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Enzymatic Saccharification and Viscosity of Sawdust Slurries Following Ultrasonic Particle Size Reduction

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Abstract Results in a previous study showed up to a 55% increase in saccharification rates when the initial particle size range decreased from $590<x<850$ μm down to $33<x<75$ μm. The smaller particle sizes also lowered the viscosity of the slurry 50-fold (for an equivalent initial solids concentration). In this study, ultrasonic irradiation was employed to further reduce the particle size of sawdust slurries below the ranges in the previous study in an attempt to further increase enzymatic saccharification rates and lower the slurry viscosity. Average particle sizes were reduced to less than 1 μm under the conditions tested. Surprisingly, the amount and rates of sugar released in this study with the ~1 μm particles is comparable (maximum glucose release of 30%) to, but no better than that seen for particle sizes in the range of $33\leq x\leq 75$ μm (maximum glucose release of 31%). Also surprisingly, the viscosity increased as the average particle sizes in the slurries decreased, which is opposite to the trend in the previous study. For example, there was an approximately threefold increase in the viscosity between unsonicated samples with a range of $10\leq x\leq 75$ μm and sonicated samples with a range of $0.05\leq x\leq 12$ μm. This is attributed to the variations in surface characteristics of the particles which were characterized here using X-ray diffraction profiles and SEM pictures.

Keywords Biomass · Sawdust · Enzymatic hydrolysis · Saccharification · Particle size · Viscosity · Ultrasonic

Introduction

Enzymatic saccharification is a key rate and cost-limiting step in the overall conversion of biomass to ethanol. To achieve an economical sugar stream via saccharification of biomass, improvements are needed in the processing efficiency of this step. Previous results by

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Dasari et al. [1] showed that the initial particle size of raw biomass substrate has a significant impact on the rate of glucose release. Tests were conducted with various particle sizes of sawdust that were obtained using a set of US standard sieves that gave the following initial particle size (x) ranges, $33<x\leq75$ μm, $150<x\leq180$ μm, $295<x\leq425$ μm, and $590<x\leq850$ μm. Overall glucose release was 50% higher for the smallest size range as compared to the biggest size range. It was recommended that further tests be conducted to determine if the saccharification rate continues to increase with decreasing particle size and if a minimum size exists at which the rate is no longer affected by particle size.

The effect of initial particle size on slurry viscosity was also investigated. Results showed smaller particle sizes result in significantly lower viscosities, which is of great benefit when processing at large scale. Viscosity directly affects power requirements which are a significant portion of the operating costs. As an example, the viscosity decreased from 3,000 cP for $150<x\leq180$ μm particle size slurries to 61.4 cP for $33<x\leq75$ μm particle size slurries [1] at a shear rate of 10 s^{-1}.

The aim of this investigation was to determine if a further reduction in particle size will continue to both increase the rate and the extent of glucose release and decrease the slurry viscosity. Particle size reduction was performed using ultrasonic irradiation with the goal to achieve particle sizes on the order of 1 μm or less. Ultrasound can be defined as sound waves above the range of human perception [2]. Currently, many ultrasonic technologies such as SONAR, medical diagnostics, and surface cleaners are available. SONAR and medical applications typically use low power and high frequency (≥1 MHz). Ultrasonic irradiation has shown to be effective in homogenizing, dispersing, extracting, degassing, and in the breaking-down of cells or other particles [2–4]. Most biological applications of ultrasonic technology have been directed towards the disruption of cell membranes [5, 6]. In the fermentation of milk by *Lactobacillus bulgaricus*, the rate of lactose hydrolysis was increased with the use of discontinuous ultrasound [7]. Recently, some studies have focused on enhancing enzymatic hydrolysis of cellulose by continuous ultrasonic irradiation to enhance desorption of the inactively adsorbed enzyme to substrate [8–10]. The changes in physical characteristics resulting from the ultrasonic irradiation were examined here using X-ray diffraction (XRD) profiles taken with an X-ray diffractometer and visually with images from a scanning electron microscope (SEM).

Materials and Methods

Substrate

The cellulose substrate used in these experiments was untreated red oak sawdust obtained from Garrard Wood Products of Lancaster, Kentucky. The major components of the sawdust are 39.7% cellulose, 18.8% hemi-cellulose, and 35% lignin which were measured following NREL Laboratory analytical procedures for determination of structural carbohydrates and lignin in biomass.

Commercial Enzyme

Commercially produced cellulase enzyme, Multifect GC Cellulase enzyme (Genencor International Inc.; Lot # 301-05336-209) was used for hydrolysis of the sawdust.

