RHEOLOGICAL CHARACTERIZATION OF LIQUEFACTION PROCESSING EFFECT ON CORN STOVER BIOMASS SLURRIES

by

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I dedicate this manuscript to my rascal little brother and to mom and dad, of course, for their unwavering love and support.

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ABSTRACT

Second-generation biofuels are being developed from non-food plant resources to address the socio-economic concerns derived from first-generation biofuels of food and land competition. However, second-generation biofuel processing confronts many processing challenges with high yield stresses at economically necessary solids loadings (>20wt.%). Various liquefaction techniques are employed to convert sugars from corn stover biomass slurries, which directly impact the rheological response through lowering solids concentrations and altering particle properties. In this study, the flow response of corn stover biomass slurries processed by enzyme liquefaction and dilute acid pretreatment are explored using a wide-gap geometry rheometer. Flow curve experiments reveal that yield stress is a function of solids loading and enzyme liquefaction further reduces the yield stress by reducing the total insoluble solids within the slurry. Combined dilute acid pretreatment and enzyme liquefaction reduce static yield stress and particle size. Drying and fractionating of particles from slurries reveal that larger particles produce a larger shear stress response than smaller particles. For successful processing on a large scale, maximizing the initial solids loading and minimizing the yield stress and viscosity of corn stover biomass slurries are essential.

1. INTRODUCTION

1.1 Research Motivation

The growth in demand for energy and the depletion of fossil fuel reserves summon the need to develop alternative resources of sustainable energy systems around the world. Energy crops have been grown and harvested for conversion of plant product to liquid fuel. Biofuel produced from starch and sugar-based plants are classified as first-generation biofuels and are most commonly used in the United States and Brazil ([1]-[3]). To offset fossil fuel consumption, bioethanol is used as an additive in gasoline. Currently, gasoline contains up to 10% bioethanol. Since first-generation biofuels are directly extracted from the food source of plants, competition of consumable food sources and land is of main socio-economic concern.

Second-generation biofuels which are derived from lignocellulosic plant waste, are being developed to address the socio-economic concerns of first-generation biofuels. Feedstock material used for second-generation biofuels includes wood, organic waste, and other plant residues, such as switchgrass and corn stover. Corn stover biomass is derived from corn waste materials consisting of corn cobs, stalks, and leaves that can be converted to ethanol with high theoretical yields of ethanol between 60-90% ([4],[5]). Although corn stover biomass is a promising solution for bioethanol, there are many issues preventing it from becoming economically feasible for immediate use.

Despite the great appeal of corn stover as a feedstock candidate, transportation of these slurries in a biorefinery is difficult because of their considerable yield stress and high viscosity. High

solids loading of biomass is necessary to reduce capital and energy costs by reducing the volume of transported material ([9]). However, with reduction of water within the system comes a repercussion of considerable yield stresses and viscosities which make slurries difficult to pump and move the material throughout a refinery to process the required ethanol conversion. Creating a solution by reducing the yield stress and viscosity of the slurry system while maintaining high solids loading is imperative for the success of second-generation biorefineries. Through enzymatic liquefaction and dilute acid pretreatment, it is possible to lower yield stress in high-solid containment systems. Understanding impact of the various processing techniques on the flow behavior of the corn stover slurry system is necessary.

While others have explored corn stover biomass and the rheological behavior of pretreated slurries, the liquefaction of corn stover pellets prior to addition into a pretreatment tank has been proposed. This liquefied slurry would reduce issues with high yield stresses and viscosities associated with the front-end of the process. In this work, slurries were liquefied with enzymes without an initial dilute acid pretreatment to explore the impact on yield stress reduction. The slurry structure varied based on the degree of processing. Various rheological techniques were employed to characterize changes in yield stress of the processed slurries.

1.2 Thesis Scope and Objectives

In the present work, the rheological behavior of corn stover biomass of various initial solids loadings (10-30wt.%) that have been processed with different techniques were characterized using a wide-gap geometry. Calibration of the wide-gap rheometer setup was completed to account for the increased gap size compared to traditional setups. The static and dynamic yield

stresses of various compositions were measured through a variety of rheological techniques. The particle size distribution of combined dilute acid pretreatment and enzyme liquefaction was measured through dynamic light scattering (DLS) to determine particle level effects on the flow response of processed slurries. The data in this work is in preparation for a soon to be submitted publication "New Strategy for Liquefying Corn Stover Pellets without Pretreatment" by Overton, J. C., Freitas dos Santos, A., Szeto, R., Patel, M. H., Gutierrez, D. M., Eby, C., ... Ladisch, M. R.

The objectives of this study were to:

1. Determine a robust rheological method to evaluate the yield stress and pumpability of corn stover biomass slurries.
2. Understand the impact of enzyme liquefaction and its effect on the flow behavior of corn stover slurries.
3. Measure the relationship between particle size and shear stress response of reconstituted slurries.

2. BACKGROUND

2.1 Corn Stover Biomass Overview

Biomass is an organic matter that is derived from plant or animal materials and can be converted to biofuels as a renewable energy resource as the materials can be replenished. First-generation biofuels are derived from food sources such as corn starch, sugar cane, or vegetable oil. Since first-generation biofuels are converted from food sources, the price of corn increases the competition with food and livestock producers and creates a "food vs. fuel" debate ([6]). To address the competition with food consumption, second-generation biofuels are being developed from lignocellulosic biomass, which include agricultural waste such as bagasse, switchgrass, and corn stover ([7]). Lignocellulosic biomass has high theoretical yield for ethanol conversion and a reduced feedstock material cost relative to first-generation biofuels.

Although lignocellulosic biomass is an attractive feedstock material for biofuels, it is recalcitrant and requires additional processing steps for ethanol conversion increasing production costs. Lignocellulosic biomass undergoes four major units of operations prior to conversion that include pretreatment, hydrolysis, fermentation, and purification ([8]). In addition to processing, it has been identified that high solids loading of feedstock (>20 wt.%) to reduce water and energy usage is necessary to lower processing costs for economic viability ([9]-[11]). At these high-solid concentrations, considerable yield stresses are developed requiring more energy for transporting material within a process. In addition to increased conversion, it is necessary to reduce the yield stress when processing lignocellulosic biomass.

2.2 Yield Stress

2.2.1 Defining Yield Stress

Yield stress is a material property that is associated with many complex fluids and material systems and can be defined as the necessary stress value to be exceeded for flow to occur (cite a source). The concept of yield stress fluids was idealized by Bingham et al. and was adopted from the plastic yield stress in metals ([12]). This property finds many uses in everyday products such as squeezing toothpaste from a tube or spreading peanut butter on bread. Quantifying the value of yield stress is necessary for the design of plants and systems because it will define the necessary pump requirements to transport material within a process. Although yield stress is an important parameter to quantify within fluid systems, there is no universal testing procedure that is standardized to determine yield stress on any material.

Many techniques have been proposed and employed to evaluate yield stress; differences in data on the order of a magnitude can occur based on material handling and technique used for the same material system ([13]). It has been proposed that yield stress materials can be identified as either simple or thixotropic ([14]). In the case of simple yield stress materials, the viscosity only depends on shear rate and the yield stress is well defined and examples of these materials include emulsions such as hair gel and shaving foam. Alternatively, thixotropic yield stress materials have a response that is dependent on the deformation history in addition to the shear rate ([15]). Since the yield stress can vary within a material system, two different yield stresses need to be defined: static and dynamic.

The static yield stress is defined as the minimum stress necessary to start flow. In thixotropic materials, the static yield stress can vary in magnitude depending on the amount of time allowed for the microstructure to build up. The dynamic yield stress is defined as the minimum amount of applied stress required before flow ceases. The value of dynamic yield stress can depend on the flow history and the amount of time a material has been sheared at different shear rates ([16]). It should be noted that the static and dynamic yield stresses are equivalent in simple yield stress fluids as their properties and structures do not depend on shear rate and structure rejuvenation ([17]).

2.2.2 Current Techniques for Yield Stress Measurements

Synonymous with the various ranges of values for yield stress that a yield stress fluid can have, there are numerous techniques that are used to determine the yield stress of a fluid. Sample handling must be noted as shear deformation from sample loading can change the response of the material. Commonly, rest periods or pre-shearing is completed prior to testing to gather consistent results.

The classical method for determining yield stress is to utilize flow curve measurements using a rheometer. A flow curve consists of a range of data points taken from shear rate or shear stress sweeps. Flow curve measurements can be a powerful tool because based on the direction of the sweep, the static or dynamic yield stresses can be measured. A measurement sweep with increasing shear rate or shear stress can be used to measure the static yield stress while a sweep with decreasing shear rate or shear stress can be used to measure the dynamic yield stress. The yield stress using this method can be determined by extrapolating the shear stress to a zero

shear-rate or by fitting rheological models to the data. A commonly used yield stress flow model is the Herschel and Bulkley model:

$$\tau < \tau_c \rightarrow \dot{\gamma} = 0\ (Solid\ Regime)$$
$$\tau > \tau_c \rightarrow \sigma = \sigma_y + K\dot{\gamma}^n\ (Liquid\ Regime) \quad \text{(Eq.1)}$$

where τ is shear stress, τ_c is a critical shear stress, σ_y is the yield stress, $\dot{\gamma}$ is the shear rate, and K and n are empirical values. Below the yield stress, the material behaves as a solid and does not flow, and above the yield stress, the material will flow based on the equation ([17]). Other yield stress models include the Bingham model, Casson model, and the Ellis and Cross model which similarly estimate a yield stress through the extrapolation of the value to a zero-shear rate ([18]).

Another rotational rheometry technique that is used to measure yield stress is through determination of the maximum torque, which corresponds to the yield stress. In this measurement, a constant shear rate is applied to the material, typically at a low shear rate. The shape of the curves from the data can be different between sample and material. Different yield stresses can be determined using this technique based on how the yield stress is defined and include the departure from linearity, maximum stress, or the value where stress reaches a constant value. In corn stover biomass slurry systems, the yield stress using this technique is by the definition of maximum stress in the profile ([19]).

Aside from rotational rheology methods, oscillatory methods are also employed to measure the yield stress through completing an oscillatory strain sweep at a fixed frequency. In this method,

the strain sweep is carried out with low values of strain to increasing values of strain. There are many ways the yield stress value is determined. The yield stress can be identified as the stress amplitude where the elastic modulus G' is lesser than the shear modulus G". The yield stress can also be identified as the maximum elastic stress through the given equation:

$$\sigma = G'\gamma \qquad \text{(Eq. 2)}$$

where σ is the elastic stress, *G'* is the elastic modulus, and γ is the strain ([20]). The last common interpretation using this oscillatory method is by fitting the data beyond the G' and G" cross over data with a power law function and identifying the yield stress point as the intersection with the extrapolation of the horizontal line from the linear viscoelastic regime ([15]). Although oscillatory methods are less commonly used for determination of yield stress, this method proves useful as this strain amplitude sweep can be used to probe other values of the sample in the same experiment and can require lower volume of the sample.

