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Rheology of Enzyme Liquefied Corn Stover Slurries:
The Effect of Solids Concentration on Yielding and Flow Behavior

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Abstract

The measurement of yield stress and shear thinning flow behavior of slurries formed from unpretreated corn stover at solids loadings of 100 to 300 g/L provides a key metric for the ability to move, pump, and mix this lignocellulosic slurry, particularly since corn stover slurries represent a major potential feedstock for biorefineries. This study compared static yield stress values and flow hysteresis of corn stover slurries of 100, 150, 200, 250, and 300 g/L, after these slurries were formed by adding pellets to a cellulase enzyme solution (Celluclast 1.5L) in a fed-batch manner. A rotational rheometer was used to quantitate relative yield stress and its dependence on processing history at insoluble solids concentrations of 4 to 21% (w/v). Key findings confirmed previous observations that yield stress increases with solids loadings and reaches ~3,000 Pa at 25% (w/v) solids concentration compared to ~200 Pa after enzyme liquefaction. While optimization of slurry forming (i.e., liquefaction) conditions remains to be done, metrics for quantifying liquefaction extent are needed. The method for obtaining comparative metrics is demonstrated here and shows that the yield stress, shear thinning and shear thickening flow behaviors of enzyme liquefied corn

stover slurries can be analyzed using a wide-gap rheometry setup with relative measuring geometries to mimic the conditions that may exist in a mixing vessel of a bioreactor while applying controlled and precise levels of strain.

Introduction

Currently, a large majority of biofuels are produced from corn grain as it can be economically converted to ethanol at an industrial scale.[1,2] Although these first-generation biofuels are a potential renewable energy source, there are concerns regarding their use and competition with the demand for food.[3] To advance the use of non-food plant products for renewable energy, second-generation biofuels made from lignocellulose biomass are being explored, such as corn stover which includes the stalks, cobs, and leaves of corn plants.[4–6]

Pelleting of biomass can increase the unit density of raw biomass resources by as much as tenfold, resulting in a flowable product that is compatible with current biomass supply system infrastructure.[7,8] Densified biomass has a lower moisture content and can be stored for longer times with minimum loss in quality.[9] In a study on the effect of die diameter on density and durability of pellets made from high moisture biomass, an estimated 10-13% (w.b.) loss of moisture was measured for woody and herbaceous biomass, including corn stover, during the pelleting and cooling process.[10] Prior work by Idaho National Laboratory on the impact of dry densification on pretreatment and enzymatic hydrolysis found that pelleting did not render corn stover more recalcitrant to dilute-acid pretreatment under low-or high-solids conditions and even enhanced ethanol yields.[8] Some previous studies on warm-season grasses have shown that the use of pellets compared to straw did not impact sugar and ethanol yields,[11] and pelleting of switchgrass allowed for less severe pretreatments and reduced enzyme loadings while achieving high hydrolysis yields.[12]

Handling and processing corn stover solids directly are technically challenging so instead, water-based slurries are created to simplify feeding into pretreatment reactors. For a biorefinery to become financially solvent, the concentration of corn stover solids in the slurry must be relatively high at 200 to 300 g/L.[13] At high solids concentrations, biomass slurries typically exhibit non-Newtonian flow behavior including shear thinning in addition to displaying considerable yield stresses and viscosities.[14–17] Such flow behavior can inhibit mixing and transport during processing and increases operational costs.[18] Thus, knowledge of a slurry's yield stress is critical for biorefinery design so that capital equipment can be properly sized to overcome this stress to initiate and effectively mix and transport the biomass.[14]

Enzyme-based biomass liquefaction processes can be successfully utilized to reduce the overall viscosity and yield stress of corn stover slurries.[14,15] The term "liquefaction" is classically used in reference to the enzymatic conversion of starch to soluble dextran[19] and more casually used to refer to a viscosity reduction or disaggregation of a concentrated suspension.[20] In this study, we build upon prior literature[21,22] that utilizes enzymes for liquefaction of lignocellulosic biomass (corn stover) in the form of pellets.[23,24] The concepts for liquefaction for cellulosic biomass materials including sugarcane bagasse and hardwoods have been established and reported previously.[23,25–28] Liquefaction and enzymatic hydrolysis can be performed to process cellulose without pretreatment (e.g., corn pericarp,[29] although this is a less recalcitrant material than corn stover and has low lignin content). On-going research to determine how lignin interferes with enzyme hydrolysis of cellulose and strategies for mitigating inhibition and deactivation (e.g., use of endoglucanse[30,31]) is expected to provide further guidance on the extent to which enzyme loadings might be reduced.

The yield stress of a slurry will impose the minimum power requirements for processing equipment in a biorefinery.[14,20] The magnitude of a slurry's yield stress is positively correlated to the packing efficiency of solid particles and, ultimately, the number of frictional contacts.[32–34] Following enzymatic liquefaction, the biomass slurry will contain a reduced concentration of insoluble solids, and the remaining solid particles will become negatively buoyant and settle if processing operations (e.g., mixing, pumping) are halted.[35–37] The static yield stress of the slurry is the necessary amount of stress to disrupt the frictional contacts between settled particles as required for the onset of flow while the dynamic yield stress is the minimum amount of stress necessary to maintain flow.