Ultrasonic Treatment and Particle Size Measurements

The sonicator used was the Hielscher Ultrasonic Processor, model UP200S (Hielscher Ultrasound Technology, Germany), that can produce a frequency of 20 kHz with an adjustable power output up to 200 W to irradiate variable ultrasonic intensities. The horn of the sonicator was placed vertically into a 25-ml glass flask for direct exposure of the substrate to the 20 kHz ultrasound.

Following ultrasonic treatment, particle size was measured using a 90 plus particle size analyzer manufactured by Beckman coulter, Inc. (CA, USA), and a Malvern Mastersizer S Particle Size Analyzer (Malvern Instruments, MA, USA).

Hydrolysis Procedure and Sugar Measurement

Methods for enzymatic saccharification of sawdust, measurement of released glucose, and measurement of viscosity of the slurries were the same as described in the paper by Dasari et al. [1]. In summary, the tests were performed at 55°C in an incubator shaker at 250 rpm for 72 h following sonication. All experiments were performed in 250-mL shake flasks with a working mass of 100 g. The initial solids concentration was 10% (wt/wt), enzyme was added to the slurry following sonication in the amount of 15 FPU/g cellulose, and the citrate buffer concentration was 0.05 M. Samples were collected every 2 h for the first 8 h and then at 24, 48, and 72 h. All tests were performed in duplicate with three samples taken from each flask at each time interval for a total of six samples per time interval. The enzymatic hydrolysis samples were centrifuged at 4,000 rpm in a Beckman GPR centrifuge for 15 min and glucose concentration was measured by a YSI-Biochemistry analyzer (Yellow Spring Instruments, OH, USA) which was calibrated daily.

Viscosity Measurement

The viscosity of the suspension after ultrasonic irradiation was measured with a Modular Compact Rheometer Physica MCR 300 (Paar-Physica). The samples were homogenized prior to the measurements and then a 30 mL slurry sample was transferred to the sample cup of the viscometer and measurements were performed at the reaction temperature (55 °C). The viscosity was measured twice for shear rates from 0.1 s^{-1} to 100 s^{-1}.

X-ray Diffraction Measurement

X-ray diffraction profiles were taken with an X-ray diffractometer (Bruker D8 Discover; Bruker AXS Co., TX, USA). The measurement conditions were as follows: drive=coupled; steptime=0.5; stepsize=0.05; stepmode=stepscan; start=10; 2 =10; radiation Cu K, 0.154 nm; detector type=scintillation counter; HV=774; voltage, 40 kV; current, 40 mA. X-ray diffraction profiles were measured as follows: An 80 mg sample was carefully loaded on a glass plate using a spatula and then dried in an oven at 373 K.

Scanning Electron Microscope

SEM pictures of the sawdust samples were taken with a Nova 600 NanoSEM (FEI Company; Hillsboro, OR, USA) scanning electron microscope. The sawdust solutions were vacuum dried in an oven before the pictures are taken.

Results and Discussion

Particle Size Reduction by Sonication

The particle size ranges obtained by Dasari et al. [1] were obtained by separating existing sawdust particles from a batch by sifting the sawdust through standard mesh screens. To reduce the size range further for this study, ultrasonic irradiation was employed. To study the ability of ultrasonic irradiation to further reduce the particle size, a batch was prepared with a sawdust concentration equal to 10%, with a range of particles sizes from 23 to 560 μm and an average particle size of 280 μm. The solution was sonicated for 12 h using an irradiation intensity of 60% (110 W). Figure 1a illustrates average particle sizes after 0 to 12 h of sonication time.

Another set of tests were performed where the batch to be sonicated initially contained smaller particle sizes which were obtained by sieving. The sawdust was sieved in a set of US standard sieves to obtain initial particles ≤295 μm in one batch (average particle size of