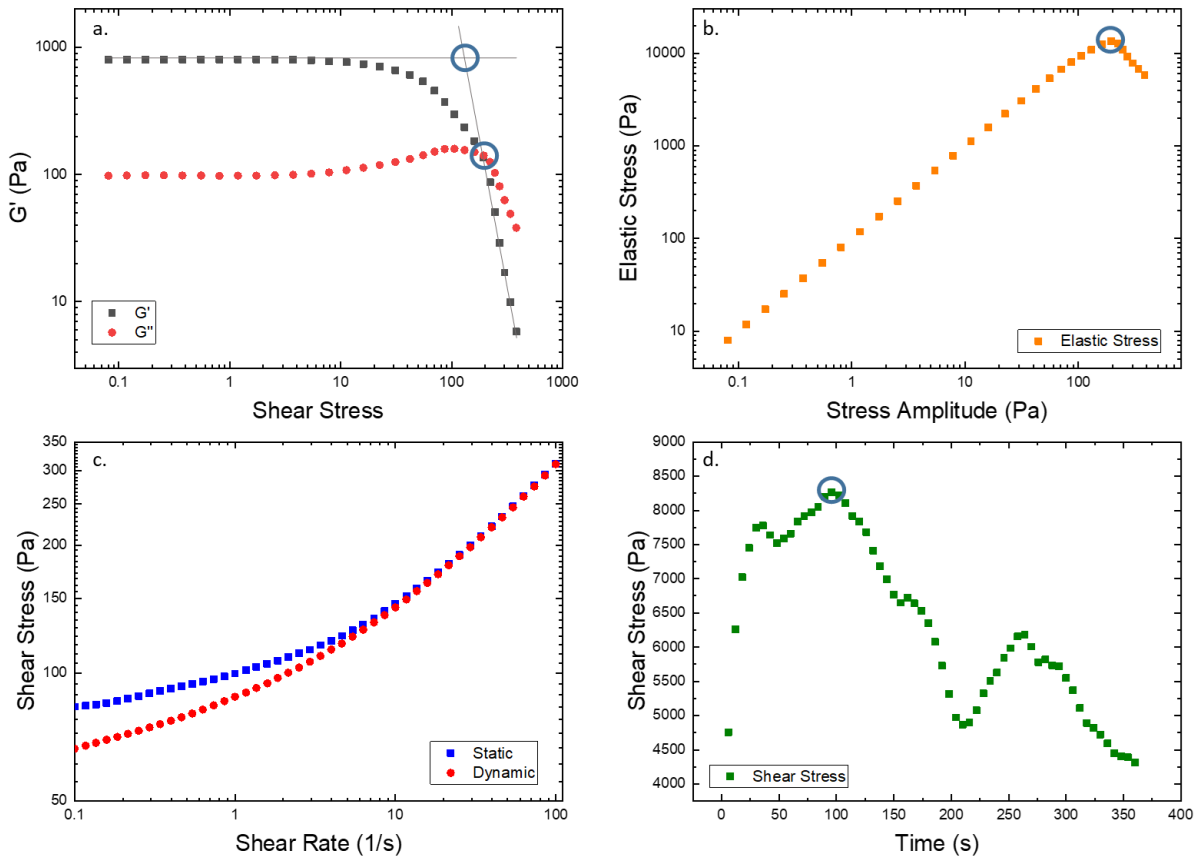


Figure 2.1. Different yield stress technique measurements: (a) Oscillatory rheometry evaluation of yield stress of mayonnaise through G'/G" crossover point and intersection of extrapolation of the linear viscoelastic regime and power-law fit. (b) Elastic yield stress method calculated from oscillatory data of mayonnaise. (c) Flow curve determination of yield stress extrapolating to a "zero-shear" rate of mayonnaise. (d) Low torque yield stress measurement of corn stover.

It is imperative for success to be aware of all the methods used to measure yield stress and how to interpret the data from these evaluations to understand whether one is exploring the static or the dynamic yield stress. Figure 2.1 shows the different yield stress measurements of mayonnaise which has been extensively studied because of its commercial important for processing ([1]). Selection of the rheological fixtures and setups must be adapted to the test sample to ensure accurate measurements. When evaluating a new material, it is advised to explore all of the common testing procedures to identify the most suited techniques for your

system. There is plenty of room for debate when measuring yield stress as demonstrated through various inter-laboratory studies using a multitude of methods and setups to simply measure yield stress ([13], [19]).

2.3 Current Efforts in Corn Stover Rheological Measurements

Measuring and understanding the rheology of corn stover biomass slurries is important to inform on pump requirements for refinery designs because transportation in the process is necessary for conversion. The yield stress has been identified as a key parameter to create a viable continuous process because it is related to the minimum power output required for a pump ([19]). There have been many efforts within the field to create the best conversion of biomass to ethanol for biofuels and alongside it, there have been numerous amount of work done to measure the yield stress of the slurries used for conversion ([19], [21], [23]-[29]). Since the conversion process of biomass corn stover modifies the composition of the sample, care must be taken to understand at what stage of processing the sample was prior to measurement to better compare data between studies. Data should not be expected to be whittled down to a precise range of values because of the array of techniques and variances in materials ([21]).

Although there is variance with materials and processes, yield stress data from various groups have found that there is a correlation between yield stress and insoluble solids concentration for their individual systems:

$$\tau_y = aC_m^b \quad \text{(Eq. 3)}$$

where T_y is the yield stress, C_m is the insoluble solids concentration, a and b are empirical fits. The reported values of the empirical constants have a large range between $0.1<a<10$Pa and $3<b<6$ ([21]). This range of values is a result from different materials and processing techniques. The power law predicts that as solids loading of corn stover increases, the yield stress will increase tremendously which is unfavorable for the necessary high solids loading for corn ethanol economic viability ([9]).

The corn stover biomass slurry created for conversion is typically a complex mixture of corn stover particles, enzymes used for conversion, and buffered solutions used to maintain pH levels. Corn stover biomass slurries contain a yield stress and are shear-thinning ([27]). Based on the insoluble solid concentration, the rheological behavior can change drastically from a dilute suspension to a thick paste as shown in Figure 2.2. In the concrete field, where the material behavior is also not consistent with varying composition, concrete can be considered one of two types of materials depending on the quantity of coarse aggregates. The first type is a concentrated suspension of solids within water; traditional fluid mechanics and rheology can be used to describe the material. In the second case, Coulomb's principle can be applied where the stress for deformation is proportional to the applied normal stress ([22]). This definition of material behavior in concrete can possibly be adopted in corn stover biomass slurries to better describe the material system.

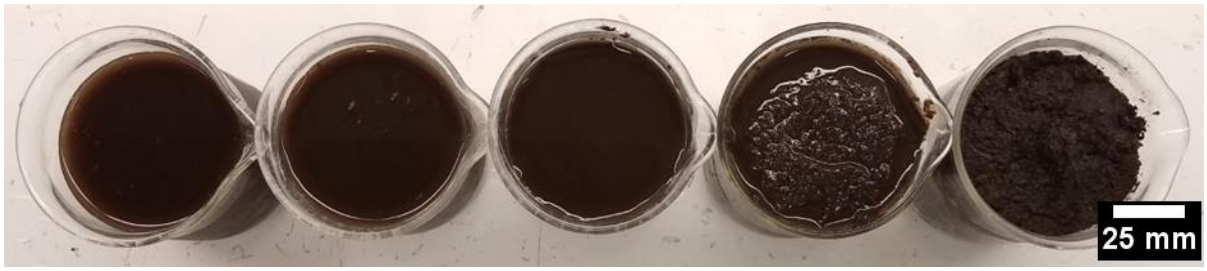


Figure 2.2. Various compositions of corn stover biomass slurries by increasing initial weight concentrations (10, 15, 20, 25, 30%) showing the difference in slurry morphology by concentration.

Corn stover biomass is not an ideal rheological sample because measurements are difficult to obtain due to large particle sizes, wall slip, sample fracture, plate ejection, and particle settling. In an attempt to measure lignocellulosic biomass slurries, many researchers have used novel rheometers and techniques in addition to traditional rheometry yield stress experiments ([19], [23]-[26]). A novel rheometer for measuring biomass at high temperatures, extreme pH, and high solids concentrations with large particle sizes has been created by Klingenberg et al. who reported consistent measurements with other equipment ([27]). Another novel measuring technique that has been used is magnetic resonance imaging of corn stover slurry flow profiles during pipe flow coupled with pressure drops to determine a yield stress ([28]). Besides novel rheometer usage, other researchers have explored corn stover using large amplitude oscillatory shear tests (LAOS) and discovered that corn stover in various stages of processing showed different rheological fingerprints ([29]). LAOS has also been reported to be useful for measuring yield stress fluids utilizing Fourier transformations and Lissajous curves for data analysis ([30]). Outside of taking raw measurements and characterizing the rheological properties of corn stover, particle properties of the biomass must be considered as they greatly influence the material response.

2.4 Particle Level Properties

Particle size of a suspension or slurry can greatly influence the rheological response of a material system. Depending on the sample and inter-particle effects, changing the particle size can either increase or decrease viscosity. In a study of silica-sand suspensions, a trend was observed where the viscosity increased with an increase in particle size and solids concentration ([31]). Conflictingly, in rheological evaluations of ball-milled mineral slurries the opposite observation was documented where a decrease in particle size led to an increase in viscosity ([32]). The increase in viscosity for the mineral slurries was attributed to particle interactions leading to an increase in solids concentrations. It should be noted that the particle sizes for both studies were different: 90-300μm in the silica-sand suspension study and 44-74μm in the mineral slurries. Particle sizes of greater than 100μm are expected in biomass slurries and the viscosity has been shown to increase with increasing particle size ([33], [34]).

Aside from observing particle size, it is necessary to take the particle size distribution into account. At a constant solid fraction, a monodisperse system will produce a higher viscosity compared to a bidisperse or polydisperse system because the maximum packing fraction is lower ([35], [36]). In a study of the liquefaction of corn stover slurries, the value of yield stress was reduced with a decrease the average particle size of the particle size distribution ([37]). Understanding the effects of both particle size and particle size distribution will lead to better comprehension of rheological behavior.

Thixotropy describes the shear-history dependent behavior of a material where response of the material depends on the structure of the material. Thixotropy is important to measure to account for the network structures that corn stover slurries can form when sedimented.

Particles in dilute corn stover slurries are known to quickly settle due to gravity ([38]). It is possible to premix the settled slurry sample to resuspend particles during the measurement, but a pre-shearing step would remove the ability to observe the bifurcated shear stress response as particles transition from a settled to resuspended state ([40]). Understanding of the flow response of the slurry in a sedimented state is important as particles will be allowed to settle during the event of a process shutdown. It has been reported that in a pipe where a thixotropic liquid is allowed to enter and rest, there is a large pressure required for the start-up flow and demands higher pump performance ([41]).

Insoluble solids concentrations have ~~been reported to have~~ the largest impact on the yield stress of corn stover slurries. This is correlated to the volume fraction of solids within the system and is a primary factor in the response of any rheological sample. However, there are secondary effects of the particles that can impact the response of the system. A greater understanding and exploration into these particle properties can be used to better inform experimental data and can be used to improve computational and model work.

2.5 Pumpability

Rheological studies are carried out to better understand the flow and deformation behavior of a material and can be used as a powerful lens to characterize a material. However, directly relating rheological data to practical processing parameters such as pumpability is not as simple as there are various factors that can affect flow such as pipe dimensions, slip layers, and pump design. Viscosity in concrete was correlated to pressure drops in pipe flow which leads to increased pump flow ([46]). Yield stress is useful to understand as it will dictate the minimum

power requirements for pumps and other processing equipment used for mixing or transport ([19]). The type of pumps employed will also impact the flow as it was suggested that yield stress is the main governing parameter in piston pumps, while viscosity is the dominating property for pumpability in a rotor pump and therefore, these two properties are key parameters to understand flow ([47]). In a study on biomass and coal slurries, pump selection is based on experimental evaluation of shear stress at specific shear rates, yield stress, and settling rate of a biomass slurry ([1]). In this same work, it was suggested that a pumpable slurry will have a maximum viscosity of 1.0 Pa·s, but do not define the shear rate or shear stress of this viscosity. While rheometry experiments can help identify relations to pumpability, there is not a clearly defined parameter that will describe the pumpability of a material as flow is dependent upon the material, the dimensions of a pipe and the power and type of pump within a system.

3. EXPERIMENTAL METHODS

3.1 Materials

Corn stover slurries

Corn stover slurries were produced by the Laboratory of Renewable Energies (LORRE), Department of Agricultural and Biological Engineering at Purdue University, West Lafayette, Indiana. Slurry formation by enzymatic liquefaction was carried in a 600mL glass beaker at 50°C for 6 hours at solids loadings of 30% (w/v) using Celluclast 1.5 L (Novozyme, Bagsværd, Denmark - 57 FPU, endoglucanase 800 UI/mL and 126 mg protein/mL). Mixing was performed by an IKA-WERKE overhead stirrer (Eurostar PWR CV S1+) out fitted with marine impellers on a 23 cm long shaft with a blade pitch of 45 degrees in an up-down configuration. The impeller was 304SS, with dimensions of 5.3 cm diameter, and with impeller spaced 5 cm apart. Rotor speed was 300 RPM for all experiments. Corn stover pellets were added in a fed batch manner. After 6 hours had elapsed from the start of corn stover addition, the slurry was transferred to a sealed storage container and stored at 4°C until further analyses.