Many biomass systems, including corn stover slurries, are notoriously difficult to evaluate due to slurry heterogeneity, both in size and shape of the solid particles as well as concentration. These structural characteristics contribute to frequently observed non-Newtonian and non-uniform flow behaviors, including shear thinning, yielding, and wall slip among others. Corn stover particles can have large sizes that can range to lengths 2 mm and greater.[14] During rheometry experiments, larger particles may settle due to gravity, migrate, and exhibit wall slip, all of which will directly impact the applied/detected torque. In dilute slurries, particle settling will initially result in a decreased torque response as deformation is localized to the low-viscosity fluid layer above the settled particles. Then upon complete sedimentation, a greater torque magnitude is typically measured from the highly viscous layer that forms at the bottom of the cell.[38] A decreased torque response can also be observed for dilute slurries as particles migrate away from the moving fixture to the stationary wall.[39] For dilute as well as concentrated slurries, large particles can also physically "bridge" the gap within absolute measuring geometries (e.g., small-gap parallel-plate and cone-plate fixtures) and generate an increased torque response. Also, it is expected that the concentrated, more "paste-like" slurries will exhibit wall slip that may be difficult to visually detect but will ultimately reduce the measured torque.[40] Sample fracture and ejection during the measurement of concentrated slurries is also possible and will reduce the measured torque.[14,41] As many of these flow behaviors ultimately lead to reductions in the measured torque, the calculated stress response will likely be artificially reduced and not a true reflection of the slurry's rheology.

Selection of an appropriate rheometer measuring geometry can potentially reduce the impact of slurry heterogeneity and the resulting measurement artifacts on the acquired data. In a comprehensive inter-lab study by Stickel et al.[14], roughened parallel-plate geometries were employed successfully for biomass slurries, although containment collars were required to prevent material ejection during shear. Wide-gap rheometer setups (e.g., gaps > 1 mm) are often recommended for use with biomass slurries to reduce the likelihood that individual large particles will span the gap and directly influence the measurements. When using a wide-gap fixture, calibrations should be performed to convert angular velocity to shear rate since the flow field varies from the more conventional small-gap fixtures.[42] Relative measuring geometries such as roughened or serrated plates and vane spindle geometries can be used to mitigate (though not entirely prevent) wall slip while multi-blade starch pasting impellers can be used to mitigate the effects of both wall slip, migration, and settling by promoting mixing during the measurement.[43,44] An additional advantage of blade-type spindles is their general resemblance to industrial mixers.[45,46] When using relative measuring geometries, however, additional calibration steps are typically performed to determine the appropriate conversion factors though some prior studies have found yield stress measurements to be independent of vane size.[40,47]

Furthermore, after selecting and calibrating the appropriate measuring geometry, the actual analysis of stress responses from sheared biomass slurries can be challenging. In particular, for a corn stover slurry, knowledge of its yield stress is considered critical to assess the slurry's relative ability to be pumped. However, there is no universal protocol or technique to determine yield stress and different strategies produce values that can vary by orders of magnitudes.[48] Methods for measuring the yield stress of corn stover slurries include: (1) decreasing and increasing shear rate ramps (flow curves) which can often be described by empirical models to determine a single yield stress value (e.g., Bingham, Hershel-Bulkley, Casson models for "simple" yield stress fluids); (2) constant low shear rate tests (also referred to as a stress growth test[47]) where the yield stress is calculated from the maximum torque as a function of time; and (3) oscillatory amplitude sweeps where the yield stress is defined as the maximum elastic stress.[14–18,35] However, corn stover slurries are not simple yield stress fluids because the measured yield stress will depend on the deformation history.[45] In these thixotropic systems, there are (at least) two yield stresses – static and dynamic – which can be measured from an increasing shear rate ramp and a decreasing shear rate ramp, respectively.[48]

In this study, the ability of an enzyme to liquefy untreated corn stover particles within slurries of varied solids concentrations (10-30%, w/v) was evaluated through rheological measurements of static yield stress given its relevance to real-world manufacturing operations (e.g., recovery after maintenance shutdowns). Measurements were conducted in a rotational rheometer with a wide-gap setup employing two relative measuring geometries (starch pasting impeller and 4-blade vane spindle) to accommodate the heterogenous, two-phase slurries, which contained solid particles up to 3 mm in size and aspect ratios of 1 to 35. Flow hysteresis measurements were also performed to assess the dependence of static yield stress on the slurry's processing history.

2.0 Methods

2.1 Materials and Enzyme Liquefaction

Corn stover (a Pioneer P0157 AMX cultivar) was harvested in Poweshiek County, Iowa, USA in the fall of 2017. Complete details on corn stover processing – harvesting, baling, moisture content adjustments, sizing, milling, pelleting, and compositional analysis – can be found in dos Santos, et al.[24] Briefly, field-dried corn stover was harvested in multiple passes by windrower and baler. Baled corn stover was then adjusted to 26% (w/w) moisture content, hammer-milled and sieved to a pre-determined particle size distribution with a geometric mean of 1.68 ± 0.22 mm. Milled stover was formed into 6-mm pellets with 15% (w/w) moisture content by a Bliss commercial ring die pellet mill (200B-350, 5 tons hr^{-1}).[9] The chemical composition of the corn stover pellets was determined according to National Renewable Energy Laboratory Analytical Protocols (LAPs)[49,50] and falls within the general ranges of corn stover compositions, although ash content was significantly higher (19.2% on a dry basis) than typical ash contents (3-4%) likely due to rainy conditions and increased entrainment of soil particles in the corn stover during conventional three pass harvesting.[46,51,52]

Enzymatically liquefied slurries were created at a scale of 300 ml with solids loadings of 10, 15, 20, 25, or 30 % (w/v). First, 0.1M citrate buffer (pH = 4.8) was added to a wide-mouth 500 mL beaker, then Celluclast 1.5L enzyme cocktail (Sigma Aldrich) was added to the beaker at a loading of 1 FPU per gram of solid material (2.2 mg protein/g dry solids). For control samples, no enzyme was added. The total amount of enzyme was added at the beginning of the reaction and adjusted throughout to maintain a concentration of 1 FPU. Buffer and enzyme were brought to 50°C in a JULABO water bath under agitation at 300 RPM. Two marine impellers were used in an up-down configuration, where the impeller at the surface of the media pushed liquid downwards and the impeller placed 1 cm from the bottom of the flask pushed liquid upwards in the reactor. After the liquefaction media reached 50°C, corn stover pellets were added in a fed-batch manner: 66 g dry solid/L was added initially followed by 47 g/L at 0.5 h and 1 h, 31 g/L at 1.5 h, and 15 g/L every 0.5 h until the desired concentration was achieved. Liquefaction was carried out for a total of 6 hours. At the end of liquefaction, agitation was stopped, and all liquids and solids were collected in a screw-top flask and stored at 4°C for further analysis.

