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The Effect of Particle Size on Hydrolysis Reaction Rates and Rheological Properties in Cellulosic Slurries

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Abstract

The effect of varying initial particle sizes on enzymatic hydrolysis rates and rheological properties of sawdust slurries is investigated. Slurries with four particle size ranges (33 μm < x ≤ 75 μm, 150 μm < x ≤ 180 μm, 295 μm < x ≤ 425 μm, and 590 μm < x ≤ 850 μm) were subjected to enzymatic hydrolysis using an enzyme dosage of 15 filter paper units per gram of cellulose at 50°C and 250 rpm in shaker flasks. At lower initial particle sizes, higher enzymatic reaction rates and conversions of cellulose to glucose were observed. After 72 h 50 and 55% more glucose was produced from the smallest size particles than the largest size ones, for initial solids concentration of 10 and 13% (w/w), respectively. The effect of initial particle size on viscosity over a range of shear was also investigated. For equivalent initial solids concentration, smaller particle sizes result in lower viscosities such that at a concentration of 10% (w/w), the viscosity decreased from 3000 cP for 150 μm < x ≤ 180 μm particle size slurries to 61.4 cP for 33 μm < x ≤ 75 μm particle size slurries. Results indicate particle size reduction may provide a means for reducing the long residence time required for the enzymatic hydrolysis step in the conversion of biomass to ethanol. Furthermore, the corresponding reduction in viscosity may allow for higher solids loading and reduced reactor sizes during large-scale processing.

Index Entries: Biomass; enzymatic hydrolysis; non-Newtonian; particle suspension; red oak wood; sawdust slurry; viscosity.

Introduction

The production of fuel ethanol from renewable lignocellulosic materials continues to receive a great deal of interest as a viable alternative fuel source. Among lignocellulosic materials, the utilization of agricultural residues has the benefit of disposal of problematic solid wastes, which usually does not have any economic alternative *(1)*. The low-cost mill residues from the sawmilling industry can be used as an alternate lignocellulosic substrate as well. Sawdust is used as the substrate because the

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material is already mechanically reduced in size as a result of a manufacturing process, and the particles can be easily classified into distinct size ranges. This material offers lower feedstock costs than that of grain and even if further milling is required, may still offset some of the higher processing costs that wood faces *(2)*. A large amount of wood wastes is produced from the lumber industries in the United States. Currently, nearly 63 million mt of this material is generated in the manufacture, use, and disposal of solid wood products each year, which can yield up to 6350 million gallons of ethanol *(3)*.

A significant amount of literature is available regarding the enzymatic hydrolysis of lignocellulosic woods based on the effect of different kinds of pretreatment methods on the enzymatic hydrolysis reaction *(4–7)* and the kinetics of the hydrolysis reactions *(8–12)*. Very few authors have reported the dependence of the course of hydrolysis reaction on the structural features of the cellulosic substrates *(13–18)*. Experiments by Peters et al. *(15)* revealed that the initial particle size over the range between 38 μm and 105 μm had no impact on the rate and the extent of reducing sugar production or on enzyme binding of the microcrystalline cellulose Avicel PH 102 (FMC Corporation, Chalfont, PA). No data appears in the literature regarding the impact of the initial particle size on the viscosity of the lignocellulosic biomass slurries.

This article explores the impact of the initial particle size of red oak sawdust on the rate and extent of enzymatic cellulose conversion to glucose and on the viscosity of the biomass slurry. Experiments were also conducted to study the change of viscosity of the slurry during the course of enzymatic hydrolysis reaction with time. The experiments aim to determine if controlling the initial substrate particle size can maximize the cellulose-to-glucose conversion and reduce the residence time for the enzymatic hydrolysis reaction to achieve maximum glucose yield. Characterization of viscosity aids in reactor design, especially at large scale. And it is necessary for predicting power consumption, which is a significant portion of the operating cost for the process.

Materials and Methods

Cellulose Substrate and Enzyme

The cellulose substrate used in these experiments is red-oak sawdust obtained from Garrard Wood Products of Lancaster, Kentucky. The carbohydrate components of the sawdust contain 39.7% cellulose and 18.8% hemicellulose. The sawdust was sieved in a set of US standard sieves for 30 min to obtain the following initial particle size (x) ranges: 33 μm $< x \leq$ 75 μm, 75 μm $< x \leq$ 104 μm, 104 μm $< x \leq$ 150 μm, 150 μm $< x \leq$ 180 μm, 295 μm $< x \leq$ 425 μm, and 590 μm $< x \leq$ 850 μm. The following sieves were used: 20, 30, 40, 80, 100, 140, and 200 mesh. The sawdust was hydrolyzed without any

pretreatment by the Multifect GC Cellulase enzyme, from Genencor International, Inc. (Rochester, NY) (Lot No. 301-04328-224).

