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The Effect of Biomass Densification on Structural Sugar Release and Yield in Biofuel Feedstock and Feedstock Blends

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Abstract In this work, we examined the behavior of feedstock blends and the effect of a specific feedstock densification strategy (pelleting) on the release and yield of structural carbohydrates in a laboratory-scale dilute acid pretreatment (PT) and enzymatic hydrolysis (EH) assay. We report overall carbohydrate release and yield from the two-stage PT-EH assay for five single feedstocks (two corn stovers, miscanthus, switchgrass, and hybrid poplar) and three feedstock blends (corn stover-switchgrass, corn stover-switchgrass-miscanthus, and corn stover-switchgrass-hybrid poplar). We first examined the experimental results over time to establish the robustness of the PT-EH assay, which limits the precision of the experimental results. The use of two different control samples in the assay enabled us to identify (and correct for) a small bias in the EH portion of the combined assay for some runs. We then examined the effect of variable pretreatment reaction conditions (residence time, acid loading, and reactor temperature) on the conversion of a single feedstock (single-pass corn stover, CS-SP) in order to establish the range of pretreatment reaction conditions likely to provide optimal conversion data. Finally, we applied the assay to the 16 materials (8 feedstocks in 2 formats, loose and pelleted) over a more limited range of pretreatment experimental conditions. The four herbaceous feedstocks behaved similarly, while the hybrid poplar feedstock required higher pretreatment temperatures for optimal results. As expected, the yield data for three blended feedstocks were the average of the yield data for the individual feedstocks. The pelleting process appears to provide a slightly positive effect on overall total sugar yield.

Keywords Biomass feedstocks · Pelleting · Densification · Recalcitrance · Pretreatment · Enzymatic hydrolysis · Response surface

Introduction/Background

One of the key challenges to the increased use of cellulosic biofuels is the logistics associated with feedstock harvesting, transportation, storage, and delivery to the gate of the biorefinery [1, 2]. Densification strategies such as pelleting [2, 3] can reduce logistics costs, although the densification itself requires specialized equipment for size reduction and grinding resulting in increased processing costs. Regardless of how densification strategies may fit into the feedstock logistics for cellulosic biofuels, it is important to understand how such strategies affect downstream biomass conversion yields. There has been recent work on the effect of pelleting on the biochemical conversion of biomass feedstocks.

Rijal et al. [4] showed that either pelleting or milling to a fine powder improved sugar release after pretreatment with either dilute acid (DA) or soaking in aqueous ammonia (SAA) followed by enzymatic hydrolysis. Pelletization of softwoods did not result in significant loss of hemicellulose and has been shown to produce a cellulosic substrate that is readily hydrolysable following steam pretreatment [5]. Shi

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et al. [6] investigated the use of ionic liquid pretreatment on both pelleted and unpelleted feedstocks. Although not the focus of their study, the data show no difference in conversion performance between the two formats. Theerarattananon et al. [7] saw consistently increased conversion performance in pelleted versus non-pelleted formats for several different herbaceous feedstocks. Ray et al. [8] investigated the effect of pelleting on dilute-acid pretreated corn stover at two different reactor scales. The results from the smaller-scale, lower-solids PT-EH assay were generally higher than in the larger-scale, higher-solids PT-EH assay. Hoover et al. [9] investigated the effect of pelleting on ammonia fiber expansion (AFEX)-pretreated corn stover. They saw little effect of pelleting on conversion performance; some conditions demonstrated improvement in conversion performance while other conditions resulted in modest reductions in conversion performance. In a separate study, the effects of densification on AFEX-pretreated biomass were examined [10] and subsequent fermentation revealed there was no adverse effect on ethanol yield for compacted biomass briquettes of AFEX-treated corn stover, switchgrass, and prairie cord grass relative to the native biomass. In general, the literature supports modest improvements in conversion performance due to pelleting.

A blended feedstock strategy has been proposed to address the high cost of feedstock access (i.e., grower payment) to enable biomass feedstock supply systems for biofuel production [11]. The blended feedstock strategy relies on the availability of multiple feedstock resources that can be blended in an economical supply radius [12] which, in turn, decreases grower payment by reducing the amount of any single biomass resource. In this manner, blending has the potential to expand the regionally available biomass resource pool to include marginal quality feedstocks at lower cost. In addition, a blended strategy offers the potential for feedstock quality upgrades and reduced variability [13]. Blending high-quality feedstocks with low-cost, low-quality feedstocks is a strategy that can be used to meet quality specifications [14] at the biorefinery, in addition to achieving volume and cost targets in the supply chain [15].

A few studies have examined the impact of blended or mixed biomass feedstocks on sugar yields from biochemical conversion. Karki et al. [4] reported on the enzymatic hydrolysis of mixtures of switchgrass and tall wheatgrass following dilute-acid and aqueous ammonia pretreatments. Switchgrass and tall wheatgrass were similar in composition before and after dilute-acid pretreatment, although tall wheatgrass had significantly higher glucose yields from enzymatic hydrolysis. Mixtures of the two species produced glucose yields that were higher than switchgrass and lower than tall wheatgrass following dilute-acid pretreatment and enzymatic hydrolysis. This