Corn stover liquefaction with Maleic Acid

Slurry formation using maleic acid was produced by LORRE lab at lab scale and Idaho National Lab (large scale samples). Lab scale liquefactions were conducted in a 50 mL total volume stainless steel reactor constructed from 1-inch stainless steel tubing sealed with Swagelok threaded endcap fittings. Corn stover pellets were loaded to 25% (w/v) solids concentration and 30 mL of maleic acid solution with different concentrations (20, 30, 40, 50, or 100 mM, respectively) was added to the reactor. The reactors were placed into a Tecam®

SBL-1 fluidized sand bath at 150°C for a total time of 36 minutes, with a 6 minute heat-up time (20°C to 150°C) and a 30 min hold time at 150°C. The reaction tubes were then placed in two liters of ice water for 10 min. The tubes were then opened, and their contents poured into a plastic screw-top bottle for storage at 4°C until needed. Larger scale maleic acid liquefaction g of pelleted corn stover were performed under similar conditions as described above for a solids concentration of 300 g/L solids and addition of 40 mM maleic acid (i.e. reaction at 150 °C (69 PSIG) for 30 minutes incubation period at temperature (± 2°C)) in a 10-L batch reactors attached to Idaho National Laboratory's chemical preprocessing system.

Slurry Formation Using Enzymes Individually and Combined to Maleic Acid Treatment

The sample procedure for enzyme liquefaction was applied for slurry formation (corn stover pellets at 30% (w/v) solids) through sequential maleic acid (large scale treated samples, liquefied at 40 mM) and enzyme treatments. However, in this case, cellulolytic enzyme (1 FPU; 2.2 mg protein/g solids) was directly added to the reactor with the maleic acid treated sample, and the resulting mixture was incubated for up to 48 h, with samples taken at 6, 24 and 48 h for rheology measurements.

3.2 Flexible Cup Holder

Rheological measurements were carried out with an Anton Paar MCR 702 rheometer using an Anton Paar Flexible Cup Holder cell. The Flexible Cup Holder allows for measuring cells of different dimensions for experimentation with proper calibration. Each unique measuring fixture and measuring cell was calibrated using a heavy mineral oil (Sigma-Aldrich 330760). Viscosity of the mineral oil was measured at constant shear rates of 0.1, 10, and 100s^{-1} in a

steel concentric cylinder setup (27mm fixture with a 1mm gap size). Desired measuring fixture and cell combinations were calibrated with the Flexible Cup Holder setup by measuring the viscosity of the mineral oil at constant shear rates of 0.1, 10, and $100s^{-1}$. Since operation of the rheometer was conducted in a shear-rate controlled mode, the CSR Factor (Controlled Shear Rate) must be changed to account for any changes to the dimensions of the rheometer. Using the equation below, the new CSR Factor for non-standard dimensions, *CSR Factor (x)*, can be calculated to ensure accurate data from measurements for gap sizes larger than the standard calibrations.

$$CSR\ Factor\ (x) = \frac{Viscosity\ (x) * CSR\ Factor\ (SI)}{Standard\ Oil\ Viscosity} \qquad \text{(Eq. 4)}$$

In this equation, *Viscosity (x)* is the viscosity measured with the desired setup, *CSR Factor (SI)* is the default value for the desired setup, and Standard Oil Viscosity is the value measured in the concentric cylinder setup. The calculated *CSR Factor (x)* is implemented into the Anton Paar software to account for changes to the measuring system and viscosity tests are run to confirm that measurements with the desired system produce equivalent oil viscosities to the concentric cylinder setup.

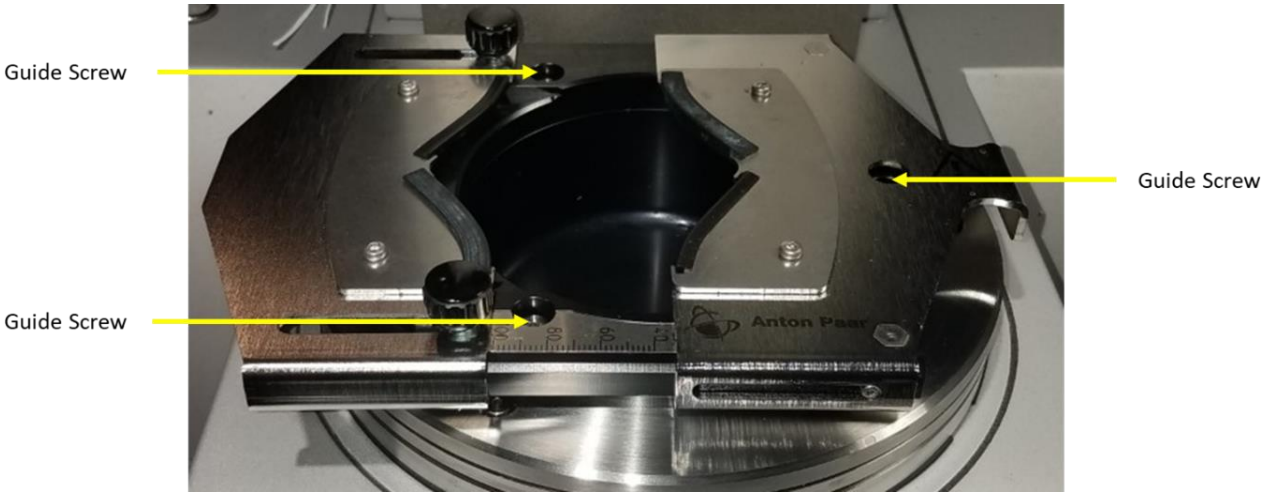


Figure 3.1. Anton Paar Flexible Cup Holder setup showing guide screws used for fixation onto MCR 702 Rheometer.

3.3 Yield Stress Measurements

The yield stress of corn stover biomass slurries were evaluated with a vane-in-glass or starch cell-in-glass rheological setup using the Anton Paar Flexible Cup Holder setup shown in Figure 3.2. The Anton Paar starch cell (ST24-2D/2V/2V-30) had a 24mm diameter and active length of 30mm. The Anton Paar vane fixture (ST24-4V-30/124) had a diameter of 24mm and active length of 30mm. After lowering of the measuring head and fixture into the sample, 15 minutes of rest was allowed to mitigate shear deformation from sample loading. Both a constant rotation rate and flow curve measurement technique were used to evaluate the static and dynamic yield stresses of the material.

Constant rotation rate experiments were conducted to evaluate the static yield stress of pasty corn stover slurry samples (initial 30wt.%) using a vane fixture. The constant applied rotation rate was $0.1s^{-1}$. The maximum measured stress or torque was identified as the static yield stress ([19]).

Flow curve experiments were conducted in a shear-rate controlled mode using a vane or starch cell setup to evaluate both the static and dynamic yield stresses of corn stover slurry samples. An initial logarithmic ramp between 0.1-1000s^{-1} was used to determine the static yield stress and a subsequent decreasing logarithmic ramp between 1000-0.1s^{-1} was used to evaluate the dynamic yield stress. The static yield stress was identified as the largest measured shear stress in the low shear-rate regime between 0.1-10s^{-1}. The dynamic yield stress was determined as the value at "zero-shear rate" from an extrapolation of the flow curve data from the decreasing shear rate ramp. For flow curve data of corn stover slurries, the static yield stress should always be larger than the dynamic yield stress data.

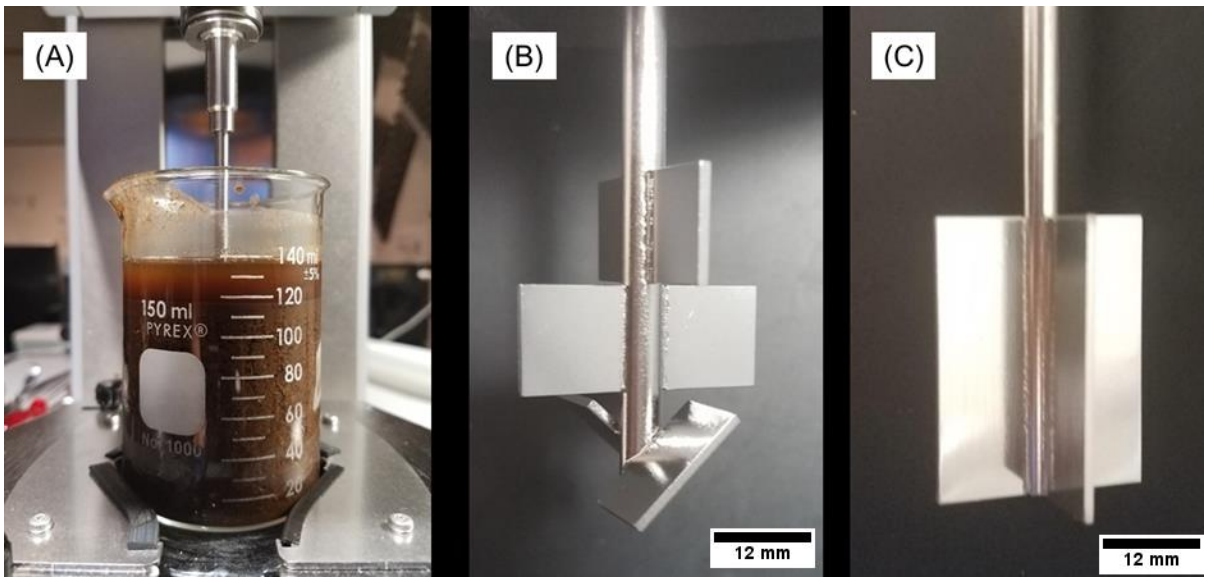


Figure 3.2. (a) Yield stress measurement setup of measuring fixture in a 15wt.% corn stover slurry sample. Elevated to show the entire setup. (b) Starch cell fixture. (c) Vane rheological fixture.

3.4 Hysteresis Studies

Hysteresis studies were conducted to better understand how the yield stress of a system recovers. These experiments were conducted on slurries with initial solids loadings of 15 and 20wt.%, with and without enzyme treatment. Flow curve experiments were conducted in a shear rate controlled mode between 0.1-1000s^{-1} with the use of a starch cell and beaker to measure yield stress as described in Section 3.3. Established periods of rest (5 minutes, 30 minutes, 1 hour, and 3 hours) were allowed after the initial shear rate ramp. The RheoCompass software was programmed to remain at a shear rate of 0s^{-1} during rest times to ensure that the measuring fixture did not move. At each period of rest, the sample was subjected to another shear rate ramp between 0.1-1000s^{-1}. The static yield stress was determined as the maximum shear stress between 0.1-10s^{-1}. The recovery of the initial yield stress was calculated as the magnitude of the measured yield stress relative to the initial yield stress.

3.5 Dynamic Light Scattering

Particle size distribution measurements for particles >1mm were evaluated using a Malvern Mastersizer 3000 HV. Deionized water was used as the liquid dispersant. The stirrer was set to 2000 RPM (revolutions per minute) to enable adequate circulation of particles during measurements. Samples were thoroughly hand mixed prior to loading then loaded with a metal spatula until an obscuration of ~10% was detected by the system with obscuration limits set between 5 and 20%. Three separate measurements from one sample were collected and averaged to generate data curves.

3.6 Optical Microscopy

Optical microscopy images were taken using a microscope and analyzed using the program ImageJ. Corn stover slurries were placed onto a glass slide and diluted with water to allow better visualization of samples. The average aspect ratio (length/width) was calculated from measurements of 25 particles with lengths greater than 1mm were measured for particles that were untreated (control), enzyme liquefied, or sequentially treated with maleic acid and enzyme for 48 hours. Particle length was identified as the longest dimension of an observed particle.

3.7 Fractionated Slurries

Fractionated slurries were produced from corn stover slurries that were either untreated or processed with an enzyme at initial solids loadings of 30wt.%. Particles were strained using a sieve with an opening size of 44μm to remove excess liquid. Separated particles were placed onto a glass dish, which was then placed on a hot plate and allowed to dry overnight. Particles were separated through a series of sieves with the aid of a RO-TAP RX-29 shaker unit shown in Figure 3.3.