Celluclast 1.5 L (Novozyme, Bagsvaerd, Denmark - 57 FPU/mL, endoglucanase - 800 UI/mL and 126 mg protein/mL) was used for the enzymatic hydrolysis of lignocellulose biomass to monomeric sugars. There are more effective commercially available enzyme preparations containing higher concentration of beta-glucosidase and beta-xylosidase activities. However, this work aims to study the formation of a slurry while minimizing production of monomeric sugars to avoid their possible loss in the subsequent step of pretreatment. Celluclast 1.5 L contains effective amounts of critical enzymes for slurry formation, i.e., endoglucanase, xylanase and cellobiohydrolases, but not beta-glucosidase and beta-xylosidase activities, and in this sense, it is an enzyme preparation with desired features for this approach.

Optical microscopy (Olympus S7X7) was used to quantify the size and aspect ratio of solid particles in the untreated control and enzyme treated corn stover slurry with solids concentrations of 30%. Samples were diluted by 300x in deionized water and droplets were placed on a glass slide for imaging. ImageJ was used to measure the major and minor axes of all visible particles that were above 10 μm in one dimension. In 10 images per sample, a minimum of 100 particles were measured of enzyme-treated and untreated (control) samples.

2.2 Yield Stress and Shear History Measurements

Rotational rheometry of corn stover slurries was performed using an Anton Paar MCR 702 operated in a shear rate-controlled mode. A starch pasting impeller (Anton Paar ST24-2D/2V/2V-30; diameter of 24 mm and an active length of 30 mm; see Figure S1) was employed low and moderate concentration slurries (10, 15, 20 and 25%). For higher concentration slurries (20, 25, and 30%), a 4-bladed vane spindle (Anton Paar ST24-4V-30/24; see Figure S1) was utilized with a spindle diameter of 24 mm and blade length of 30 mm. The starch cell impeller was selected for use given its similarity to equipment used for industrial mixing; the vane spindle was used given its ubiquity in most processing labs. All measurements were conducted in a glass beaker setup with an inner diameter of 56 mm (gap = 16 mm) using the Anton Paar Flexible Cup Holder shown Figure S1. As this is a non-standard and relative measuring system, rheometer software adjustments and calibrations were performed using a heavy mineral oil (details in Supporting Information). Calibrations, though not always required for yield stress measurements with vane geometries,[47] ensure that data collected using the pasting impeller and the vane spindle can be directly compared in this study. And unlike parallel-plate geometries,[14] the use of a cup-style geometry better contained the solid particles in the slurry as well as free fluid during measurements over a wide range of shear rates.

After hand mixing and loading a slurry sample into the glass beaker, the rheometer measuring fixture (vane spindle or starch pasting impeller) was lowered into the sample and 15 minutes of rest was allowed to account for shear effects from loading. Increasing shear rate ramp (flow curve) experiments were conducted at room temperature on the corn stover slurries between shear rates of 0.1-1000 s^{-1} to determine the apparent static yield stress, quantified as the maximum shear stress experienced at low shear rates during testing.[17,46,53] From the many ways of measuring yield stress described in the background section, increasing shear rate ramps were selected for this study given their practical relevance to industrial operations (e.g., resuming processing after maintenance shutdowns). The static yield stress values determined from increasing shear rate ramps are typically larger than dynamic yield stress values[48] and thus represent a kind of "worst case" scenario that could be encountered during operations. Note that shear rates of 0.1-100 s^{-1} were previously found to be similar to impeller speed (1-400 RPM) in a 1 L Bioflo stirred reactor.[46] For 30% concentration slurries, an additional constant low shear rate test was conducted at room temperature using the vane spindle at a shear rate of 0.1 s^{-1}, and the static yield stress was identified as the maximum shear stress observed. Flow curve and constant rate tests were repeated with fresh samples a total of three times. The effects of shear history on the behavior of 15% and 20% concentration slurries were evaluated by a series of sequential flow curve measurements (starch pasting impeller; increasing shear rates from 0.1-1000 s^{-1}) separated by progressively increasing zero-shear "resting" periods of 1-360 minutes in duration.

3.0 Results and Discussion

3.1 Slurry Heterogeneity

Shown in Figure 1 are images of selected slurry samples following enzyme liquefaction to highlight the visible differences with increasing solids concentration. The slurry transitioned from a settled two-phase mixture (Figure 1a of a 10% slurry) to a thick paste (Figure 1c of a 30% slurry). Milled and pelleted corn stover is expected to have a large particle size range that will change during processing due to a combination of mechanical breakage and enzyme action during the fed-batch processing.[24] The optical microscope image shown in Figure 2 confirms that the untreated corn stover slurries were heterogeneous in solid particle size and shape. Additionally, the aspect ratio of the solid particles varied between 1 to 35. Particle size characterization by optical image analysis of diluted 30% slurry samples (Figure 3) revealed that the enzyme-treated sample contained a greater population of particles that were 200-500 microns in size compared with the untreated (control) sample. The aspect ratio range was unchanged.