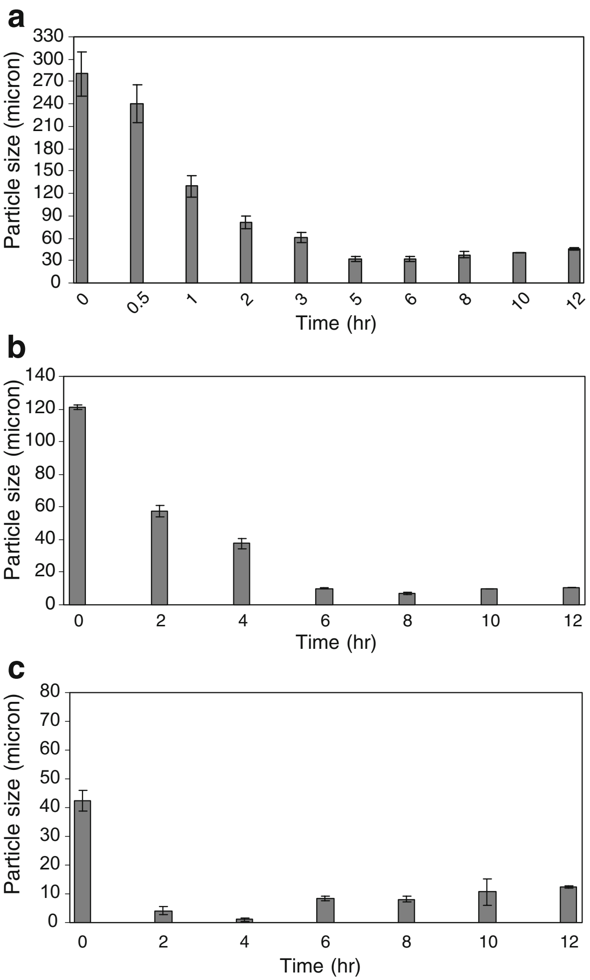


Fig. 1 **a** Average particle sizes following sonication (initial particle sizes 23≤x≤560 μm). **b** Average particle sizes following sonication (initial particle sizes ≤295 μm). **c** Average particle sizes following sonication (initial particle sizes ≤75 μm)

120 μm) and ≤75 μm in another batch (average particle size of 45 μm). The solid concentration was again equal to 10% and the solution was sonicated for 12 h. Figure 1b, c shows average particle sizes following sonication for samples with initial particle sizes that are ≤295 and ≤75 μm. Figure 2 shows the particle size distributions before (Fig. 2a) and after (Fig. 2b) sonication.

All the results clearly show the effectiveness of ultrasonic irradiation in reducing the particle size of sawdust. The average initial particle size of the initial batch was reduced from 280 μm to 40 μm after 5 h. The average initial particle sizes of the two sifted batches reduced from 120 to 10 μm and from 45 to 1 μm. In all cases, the average particle size appears to increase after reaching some minimum value. This is attributed to agglomeration of particles that pack together from the impact in the high energy sonication environment. Agglomeration occurs often for micron (<10 μm) or nano-sized particles, even if they are dry. In a wet environment, wet aggloromerates are bonded by the effects of surface tension and capillary forces of the liquid binder [11]. Results (shown later) indicate the agglomerations unpack during enzymatic hydrolysis.

The effect of ultrasonic intensity on particle size reduction was also investigated (Fig. 3a, b, c). There appears to only be modest enhancement as ultrasonic intensity increases from 20% to 60% (40 to 110 W) and mostly in terms of the amount of time to reach the minimum size rather than in obtaining smaller particles. At 20% intensity, the initial average particle size was 48 μm and was decreased to an average size of ~1 μm after 8 h of irradiation. At 40% intensity, the initial average particle size was 53 μm and was decreased to ~0.9 μm after 8 h of irradiation. At 60% intensity, the initial average particle size was 45 μm and was decreased to ~1 μm after 4 h of irradiation.

Enzymatic Saccharification

To examine the effect of the particle size reduction on the enzymatic saccharification of the sawdust, the amount of glucose released was measured. The material subjected to 60% intensity was used as a test case. By testing the sample subjected to sonication for 12 h, which is longer than Fig. 3c shows it takes for the particles to reach a minimum average size, the idea that the apparent increase in average particle size for longer sonication times is due to agglomeration can also be tested. If the particles are, in fact, smaller following 12 h of sonication as compared to 4 h, then more glucose release should occur since it has been previously proven that saccharification rates are higher for smaller substrate particles.

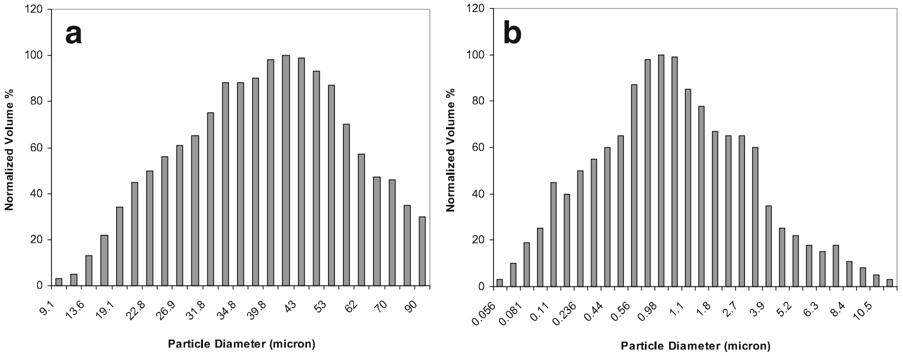


Fig. 2 Particle size distribution of sawdust with initial particle sizes of ≤75 μm **a** before sonication **b** after sonication (irradiation intensity of 110 W and 4 h sonication time)