study also demonstrated hydrolysis yields for mixtures could be predicted based on results of the individual components. Shi et al. [6] examined the impact of mixed and densified feedstocks on conversion efficiency using ionic liquid (IL) pretreatment. Switchgrass, lodge pole pine, corn stover, and eucalyptus were mixed equally in flour and pelleted forms. Sugar yields for flour and pelleted forms of mixed feedstocks reached 90% within 24 h of enzymatic hydrolysis. Another study examined the sugar yields from blends of municipal solid waste (MSW) paper materials and corn stover following IL pretreatment and enzymatic hydrolysis [16]. Glucose yields of up to 84% were achieved following enzymatic hydrolysis of the IL-pretreated corn stover-MSW blends. These studies demonstrate the efficient conversion of blended or mixed feedstocks to fermentable sugars and highlight the potential for blending to expand the available resources for biofuel production. In addition, a recent study has also demonstrated that blended feedstocks exhibited improved flow behavior, as compared to a single constituent, like *Miscanthus* [17]. This work demonstrated that the flowability of a feedstock blend could be predicted from knowledge of the flowability of the pure feedstocks. This study further demonstrates that blending has the potential to offer significant benefits to the supply chain, as well as feeding systems at the refinery.

In this work, we use a laboratory-scale two-stage dilute acid pretreatment (PT) and enzymatic hydrolysis (EH) assay to compare pelleting and blending. We have recently published a study comparing the results of PT/EH experiments at different scales using different pretreatment reactors [18]. In that work, we demonstrated that because the pretreatment reaction is a complex multiphase reaction, optimal conditions vary among pretreatment reactors, and simple strategies such as matching reactor temperatures and residence times between reactors are unlikely to produce similar results. Thus, we caution that the data presented in this work should be used primarily to compare the effects of pelleting and blending on feedstock conversion. Thus, this work builds on the previous literature in two ways. First, we explicitly investigate the reaction space of a dilute pretreatment assay used in order to ensure we are performing screening experiments near the optimal conversion conditions for the reactor system we employed. Second, we examine four different herbaceous materials and one woody material both as single feedstocks and feedstock blends to understand the effects of blending and pelleting on total sugar release in the PT-EH assay. We demonstrate that (1) within the uncertainty of the assay used in this work, pelleting does not affect the conversion of the biomass feedstocks tested and (2) the conversion performance of blended materials can be accurately predicted as the weighted average of the conversion performance of the blend constituents.

Materials and Methods

Materials

Corn stover harvested in a single-pass (CS-SP) was harvested in Ames, IA in October 2012; corn stover harvested in multiple passes (CS-MP) was harvested in Ames, IA in October 2013. Bales of both corn stover materials were received at Idaho National Laboratory (INL) in December 2013. No information on the pedigree of the corn hybrid is available. The hybrid poplar used in this study was a mixture of DxN hybrid (OP3367 bred by Oxford Paper) and TxD hybrid (195-529 bred by University of Washington). Trees were 11–13 years at the time of harvest. Clean chips of hybrid poplar (HP) were obtained from Boardman, OR, USA in November 2013. Switchgrass (SW) was harvested in Garvin County, OK, USA in January 2012 and collected by Oklahoma State University in July 2012; these bales were received at INL in November 2013. No information on the pedigree of the switchgrass is available. *Miscanthus* (MS) was harvested in Tifton, GA, USA in early January 2014, was chopped to 1″ using a Class Forage Chopper, and then arrived at INL in late January 2014. The miscanthus was provided by Repreve® Renewables; it was their patented Freedom® Giant Miscanthus variety (Miscanthus x giganteus).

Bales of corn stover and switchgrass, chopped miscanthus, and chips of hybrid poplar were fed through a two-stage, full-scale grinding process using the Feedstock Process Demonstration Unit (PDU) as part of DOE's Biomass Feedstock National User Facility (BFNUF) that is located at INL. First, material was processed through a Vermeer BG-480 (Pella, IA, USA) grinder, which has two horizontal grinding drums with swinging hammers powered by two 200-HP motors (19), and passed through a 2-in screen. Then, the 2-in screened material was processed through a Bliss hammer mill (Ponca City, OK) with a ¼-in screen. Two 5-gal buckets of ground material were sent to the National Renewable Energy Laboratory (NREL) in Golden, CO, USA for compositional analysis and conversion studies. Ground biomass was mixed on a dry weight basis at the specified ratio for each of blends at the INL BFNUF: a blend of 1:1 single pass corn stover and switchgrass (CS-SW); a 1:1:1 blend of single-pass corn stover, switchgrass, and miscanthus (CS-SW-MS); and a 1:1:1 blend of single-pass corn stover, switchgrass, and hybrid poplar (CS-SW-HP).

Biomass Pelleting

Pellets were generated from biomass as described previously by Crawford et al. [17] Briefly, super sacks of ground biomass were sent to Bliss Industries, LLC (Ponca City, OK, USA) for pelleting. Ground biomass was pelleted using a Bliss Pioneer Pellet Mill (B35A-75) which has two 40-HP motors. The mill has a rated output of ~1000 kg/h for herbaceous biomass, although the actual capacity is material dependent. The die diameter used for all materials was 6 mm, and the length-to-diameter ratio of the pellet mill die varied. The L/D ratio was 9 for the blends and the hybrid poplar, 12 for the corn stovers and miscanthus, and 10 for the switchgrass.

Thus, a total of eight materials (five feedstocks and three feedstock blends (single-pass corn stover and switchgrass, CS-SW, single-pass corn stover-switchgrass-hybrid poplar, CS-SW-HP, and single-pass corn stover-switchgrass-miscanthus, CS-SW-MS)) were provided for subsequent analysis.

