

# Kinetics and Rheological Behavior of Higher Solids (solids>20%) Enzymatic Hydrolysis using Both Dilute Acid (DA) and Deacetylation and Disk Refining (DDR), and Mechanical Refining (DMR) Pretreated Corn Stover Slurries

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## ABSTRACT

Many potential biochemical pathways that produce advanced hydrocarbon fuels from renewable lignocellulosic biomass require hydrolysates with sugar high concentrations and low toxicity, enabling flexible fermentation strategies such as fed-batch fermentations producing high product titers and production rates during fermentation of the sugars. High sugar concentrations also increase the osmotic pressure in the hydrolysate, thus helping to decrease contamination issues. Direct production of high sugar concentrations from biomass without the need for concentration, conditioning, and/or purification unit operations may significantly decrease the overall energy demand. In our previous work we successfully demonstrated high biomass sugar concentrations (over 230 g/L fermentable sugars) produced from high solids (> 20wt.%) enzymatic hydrolysis of dilute alkali Deacetylated and Mechanically Refined (DMR) corn stover slurries. The goal of this work was to understand the effects of initial solids loadings of corn stover using different pretreatment methods on the rheological property changes of enzymatic hydrolysate slurries as well as the rate and yield of the enzymatic hydrolysis reaction. We performed high-solids enzymatic hydrolysis of Deacetylated and Disk Refined (DDR), Deacetylated and multi-stage Mechanical Refined (DMR) and Dilute Acid Pretreated (DAP) corn stover at four different initial total solids loadings: 17%, 22%, 27%, and 32%. Slurry samples were collected at regular intervals for measurement of monomeric and oligomeric sugar concentrations (glucose and xylose) particle size distributions, viscosities, and yield stress. We produced over 270 g/L of fermentable, monomeric sugars (157 g/L of glucose and 114 g/L of xylose) at 32% total insoluble solids during enzymatic hydrolysis of DMR substrates. The pumpability of the digested, high-solids enzymatic hydrolysates was shown to be comparable to other commercially relevant slurry streams such as honey, peanut butter and ketchup from the food industry. The conversion and rheology results indicate that high-solids enzymatic hydrolysis

(~33wt.%) of DMR substrates is a promising and scalable technology for producing high sugar concentration slurries from biomass.

## INTRODUCTION

According to the March 2016 update of the Department of Energy (DOE) Bioenergy Technologies Office's Multi-Year Program Plan, the U.S. Department of Energy is leading an effort to produce cost-effective advanced drop-in fuels from lignocellulosic biomass. One of the research efforts in support of achieving this goal presented in Section 2 of the report is biological conversion of biomass to intermediates and products with the primary objective of identifying and developing robust microorganisms capable of converting complex intermediates to desired target molecules in the presence of inhibitors at high rates, titers, selectivity, and yields.<sup>1</sup> In a review of inhibitory by-products of various lignocellulose treatment strategies a list of options for achieving high yields, rates, and titers were presented such as removal of the inhibitory compounds (e.g., acetate) prior to biological upgrading or developing a deconstruction strategy that does not produce certain inhibitors such as furans resulting from dilute acid pretreatment processes.<sup>2</sup>

One approach to produce high sugar concentration, low toxicity lignocellulosic hydrolysates is the Deacetylation and Mechanical Refining (DMR) process where acetate is removed in the mild alkali deacetylation step and the resulting solids undergo physical disruption in a disc refiner and Szego mill prior to enzymatic hydrolysis.<sup>3</sup> A previously reported techno-economic analysis investigated the production of a diesel blendstock produced from lignocellulosic sugars derived from deacetylation and dilute acid pretreatment followed by enzymatic hydrolysis for a 2000 MT/d (dry-basis) biorefinery.<sup>4</sup> In the techno-economic analysis, it was assumed that the enzymatic hydrolysis glucose and xylose yields were 90% and 93%, respectively, with an enzyme loading of 10 mg protein/g cellulose, and the resulting hydrolysate was 20% (w/w) fermentable sugars prior to concentration to 50% (w/w) for fed-batch fermentation.<sup>4</sup> Because of the high energy demand for concentrating sugar solutions, production costs are substantially increased due to need for larger reactor volumes and electricity may need to be imported

from non-renewable sources impacting the biorefinery's carbon footprint. Thus, concentrating sugars decreases economic viability and raises environmental concerns.