Figure 4 shows glucose release as a percent of available glucan during saccharification following different sonication times at 60% irradiation intensity. Saccharification without ultrasonic irradiation as a control is also shown. The amount of released glucose was 53% higher for the 12-h sonicated sample, 43% higher for the 4-h sonicated sample, and 22% higher for the 2-h sonicated sample compared to the unsonicated sample. The higher glucose release rate for the 12-h sample supports the hypothesis that the particles are actually smaller but become unpacked during saccharification. The average particle size is, then, likely much less than the 1 μm measured and shown in Figs. 1 and 3.

The amount and rates of sugar released in this study with the ~1 μm particles is comparable (maximum glucose release of 30%) to, but no better than that seen for particle sizes in the range of $33 \leq x \leq 75$ μm (maximum glucose release of 31%) in our previous study (Dasari et al. [1]). This indicates a limit in the improvement to sugar release that may be realized when particle sizes are reduced below at least 33 μm. This appears to be counterintuitive since smaller particles have larger surface area per unit volume and,

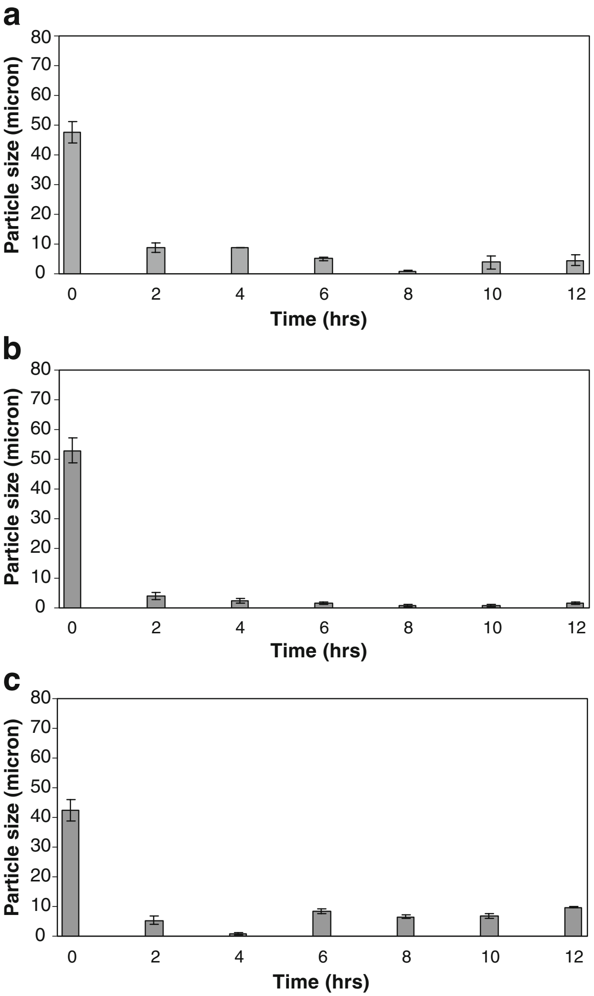
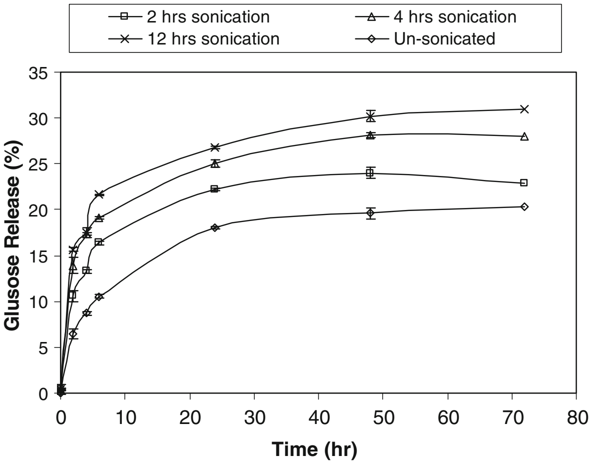


Fig. 3 **a** Average particle sizes throughout 12 h of sonication using 20% intensity. **b** Average particle sizes throughout 12 h of sonication using 40% intensity. **c** Average particle sizes throughout 12 h of sonication using 60% intensity

Fig. 4 Effect of sonication on glucose release (10% solid concentration, 60% sonication intensity, initial particle size range ≤75 μm)



therefore, more cellulose may be accessible for the enzyme to reach and at a faster rate. The smaller particles have also been exposed to more grinding at the surface resulting in a reduction of crystallinity and an increase in amorphous nature at the surface [12, 13]. The higher amount of amorphous fibers should also tend to increase the rate and extent of glucose release.