Figure 3.3. RO-TAP RX-29 sieve shaker unit fixated to table for use of particle separation with a sieve stack.

Slurries were reconstructed using particles isolated by sieves to the desired solids concentrations. The solids concentrations used in this section can be considered insoluble solids concentration as the samples did not undergo further liquefaction after sample manufacture. Initial flow curve experiments between shear rates of 0.1 and $100s^{-1}$ were conducted in a conventional steel concentric cylinder setup with a gap size of 1mm to probe the effect of particle size. These initial samples were produced to a weight fraction of 15% in water assuming that the density of water is 1g/mL from particles from Sieve No. 50 and 80 with opening sizes of 177 and 297µm, respectively.

Other reconstituted slurries were constructed to weight fractions of 15, 20, and 30wt.% solids to a total mass of 100g in water. Flow curve measurements of these reconstructed slurries were conducted in the wide-gap rheometry setup with the vane fixture between shear rates of 0.1 and $1000s^{-1}$ for static yield stress determination and 1000 and $1s^{-1}$ to measure the dynamic yield stress. The measured yield stresses were fitted to power laws with concentration as the variable.

4. RESULTS AND DISCUSSION

4.1 Calibration of Flexible Cup Holder Set-ups

All of the wide-gap rheometry setups in this study were calibrated to ensure accurate data collection using non-factory standard dimensions. Shear viscosity tests were conducted using a conventional concentric cylinder setup with a Newtonian mineral oil. At all shear rates (1, 10, and 100s^{-1}), the measured viscosity was 152mPa*s in the concentric cylinder setup. The glass beaker used as the sample cell had an inner diameter of 46mm. The starch cell and vane fixtures had outer diameters of 24mm, allowing the combined setup with both fixtures and the glass beaker to have a measuring gap size of 11mm. The initial measured viscosities with the starch cell and vane were 90.7mPa*s and 73.3, respectively. The calculated values for the corrected (calibrated) CSR Factors were 33.7s/s for the starch cell and 27.0s/s for the vane setups with the glass beaker. Initial and calibrated viscosity values with corrected readings are shown in Table 4.1. Measured viscosity values at 100s^{-1} using the wide-gap setups with calibrated CSR Factors were higher than 170mPa*s due to secondary flow effects from the large gap size. This effect should be noted when making measurements at high shear rates.

Table 4.1. Calibrated values and corresponding viscosities for measuring setups with 46mm inner diameter glass beaker

Fixture	CSR (Factory) [s/s]	CSR (Calibrated) [s/s]	Viscosity @ 10s^{-1} [mPa*s]	Calibrated Viscosity @ 10s^{-1} [mPa*s]
Concentric Cylinder	78.0	-	152	-
Starch Cell	60.0	33.7	90.7	152
Vane	60.0	27.0	73.3	152

4.2 Effect of Solids Loading and Enzyme Liquefaction

The yield stress data of various solids loadings and enzyme liquefaction are shown in Figure 4.1. In this study, the starch cell setup was used to measure samples with an initial solid loading between 10-20wt.% and the vane setup was used to measure samples with solids loading between 20-30wt.%. The variances in the setups were to account for particle settling, slip, and sample structure as displayed in Figure 2.2. Difficulties in loading the vane fixture may occur as the rheometer's moving profile ceases upon reaching normal force values of 15N. When 15N is reached, the vane will not proceed to the designated loading position of 0mm in the Anton Paar RheoCompass software. When working with samples of solely pasty nature, it is advised to use a parallel plate setup as detailed in other rheometry studies to avoid issues with sample loading ([19]).

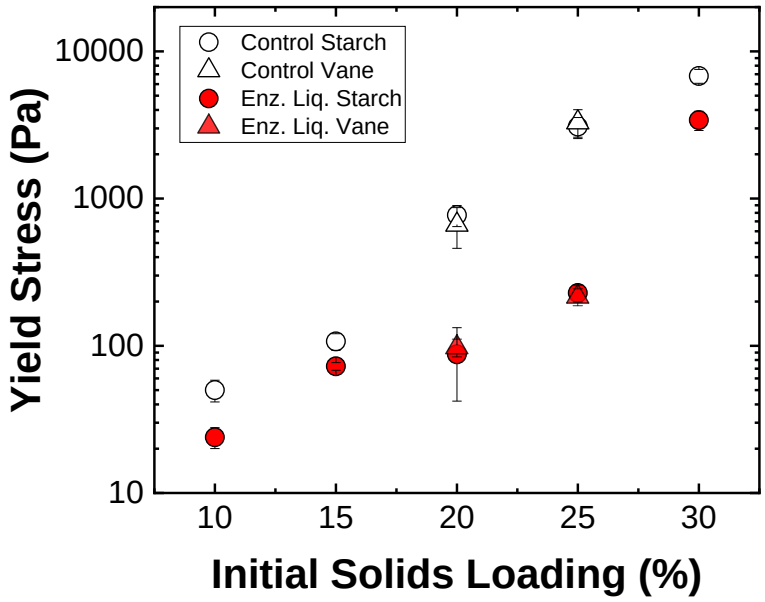


Figure 4.1. Static yield stress data from increasing flow curves of corn stover biomass slurries. Standard deviation is calculated from a sample size of three separate measurements.

As the percentage of initial solids loading increased, the yield stress also increased producing an upward trend as observed in Figure 4.1. This result was expected due to the increased volume fraction of solids. Similar results were reported in other research on corn stover slurries ([21], [23]-[27]). Across all solids loading, the yield stress of enzymatically liquefied particles decreased as a result of the reduction in insoluble solids concentration. The insoluble solids concentration was related to the volume fraction of the slurry as shown in Table 4.2; data for insoluble solids concentrations for both untreated and enzyme liquefied samples are also summarized. Insoluble solids data was collected by the LORRE (Laboratory of Renewable Resources Engineering) team at Purdue University. Data for the enzyme liquefied sample with 15wt.% initial solids loading is not available (N/A) as the sample was visibly contaminated by fungus prior to testing. Of note, shear processes involved in slurry creation will mechanically breakdown some biomass to reduce crystallinity and particle size ([38]).

Table 4.2. Insoluble solids concentration of corn stover slurry samples post-processing

Initial Solids Loading (wt.%)	Control (wt.%)	Enzyme (wt.%)
10	6.95	4.04
15	9.38	N/A
20	14.2	11.76
25	17.17	14.2
30	21.32	17.8

There are three distinct behaviors at different shear rates: initial regime ($0.1\text{-}1s^{-1}$), transition regime ($1\text{-}100s^{-1}$), and suspended regime ($100\text{-}1000s^{-1}$). In the initial regime, corn stover particles are fully settled and hold random orientation. A yield stress needs to be reached to break down this structure and initiate flow ([19]). Once the structure is broken, at higher shear

rates, the transition regime is reached, and shear thinning behavior is observed as particles align in the shear direction ([17]). The shear stress response is controlled by the frictional contacts between particles in these first two regimes. At sufficient shear rates (<100s^{-1}) in the suspended regime, particles lose frictional contacts and the flow becomes turbulent and the shear stress response is a measure of particle collisions and secondary flows ([40]). It should be noted that flow curve data from higher solids loading cannot be used outside of a yield stress, which produces data equivalent to a low-torque measurement. High solids loading samples will fracture and measurements will be a reading between a sample plug and remaining biomass. Figure 4.2 shows the flow response curve of a dilute slurry at 15wt.% initial solids loading that had been liquefied with enzyme for 6 hours. The hypothesized structure during testing is also illustrated in Figure 4.2.

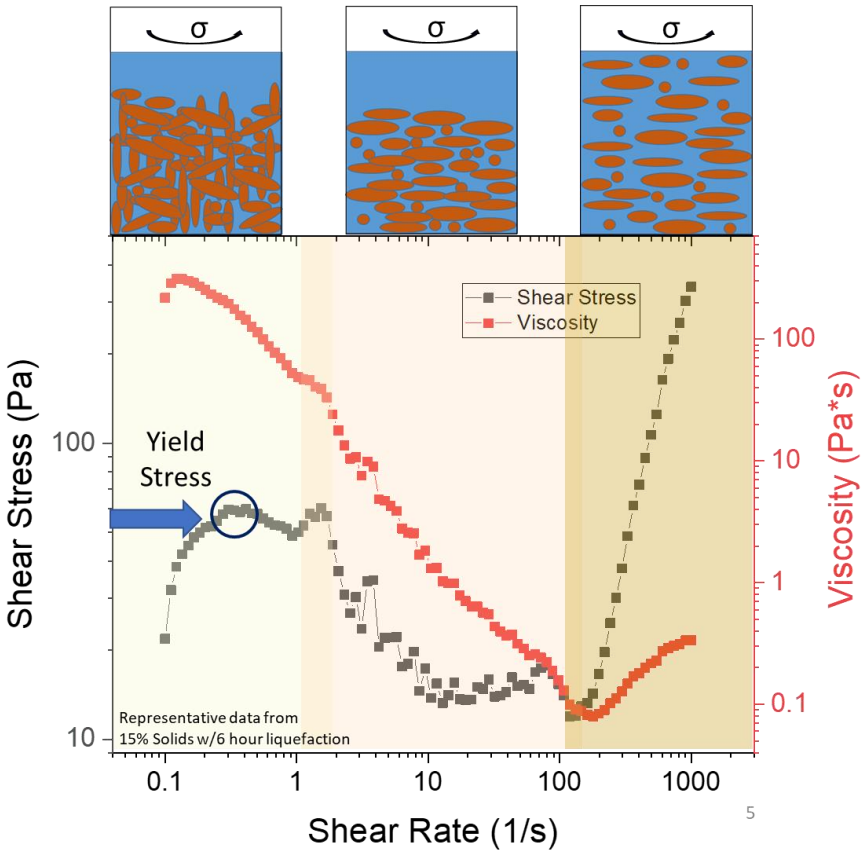


Figure 4.2. Flow curve data of an enzyme liquefied 15wt.% initial solids loading corn stover slurry. The hypothesized structure is shown: initial regime (0.1-1s^{-1}), transition regime (1-100s^{-1}), and suspended regime (100-1000s^{-1}).

4.3 Hysteresis Studies

Hysteresis flow curve measurements of 15wt.% untreated and 15wt.% enzyme-liquefied slurries are shown in Figure 4.3. Results from hysteresis flow curve measurements showed the enzyme-liquefied 15wt.% slurry sample fully recovering its initial yield stress after 3 hours of rest time between flow curve retests. Other observations showed that after a set period of rest, the subsequent flow curve had lower yield stress than the initial curve because the structure has not fully rested. It has also been reported that for systems with wide-gap geometries, the flow curve response based on the direction of the test will produce different viscosities, arising from different packing of the particles when fully settled ([40]).

As summarized in Table 4.3, the 15wt.% control and both 20wt.% samples did not fully recover their initial yield stress, which is likely a result of particles permanently migrating to the outer wall of the glass beaker setup. This migration would leave a depleted zone of particles near the measuring fixture resulting in a decreased yield stress.