The ability to increase solids loading in an enzymatic hydrolysis is process dependent. For example, dilute-acid pretreated (DAP) biomass slurries often result in lower sugar yields and/or titers because of the strong enzyme inhibition present at higher solids concentrations potentially due to high solubilized sugar concentration and solubilized aromatics produced from the lignin during DAP.<sup>2, 5</sup> The possibility exists to improve sugar titers to above 20% (w/w) by increasing the biomass concentrations, which can be achieved by the DMR process. In a previously published paper, we successfully demonstrated over 230 g/L fermentable monomeric sugars produced using Deacetylated and Disk Refined (DDR) and Deacetylated and Mechanical Refined (DMR) corn stover substrates without any evaporation, concentration, conditioning, or purification steps, which opens the door to producing high sugar titer (>200g/L) hydrolysate as well as high ethanol titer (> 10% v/v) slurries from lignocellulosic biomass.<sup>3</sup> However, enzymatic hydrolysis at higher insoluble biomass solids (>20%) will lead to higher yield stress and higher viscosity of sugar syrups, causing potential mixing and pumping issues when scaling up the process<sup>6</sup>, which necessitates research on rheology and kinetics of higher solids content hydrolysates.

The goal of this work was to understand the effects of initial solids loadings during enzymatic hydrolysis of DDR- (single stage disk refining) , DMR- (multi-stage disk refining and Szego milling) and DAP-treated corn stover on the rheology of the slurries and reaction kinetics. We conducted high-solids enzymatic hydrolysis experiments for each material at four different initial solids loading (17%, 22%, 27%, and 32%). Slurry samples were collected at regular intervals for kinetics and rheological measurements, including glucose and xylose concentrations, total solids, total soluble solids in the liquors, total insoluble

solids, particle size distributions, viscosities, and yield stress measurements. In this work, we produced over 270 g/L of monomeric fermentable sugars (157 g/L of glucose and 114 g/L of xylose) at 33% (w/w) total insoluble solids loading in enzymatic hydrolysis using DMR substrates. In addition, a simple but vivid demonstration of pumpability of the high-solids enzymatic hydrolysate slurries were compared to other commercial food industry slurry streams such as honey, peanut butter and ketchup.

## METHODS AND MATERIALS

### *Feedstock Material and Pilot-Scale Deacetylation and Disc Refining (DDR)*

Corn stover used in this study was also used in our previous research<sup>3</sup>. The native corn stover contain 36.4% glucan, 30.8% xylan, 14.9% lignin, 1.8% galactan, 3.4% arabinan, and 2.7% acetate.

The same procedure was used for the dilute alkali deacetylation of corn stover and described elsewhere<sup>3</sup>. Briefly, it was performed in a 1900-L paddle mixer (American Process Systems, Gurnee, Illinois), where dry corn stover (100 to 120 dry kg) was added to the paddle mixer along with a dilute (0.1 M) sodium hydroxide solution with a 12:1 liquid to solid ratio (8 wt% total insoluble solids) slurry. After heating at 80°C for 2 hours, the black liquor was separated from corn stover slurry by overnight draining. The drained solids were pumped to a continuous screw press (Vincent Corp. Model CP10, Tampa, Florida) and dewatered to between 45% and 50% (w/w) total insoluble solids. The dewatered corn stover slurry was subjected to disk refining at the Andritz R&D facility in Springfield, Ohio, with the Sprout Model 401, 91-cm (36-inch) commercial-scale disc refiner. The atmospheric refiner trials were conducted at two different feed mass flow

rates. The motor load target for each refining energy level in the series was adjusted to maximize the refiner motor load for the given feed mass flow rate. The refiner energy consumptions used in the current study were measured at 128 and 212 kWh/oven dry metric ton (ODMT) as shown in Table 1.