Compositional Analysis

The chemical composition of both the whole and pelleted biomass was measured according to the National Renewable Energy Laboratory's (NREL) laboratory analytical procedures (LAPs) for biomass analysis [20]. The history and precision of these methods have been previously reported [21, 22]. Briefly, biomass was sequentially extracted with water and ethanol using an automated solvent extractor. The extracted materials then underwent two-stage acid hydrolysis. The resulting hydrolysis liquor was analyzed for monomeric sugars and organic acids using high-performance liquid chromatography (HPLC) with a refractive index detector. Sugars were analyzed on a Shodex SP0810 column with a mobile phase of 18.2 MΩ Nanopure water at a flow rate of 0.6 mL/min at 85 °C, and organic acids were analyzed on an Aminex HPX-87P column with a mobile phase of 0.005 M sulfuric acid at a flow rate of 0.6 mL/min and column temperature of 55 °C. Acid-soluble lignin fractions were analyzed using ultraviolet-visible spectroscopy. Acid-insoluble lignin was measured as the residual solid material after analytical hydrolysis (after correction for ash and protein content). The total lignin content was the sum of the acid-insoluble and acid-soluble fractions. All analyses were performed in duplicate, and the results are the average of these duplicates.

Experimental Assay

The two-stage dilute acid pretreatment and enzymatic hydrolysis (PT-EH) assay used in this work has been described previously [23]. In brief, dilute acid pretreatment was performed on a small (3 g oven dry weight, ODW) biomass sample using an Accelerated Solvent Extractor (ASE 350, Dionex). A total of 30 mL of water containing a variable amount of sulfuric acid was contacted with the biomass sample in an acid-resistant steel cell under elevated pressure at a range of temperatures and time periods. At the conclusion of the extraction, the biomass sample was rinsed multiple times with deionized water to remove any residual water. The composition of the collected rinsate was analyzed for soluble

carbohydrates with HPLC, and the washed biomass sample was removed from the cell and reserved for subsequent enzymatic hydrolysis. During enzymatic hydrolysis, a ~10% (w/v) slurry containing approximately 1 g of the washed acid-pretreated solids was contacted with 20 mg/g (ODW) cellulose enzyme cocktail (Cellic CTec2, Novozymes) along with suitable buffers for 6 days at a temperature of 48 °C and a pH value of 4.8. The liquid fraction was then filtered and analyzed for soluble carbohydrate with HPLC. The total sugar yield is calculated as the fraction of structural carbohydrate present in the feedstocks that was released during pretreatment or enzymatic hydrolysis.

Primary experimental and analytical data were collected in Excel spreadsheets. Data reduction and analysis were performed in R [24]. Sugar release (e.g., the mass of soluble carbohydrates formed per gram of biomass) was calculated for both the pretreatment and enzymatic hydrolysis assays, as well as the overall release for both steps. Since the composition of the feedstocks were known, the sugar yields (the mass of either soluble glucose and xylose formed per gram of the corresponding structural carbohydrate after pretreatment and enzymatic hydrolysis, corrected for the water of hydration in the structural carbohydrate) were also calculated in a similar fashion. The main variable reported in this work is the total sugar yield, the fraction of all glucan and xylan released as monomeric sugars.

Results and Discussion

The goals of this work were to determine the effects of (1) a specific feedstock densification strategy (pelleting) and (2) blending feedstocks on sugar release and yield as measured by a laboratory-scale PT-EH assay. In order to answer these questions, we first investigate the effect of variable PT reaction conditions on the conversion of a single feedstock (single-pass corn stover, CS-SP) in order to establish the range of reaction conditions likely to provide useful conversion data. Once we show that the range of reaction conditions used for subsequent work to be relevant, we then apply the assay to the 16 materials (8 feedstocks in 2 formats) over a more limited range of experimental conditions.

Compositional Analysis Results

The compositional analysis results are shown in Table 1. As mentioned previously, all analyses were performed in duplicate, and the data in Table 1 are the average of these duplicates. We saw no differences in duplicate analyses greater than 0.9 for glucan measurements, greater than 0.5 for xylan measurements, and greater than 0.3 in lignin measurements. This is consistent with the historical uncertainties seen in compositional analysis [22].

Table 1 Composition of the biomass feedstocks and feedstock blends used in this study. All analyses were performed in duplicate and the results shown are the average of these duplicates. The composition of the feedstock blends were essentially the weighted average of the composition of the individual feedstocks (see text)

Feedstock	Format	Glucan	Xylan	Lignin	Ash
Single-pass corn stover (CS-SP)	Pellet	35.5	19.8	15.9	7.1
	Whole	35.6	20.4	16.0	6.6
Multi-pass corn stover (CS-MP)	Pellet	35.5	18.2	15.6	9.6
	Whole	38.1	20.6	17.0	6.5
Miscanthus (MS)	Pellet	39.5	20.2	19.8	2.6
	Whole	39.6	20.9	20.4	2.1
Hybrid poplar (HP)	Pellet	45.5	13.2	25.7	0.8
	Whole	45.1	13.1	26.1	0.9
Switchgrass (SW)	Pellet	35.7	22.8	18.6	4.7
	Whole	35.2	23.0	18.8	4.1
1:1 CS-SW blend	Pellet	35.2	21.1	17.4	5.4
	Whole	35.3	20.4	16.6	6.6
1:1:1 CS-SW-MS blend	Pellet	37.3	21.1	18.2	4.1
	Whole	37.0	21.7	18.3	4.6
1:1:1 CS-SW-HP blend	Pellet	39.1	18.3	18.7	4.0
	Whole	39.6	17.9	19.2	4.0