An explanation may be attributed to the effect that the lignin that is likely sheared off during sonication would have. Results by Sun et al. [8] showed that ultrasonic treatment of bagasse led to a release of over 90% of the original hemicelluloses and lignin. If lignin is releasing during sonication and floating free in the solution, then lignin adsorption of enzyme is higher than the substrate since it has a higher affinity to enzyme than the substrate [14], accounting for the loss in activity.

Since the lignin has an amorphous structure [15, 16], any changes in the percentage of the amorphous portion will change the crystallinity percentage. If amorphous lignin is removed, then the crystallinity percentage should increase during sonication. To follow-up on this hypothesis, X-ray diffraction was employed to measure the relative amounts of crystalline and amorphous structure of the substrate following sonication.

Structural Analysis Using XRD

X-ray diffraction patterns of the sonicated sawdust from 0–12 h of sonication is presented in Fig. 5. XRD spectra of red oak wood is deconvoluted at 15, 18, 22, 25, and 34°. Degree of crystallinity was determined from $X_c = A_c/(A_c + A_a)$ (Nara et al. [17]) where X_c represents the degree of crystallinity, A_c represents the area under the crystalline regions, and A_a represents area under the crystalline and amorphous region of the spectrum. Changes in degree of crystallinity over time is shown in Fig. 6.

The results indicate that the degree of crystallinity rises with time as seen in Fig. 6. If all or some of the amorphous volume is removed, the percentage of crystallinity, which is what XRD measures, would increase. These results suggest the amorphous fibers at the surface, as well as amorphous lignin, have been sheared off during sonication. A small amount of glucose, about 0.17 g/L, was released during sonication indicating some evidence of the shearing off of some amorphous cellulose. It is, therefore, reasonable to expect that some amorphous lignin could be sheared off in this process as well.

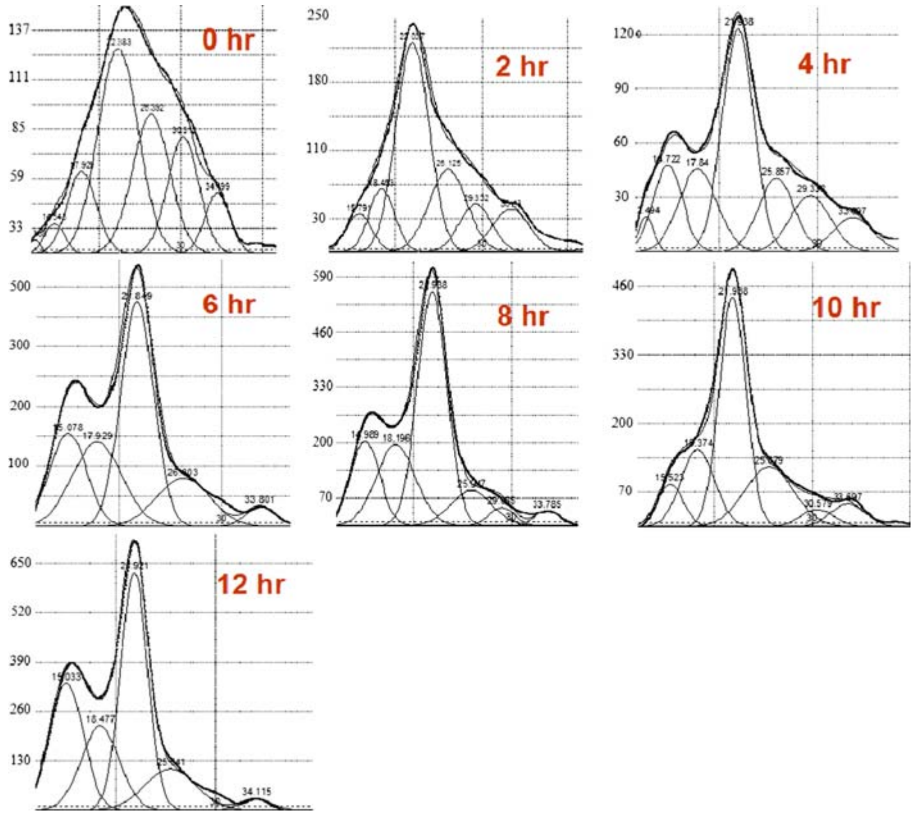
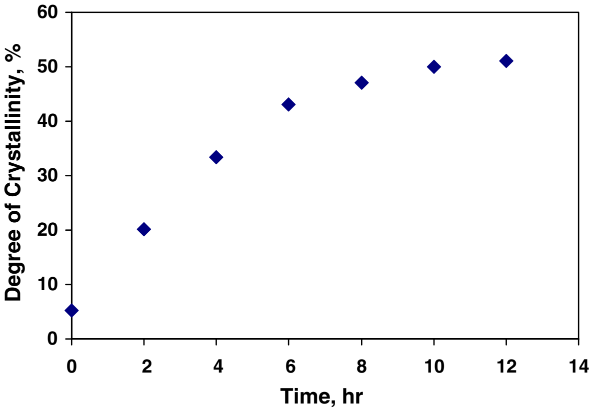