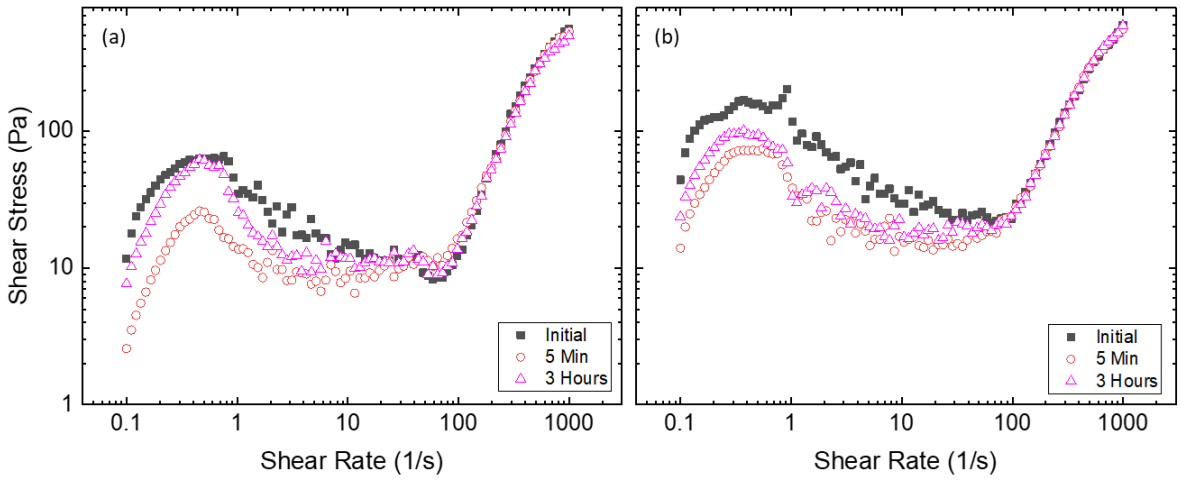


Figure 4.3. Hysteresis flow curve measurements of (a) 15% enzyme liquefied corn stover slurry and (b) 15% untreated corn stover slurry. Time is amount of rest time between sequential flow curve evaluations after the initial flow curve measurement.

Table 4.3. Yield stress %recovery with increasing rest time in hysteresis flow curve measurements

Sample	Initial (Pa)	5 Minutes	30 Minutes	1 Hour	3 Hours
15% Enzyme	66	40%	78%	75%	95%
15% Control	205	36%	46%	42%	59%
20% Enzyme	165	27%	44%	52%	58%
20% Control	606	34%	27%	28%	35%

4.4 Impact of Dilute Acid Pretreatment and Enzyme Liquefaction

Dilute acid pretreatment was used to promote hydrolysis through the breakdown of hemicellulose and lignin ([8]). Enzyme liquefaction was then performed after sequentially to perform further hydrolysis and liquefaction of the slurry ([42]). Combining the two methods significantly lowered yield stress relative to the untreated slurry control as shown in Figure 4.4.

A 100-fold reduction in yield stress from 5000Pa to 50Pa was observed between the control and maleic acid + 48hr enzyme liquefied samples. The data also followed an exponential decay that is similar to another research with corn stover that combined dilute acid pretreatment and enzyme liquefaction ([37]).

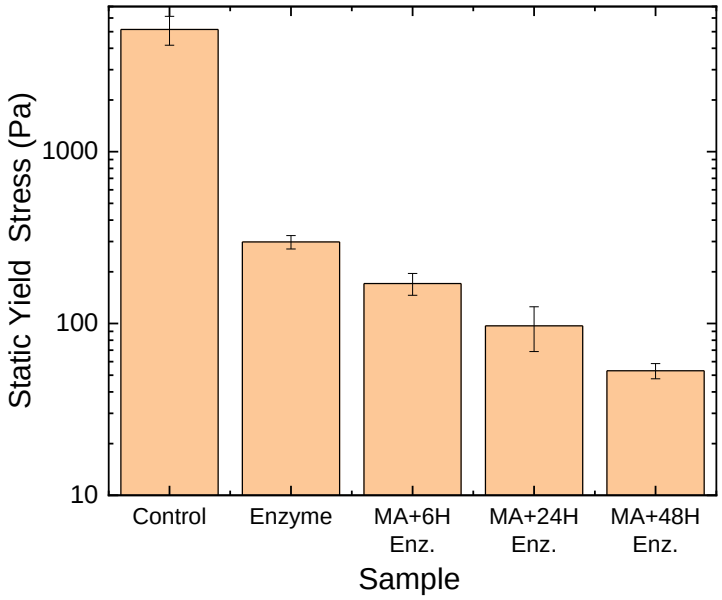


Figure 4.4. Static yield stress data of various treatments of corn stover with maleic acid and enzyme liquefaction.

Dynamic yield stress experiments were conducted on corn stover slurries with combined treatments of maleic acid and enzyme liquefaction. As shown in Figure 4.5, the dynamic yield stress was lower than the static yield stress, which was determined as the largest shear stress value between shear rates of 0.1-10s^{-1}. This was expected as the dynamic yield stress should always be lower than the static yield stress as particles are fully suspended and particles retain some orientation from shear during testing. Dynamic yield stress values from extrapolation to a zero-shear rate using the Herschel-Bulkley yield stress model revealed yield stresses of 9, 13, and 14Pa for combined maleic acid and enzyme liquefaction of 6, 24, and 48 hours,

respectively. Results using the Herschel-Bulkley model also showed that increased time of enzyme liquefaction resulted in decrease in the shear thinning index indicating that samples have more shear thinning behavior ([43]).

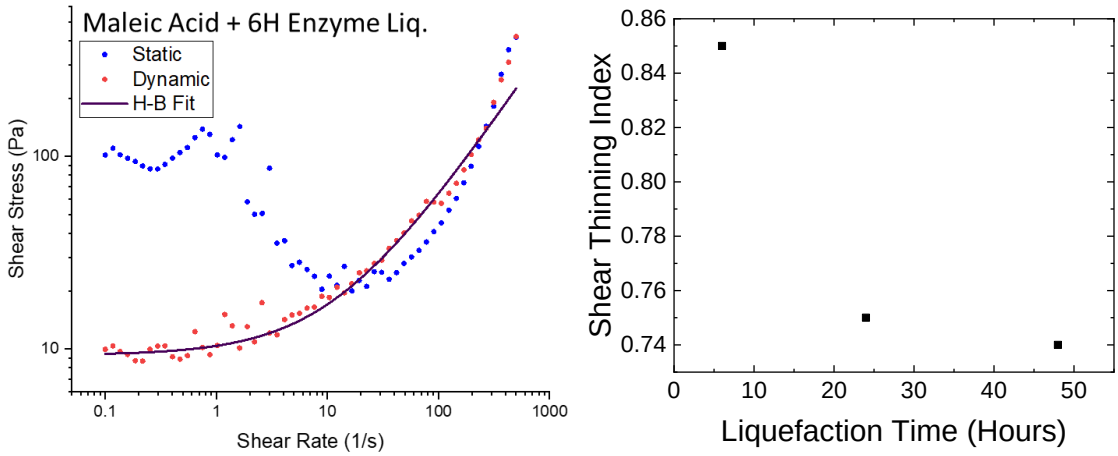


Figure 4.5. (Left) Static and dynamic flow curve data of a corn stover slurry pretreated with maleic acid and then enzyme liquefied for 6 hours. H-B Equation for fit: $9 + 1.1\dot{\gamma}^{0.85}$ (Pa) (Right) H-B model shear thinning index of corn stover slurries of combined maleic acid and enzyme liquefaction.

Dynamic light scattering (DLS) measurements were conducted on samples to determine the changes in particle size distribution with various processing techniques. In Figure 4.6, it was observed that the particle size distribution for the control and the enzyme liquefied samples had average sizes larger than the limits of the measuring technique (>1mm) and would need to be observed through optical microscopy. A reduction in particle size was observed between the combined maleic acid pretreatment and enzyme liquefaction of 6 hours and 48 hours. The average particle sizes were reduced from 425 and 55.2µm for 6 hours of enzyme liquefaction to 330 and 33.2µm for 48 hours of enzyme liquefaction. This reduction in particle size may also be attributed to the observed reduction in yield stress as smaller hydrodynamic size may result in a reduced yield stress as found in other biomass slurry studies ([37]).

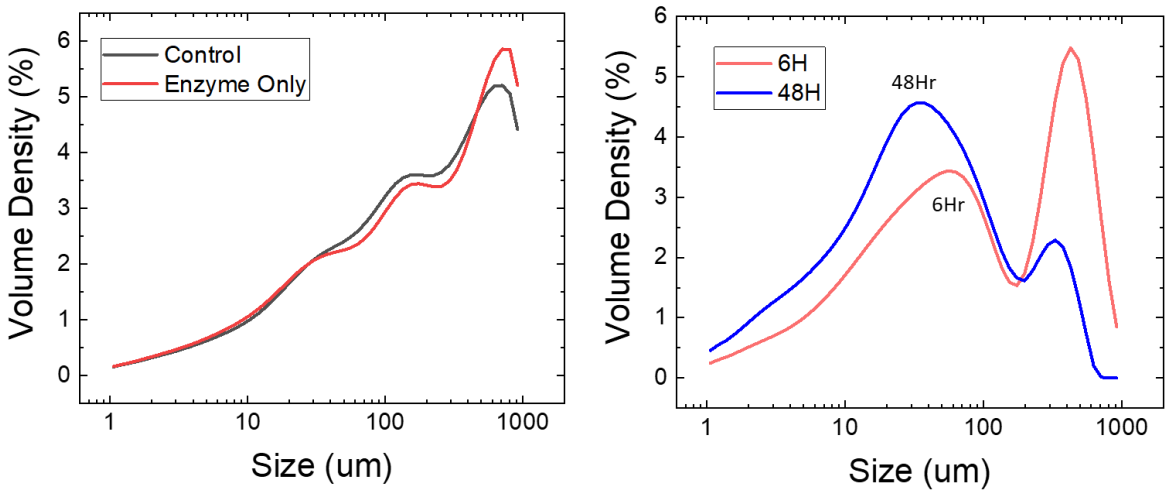


Figure 4.6. Particle size data obtained from DLS measurements. (Left): Particle size data of corn stover slurry particles without treatment (control) and with enzyme liquefaction (enzyme only) showing peaks approaching the maximum size detection limits. (Right): Particle size data of combined maleic acid pretreatment with 6 and 48 hours of enzymatic liquefaction.

Optical microscopy images were obtained for diluted samples of various treatments of corn stover slurries to determine the aspect ratio of particles larger than 1mm. Figure 4.7 showcases the heterogeneity of the particles within the system with regards to both particle size distribution and particle shape. The longest dimension of particles was measured as the length of the particle and the average aspect ratio of particles were 6.0, 6.5, and 6.1 for control, enzyme only, and maleic acid + 48 hours of enzyme liquefaction respectively as shown in Figure 4.8. The average length measured was 1.5mm. Larger particles in corn stover slurries have been reported to increase the yield stress of slurries and a more thorough understanding of these particles is necessary to understanding the rheological response at these particle sizes ([33]). Although the aspect ratio of these particles is similar, it is suggested in other work that the roughness of the particles might change as a result of treatment which can impact the rheology and that particle size change does not always equate to reduction in yield stress ([44]). Further understanding of particle properties will provide better insight into slurry flow behavior.

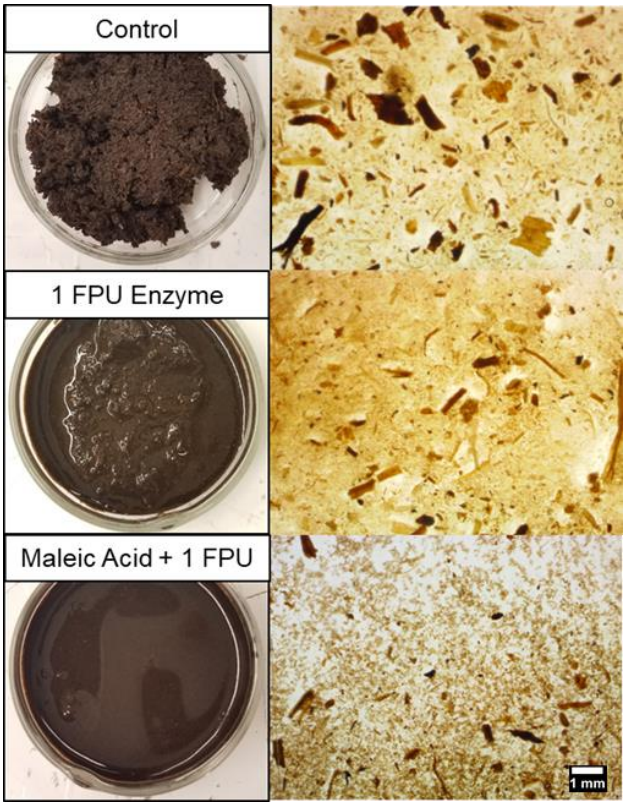


Figure 4.7. Optical microscopy image of corn stover particles taken at 1x magnification showcasing particle heterogeneity. Scale bar indicates a 1mm length.