**Table 1.** Deacetylation and disc/mechanical refining (DDR/DMR) conditions.

Sample ID	Deacetylation Conditions	S.E*. in Disk Refining (kWh/ODMT)	S.E*. in Szego milling (kWh/ODMT)	Total S.E. applied in refining (kWh/ODMT)
DDR	50 g/kg NaOH, 80°C, 2 h	212	0	212
DMR		128	~70	198

\*S.E. = Specific Energy

#### *Szego Milling*

The DMR process is a step forward of DDR process where the deacetylated and disk refined (DDR) corn stover is further milled in a so called Szego mill. The milling apparatus and process are also described in our previous publications<sup>3, 7</sup>. Briefly, DDR corn stover was further refined in a Szego SM-160 mill (The Szego Mill, Toronto, ON, Canada) constructed with three 60 mm diameter by 160 mm long steel rollers. The energy consumption for first stage DDR process was 128 kWh/ODMT, while the specific energy for Szego milling was about 70 kWh/ODMT (Table 1).

#### *Pilot-Scale Dilute Acid Pretreatment*

Dilute acid pretreatment was conducted in a 200 kg/day continuous, horizontal pretreatment reactor (Metso Inc., Norcross, GA, USA) at 158°C and a 5 minute residence time as previously described.<sup>8-9</sup> Prior to pretreatment, the corn stover was presoaked with 2% (w/w) sulfuric acid in the 1,900-L paddle mixer. After a 2 h soak time, the acid-impregnated corn stover was dewatered to 45–50% solids using the continuous screw press.

### *Enzymatic Hydrolysis*

Enzymatic hydrolysis was performed in 1-L stainless steel roller bottles loaded with 150 g of neutralized slurry at targeted 15%, 20%, 25%, and 30% (w/w) total solids loadings (solid loadings will be reported on a weight percent basis throughout this document), with actual total solids measured at 17%, 22%, 27%, and 32% total solids. The roller bottles employ gravitational tumbling as a mixing mechanism to homogenize solids by horizontally rotating the bottles at 4 rpm on a 3-deck roller apparatus for mini bottles (Wheaton Industries Inc., Millville, NJ).<sup>10</sup> Each of the bottles was loaded with five 2.54 cm (1 in) stainless steel balls to enhance mixing. The setup with roller bottles and stainless steel balls to hydrolyze high solids biomass could be scaled up using industrial equipment, such as peg mixer (often used in pulping industry), cement mixer and so on<sup>11</sup>. The roller apparatus was placed in a general purpose incubator (Model 1545, VWR International, LLC, West Chester, PA) that was controlled at 48.5°C. Novozymes' cellulase, Cellic<sup>®</sup> CTec3, and hemicellulase, Cellic<sup>®</sup> HTec3 preparations were used at enzyme loadings of 16 mg CTec3 and 4 mg HTec3 (protein basis) per gram of cellulose.

Enzymatic hydrolysis was performed for 144 hours and samples were taken on a 6, 24, 48, 72 (optional), 98 (optional), 120 and 144 hrs (optional). Upon completion of enzymatic hydrolysis, the slurries were subjected to Fraction of Insoluble solids (FIS), solids compositional, liquor analysis and rheological property tests.

### *Rheology*

Rheological analysis of the pretreated and enzymatically hydrolyzed corn stover samples were conducted using a Bohlin Gemini HR Nano stress-controlled rheometer (Malvern Instruments, Westborough, MA, USA). All experiments were performed using roughened 40 mm diameter stainless steel parallel plates. The roughened plates were machined to have surface



serrations of approximately 1 mm, which help prevent slippage at the sample-tool interface. The gap height between the parallel plates was set and maintained at 1 mm. Also, a clear plastic collar (diameter  $\sim 42$  mm) was placed around the parallel-plate geometry to prevent sample ejection during shearing. A Peltier temperature controller was used to maintain a constant temperature of  $25.0 \pm 0.1$  °C during rheological testing.