There are large differences in composition among the different feedstocks, but essentially no difference between whole and pelleted material within feedstocks. Of the 64 measurements shown in Table 1, only the glucan content of the multi-pass corn stover between the whole and pelleted formats differs by a value greater than the historical uncertainty of the analytical method [22]. The measured compositions of the blended materials are very close to the weighted average of the constituent materials. The largest difference between the measured and predicted composition of the blended materials for any constituent was 1.3% for the xylan content of CS-SW blend. The measured value was 20.4%, while the predicted value was 21.7 (the average of the CS-SP xylan content of 20.4 and the SW xylan content of 23.0). From these data, we conclude that the pelleting process does not appear to affect composition, and that the composition of blended materials appears to be the weighted average of the compositions of the constituent materials.

Optimization Experiments

The goal of the optimization experiments was to develop a comprehensive understanding of the effect of reaction conditions in the pretreatment assay on the overall conversion performance of the combined PT-EH assay. By doing this, we could then select a subset of these reaction conditions to screen the materials described above.

We used the whole (unpelleted) CS-SP as the feedstock for the optimization experiments. We varied the pretreatment reactor temperature from 135 to 185 °C, the reactor static time from 4 to 8 min, and the sulfuric acid concentration from 0.25 to 1.75% (w/v). These ranges were determined from prior knowledge of the behavior of a wide variety of biomass samples in the PT-EH assay. All other experimental conditions, including conditions for the EH assay, were kept constant. We used an inscribed central composite design (CCD) to develop the experimental plan. A total of 29 unique conditions were examined, with the central point at a PT reactor temperature of 160 °C, a static time of 6 min, and an acid concentration of 1.0%. The experimental design was executed in a stepwise manner over four PT batches. Control materials were included in each PT and EH batches. We noticed a bias in the EH results for the control materials in the latter two EH batches; we have normalized the data for this bias. Note that we blocked the experimental design by PT temperature so each PT batch contained all 16 materials pretreated at a single temperature. Thus, the relative performance of the materials was not affected by the bias in two of the EH batches. We include a detailed discussion of this bias and the correction as Supplementary Material.

The results of these experiments are shown in response surface model contour plots in Figs. 1 and 2. In these plots, the modeled value of the response variable is displayed as contours (similar to elevation contours in a topographic map) as the experimental parameters are varied on the x- and y-axes. In all cases displayed in this work, the models showed no statistically significant lack of fit; the models are acceptable descriptions of the underlying experimental data.

In Fig. 1, we show the response surface contour plot of total sugar yield after PT-EH as a function of all three experimental variables: time, temperature, and acid concentration. The top figure (at fixed time) shows a wide optimum (above 0.95) in total sugar yield, which occurs along a line from the top left (high acid concentration and low temperature in the PT reactor) to the bottom right (low acid concentration and high temperature in the PT reactor). A broad range of acid/temperature values would provide similar conversions. At low-acid/low-temperature conditions, there is less conversion (as low as 0.40) due to "undercooking" the biomass, while at high-acid/high-temperature conditions, the lower conversion (as low as 0.50) is due to "overcooking" the biomass. The middle and lower figures show optimum conditions for time vs. temperature at fixed acid concentration and time vs. catalyst concentration at fixed temperature. Close inspection of these two plots show that while an optimal value is apparent, the range in conversion as the reactor time varies is quite small; over the range in reactor times studied (4–8 min), there is very little effect of reactor time on conversion. The results of the response surface model (a linear model including first- and second-order terms and interactions) support this conclusion.

The only statistically significant terms in the three-variable model were the temperature/catalyst concentration interaction and the second-order temperature terms.

The results of a response surface model also provide a "stationary point"; the values of the independent variables which maximize the modeled variable. For the three-variable model, the stationary point values are a reactor temperature of 163 °C, a catalyst concentration of 1.0% (w/v), and a static time of 5.8 min. These values are very close to the central point of the central composite design discussed above. We strongly believe that this result is due in large part to our familiarity with the PT-EH assay; good experimental design requires good knowledge of the system being studied.

Because of the limited influence of the reactor time in the three-variable response surface model results, we investigated two-variable (reactor temperature and catalyst concentration) response surface models of the same dataset. In Fig. 2, we present the results of two-variable response surface models for the yields of total sugar, glucose, xylose, and furfural. The response surface for total sugar yield shown in Fig. 2a is quite similar to the one shown in the top plot in Fig. 1; a broad optimum in yield stretching from high-acid/low-temperature to low-acid/high-temperature conditions. Since the total sugar yield depends on the yield of both glucose and xylose, it is valuable to inspect response surface models for these sugar yields independently. These plots are shown in Fig. 2b, c, respectively. Both of these plots show similar behavior to the total yield plot, but the glucose yield plot shows an optimum at low-acid/high-temperature conditions while the xylose yield plot shows an optimum at high-acid/low-temperature conditions. This is consistent with the chemistry of biomass pretreatment; the xylan content is more labile than the glucan content, and conditions for optimal overall sugar yield are a compromise between conditions that optimize either glucose or xylose yield separately.