Fig. 5 X-ray diffraction patterns of sawdust sonicated for 0–12 h

Viscosity Trends

Dasari et al. [1] showed an unexpected benefit of processing with smaller particles in that the viscosity of the biomass slurries dropped significantly as particle sizes in the slurry were reduced. The reduction was 50-fold in study by Dasari et al. [1] when the particle size range

Fig. 6 Changes in degree of crystallinity over time



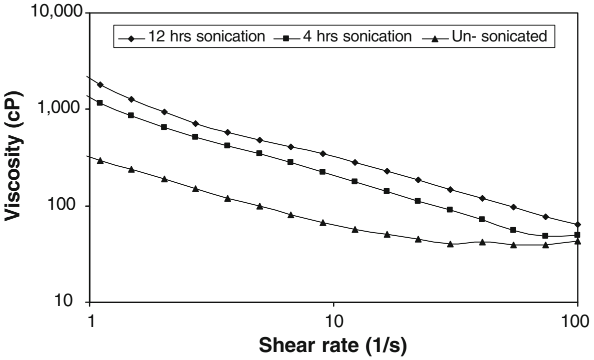


Fig. 7 Viscosity vs. shear rate (10% initial solids concentration, un-sonicated particles size range=$10 \leq x \leq 75$ µm; 4-h sonicated particle size range=$0.05 \leq x \leq 12$ µm; 12-h sonicated particle size range=$1.5 \leq x \leq 25$ µm)

decreased from $150 < x \leq 180$ µm to $33 \leq x \leq 75$ µm. This has important implications when operating at large scale. It was hoped that by further reducing the size, this trend would continue and result in even a greater reduction in viscosity when the average particle size was on the order of ~1 µm.

The rheological behavior of unhydrolyzed [18] and hydrolyzed [1] biomass slurries has been shown to be non-Newtonian in nature, which is also the case here (Fig. 7). Unexpectedly though, the viscosity increased as the particle size decreased in the range tested here. There was an approximately threefold increase in the viscosity between unsonicated samples with a range of $10 \leq x \leq 75$ µm and samples sonicated for 4 h that ended up with a range of $0.05 \leq x \leq 12$ µm. The viscosity increased further when the samples were sonicated for 12 h and the particles were further reduced in size.

However, the trend observed here actually is the same trend observed when comparing the viscosity of other types of slurries with varying particle size ranges. During an investigation of rheological properties of coal–oil–water suspensions containing solids of different sizes by Majumder et al. [19], it was found that suspensions of coal–oil–water show an increase in viscosity with a decrease in the coal particle size. Smaller particles provide more surface area per unit volume leading to an increase in friction and resistance to flow which would explain the observed higher viscosity for smaller size particle slurries. They also stated that the increase in viscosity is attributed to the fact that a decrease in size leads to an increase in the irregularity and non-free flow behavior. The fluidity decreases with irregular shaped solids because of unevenness and sharpness of the edges of the surface.