A common rheological property that describes a material's flow behavior is its yield stress. Yield stress is defined as the amount of force required to initiate the flow of a material (i.e., the force needed for a material to transition from a stationary state to a dynamic flowing state). Here, the yield stress of the corn stover materials was measured by slowly rotating the top plate under a controlled rotation rate (0.01 rad/s). The material's torque response (or shear stress) was monitored as a function of time. The maximum in the torque profile was then deemed the dynamic yield stress of the material ( $\tau_y$ , see Supporting Information Figure S1). An extensive, inter-laboratory study by Stickel *et al.* (2009) showed that measuring the yield stress via constant rotation rate was comparable to other common yield stress measurement methods (namely oscillatory measurements and model fitting of viscosity curve data) for corn stover slurries at various concentrations of insoluble solids. Due to its simplicity and minimal data analysis requirements, the constant rotation rate method for determining yield stress was used for this study.<sup>12</sup>

Once the pretreated corn stover materials were sufficiently enzymatically hydrolyzed and were "flowable," an apparent viscosity as a function of shear rate was measured. In order to determine a material's viscosity, the shear rate was increased from 1 to 1000  $s^{-1}$  using a stepped steady state profile. Each shear rate was held constant until the material's torque response deviated by less than 3% over a 5 second time interval. Once the material's response had

“steadied,” the collected data during the stable 5 second period was averaged and the instrument began measuring at the next shear rate in the predetermined window of shear rate values.

## RESULTS AND DISCUSSIONS

### *Effect of Solids on Sugar Concentrations of Enzymatic Hydrolysis*

Figure 1

Figure 1. Effect of initial solids loading on monomeric sugar concentration at the end of enzymatic hydrolysis

Figure 1 shows the monomeric glucose and xylose concentration upon completion of enzymatic hydrolysis using DDR, DMR and DAP substrates at different initial solids loadings. When initial solids loading equal or below 22%, the total amount of glucose and xylose concentrations are similar for all three processes, with DMR having slightly higher sugar titers than the other two. Increasing the solids over 22%, DMR and DDR show much higher sugar concentrations compared to DDR, indicating a decrease of sugar yields at higher solids (>22%) for dilute acid pretreated corn stover due to higher concentration of inhibitors. The highest glucose and xylose concentration are 157 and 114 g/L and both occurred at 32% initial solids using DMR substrates, reaching a total of fermentable sugars of 271 g/L. This number is 40 g/L higher than the one we previously reported at 28% initial solids using DMR substrates<sup>3</sup>. In addition, DDR and DMR substrates maintain very stable glucose and xylose ratio at 1.37 and 1.41, respectively, while that ratio of DAP substrates drops from 1.71 to 1.45 when solids increased from 16% to 33%, indicating cellulose hydrolysis is significantly inhibited at higher solids. For DAP substrates, xylose and xylo-oligomer are solubilized during acid pretreatment and proved to be strong inhibitors to cellulases<sup>13</sup>. The increasing of initial solids could result in more concentrated xylose and xylo-oligomers in the slurry, leading to stronger inhibition and lower glucose yield.

### *Kinetics of Enzymatic Hydrolysis Reactions at Higher Solids*

Figure 2 shows the reaction kinetics of monomeric glucose and xylose formation during enzymatic hydrolysis of the three different substrates at different total solids loadings. All substrates successfully liquefied after 120 hours, even at the highest initial solids loading of 33%. The DMR substrates hydrolyzed slightly faster in the beginning for solids equal or over 22%, and also achieved slightly higher glucose and xylose yields than DDR substrates. DAP substrates at lower initial solids achieved the highest glucose yield of 89%, which is 5% higher than DMR and 10% higher than DDR, but decreases down to 73% when solids loading increased from 16% to 33%. The impact of solids on sugar yields for DMR and DDR substrates are minimal, but causes significant decrease for DAP substrates as discussed previously. For all the substrates, increasing solids loading will reduce the initial monomer sugar formation rate, showing the reaction rate may be controlled by water that is accessible to the cellulose. Lipsinsky et al. has shown that the hydrolysis of crystal cellulose involves the disengaging the inter-chain hydrogen bonding between cellulose layers followed by swelling of the cellulose chain with water facilitating accessibility of the enzymes<sup>14</sup>. The higher solids ( $\geq 20\%$ ) enzymatic hydrolysis result in much less water involved in the hydrolysis process compared to lower solids ( $< 20\%$ ) enzymatic hydrolysis and might lead to the slowdown of the hydrolysis in the beginning. However, this slowdown may not affect the final sugar yields as with the hydrolysis progress forward, the rate controlling step switches to the accessible cellulose which become less available in lower solids (used in the initial stage) and more available in higher solids. Therefore, the reaction rate for higher solids after 24 or 48 hours starts to pick up and eventually help the higher solids enzymatic hydrolysis to achieve the same sugar yields by given an enough time period (144 hours).