3.2 Flow Behavior of Low Concentration Slurries (10-15%)

For relatively low loadings of corn stover, the slurry contained two distinct regions with a settled bed of corn stover particles surrounded by liquid (see Figure 1a). Figure 4 shows the shear stress and viscosity responses measured using the starch pasting impeller for slurries initially containing 10% and 15% solids. The low concentration slurries displayed distinct behaviors dependent on shear rate. At very low shear rates (<1 s^{-1}), an increase in shear stress was observed as the settled particles formed frictional contacts with each other. As the shear rate was increased to 1 s^{-1}, the measured shear stress increased to a local maximum which was defined here as the yield stress. For both the 10% and 15% concentration slurries, the enzyme-treated samples displayed lower yield stress values than the untreated (control) slurry samples (Figure 4a). With enzyme liquefaction, the average yield stress of the treated 10% slurry samples was reduced by ~ 53%, from 50.0 ± 8.4 Pa for the untreated sample to 23.9 ± 3.9 Pa. Similarly, with enzyme liquefaction, the average yield stress of the treated 15% slurry samples was reduced by 32%, from 107.0 ± 13.8 Pa for the untreated slurry to 72.5 ± 4.4 Pa.

After yielding, decreasing shear stress and viscosity values were observed with increasing shear rates (1-100 s^{-1}). This shear thinning behavior clearly displayed in Figure 4b likely resulted from particle alignment and rearrangement. With increased shear, it is expected that particles align in the direction of the applied shear forces.[54,55] At very high shear rates (>100 s^{-1}), increasing shear stress and viscosity values were observed with increasing shear rates. It is likely that this shear thickening behavior resulted from particles being resuspended by the applied shear and increasing particle-particle interactions (interparticle friction) at these fast shear rates.[56] The thinning-to-thickening inflection point in the shear stress response of these slurries was the point at which particles transition from a settled state to a resuspended state, as reported by Crawford et al.[45] in a system with settled α-cellulose particles. In such a transition, the settled particle bed begins to erode starting from the top layer into the liquid phase of the slurry until all the particles were suspended throughout the entirety of the liquid volume.

3.3 Flow Behavior of Moderate Concentration Slurries (20-25%)

Figure 5 shows the shear stress and viscosity responses measured using the starch pasting impeller for slurries initially containing 20% and 25% solids. Similar to the low concentration slurries, for both the 20% and 25% concentration slurries, the enzyme-treated samples displayed lower yield stress values than the untreated (control) slurry samples (Figure 5a). With enzyme liquefaction, the average yield stress of the treated 20% slurry samples was reduced by 89%, from 772 ± 127 Pa for the untreated slurry to 87.4 ± 45.3 Pa. Similarly, with enzyme liquefaction, the yield stress of the treated 25% slurry was reduced by 93%, from 3,080 ± 473 Pa for the untreated slurry to 229 ± 30 Pa.

After yielding, the observed decreases in shear stress and viscosity were due to corn stover solids migrating away from the moving rheometer fixture as the shear rate was increased. Such particle migration resulted in a depletion layer near the fixture that contained a lower concentration of particles, resulting in reduced measured torque values. The appearance of a depletion layer is common in wide-gap rheometer cells during measurement of two-phase materials[45,57] and has been verified via magnetic resonance imaging.[58] The increase in shear stress at high shear rates (Figure 5a, >100 s^{-1}) was attributed to shear-induced turbulence within the low-viscosity depletion layer, and in comparison to the low concentration slurries (Figure 4b), minimal shear thickening behavior was detected for the moderate concentration slurries due to the reduced stress responses resulting from depletion layer formation (Figure 5b).

3.4 Flow Behavior of High Concentration Slurries (30%)

To determine the yield stress of the paste-like high concentration corn stover slurries, both a flow curve and a constant shear rate test were performed using the vane spindle; representative curves are shown in Figure 6a and b, respectively. Compared to the lower concentration slurries (Figures 4 and 5), greater yield stress values were observed for these slurries, attributed to the large number of frictional contacts between solid particles and the relatively low amount of free water available for lubrication.[17] Similar to the low and moderate concentration slurries, for the 30% concentration slurry, the enzyme-treated samples displayed a lower average yield stress value of 3,410 ± 511 Pa than the untreated (control) slurry with an average yield stress of 6,810 ± 737 Pa, which is a 50% reduction with enzyme liquefaction. The yield stress values determined from the two rheometry tests as well as from multiple trials of each test were found to be similar: e.g., for the enzyme-treated samples, a yield stress value of 3,600 Pa was determined from the flow curve (Figure 6a) and a yield stress value of 3,700 Pa was determined from the constant shear rate test (Figure 6b).

3.5 Comparison of Yield Stress Results

As shown in Figure 7a, slurries that were treated with enzymes displayed significantly reduced values of yield stress compared to untreated slurries at every concentration of initial solids.

The yield stress for low and high concentration slurries displayed reductions of approximately 30-50% with liquefaction while moderate concentration slurries displayed reductions of 90%. The greater yield stress reduction that was observed for moderate concentration slurries was likely due to more efficient mixing of these slurries relative to lower and higher concentrations.

Figure 7a includes average yield stress values measured using both the starch pasting impeller and vane spindle. Data points almost completely overlapped, indicating that the measured stress responses of these slurries were independent of the rheometer fixture type. Both the starch pasting impeller and vane spindle have the same diameter and total height which likely resulted in the generation of similar shear gradients during rheometry measurements.

The majority of the data plotted in Figure 7a was generated from increasing shear rate ramp tests (flow curves). However, for the 30% concentration samples, the yield stress values indicated by the diamond points (labelled "Fixed shear rate" in the legend) were determined by constant shear rate tests performed at 0.1 s^{-1} (e.g., displayed in Figure 6b). The overlapping data points in Figure 7a for the 30% slurries indicate the independence of the measured yield stress values from the particular rheometry test.