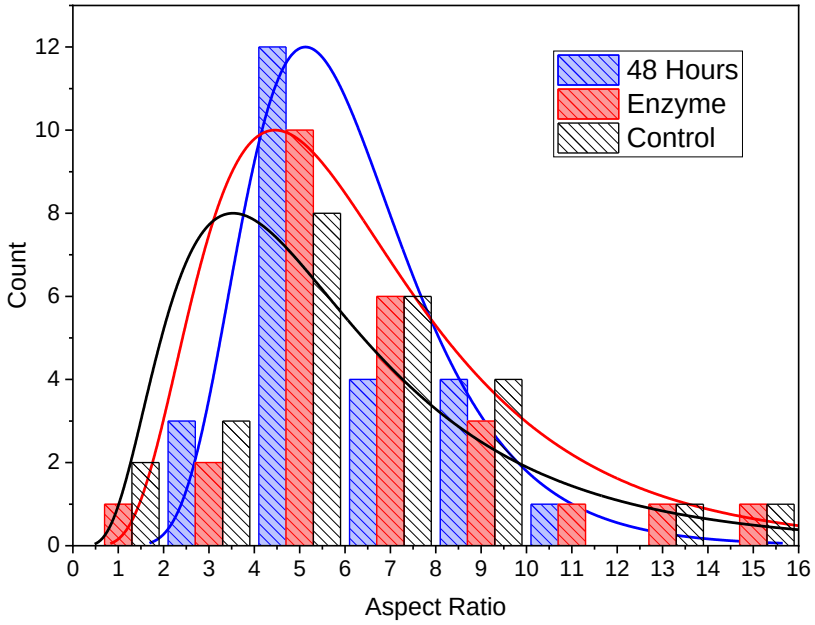


Figure 4.8. Aspect ratio of corn stover particles with lengths greater than 1mm. Distributions of aspect ratios are lognormal fits.

4.5 Fractionated Slurries

Untreated and enzyme liquefied corn stover slurries were dried to isolate particles to explore the effect of particle size and the effect of enzymatic treatment. The particle size distribution of corn stover particles separated through a sieve stack and measured by mass is shown Figure 4.9.

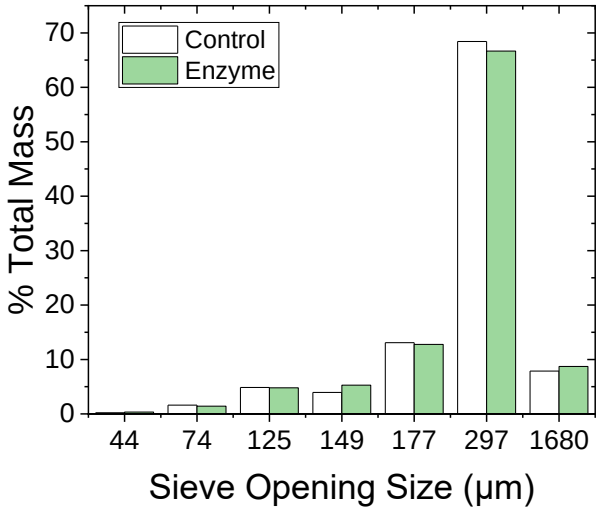


Figure 4.9. Particle size distribution by weight fraction of corn stover particles separated by a series of sieves.

Isolated particles from Sieves No. 50 (>297um) and No. 80 (>177um) were used to a generate slurries of 15wt.% from the dry particle mass. The flow curves of these initial reconstituted slurries measured within a steel cylinder and vane fixture (1mm gap) are shown in Figure 4.8. These experiments showed that larger particles from Sieve No. 50 produced a stronger shear stress response than slurries created from corn stover particles in Sieve No. 80 regardless of processing technique. Interestingly, from slurries created from Sieve No. 50, enzymatically treated particles produced a larger shear stress than untreated particles. It is hypothesized that the enzymatically treated particles are more porous as the processing will convert some of the solids to solubilized sugars. In a drying study, measuring the amount of absorbed water showed

that this was the case as enzymatically treated particles absorbed more water than untreated particles. This larger absorbance of water reduced the available free water within the system and increased the shear stress response as the solid volume fraction was larger. The measurements from these fractionated slurries supported the findings in the combined maleic acid and enzyme liquefaction slurry samples that decreasing particle size reduces the system's yield stress.

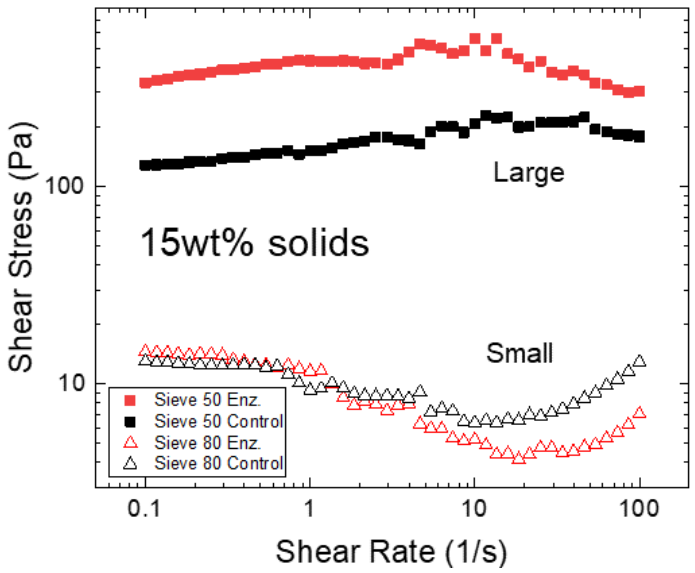


Figure 4.10. Larger particles will produce a larger shear stress response because their larger hydrodynamic size offers more flow resistance.

Figure 4.11 shows the static and yield stress data of reconstituted slurries created from particles sieved with Sieve No. 50. Particles were pre-hydrated to account for absorption and the wet weight was used as the mass to create slurries of different weight fractions with water. The measured static yield stress was greater than the dynamic yield stress across all compositions, which was consistent with other works. Additionally, each yield stress followed a power law in terms of growth with weight fraction, which has been reported by many other groups on cellulosic biomass slurries studying yield stress. The exponent for the dynamic yield stresses

was 5.2 and 4.6 for the control and enzyme treated particle slurries respectively. The exponent for the static yield stress was 6.5 and 7.4 for the control and enzyme treated particle slurries respectively. The lower exponent in the dynamic yield stress power law can be attributed to the nature of the measurement as particles are aligned in the shear direction, unlike a random settled state in the static yield stress measurement.

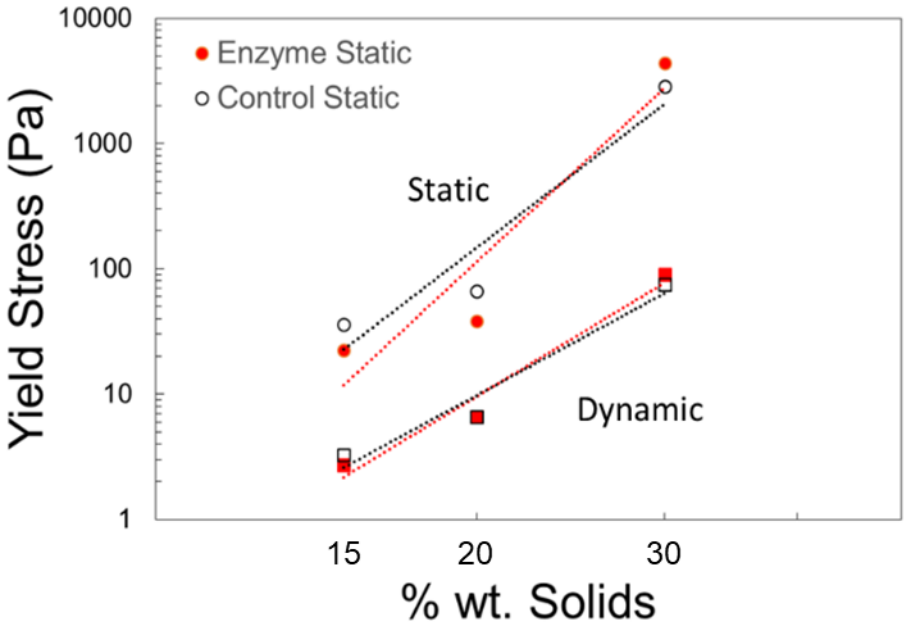


Figure 4.11. Static and dynamic yield stress data of reconstituted slurries with power law fits showing an increase in magnitude with solids concentration.

5. CONCLUSIONS

5.1 Summary and Main Conclusions

In this study, corn stover slurries with various solids concentrations and liquefaction processing were characterized through the use of a rheometer equipped with a wide-gap geometry to identify the yield stress and understand flow properties. Solid concentrations of produced slurries were between initial weight compositions of 10-30% with various processing techniques used to lower the yield stress. The shear stress response of the system was measured through flow curve experiments to determine how the system responded to different shear rates. The yield stress of slurries processed with combined liquefaction techniques of dilute acid pretreatment and enzymatic liquefaction was measured to show a further decreased yield stress. Corn stover particles were dried and fractionated by particle size and slurries were reconstructed in water to probe the effect of particle size. The main experimental outcomes were the following:

1. The yield stress of corn stover slurries is primarily a function of the volume fraction of solids within the system and is directly impacted by the initial solids loading during processing and the degree of liquefaction during mixing. Slurries between 10-30wt.% initial solids loading were evaluated with a rheometer and the yield stress was shown to increase with the solid fraction and enzymatically treated slurries were shown to decrease the yield stress across all weight fractions showing that the enzyme was not inhibited despite the increase in pasty structure at higher solids concentrations.

2. Corn stover slurries were created using an additional dilute acid pretreatment prior to enzymatic liquefaction to further hydrolyze the corn stover. Slurries that were liquefied for 48 hours after dilute acid pretreatment showed an approximate 100-fold reduction in yield stress relative to a slurry created without any liquefaction. The shear thinning index of these slurries was also shown to decrease with liquefaction time indicating more shear thinning behavior. The average particle size and particle size distribution was shown to decrease in samples from 425 and 55.2μm for 6 hours of enzyme liquefaction to 330 and 33.2μm for 48 hours of enzyme liquefaction. It is possible that the reduction in average particle size has a secondary effect in the yield stress reduction aside from a decrease in solids volume fraction.

3. Drying and fractionating particles though a series of sieves and reconstructing slurries from isolated particles by size shows that slurries produced from larger particles will have a larger shear stress response regardless of particle treatment. Enzymatically treated particles were also shown to absorb more water suggesting that the particles were more porous. Reconstructing slurries from prewet particles and measuring their shear stress response demonstrates that volume fraction is a primary factor in determining the yield stress and shear stress response of a slurry. No difference in yield stress was found between reconstituted slurry samples created from enzyme treated particles or untreated particles.

5.2 Future Work

The implications of this work are to establish an experimental method that can be used to measure corn stover slurries at multiple solids concentrations that vary in character from a dilute slurry to a thick paste. Future experimental work in this field should be directed towards measuring and evaluating physical particle properties such as roughness, aspect ratio, and modulus. Rheometry experiments can probe the changes in particle size distribution, particle surface properties, and changes to the liquid medium for corn stover slurries. Specifically, scanning electronic microscopy could be used to observe the qualitative effects of treatment on surface roughness and atomic force microscopy can give insight on the quantitative data of treated biomass particles. Connections between particle size distribution and confirmation of the data from these techniques have also been discussed in other work ([44]). These particle properties can be applied as variables in computational studies and can help improve the ability of models to predict the response of the material with more data.

APPENDIX – FLEXIBLE CUP CALIBRATION PROCEDURE

Section 1 – General Safety

To ensure safety of an individual while conducting experiments, it is required that a researcher must undergo the required lab safety training to gain access to the labs. The rheometer utilizes moving parts to characterize material and any contact with the rheometer should be avoided during an experiment. If at any time, an experiment needs to be terminated during an unforeseen event, the experiment can be aborted by clicking a red "Stop" icon on the top of the Rheocompass software screen.