In Fig. 2d, we show the response surface model for furfural yield from xylan. Both high-temperature and high-acid concentrations are required for optimal furfural yield, and no furfural is seen at either low-acid or low-temperature conditions. This plot also reinforces the decrease in total sugar yield seen at high-temperature high-acid conditions as in Fig. 3a; excessively harsh pretreatment conditions cause a loss of xylan to furfural (and to degradation products beyond furfural).

Blending/Pelleting Conversion Experiments

The optimization experiments described above consisted of a total of 41 separate experimental samples at 29 unique conditions along with appropriate replication. Such a thorough investigation of reactor conditions is valuable, but completely inappropriate for the rapid screening of large numbers of samples. Based on the optimization experiments described above, we chose to compare the conversion performance of the whole
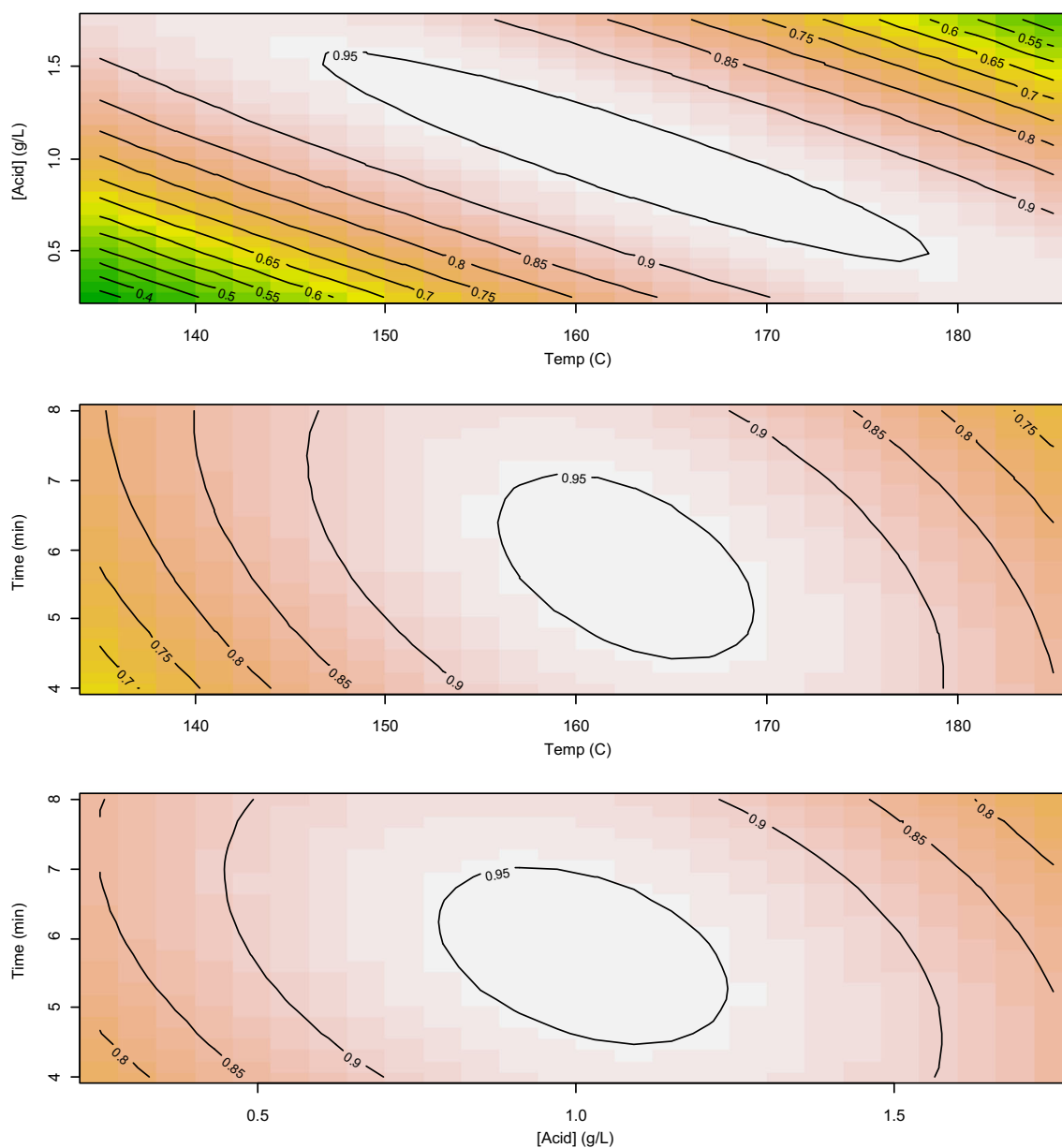
Fig. 1 Response surface method (*RSM*) plot of single-pass corn stover variability experiment with three independent variables catalyst concentration, reaction time, and reaction temperature, and one response variable: total sugar yield. **a** Acid concentration vs. temperature (fixed reaction time = 5.75 min). **b** Reaction time vs. temperature (fixed acid concentration = 1 g/L). **c** Reaction time vs. catalyst concentration (fixed temperature = 162 °C). There is little effect of reaction time over the range tested (see text)

and pelleted materials across a range of pretreatment temperatures while holding the values of the reactor static time and the acid concentration constant at 6.0 min and 1.0% (*w/v*), respectively. We varied the reactor temperature between 145 and 180 °C. The reaction space used for the conversion experiments can be visualized as the horizontal red line in Fig. 2a, which passes through the maximum in total sugar yield for the CS-SP material. All conversion experiments were performed once, except for two whole hybrid poplar runs, which were triplicated. The full dataset is included as Supplementary Material.