To better determine the impact of liquefaction, the total amount of insoluble solids was determined for each slurry and is reported in Table 1. Unfortunately, due to sample contamination, data was not recovered for the 15% treated slurry. Reduced amounts of insoluble solids were measured in the untreated slurries, likely due to water released from mechanical breaking of pellets during processing as well as solubilization of parts of the pellets (e.g., soluble ash and other extractives) due to the mixing maintained during the 6-hour liquefaction. The solubilization would release soluble solids previously contained in the insoluble unbroken pellet and, to a smaller extent, oligosaccharide originated by collision, friction, and shear as described by Liu, et al.[59] Accordingly, the measurement of insoluble solids was crucial to distinguish the solubilization of already soluble solids from the effects of the enzyme liquefaction. Indeed, as reported in Table 1, the amount of insoluble solids decreased further following enzymatic liquefaction. Thus, the reduced yield stress values of the enzyme liquefied samples displayed in Figure 7a compared to the untreated samples were likely due in part to the reduction in the total amount of insoluble solids in each slurry. Practically, to monitor the efficiency of the liquefaction, aliquots of slurry could be tested at different time points during processing to measure the change in viscosity and yield stress, which would mirror the efficiency of the liquefaction.

Figure 7b displays the yield stress values of Figure 7a as a function of the concentration of insoluble solids for each slurry. Others[14–17,60] have observed yield stress increases with concentration of insoluble solids following a power law function:

$$\tau_y = aC_m^b \ ,$$

where C_m is the insoluble solids concentration in a biomass slurry and values for a and b are empirical. The untreated slurries were better described by a power law function (b = 4.9 ± 0.3) compared to the enzyme liquefied slurries (b = 1.8 ± 0.7). Fitting parameters are known to differ based on feedstock characteristics and processing parameters and, in particular, the exponent b can vary from ca. 2-8.[14,61] Stickel, et al.[14] compiled round-robin data from multiple laboratories on sheared pretreated corn stover slurries containing 5-30 wt.% insoluble solids; yield stress values ranged from ca. 1-40,000 Pa and were best described by a power law function with b = 5.7 ± 0.5. The authors suggested that smaller power law values (e.g., 2-4 for wood fiber systems[60,62–64] and in simulations[65]) might be due to the more uniform morphology of the solids in the slurry.

For the corn stover slurries investigated in this study, the observed reductions in relative yield stress following enzymatic liquefaction were likely due to a combination of physical and chemical factors. In general, pretreatment and enzyme liquefaction processes modify the structure of the biomass to allow for more access to fermentable sugars.[5,66,67] In addition to decreases in total insoluble solids content due to liquefaction (Table 1), reduced yield stress ~~could~~ can also result from decreases in particle size and variation of surface properties. Viamajala, et al.[17] observed similar behavior for corn stover slurries and suggested that smaller particles would entrain less water in their porous structures, resulting in a decreased stress response. While a greater population of small (< 500 microns) particles was observed in Figure 3 for the enzyme-treated samples, these particles amount to a relatively small volume fraction of the liquefied slurry and are thus expected to have a small or negligible impact on the bulk stress response of the slurry. Thus, the reduced yield stress of the most concentrated slurry to undergo liquefaction in this study was not simply due to the formation of smaller corn stover solids. Additionally, particle surface roughness was likely modified during liquefaction, and increases in surface roughness can lead to increased friction coefficients.[68] Further evaluation of physical property changes (e.g., elastic or bending moduli of solids) following enzyme liquefaction should be conducted in the future to gain a more complete understanding of the flow properties of corn stover slurries.

3.6 Dependence of Yield Stress on Flow History

The effect of shear flow history on slurry yield stress was evaluated for untreated (control) and enzyme-treated slurry samples with solids concentrations of 15% and 20%. The sequential flow curve measurements separated by various resting times ranging from 5 minutes to 3 hours are shown in Figure 8 for 15% slurry samples. All yield stress percent recovery values following each rest duration are reported in Table 2 with complete flow responses in Figure S2. The enzyme-treated sample (Figure 8a) displayed near full recovery (95%) of its "initial" yield stress after 3 hours of rest. In contrast, the untreated 15% sample displayed reduced yield stress values even after 6 hours of rest, reaching only 50% of its initial yield stress value after 3 hours of rest (Figure 8b). The persistent reduction of yield stress for the untreated 15% slurry sample was likely due to the solid particles retaining some alignment from the previous flow curve tests. Indeed, shear-induced alignment may be more lasting for untreated corn stover particles due to their overall larger size and greater aspect ratios relative to enzyme-treated particles.[17,35,36]

As shown in Table 2 (and Figure S2), both the untreated and enzyme-treated 20% slurry samples did not fully recover their initial yield stress values within the timescale of the experiment. After 3 hours of rest, the untreated and enzyme treated samples only recovered 35% and 58%, respectively, of their initial yield stress values. This prolonged reduction in yield stress was a result of particles permanently migrating to the outer wall of the glass beaker setup. As previously discussed, such particle migration would result in a depleted zone near the measuring fixture, resulting in a decreased torque response and subsequent stress response. Data from slurry samples containing 25% and 30% solids concentrations were not analyzed due to extreme particle migration resulting in very low values of torque (and significant noise) for subsequent flow tests following the initial response.

4.0 Conclusions

Rotational rheometry experiments were conducted to evaluate the flow behavior of enzymatically liquefied corn stover slurries containing 10 to 30% solids concentrations. Yield stress values were determined from increasing shear rate ramps (flow curves) utilizing a starch pasting impeller or vane spindle within a glass beaker. The advantage of using a wide-gap rheometry setup with relative measuring geometries was the ability to recreate conditions that may exist in a mixing vessel of a bioreactor while applying controlled and precise levels of strain. The effect of flow history on 15% and 20% concentration slurries was quantified from sequential flow curve experiments separated by various resting times.