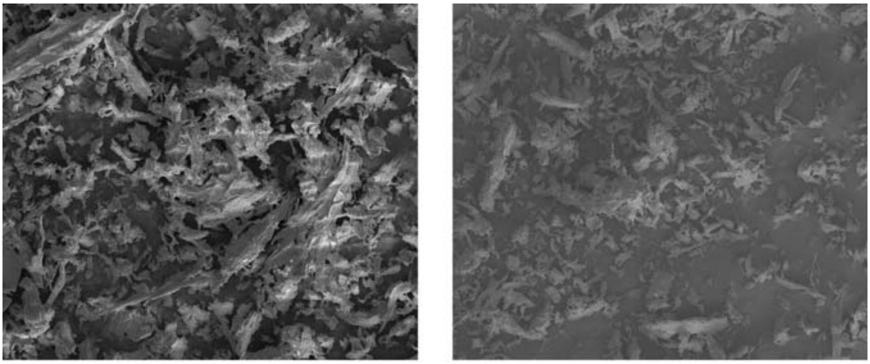
The difference in the viscosity trends between the size ranges in the paper by Dasari et al. [1] and the range studied here may be attributed to the surface features on the particles. Nguyen et al. [20] reported that the viscosity of slurries is related to the nature of particle-particle interactions. Amorphous fibers on the surface of the sawdust particles used in this study will affect slurry viscosity as they interact with neighboring particles. The viscosity trends, then, will be a function of the length of the surface fibers and degree of entanglement with neighboring particles as they move past each other in a flow field, such as when subjected to shear stress in a viscometer cup.

Morphological Analysis Using SEM

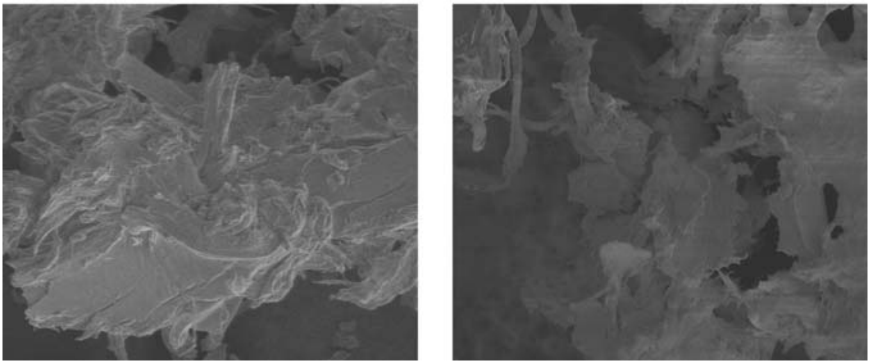
SEM pictures of the unsonicated and sonicated sawdust samples were taken in order to examine the physical changes of the surface features of the sawdust particles following sonication. Figure 8 shows the SEM images prior to sonication and after 4 h of sonication, both at ×400 and ×5,000 magnification. The unsonicated sample shows a more rigid and connected structure with larger particles, while the sonicated sample appears to have smaller, disconnected particles. The surfaces of the sonicated particles also appear to be smoother. An explanation of how these changes affect the viscosity follows.

Figure 9 illustrates how the size may affect viscosity differently for particles with different surface features. In Fig. 9a, larger biomass particles have larger fibers on the surface which are more easily entangled as they move past other particles. In Fig. 9b, smaller particles have smaller fibers on the surface and become less entangled. This explains why the viscosity decreased when particle size decreased. Fig. 9c, d show how the dominating effect on viscosity for particles without surface fibers, such as the coal slurries, is the increased surface area per unit volume of particle which leads to more friction and, hence, greater viscosity.

Biomass slurries with very small particle sizes following sonication behave more like the coal–oil–water suspensions since the surface fibers have likely been sheared off. Figure 10 illustrates the relative amount of surface fibers decreasing as the particle size decreases. The smallest particles, like in the range used here, have a surface that is somewhat smoother such that the predominate effect on viscosity is the surface area rather than the surface



(a) Un-sonicated sawdust (mag 400 x) [1] (b) 4 hrs sonicated sawdust (mag 400 x)



(a) Un-sonicated sawdust (mag 5000 x) (b) 4 hrs sonicated sawdust (mag 5000 x)

Fig. 8 SEM images of **a** un-sonicated sawdust compared to **b** sonicated sawdust