The results of these screening experiments for the eight materials (expressed as the total sugar yield after PT-EH) in both formats are shown in Figs. 3 and 4. In Fig. 3, we show the results for single pass corn stover (CS-SP, Fig. 3a), multi-pass corn stover (CS-MP, Fig. 3b), miscanthus (Fig. 3c), and switchgrass (Fig. 3d). In Fig. 4, we show the results for hybrid poplar (Fig. 4a), the corn stover-switchgrass blend (CS-SW, Fig. 4b), the corn stover switchgrass-miscanthus blend (CS-SW-MS, Fig. 4c), and the corn stover-switchgrass-hybrid poplar blend (CS-SW-HP,

Fig. 2 Response surface method (*RSM*) plot of single-pass corn stover variability experiment with two independent variables: catalyst concentration and reaction temperature. **a** Total sugar yield. **b** Glucose yield. **c** Xylose yield. **d** Furfural yield (from xylose). The total sugar yield plot clearly shows an optimal condition. The *red horizontal line* in (**c**) shows the fixed acid concentration used later in this work
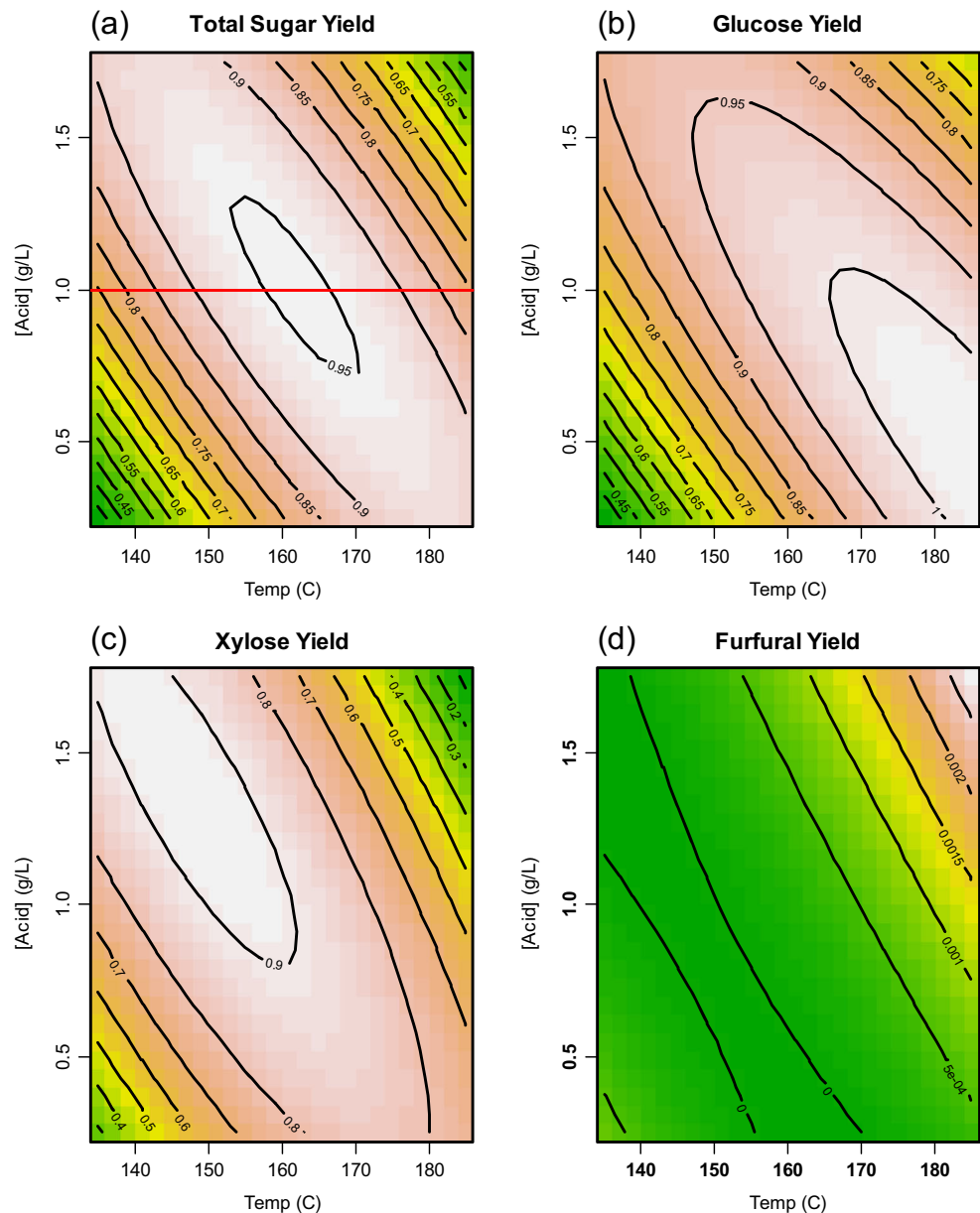

Fig. 4d). In Fig. 3a, we show a smooth curve through the total sugar yield data for the CS-SP whole feedstock. This curve is a cubic spline fit to the total yield data, and is included not as a mechanistic model, but to guide the reader's eye.