The yield stress of corn stover biomass slurries increased with the concentration of solid particles. Similar to other reports, yield stress values were modelled by a power law as a function of insoluble solids concentrations. Following enzymatic liquefaction with Celluclast 1.5L, yield stresses of all enzyme-treated slurries were significantly reduced compared to untreated slurries, by at least ~30-50% for low and high concentration slurries and at most ~90% for moderate concentration slurries. Flow history experiments revealed that reduced yield stress values continued to be observed following an initial period of shearing after more than 6 hours of resting for some slurries. Practically, sequential flow curve measurements could be used to inform plant operators as to what could happen within a biorefinery following operational shutdowns.

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Declarations

MRL was CTO of Mascoma from 2007 to 2013, and consultant in 2014. Mascoma developed and scaled-up consolidated bioprocessing of hardwood slurries. MRL holds equity in Mascoma (acquired by Lallemand and Renmatix, respectively), which is managed by Enchi.

Supporting Information

Includes details on the use and calibration of the Anton Paar Flexible Cup Holder; complete shear stress responses for the sequential flow curve experiments.

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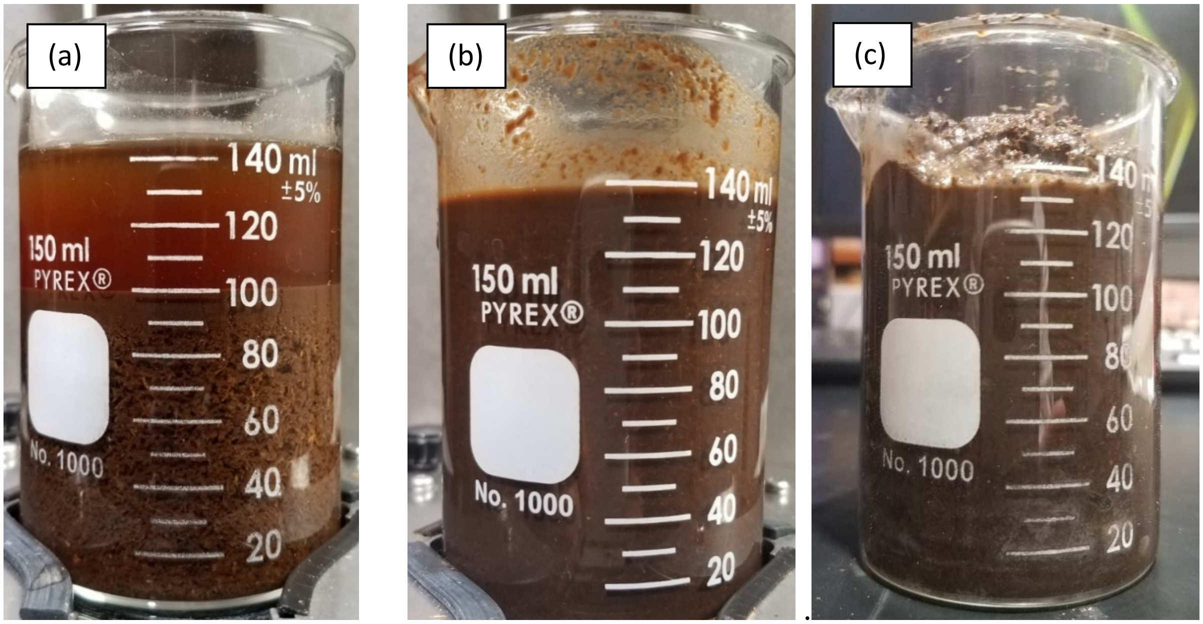

Figure 1: Images of corn stover slurries at different solids concentrations after enzymatic liquefaction: (a) 10%, (b) 20%, (c) 30% (w/v).

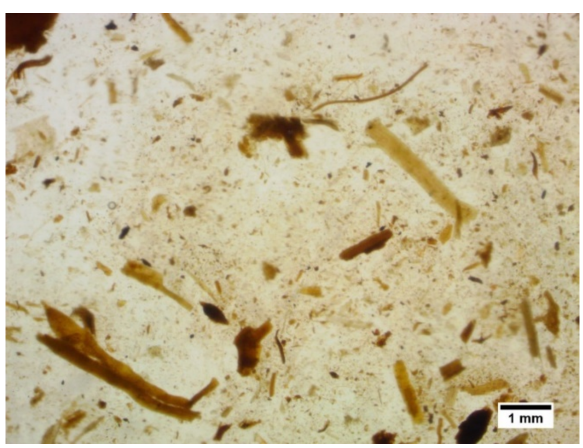

Figure 2: Optical image of solid particles in a diluted, untreated (control) corn stover slurry.

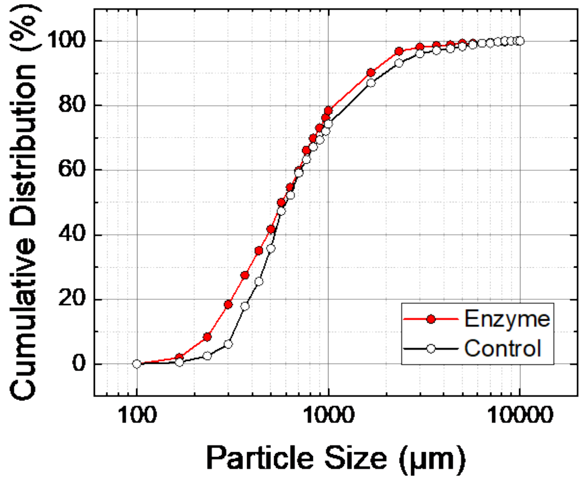

Figure 3: Cumulative particle size distribution of diluted samples of untreated (control) and enzyme-treated corn stover slurries with solids concentrations of 30% (w/v).