Figure 2

**Figure 2.** Effects of initial solids loadings on monomeric sugar concentrations during enzymatic hydrolysis of A) DDR, B) DMR, C) DAP pretreated solids. Total solids were measured for each slurry as shown in the figure legends.

It should be noted that Panel C2 in Figure 2 is not xylose yield for DAP hydrolysis instead it shows the xylose concentration that keep almost unchanged during the hydrolysis, showing that the residual xylan in DAP substrates cannot be further converted to xylose during enzymatic hydrolysis.

#### *Liquor and Total Solids Changes in Enzymatic Hydrolysis Reactions at Higher Solids*

The total solids and dissolved liquor solids concentration of DAP, DDR, and DMR slurries during enzymatic hydrolysis are shown in Figure 3. Dissolved liquor solids content increased by 5% to 15% during the course of enzymatic hydrolysis, resulting in as much as a 6% rise in the absolute solids concentration. This effect was due to water being incorporated in the soluble sugars produced from polysaccharides catalyzed by enzymes<sup>15</sup>. The effect was more pronounced at higher total solids concentrations because there was less available water at the beginning of enzymatic hydrolysis. The initial liquor solids content was nearly zero for the DDR and DMR processes, and the liquor solids content post enzymatic hydrolysis was the same as the initial total solids concentration (32%) before enzymatic hydrolysis indicating very high conversion yields of the polysaccharides into component glucose and xylose sugars. The takeaway message here is that the total solids of higher solids enzymatic hydrolysis slurries does not remain unchanged throughout the hydrolysis, which increases due to the consumption of water during polysaccharides converting to monomeric sugars. The change is not negligible as at 33% initial solids the total solids increase could be as much as 6%, which could significantly affect final sugar yield calculations<sup>15</sup>.

Figure 3

**Figure 3.** Effects of time on enzymatic hydrolysis reactions on total solids concentration and liquor solids concentration using DDR, DMR, and DAP samples. The measured total solids for each slurry is shown in the figure legends.

*Particle Size Distributions Changes in Enzymatic Hydrolysis Reactions at Higher Solids Loadings*

Figure 4 shows the effect of time and initial solids ( $t=0$ ) on particle size distribution (Panel A) as well as mean particle size (Panel B) of the DDR, DMR and DAP substrates during enzymatic hydrolysis. The initial particle size distribution of DDR substrates (Panel A-1) shows a very similar double-peak behavior compared to untreated corn stover as reported previously.<sup>16</sup> This suggests that single stage high solids disk refining at a refining energy of 200 kWh/ODMT may not be sufficient to reduce all the large particles to below 200  $\mu\text{m}$  as the demanded particle size range. With the hydrolysis progressing, the right peak that stands for large particles with particle size ranging approximately from 100-3000 starts to shrink and the whole particle size distribution migrates to the left suggesting an overall decrease of particle size due to sugar release. Panel B-1 reflects such observation by showing the mean particle size decreases from approximately 200-300  $\mu\text{m}$  at  $t=0$  to about 30  $\mu\text{m}$  at the end of the hydrolysis. The rise of particle size for 27% and 33% initial solids from 6 hrs to 24 hrs are mainly caused by the difficulty in sampling evenly over the double-peak distributed particles. Panel A-2 shows the distribution of DMR substrate at 33% initial solids in a time-course manner as well. Thanks to the two-stage refining with different refining mechanism<sup>17</sup> and higher total refining energy, the double peak particle size distribution is much weakened for DMR substrates and also showing lower mean particle size around 100-160  $\mu\text{m}$  (Panel B-2). The DMR substrates are showing similar particle size reduction performance as DDR substrates, with final particle sizes landed around 30  $\mu\text{m}$ . The higher initial solids concentration of the hydrolysis longer the time is needed to reduce the particle sizes to that level. The DAP substrates have the lowest mean particle size (Panel B-3) before hydrolysis, ranging from