The variability of the overall assay can be estimated from the replicates of the single-pass corn stover (CS-SP) sample at 160 °C shown in Fig. 3a. This condition was the central point of the central composite design, and were performed a total of seven times. The mean and standard deviation of the overall total sugar yield is 96.2 ± 2.6%. This variability serves as a limit to the precision of the assay. We can calculate the pooled standard deviation (by reaction temperature) of another

sample with sufficient replication, the whole hybrid poplar feedstock sample (Fig. 5a). The pooled SD for this sample is 1.3%, or half the SD of the CS-SP sample.

The total sugar yield for all materials tested (in both formats) except for the hybrid poplar have broad but clear maxima with respect to pretreatment temperatures. The optimal temperatures vary slightly with the feedstock type, but not between feedstock formats. The hybrid poplar feedstock requires higher pretreatment temperatures and achieved the lowest total sugar yield (<80%) of any of the feedstock and feedstock blends. This is consistent with our previous results [23].

For all feedstocks except the single-pass corn stover (CS-SP, Fig. 3a), the total sugar yield appears slightly higher for the

Fig. 3 Total sugar yield from PT-EH assay versus PT temperature for whole (non-pelleted) and pelleted biomass feedstocks. **a** Single-pass corn stover (CS-SP). **b** Multiple-pass corn stover (CS-MP). **c** Miscanthus and **d** switchgrass. The *red line* in plot (**a**) is a cubic spline fit to the data
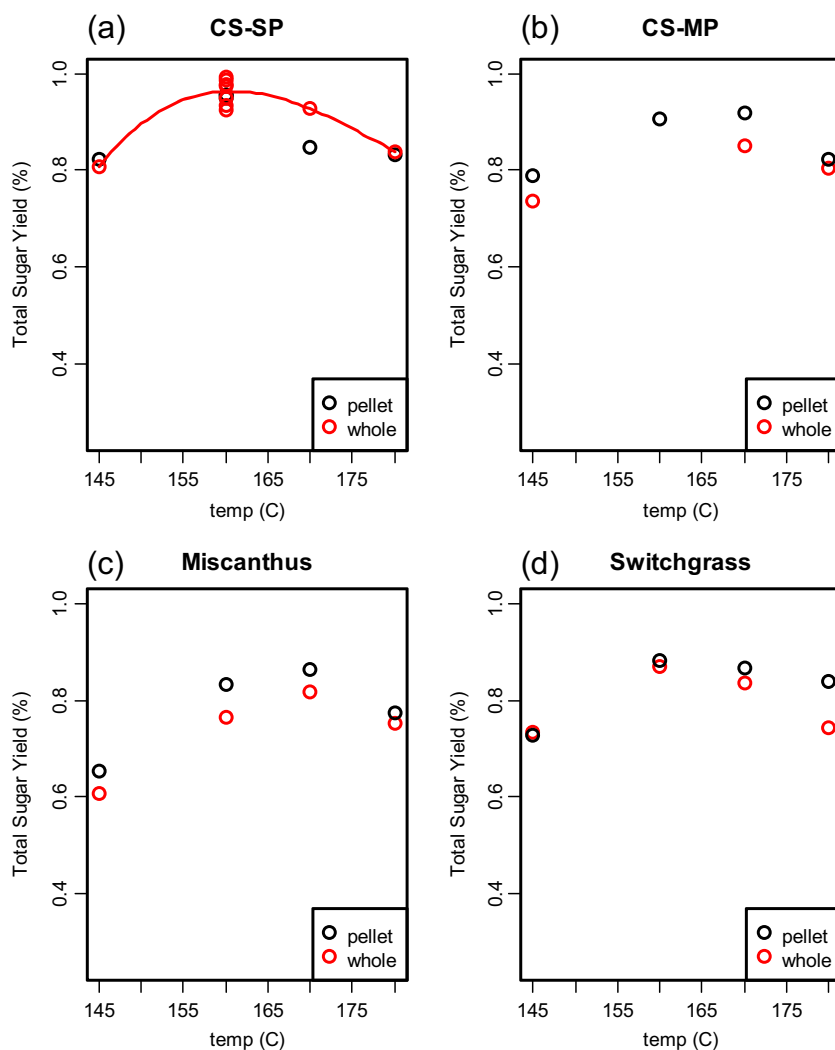
pelleted format than for the non-pelleted (whole) format. However, because of the variability of the assay itself, this positive effect is not statistically significant.

We performed an analysis of variance (ANOVA) on the data shown in Figs. 3 and 4. The results indicate that both reactor temperature and feedstock type are statistically significant factors in the variance of the total overall sugar yield, but the feedstock format (pelleted vs. non-pelleted) is not ($p = 0.05$). If we limit the analysis to the optimal conversion value (e.g., the highest yield for each feedstock/format combination, regardless of temperature), the results are the same. There are differences among the feedstock types, but no difference between pelleted and whole materials.

As discussed above, we found that the composition of the blended components was simply the weighted average of the composition of the individual feedstocks. We can use cubic spline fits like the one shown in Fig. 3a to assess whether the conversion performance of the blended materials follows the same behavior. We determined cubic spline fits to the miscanthus, switchgrass, and hybrid poplar data in Figs. 3 and 4, and used the weighted averages of these fits to predict the conversion performance of the three feedstock blends. The red curves in Fig. 4b–d are these predictions for the whole (un-pelleted) blends. While not exact fits, the curves describe the conversion performance of the blends quite well, given that the primary assay has a precision of approximately 3%.