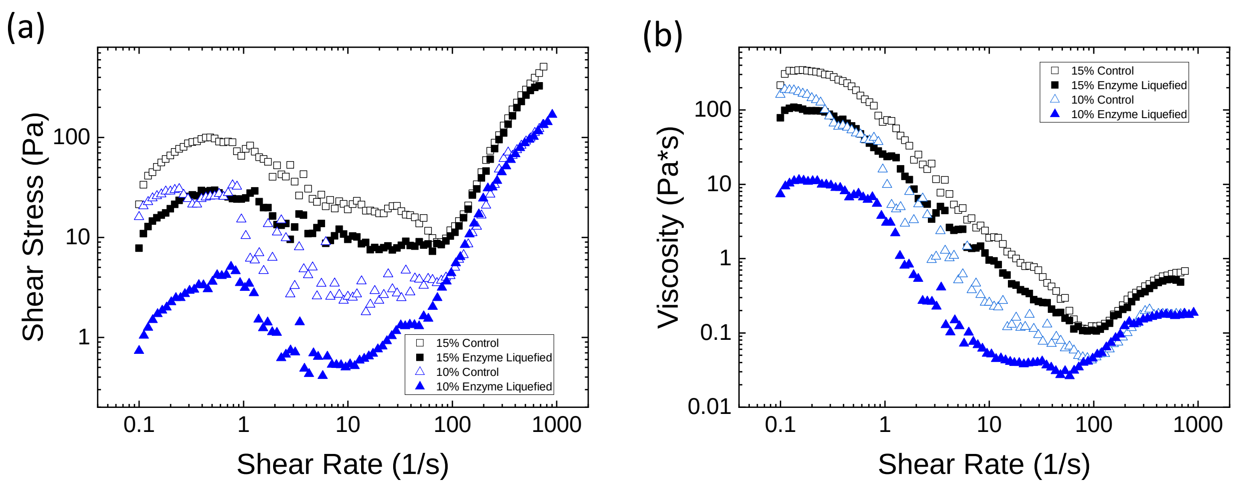

Figure 4: Representative relative (a) shear stress and (b) viscosity responses for low concentration slurries (10 and 15% w/v solids) sheared in a wide-gap rheometer setup with a starch pasting impeller.

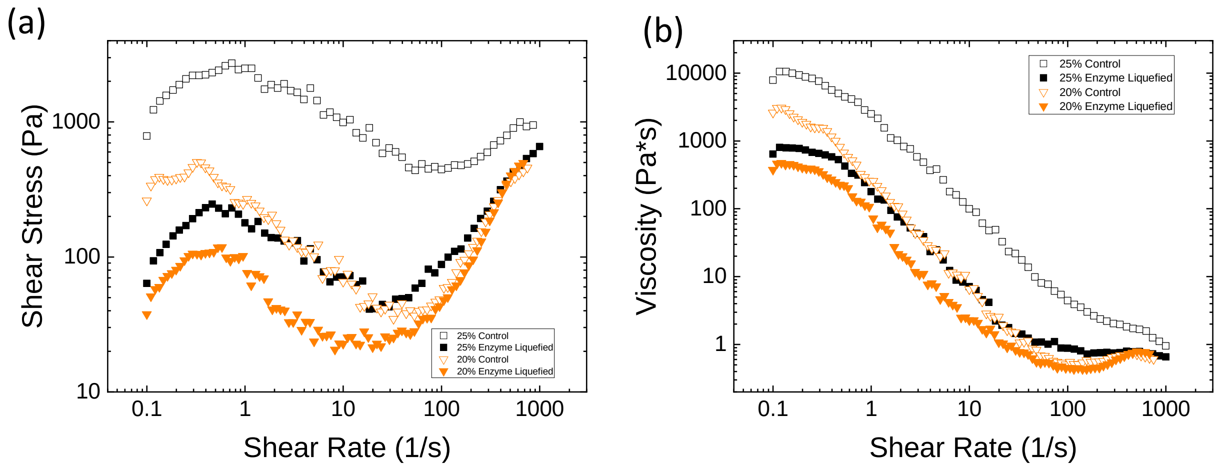

Figure 5: Representative relative (a) shear stress and (b) viscosity responses for moderate concentration slurries (20 and 25% w/v solids) sheared in a wide-gap rheometer setup with a starch pasting impeller.

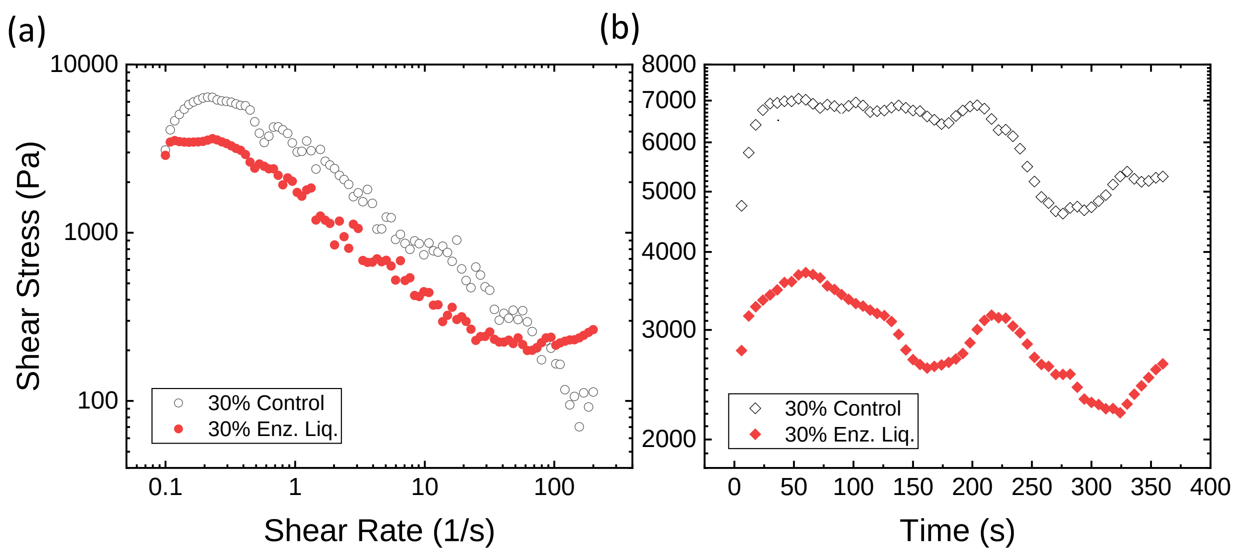

Figure 6: Representative relative shear stress response of high concentration slurries (30% w/v solids) from: (a) an increasing shear rate ramp (flow curve) and (b) a constant shear rate test ($0.1 s^{-1}$), both performed in a wide-gap rheometer setup with the vane spindle.