30-50  $\mu\text{m}$ . The double peak distribution is weakened most among all three pretreatment methods as a consequence of severe acid pretreatment. The mean particle sizes are reduced to 10-20  $\mu\text{m}$  with the completion of enzymatic hydrolysis. Interestingly, Panel A-3 shows a trend of accumulation of small particles with particle size around or less than 1  $\mu\text{m}$ . The generation of double peak at lower particle size region provides the evidence that the enzymatic hydrolysis could produce small lignin/carbohydrate fragments that separate from large particles.

Figure 4

*Figure 4. Effect of time on particle size distribution (Panel A) and effect of time and initial solids concentration on mean particle size (Panel B)*

The small particle sizes of the residual solids after enzymatic hydrolysis (primarily lignin) pose solid/liquid separation challenges. The residual lignin can be burned for cogeneration of steam and power or recovered and converted to a valuable by product. However, we have found that DDR and DMR lignin residual solids are much more difficult to separate from the liquor stream using centrifugation or filtration than DAP-EH lignin residual solids, even though, the latter has a 3-fold smaller particle size and should be more difficult to separate. We speculate that this is possibly caused by the negative charge of lignin from alkali pretreated DDR and DMR substrates, which produces an electrostatic repulsion force between adjacent particles<sup>18</sup>. The charge appears to be sufficiently high that the lignin residual solids from the DDR and DMR enzymatic hydrolysis remain discrete, dispersed, and in suspension.

*Rheological Changes during the Enzymatic Hydrolysis Reactions at Higher Solids Loadings*

Figure 5 shows the measured yield stress as a function of enzymatic hydrolysis reaction time (Panel A) and yield (Panel B) for the DDR, DMR, and DAP substrates. Regardless of solids concentration and pretreatment strategy, the yield stress of the corn stover slurry decreased as hydrolysis progressed. The initial yield stress for DMR and DDR corn stover prior to the beginning of hydrolysis were found at approximately  $10^5$  Pa, regardless of the solids concentrations in the reactions, because the samples were primarily wet biomass. However, the DAP substrates had an initial yield stress of approximately 800 Pa when the total solids concentrations were below 25%, because the samples were partially hydrolyzed in the dilute acid pretreatment step. Increasing the total solids concentrations of the dilute acid pretreated substrates to 30% increased the initial yield stress to approximately 5000 Pa. After hydrolysis began, yield stress was found to decrease. The decreasing rate of yield stress was directly correlated with solids concentration – the higher the solids concentration, the lower the rate that the yield stress decreases.

In addition, Figure 5A-1 compares the yield stress of DDR hydrolysate slurries to a variety of food industry slurries such as peanut butter, jam, mayonnaise and ketchup. A common rule of thumb is that a material becomes “pumpable” when its yield stress is less than about 10 Pa (denoted by the horizontal black dashed lines in Figure 5 Panel A). Most of the hydrolysate slurries tested were found to be pumpable after 144 hours of digestion. We found that the single-stage DDR and DAP substrates had yield stresses right on the pumpable line for 30% TS slurries. We speculate that increasing the enzyme loading slightly could accelerate the liquefaction process and thus reduce the yield stress to a pumpable level sooner. In contrast, the multi-stage DMR substrate at 30% insoluble solids loadings showed that the yield stress decreased to below 10 Pa after approximately 60 hours of enzymatic hydrolysis, and approached 1 Pa after 144 hour hydrolysis.

Figure 5

**Figure 5.** Yield stress as a function of enzymatic hydrolysis reaction time for (a) DDR, (b) DMR, and (c) DAP corn stover residues at different solids loadings. The solid lines are power-law function fits to the measured yield stress data, while the horizontal dashed lines indicate a yield stress of 10 Pa, a threshold value at or below slurries are typically deemed “pumpable”.