Our interpretation of these results is quite simple. For the feedstock samples investigated using the laboratory-scale PT-EH assay used in this work, pelleting appears to have a slightly positive (although not statistically significant) effect on the overall total sugar yield for both feedstocks and feedstock blends. The composition and conversion performance of blended materials (either whole or pelleted) can be well-predicted from knowledge of the characteristics and blending ratios of the constituent feedstocks comprising the blend.

Fig. 4 Total sugar yield from PT-EH assay versus PT temperature for whole (non-pelleted) and pelleted biomass feedstocks. **a** Hybrid poplar (HP). **b** Two-component blend: single-pass corn stover and switchgrass (CS-SW). **c** Three-component blend: single-pass corn stover, switchgrass, and miscanthus (CS-SW-MS). **d** Three-component blend: single-pass corn stover, switchgrass, and hybrid poplar (CS-SW-HP). The *red lines* in plots (**b**–**d**) are the predicted total sugar yields calculated as the weighted average of the sugar yield data from the individual feedstocks (see text)
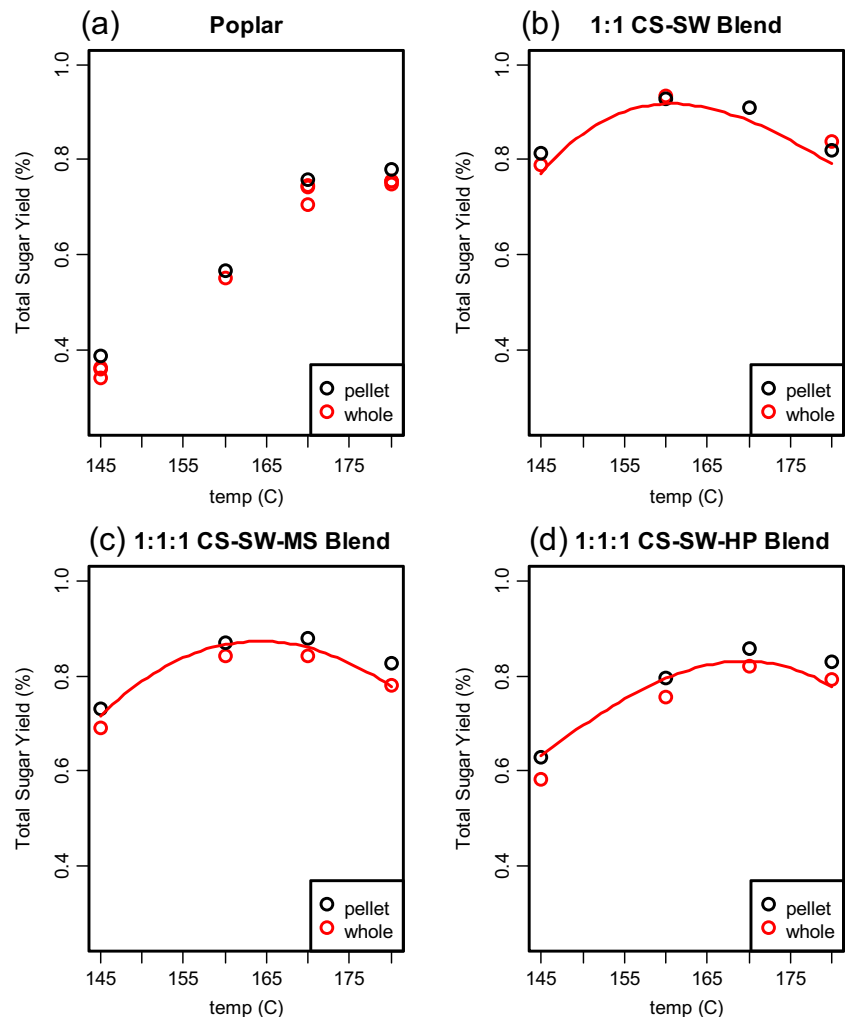
Conclusions

The overall goal of this work was to determine the effect of blending and pelletization on the release and yield of structural carbohydrates in a two-stage dilute acid pretreatment enzymatic hydrolysis (PT-EH) assay. The combined PT-EH assay has a limit of precision of approximately 3% as measured by replicate experiments at the center point of the central composite design (CCD). This sets a limit on the ability of the assay to detect differences among different feedstock samples, formats, or operating conditions.

A response surface model (RSM) approach to experimental design identified the experimental conditions which provide optimal biomass conversion as measured by the total overall sugar yield. While we investigated three pretreatment assay variables (reactor temperature, residence time, and dilute acid catalyst concentration) over the range of these variables used in this work, only reactor temperature and acid concentration affected total overall sugar yield. To screen the eight feedstock samples, we held all conditions except for pretreatment reactor temperature constant.

For the eight feedstock samples investigated in the laboratory-scale PT-EH assay used in this work, feedstock blends behave (both in terms of composition and conversion performance) as the weighted average of the behavior of the individual feedstock constituents. Pelleting appears to have a slightly positive although not statistically significant effect on overall total sugar yield. The use of a more precise PT-EH may have been able to demonstrate a statistically significant positive effect of pelleting.

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