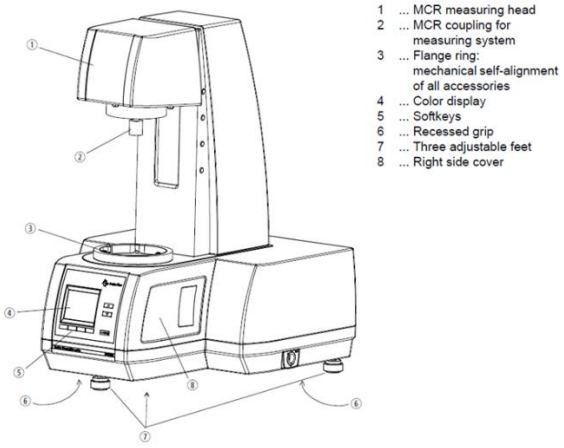
Section 2 – Overview

This Standard Operating Procedure (SOP) will serve as the guidelines for best practices for measuring yield stress of corn stover slurries. Yield stress is defined as the minimum stress necessary to initiate or maintain flow.

Section 3 – Equipment

Anton Paar Modular Compact Rheometer (MCR) 702 MultiDrive

The MCR 702 is a rheometer is equipped with a single motor/transducer in the measuring head. This duality enables the user to operate the rheometer in either controlled strain mode or a controlled stress mode.

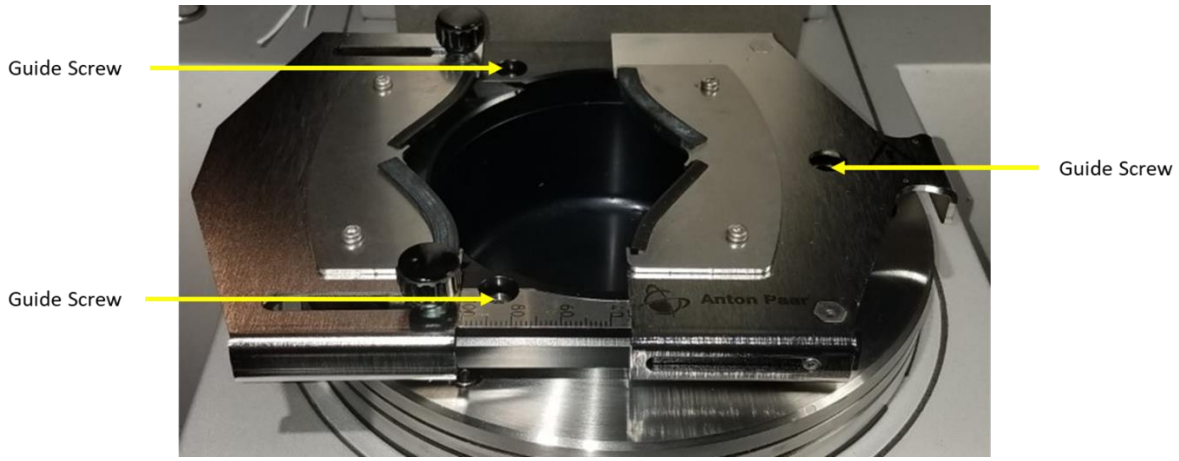


Minimum Torque	1 nNm
Maximum Torque	230 mNm
Torque Resolution	0.1 nNm
Deflection Angle	0.05 to ∞ μrad
Maximum Speed	314 rad/s
Frequency Range	10^{-7} to 628 rad/s
Normal Force Range*	0.005 to 50 N
Normal Force Resolution	0.5 mN

*Note: Even though this is the limit of the rheometer, a moving profile has been set to restrict the rheometer to only reach 15N to protect the transducer from damage. DO NOT override this setting.

Anton Paar Flexible Cup Holder

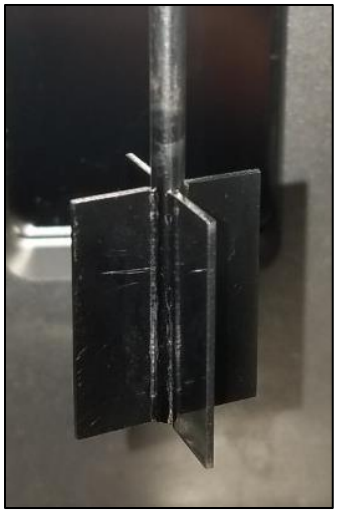
The flexible cup holder is a device that can be mounted and aligned directly onto the MCR 702 unit using the guide and fixture screws on the rheometer. The flexible cup holder can affix any cylindrical measuring vessel with diameters between 40 - 100 mm.

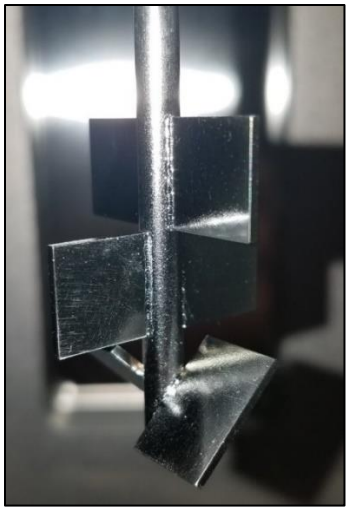


Measuring Fixtures

The MCR 702 can utilize a variety of measuring systems using the modularity of the system and its components. Vane and Starch Cell fixtures have been identified as the most effective fixtures for the corn stover slurry system. The vane is typically used for samples of more paste/solid-like character and the starch cell is better employed for sedimenting systems.

Fixture	Vane (ST24-4V-30/124)	Starch Cell (ST24-2D/2V/2V-20)
Diameter (mm)	24.000	24.000
Length (mm)	30.000	30.000
CSS Factor (SI) [Pa/Nm]	76220.000	138000.00
CSR Factor (SI) [s/s]	60.000	60.000
Active Length (mm)	124	108.5
Positioning Length (mm)	72.5	72.5





Anton Paar Rheocompass

Rheocompass is software developed by Anton Paar which creates an interface between the user and equipment. The user can select and change any specific measuring parameters for an experiment and collect data using this software.

Section 4 – Initial Start Up

Section 4.1 – General Setup

1. Turn on the power for the MCR 702 unit and allow the system to remain on for 1 hour prior to any measurements or calibrations to allow the system to adequately warm up and ensure accuracy of measurements.
2. After an hour or more has passed, open the Anton Paar Rheocompass Software and enter a registered Username and Password to access the program.
3. Link the program and rheometer by selecting “initialize” in the control panel on the top-right corner of the window. Allow rheometer to adjust and align with the software.
4. Under “Measuring Set”, select “Flexible Cup” as the Current configuration and click “Check communication”. Proceed when receiving an “Ok” message in the new pop-up window. This will notify the program that the Flexible Cup Holder has been mounted and will use the moving profile associated with this setup.
5. Remove the guard ring on the measuring head by unscrewing counterclockwise.
6. Affix desired measuring system to the measuring head and allow the rheometer to identify the specific fixture. An audible ring and status message will appear when the fixture has been identified.

Section 4.2 – Verifying Sample Measuring Vessel

When it is desired, to change the dimensions of the measuring cylinder based on particle size for characterization, a set of validation experiments need to be run to obtain accurate measurements. The rheometer will convert measured values of torque based on the gap size between the outer diameter of the measuring fixture and inner wall of the measuring cup. A series of constant shear rate experiments using a Newtonian-viscosity fluid will be used to validate any changes in dimensions.

1. Obtain a Newtonian-viscosity fluid (Typically mineral oil) and measure its viscosity in a measuring setup with calibrated values at shear rate values $\dot{\gamma}$=0.1, 1, 10, 100s^{-1} (e.g. Stainless-steel concentric cylinder).
2. Repeat step 1 with the desired measuring fixture and measuring cup.
3. Calculate the CSR factor (x) for the new dimensions of the cup using the average measured viscosity using the following equation:

$$CSR\ Factor\ (x) = \frac{Viscosity\ (x) * CSR\ Factor\ (SI)}{Standard\ Oil\ Viscosity}$$

4. Go to: Setup → Measuring systems → Select measuring fixture → Dimensions, Factors → input calculated CSR factor (SI)

Section 5 – Measuring Head Calibration

The system must be calibrated to obtain robust and accurate data. Calibration ensures that the motor and air bearing will record torque and rotation accurately through inertial measurements. Each individual fixture must be calibrated separately. It is recommended to recalibrate the system every 30 days for each fixture in use.

1. Connect the Rheometer and Rheocompass software as outlined in Section 3 and ensure that the rheometer has been powered on for a minimum time of an hour.
2. In the program, open the sequence for calibration by clicking through the following tabs:

   My Apps → Verification & Adjustment → Motor adjustment (CC/CP/DG/PP/ST), Air bearing
3. Place desired measuring container into the flexible cup holder.
4. Click “Start” and enter the fixture and date into Test name. Then press Continue.
5. Define waiting position as “0 mm” and then press continue.

6. Ensure that the calibration has begun by confirming that data is being populated on the diagram. It is recommended to leave the room until calibration has been completed as the system is sensitive to vibrations or other disturbances.
7. A report will be generated ensure that the torque is within -0.025 uN*m and 0.025uN*m. If the torque is within these limits, the calibration is sufficient for future measurements.
8. The report can be exported if necessary. Click Continue to complete the calibration.

Section 6 – Yield Stress Measurement

Yield stress measurements are conducted through flow curves which are ramps of increasing or decreasing shear rates[1]. For corn stover biomass slurries, multiple measurement methods have been evaluated and found to produce similar values[2]. This protocol will determine both the static and dynamic yield stresses. Static yield stress is the minimum stress necessary to initiate flow and dynamic yield stress is the minimum stress necessary to maintain flow[3].

Section 6.1 – Establishing Experiment Parameters

1. Open a measurement file from the home page of the Rheocompass software.
2. Select the Measurement tab and in the new window, right click and add a set variable. Select shear rate in the drop down menu.
3. Click into the first interval that appears and set each data point to be collected every 2 seconds.
4. Set the Profile to "Ramp Logarithmic" and set the shear rate range between 0.1 and $1000s^{-1}$.
5. For convenience, use the calculator tab and set the measurement to 15 pt./dec. (points per decade) and click apply.
6. To set up the dynamic flow measurement, right click the main display and select append interval create a measurement immediately after the static yield stress measurement.
7. Click into the second interval that appears and set each point to be collected every 2 seconds.
8. Set the Profile to "Ramp Logarithmic" and set the shear rate range between 1000 and $0.1s^{-1}$.
9. Use the calculator tab and set the measurement to 15pt./dec. and click apply.

Section 6.2 – Sample Loading

1. Attach desired measuring fixture to measuring head and wait for an audible tone to play to signal that the rheometer has identified the fixture.
2. Raise the measuring head to a position of 160mm using the control panel to allow space for the measuring cylinder and sample to be loaded.
3. Hand-mix slurry sample to ensure adequate particle dispersion and pour into the glass cylinder.
4. Pull the right side of the flexible cup holder to open the chamber and place the sample container. Release the flexible cup holder gently to lock the sample-containing glass cylinder in place.
5. Lower the measuring head to a position of 0mm to place the vane into the sample.
6. Allow the sample to equilibrate for 15 minutes.

Section 6.3 – Characterization

1. Click Start on the top-left corner of the measurement screen and type in the desired sample file name and fill out any other desired information.
2. Click Continue and the measurement will begin with the parameters outlined in Section 6.1. The data will be plotted in the software in real time.
3. Once the measurement has been completed, click the Table tab to access the raw data. Select the sample and check the curves that you want to extract.
4. Click into the table, Press "Ctrl + A" to select all of the data for the test and then paste the data into an excel file for data analysis. Repeat for all desired data.
5. Raise the measuring head to a position of 160mm using the control panel to remove the vane from the sample.
6. Remove sample from the glass measuring cylinder and flexible cup holder. Remove the vane from the rheometer.
7. Wash rheometry fixture and glass cylinder with water. Dry with compressed air or dry paper towels.