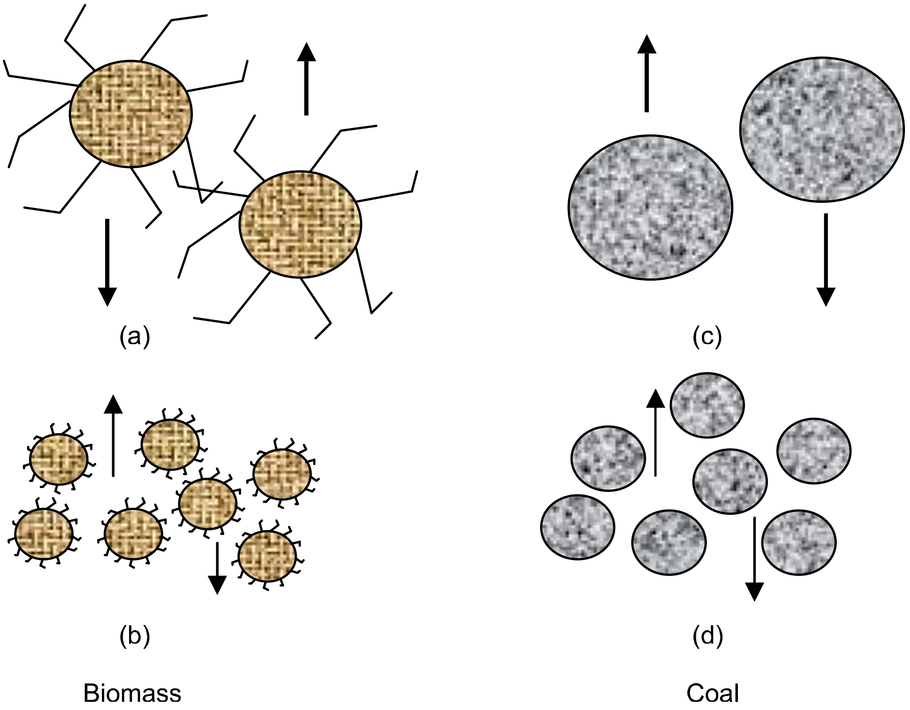


Fig. 9 Schematic diagram of larger and smaller (*a* and *b*) biomass and (*c* and *d*) coal particles

fibers. The structural features seen in Figs. 8, 9, and 10, particularly the SEM images, help support the discussion on the effect of sonication and particle size on both: (1) the viscosity changes due to morphological changes of the particles and (2) the increased glucose release due to increased substrate surface area.

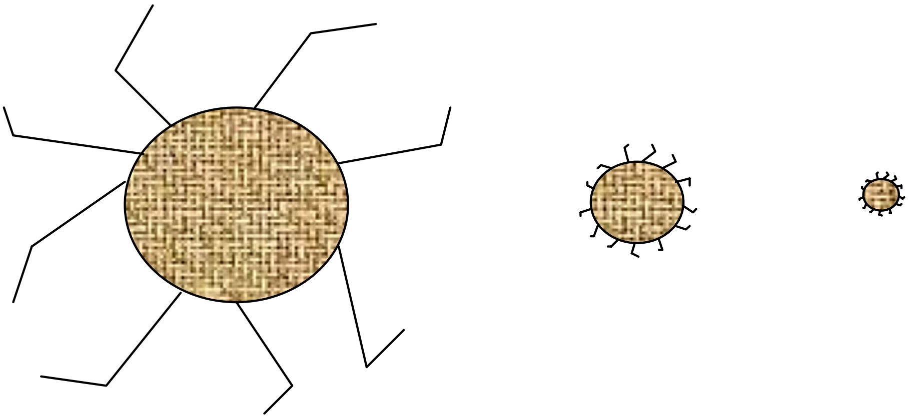


Fig. 10 Schematic diagram showing surface fiber size relative to particle size

Conclusions

Ultrasonic irradiation was able to reduce the average particle size of sawdust particles to well under 1 μm. Previous trends showed a significant increase in the saccharification rate and amount of glucose released with decreasing particle size, but results here show that there appears to be a limit to the increase when the particle size is further reduced below at least 33 μm. The maximum glucose released seen here during saccharification of ~1 μm particles was equivalent to the previously seen amount released during saccharification of $33 \leq x \leq 75$ μm particles.

Furthermore, the viscosity trend for the slurries tested here was opposite to the trend previously observed for slurries with particles at higher size ranges than the ranges tested here. Whereas a 50-fold decrease was previously observed when the particle sizes decreased from a range of $590<x<850$ μm down to $33<x<75$ μm, the viscosity here increased threefold when the particle sizes decreased from a range of $10 \leq x \leq 75$ μm down to $0.05 \leq x \leq 12$ μm. This is attributed to the differences in the surface features of the particles between the two sets of slurries; larger particles have more and longer surface fibers that become entangled, thereby increasing the viscosity, whereas the smaller particles that have had the fibers sheared off behave more like particles in slurries with smoother surfaces where increased surface area and friction are the controlling phenomena.

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