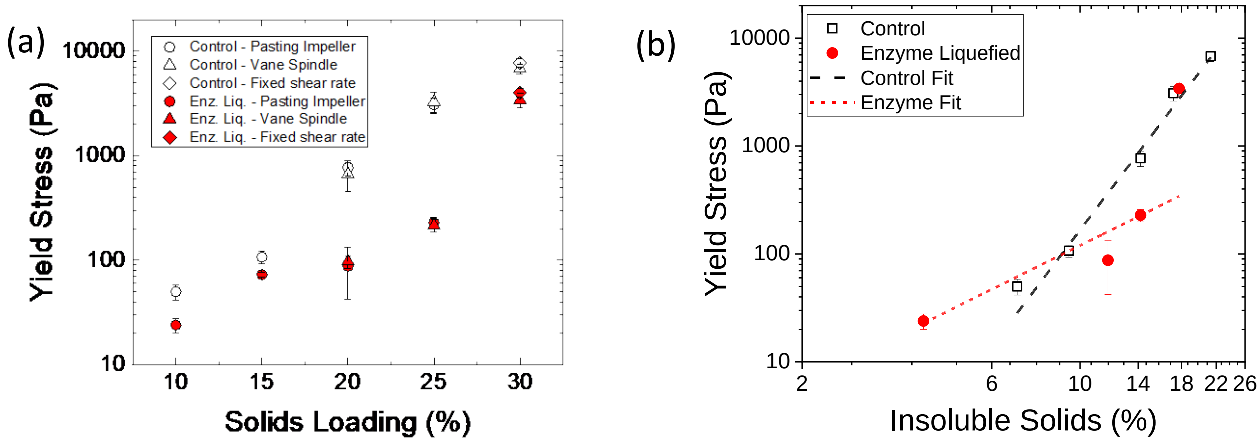

Figure 7: Average yield stress values (± 1 standard deviation; n = 3) as a function of (a) concentration of corn stover solids and (b) concentration of insoluble corn stover solids, as reported in Table 1.

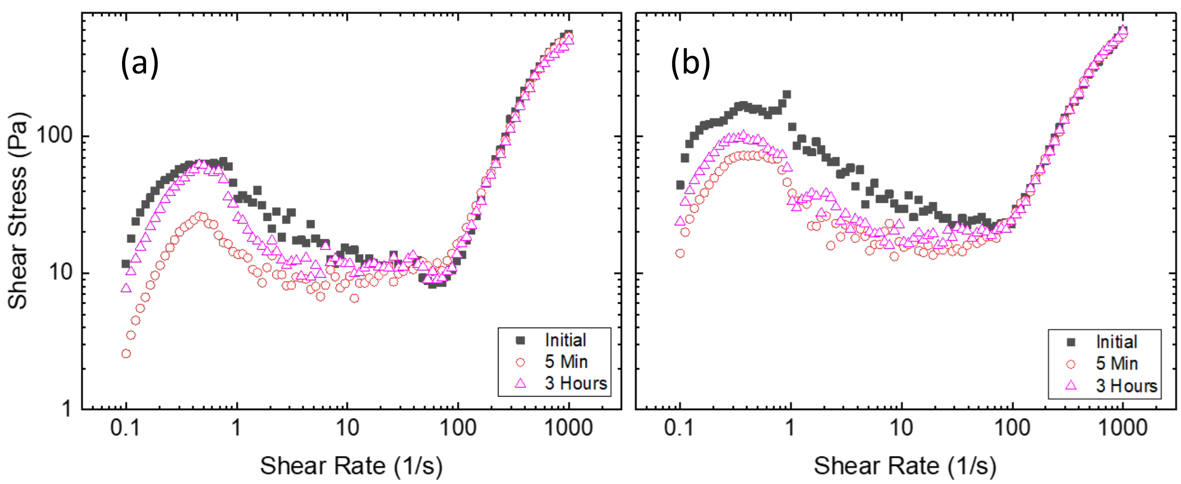

Figure 8: Sequential flow curve tests for: (a) enzyme-treated and (b) untreated (control) slurries with solids concentrations of 15% (w/v) measured using the starch pasting impeller.

Table 1: Insoluble solids concentration (w/v).

Starting Slurry Concentration (w/v)	**10%**	**15%**	**20%**	**25%**	**30%**
Enzyme Liquefied Slurry	4.04%	-	11.76%	14.20%	17.75%
Untreated Control Slurry	6.95%	9.38%	14.20%	17.17%	21.32%

Table 2: Yield stress recovery (%) of corn stover slurries from sequential flow curve measurements.

Slurry Sample (solids conc., w/v)	**Initial Yield Stress (Pa)**	**5 min. rest**	**1 hr. rest**	**3 hrs. rest**
15% Untreated Control	205	36%	42%	50%
15% Enzyme Liquefied	66	40%	75%	95%
20% Untreated Control	606	34%	28%	35%
20% Enzyme Liquefied	165	37%	52%	58%