REFERENCES:

[1] Jagger, A. (2009). Brazil invests in second-generation Biofuels. *Biofuels, Bioproducts and Biorefining, 3*(1), 8-10. doi:10.1002/bbb.127

[2] Gomez, L. D., Steele-King, C. G., & McQueen-Mason, S. J. (2008). Sustainable liquid biofuels from biomass: The writing's on the walls. *New Phytologist, 178*(3), 473-485. doi:10.1111/j.1469-8137.2008.02422.x

[3] Mumm, R. H., Stein, H. H., Rausch, K. D., & Goldsmith, P. D. (2015). Land usage attributed to corn ethanol production in the united states: Sensitivity to technological advances in corn grain yield, ethanol conversion, and co-product utilization. *Fuel Production from Non-Food Biomass,* 272-307. doi:10.1201/b18437-18

[4] Mensah, M. B., Jumpah, H., Boadi, N. O., & Awudza, J. A. (2021). Assessment of quantities and composition of Corn STOVER in Ghana and their conversion Into bioethanol. *Scientific African, 12*. doi:10.1016/j.sciaf.2021.e00731

[5] Zhang, M., Wang, F., Su, R., Qi, W., & He, Z. (2010). Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. *Bioresource Technology, 101*(13), 4959-4964. doi:10.1016/j.biortech.2009.11.010

[6] Martin, M. A. (2010). First generation biofuels compete [Abstract]. *New Biotechnology, 27*(5), 597-608. doi:doi.org/10.1016/j.nbt.2010.06.010

[7] Zoghlami, A., & Paes, G. (2019). Lignocellulosic Biomass: Understanding Recalcitrance and Predicting Hydrolysis. *Frontiers in Chemistry, 7*(874), 1-11. doi:doi.org/10.3389/fchem.2019.00874

[8] Mosier, N. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresource Technology, 96*(6), 673-686. doi:10.1016/j.biortech.2004.06.025

[9] Jørgensen, H., Kristensen, J. B., & Felby, C. (2007). Enzymatic conversion of lignocellulose into fermentable sugars: Challenges and opportunities. *Biofuels, Bioproducts and Biorefining, 1*(2), 119-134. doi:10.1002/bbb.4

[10] Galbe M, Zacchi G. 2007. Pretreatment of Lignocellulosic Materials for Efficient Bioethanol Production. In: Olsson, L, editor. Biofuels. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 41–65. https://doi.org/10.1007/10_2007_070.

[11] Humbird D, Mohagheghi A, Dowe N, Schell DJ. 2010. Economic impact of total solids loading on enzymatic hydrolysis of dilute acid pretreated corn stover. Biotechnol. Prog. 26:1245–1251

[12] Barnes, H. A. (1999). The yield stress—a review or ‘παντα ρει’—everything flows? *Journal of Non-Newtonian Fluid Mechanics, 81*(1-2), 133-178. doi:10.1016/s0377-0257(98)00094-9

[13] Nguyen, Q. D., Akroyd, T., Kee, D. C., & Zhu, L. (2006). Yield stress measurements in suspensions: An inter-laboratory study. *Korea-Australia Rheology Journal, 18*(1), 15-24.

[14] Bonn, D., & Denn, M. M. (2009). Yield Stress Fluids Slowly Yield to Analysis. *Science, 324*(5933), 1401-1402. doi:10.1126/science.1174217

[15] Dinkgreve, M., Paredes, J., Denn, M. M., & Bonn, D. (2016). On different ways of measuring “the” yield stress. *Journal of Non-Newtonian Fluid Mechanics, 238*, 233-241. doi:10.1016/j.jnnfm.2016.11.001

[16] Coussot, P., Nguyen, Q. D., Huynh, H. T., & Bonn, D. (2002). Viscosity bifurcation in thixotropic, yielding fluids. *Journal of Rheology, 46*(3), 573-589. doi:10.1122/1.1459447

[17] Coussot, P. (2014). Yield stress fluid flows: A review of experimental data. *Journal of Non-Newtonian Fluid Mechanics, 211*, 31-49. doi:10.1016/j.jnnfm.2014.05.006

[18] Mezger, T. G. (2011). *The Rheology Handbook* (3rd ed.). Hanover, Germany: Vincentz Network.

[19] Stickel, J. J., Knutsen, J. S., Liberatore, M. W., Luu, W., Bousfield, D. W., Klingenberg, D. J., . . . Monz, T. O. (2009). Rheology measurements of a biomass slurry: An inter-laboratory study. *Rheologica Acta, 48*(9), 1005-1015. doi:10.1007/s00397-009-0382-8

[20] Walls, H. J., Caines, S. B., Sanchez, A. M., & Khan, S. A. (2003). Yield stress and wall slip phenomena in colloidal silica gels. *Journal of Rheology, 47*(4), 847-868. doi:10.1122/1.1574023

[21] Volynets, B., Ein-Mozaffari, F., & Dahman, Y. (2019). Biomass processing into ethanol: Pretreatment, enzymatic hydrolysis, fermentation, rheology, and mixing. *[Set Bioenergy, Vol. 1+2]*. doi:10.1515/energyo.0137.00001

[22] Schutter, G. D., & Feys, D. (2016). Pumping of Fresh Concrete: Insights and Challenges. *RILEM Technical Letters, 1*, 76. doi:10.21809/rilemtechlett.2016.15

[23] Knutsen, J. S., & Liberatore, M. W. (2009). Rheology of high-solids biomass slurries for biorefinery applications. *Journal of Rheology, 53*(4), 877-892. doi:10.1122/1.3143878

[24] Pimenova, N. V., & Hanley, T. R. (2004). Effect of Corn Stover Concentration on Rheological Characteristics. *Applied Biochemistry and Biotechnology, 114*(1-3), 347-360. doi:10.1385/abab:114:1-3:347

[25] Ehrhardt, M. R., Monz, T. O., Root, T. W., Connelly, R. K., Scott, C. T., & Klingenberg, D. J. (2009). Rheology of Dilute Acid Hydrolyzed Corn Stover at High Solids Concentration. *Applied Biochemistry and Biotechnology, 160*(4), 1102-1115. doi:10.1007/s12010-009-8606-z

[26] Samaniuk, J. R., Scott, C. T., Root, T. W., & Klingenberg, D. J. (2012). Rheological modification of corn stover biomass at high solids concentrations. *Journal of Rheology, 56*(3), 649-665. doi:10.1122/1.3702101

[27] Klingenberg, D. J., Root, T. W., Burlawar, S., Scott, C. T., Bourne, K. J., Gleisner, R., . . . Subramaniam, V. (2017). Rheometry of coarse biomass at high temperature and pressure. *Biomass and Bioenergy, 99*, 69-78. doi:10.1016/j.biombioe.2017.01.031

[28] Lavenson, D. M., Tozzi, E. J., McCarthy, M. J., & Powell, R. L. (2011). Yield Stress of Pretreated Corn Stover Suspensions Using Magnetic Resonance Imaging. *Biotechnology and Bioengineering, 108*(10), 2312-2319. doi:10.1002/bit.23197

[29] Ghosh, S., Holwerda, E. K., Worthen, R. S., Lynd, L. R., & Epps, B. P. (2018). Rheological properties of corn stover slurries during fermentation by Clostridium thermocellum. *Biotechnology for Biofuels, 11*(1). doi:10.1186/s13068-018-1248-z

[30] Ewoldt, R. H., Winter, P., Maxey, J., & Mckinley, G. H. (2009). Large amplitude oscillatory shear of pseudoplastic and elastoviscoplastic materials. *Rheologica Acta, 49*(2), 191-212. doi:10.1007/s00397-009-0403-7

[31] Mangesana, N., Chikuku, R. S., Mainza, A., Govender, I., Westhuizen, A., & Narashima, M. (2008). The effect of particle sizes and solids concentration on the rheology of silica sand based suspensions. *The Journal of The Southern African Institute of Mining and Metallurgy, 108*, 237-243.

[32] Kawatra, S., & Eisele, T. (1988). Rheological effects in grinding circuits. *International Journal of Mineral Processing, 22*(1-4), 251-259. doi:10.1016/0301-7516(88)90067-1

[33] Viamajala, S., McMillan, J. D., Schell, D. J., & Elander, R. T. (2009). Rheology of corn stover slurries at high solids concentrations – Effects of saccharification and particle size. *Bioresource Technology, 100*(2), 925-934. doi:10.1016/j.biortech.2008.06.070

[34] Dasari, R. K., & Berson, R. E. (2007). The effect of particle size on hydrolysis reaction rates and rheological properties in cellulosic slurries. *Applied Biochemistry and Biotechnology, 137*, 289-299. doi:doi-org.ezproxy.lib.purdue.edu/10.1007/s12010-007-9059-x

[35] Pednekar, S., Chun, J., & Morris, J. F. (2018). Bidisperse and polydisperse suspension rheology at large solid fraction. *Journal of Rheology, 62*(2), 513-526. doi:10.1122/1.5011353

[36] Farris, R. J. (1968). Prediction of the Viscosity of Multimodal Suspensions from Unimodal Viscosity Data. *Transactions of the Society of Rheology, 12*(2), 281-301. doi:10.1122/1.549109

[37] Chen, X., Crawford, N., Wang, W., Kunh, E., Sievers, D., Tao, L., & Tucker, M. (2018). Kinetics and Rheological Behavior of Higher Solid (Solids 20%) Enzymatic Hydrolysis Reactions Using Dilute Acid Pretreated, Deacetylation and Disk Refined, and Deacetylation and Mechanical Refined (DMR) Corn Stover Slurries. *ACS Sustainable Chemistry & Engineering, 7*(1), 1633-1641. doi:10.1021/acssuschemeng.8b05391

[38] Berson, R. E. (2009). *Ethanol Production from Biomass: Large Scale Facility Design Project* (Rep.). Louisville, KY.

[39] Kim, S. M., Dien, B. S., & Singh, V. (2016). Erratum to: Promise of combined HYDROTHERMAL/CHEMICAL and mechanical refining for pretreatment of Woody and herbaceous biomass. *Biotechnology for Biofuels, 9*(1). doi:10.1186/s13068-016-0643-6

[40] Crawford, N. C., Sprague, M. A., & Stickel, J. J. (2016). Mixing behavior of a model cellulosic biomass slurry during settling and resuspension. *Chemical Engineering Science, 144*, 310-320. doi:10.1016/j.ces.2016.01.028

[41] Barnes, H. A. (1997). Thixotropy - a review. *Journal of Non-Newtonian Fluid Mechanics, 70*(1-2), 1-33. doi:https://doi.org/10.1016/S0377-0257(97)00004-9

[42] Ladisch, M. R., Ladisch, C. M., & Tsao, G. T. (1978). Cellulose to sugars: New path gives quantitative yield. *Science, 201*(4357), 743-745. doi:10.1126/science.201.4357.743

[43] Polachini, T. C., Mulet, A., Cárcel, J. A., & Telis-Romero, J. (2019). Rheology of acid suspensions containing cassava bagasse: Effect of biomass loading, acid content and temperature. *Powder Technology, 354*, 271-280. doi:10.1016/j.powtec.2019.05.086

[44]

[45] Dibble, C. J., Shatova, T. A., Jorgenson, J. L., & Stickel, J. J. (2011). Particle morphology characterization and manipulation in biomass slurries and the effect on rheological properties and enzymatic conversion. *Biotechnology Progress, 27*(6), 1751-1759. doi:10.1002/btpr.669

[46] Jacobsen, S., Mork, J. H., Lee, S. F., & Haugan, L. (2008). Pumping of concrete and mortar – State of the art. *COIN Project Report 5*.

[47] Vikan, H., & Jacobsen, S. (2010). Influence of rheology on the pumpability of mortar. *COIN Project Report 21*.

[48] He, W., Park, C. S., & Norbeck, J. M. (2009). Rheological study of comingled biomass and coal slurries with hydrothermal pretreatment†. *Energy & Fuels, 23*(10), 4763-4767. doi:10.1021/ef9000852

[49] Ma, L., & Barbosa-Cánovas, G. (1995). Rheological characterization of mayonnaise. part ii: Flow and viscoelastic properties at different oil and xanthan gum concentrations. *Journal of Food Engineering, 25*(3), 409-425. doi:10.1016/0260-8774(94)00010-7