A.; Bonner, I. J.; Kenney, K. L.; Wendt, L. M. Practical considerations of moisture in baled biomass feedstocks. *Biofuels* **2013**, *4* (1), 95–110.
- (6) Darr, M. J.; Shah, A. Biomass storage: an update on industrial solutions for baled biomass feedstocks. *Biofuels* **2012**, *3* (3), 321–332.
- (7) Krigstin, S.; Wetzel, S. A review of mechanisms responsible for changes to stored woody biomass fuels. *Fuel* **2016**, *175*, 75–86.

- (8) Brand, M. A.; Bolzon de Muñiz, G. I.; Quirino, W. F.; Brito, J. O. Storage as a tool to improve wood fuel quality. *Biomass Bioenergy* **2011**, *35* (7), 2581–2588.
- (9) Himmel, M. E.; Ding, S.-Y.; Johnson, D. K.; Adney, W. S.; Nimlos, M. R.; Brady, J. W.; Foust, T. D. Biomass Recalcitrance: Engineering Plants and Enzymes for Biofuels Production. *Science* **2007**, *315* (5813), 804–808.
- (10) McCann, M. C.; Carpita, N. C. Biomass recalcitrance: a multi-scale, multi-factor, and conversion-specific property. *J. Exp. Bot.* **2015**, *66* (14), 4109–4118.
- (11) Hosoya, T.; Kawamoto, H.; Saka, S. Pyrolysis behaviors of wood and its constituent polymers at gasification temperature. *J. Anal. Appl. Pyrolysis* **2007**, *78* (2), 328–336.
- (12) Zhao, J.; Xiuwen, W.; Hu, J.; Liu, Q.; Shen, D.; Xiao, R. Thermal degradation of softwood lignin and hardwood lignin by TG-FTIR and Py-GC/MS. *Polym. Degrad. Stab.* **2014**, *108*, 133–138.
- (13) Chen, H. Lignocellulose biorefinery feedstock engineering. In *Lignocellulose Biorefinery Engineering Principles and Applications*; Elsevier: Amsterdam, 2015; Vol. Woodhead Publishing Series in Energy: Number 74, pp 37–86. DOI: 10.1016/C2014-0-02702-5.
- (14) Gu, X.; Ma, X.; Li, L.; Liu, C.; Cheng, K.; Li, Z. Pyrolysis of poplar wood sawdust by TG-FTIR and Py-GC/MS. *J. Anal. Appl. Pyrolysis* **2013**, *102*, 16–23.
- (15) Liu, M.; Yang, J.; Liu, Z.; He, W.; Liu, Q.; Li, Y.; Yang, Y. Cleavage of Covalent Bonds in the Pyrolysis of Lignin, Cellulose, and Hemicellulose. *Energy Fuels* **2015**, *29* (9), 5773–5780.
- (16) Nowakowski, D. J.; Bridgewater, A. V.; Elliott, D. C.; Meier, D.; de Wild, P. Lignin fast pyrolysis: Results from an international collaboration. *J. Anal. Appl. Pyrolysis* **2010**, *88* (1), 53–72.
- (17) Jin, W.; Shen, D.; Liu, Q.; Xiao, R. Evaluation of the co-pyrolysis of lignin with plastic polymers by TG-FTIR and Py-GC/MS. *Polym. Degrad. Stab.* **2016**, *133*, 65–74.
- (18) Moraes, M. S. A.; Migliorini, M. V.; Damasceno, F. C.; Georges, F.; Almeida, S.; Zini, C. A.; Jacques, R. A.; Caramão, E. B. Qualitative analysis of bio oils of agricultural residues obtained through pyrolysis using comprehensive two dimensional gas chromatography with time-of-flight mass spectrometric detector. *J. Anal. Appl. Pyrolysis* **2012**, *98*, 51–64.
- (19) Tassarolo, N. S.; dos Santos, L. R. M.; Silva, R. S. F.; Azevedo, D. A. Chemical characterization of bio-oils using comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *J. Chromatogr. A* **2013**, *1279*, 68–75.
- (20) Tassarolo, N. S.; Silva, R. V.S.; Vanini, G.; Casilli, A.; Ximenes, V. L.; Mendes, F. L.; de Rezende Pinho, A.; Romão, W.; de Castro, E. V.R.; Kaiser, C. R.; Azevedo, D. A. Characterization of thermal and catalytic pyrolysis bio-oils by high-resolution techniques: ¹H NMR, GC X GC-TOFMS and FT-ICR MS. *J. Anal. Appl. Pyrolysis* **2016**, *117*, 257–267.
- (21) Sfetsas, T.; Michailof, C.; Lappas, A.; Li, Q.; Kneale, B. Qualitative and quantitative analysis of pyrolysis oil by gas chromatography with flame ionization detection and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry. *J. Chromatogr. A* **2011**, *1218* (21), 3317–3325.
- (22) Fullana, A.; Contreras, J. A.; Striebich, R. C.; Sidhu, S. S. Multidimensional GC/MS analysis of pyrolytic oils. *J. Anal. Appl. Pyrolysis* **2005**, *74* (1), 315–326.
- (23) Groenewold, G. S.; Johnson, K. M.; Fox, S. C.; Rae, C.; Zarzana, C. A.; Kersten, B. R.; Rowe, S. M.; Westover, T. L.; Gresham, G. L.; Emerson, R. M.; Hoover, A. N. Pyrolysis Two-Dimensional GC-MS of Miscanthus Biomass: Quantitative Measurement Using an Internal Standard Method. *Energy Fuels* **2017**, *31* (2), 1620–1630.
- (24) Stein, S. E. Estimating probabilities of correct identification from results of mass spectral library searches. *J. Am. Soc. Mass Spectrom.* **1994**, *5* (4), 316–323.
- (25) Sluiter, J. B.; Ruiz, R. O.; Scarlata, C. J.; Sluiter, A. D.; Templeton, D. W. Compositional Analysis of Lignocellulosic Feedstocks. 1. Review and Description of Methods. *J. Agric. Food Chem.* **2010**, *58* (16), 9043–9053.
- (26) Lacey, J. A.; Aston, J. E.; Thompson, V. S. Wear Properties of Ash Minerals in Biomass. *Front. Energy Res.* **2018**, *6* (119), 1–6.
- (27) Krumm, C.; Pfaendtner, J.; Dauenhauer, P. J. Millisecond Pulsed Films Unify the Mechanisms of Cellulose Fragmentation. *Chem. Mater.* **2016**, *28* (9), 3108–3114.