In Figure 5 Panel B, yield stresses are plotted against insoluble solids. Overall, yield stresses of DDR and DMR substrates collapse onto a single curve showing similar performance of dilute acid pretreated corn stover which is also in agreement with the conclusion previously described by Roche et al. who further elucidate that yield stress is monotonically correlated with underlying physical parameter of volume fraction which in turn directly correlates to insoluble solids<sup>6</sup>. The conclusions are further verified here on substrates that are subjected to completely different chemical and mechanically pretreatment with the raise of initial solids of enzymatic hydrolysis substrates from 17% to 33%, showing yield stress is directly correlated with insoluble solids.

Figure 6 shows the measured apparent viscosity of DDR and DMR substrates versus time. The viscosity also decreased as the enzymatic hydrolysis process progressed. The decrease of viscosity is attributed to the hydrolysis and depolymerization of the dissolved polysaccharides, especially xylooligomers. However, the viscosity tends to reach a minimum value and plateaus after a given amount of hydrolysis time. The minimum viscosity of the liquefied corn stover slurry increased as the solids loading increased, which was determined by the amount of soluble sugars in the slurry. The time required to reach the plateau also increased with increasing solids concentration: for DDR at 17% solids, the required time was 6 hours; at 33% solids, that time became 144 hours.

Figure 6



**Figure 6.** Apparent viscosity of (Panel A) DDR and (Panel B) DMR corn stover residues measured as a function of enzymatic hydrolysis reaction time.

## Conclusions

We investigated the kinetics and rheological behavior of higher solids (solids > 20%) enzymatic hydrolysis reactions using three deconstruction and pretreatment processes (dilute acid, DDR, and DMR) at four levels of initial solids loading (17%, 22%, 27%, and 33%). We demonstrated over 270 g/L fermentable sugars (157 g/L of glucose and 114 g/L of xylose) produced at 32% total insoluble solids in enzymatic hydrolysis reactions using DMR substrates. For DDR and DMR hydrolysates, liquor densities increased by 12% while viscosity decreased by almost 10-fold when comparing the 144 hour enzymatic hydrolysis samples to the initial starting slurry mixtures. For DMR substrates at 32% initial total insoluble solids, yield stress was reduced to below 10 Pa after 60 hours of hydrolysis, indicating the slurry is pumpable. In contrast, the monomeric sugar yields decreased with increasing total solids concentrations in enzymatic hydrolysis for the dilute acid pretreated corn stover substrate. The kinetics of the enzymatic hydrolysis reactions showed different behavior for the dilute acid pretreated samples compared to DMR samples, with DMR showing higher rates of enzymatic hydrolysis. The measured rheological properties of the DMR corn stover showed the most rapid decrease in viscosity of the high (32 wt%) insoluble solids enzymatic hydrolysis substrate of the three deconstruction processes tested with the yield stress showing a pumpable slurry in 60 hours. All of the high insoluble solids enzymatic hydrolysis slurries (>30%) observed a viscosity decrease to that of water after 7 days enzymatic hydrolysis reaction time. The DMR process can produce substrates that are digestible at high yields in enzymatic hydrolysis (90%) with high hydrolysate sugar concentrations (270 g/L combined monomeric glucose and xylose) at high initial insoluble solids loadings (32%) that are also pumpable after 60 hours of enzymatic hydrolysis (yield stress  $\leq 10$  Pa).

## SUPPORTING INFORMATION

- Yield stress measurement (Figure S1)
- Visual Observation of Pre- and Post- Enzymatic Hydrolysis of DAP, DDR and DMR Substrates (Figure S2)

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### **Author Contributions;**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. ‡These authors contributed equally. XC and EK led and designed the experiment. XC also conducted the hydrolysis work and chemical analysis. NC led and designed the rheology experiment and performed data analysis and modeling. EK conducted hydrolysis work and chemical analysis. WW designed and conducted particle size measurement. DS and LT reviewed and revised the manuscript. MPT led the whole project and help conducted deacetylation and mechanical refining work.

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