Hydrolysis Procedure

The initial particle size ranges used for the hydrolysis studies were: 33 μm < $x \leq 75$ μm, 150 μm < $x \leq 180$ μm, 295 μm < $x \leq 425$ μm, and 590 μm < $x \leq 850$ μm. The enzyme loading was 15 filter paper units per gram of cellulose. 1 *M* citrate buffer was prepared by adjusting the pH to 4.8 with NaOH, and was used as 5% of the total mass to yield an effective molality of 0.05 mol/kg. All the materials were sterilized in an autoclave at 121°C before use. Enzymatic hydrolysis was performed at 50°C in an Innova 4230 incubator shaker (New Brunswick Scientific Co., Inc., Edison, NJ) at 250 rpm for 72 h. All experiments were performed in 250-mL shake flasks with a working mass of 100 g, and for both 10 and 13% (wt/wt) initial solids concentrations. Samples were collected every 2 h for the first 12 h and every 24 h afterwards for glucose concentration determination. All enzymatic hydrolysis experiments were performed in duplicate and average results were given.

The enzymatic hydrolysis samples were centrifuged at 4000 rpm in a Beckman GPR centrifuge (Beckman Instrument, Inc., Palo Alto, CA) for 15 min, and the glucose concentration was measured by a YSI-Biochemistry analyzer (Yellow Spring Instruments, OH), which was calibrated daily.

Viscosity Measurements

The viscosity of wood particle slurries was measured with a Physica MCR 300 modular compact rheometer from Anton Parr (Ashland, VA) containing a six-bladed vane in a 40-mL cup. The vane dimensions are 1.6 cm long by 0.9 cm wide by 1 mm thick. Sample size used in the cup is 30 mL, which is enough volume to cover the impeller blades. The viscosity of the slurries with the following initial particle size ranges was measured: 33 μm < $x \leq 75$ μm, 75 μm < $x \leq 104$ μm, 104 μm < $x \leq 150$ μm, and 150 μm < $x \leq$ 180 μm. Two kinds of viscosity measurements were performed, discrete and continuous. In discrete measurements, the viscosity of the slurries was measured for all four size ranges at an applied shear rate of 10.8/s and at different time intervals of the enzymatic hydrolysis (0, 24, 48, and 72 h). Measurements were made after 10 min of stirring in the viscometer cup, which is the amount of time needed to overcome time dependent changes in viscosity. In continuous measurements, the viscosity of the slurries was measured for the first 12 h of the enzymatic hydrolysis. For these 12 h viscosity tests, the enzymatic hydrolysis reaction was performed directly in the viscometer cup so that the viscosity could be continuously measured. Viscosity data was collected at 10 min intervals. The cup of the viscometer was covered with parafilm to avoid evaporation. The buffer and enzyme concentrations were the same as those in the enzymatic hydrolysis test.

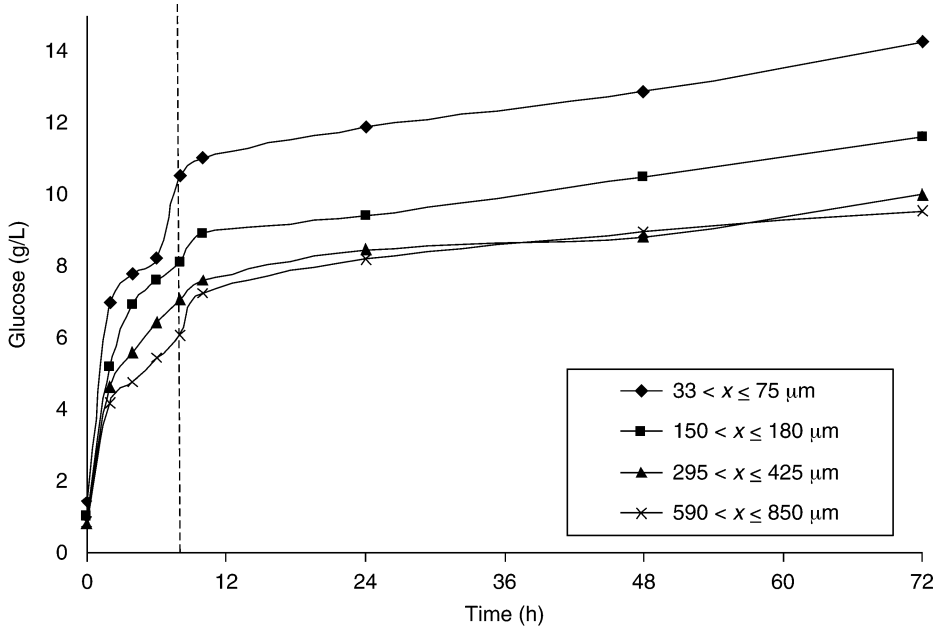

Fig. 1. The effect of initial particle size on glucose production with 10% initial solids concentration (glucose ± 0.15 g/L).

Results and Discussions

The extent of glucose release from cellulose during the course of an enzymatic hydrolysis reaction for four particle size ranges (33 μm < x ≤ 75 μm, 150 μm < x ≤ 180 μm, 295 μm < x ≤ 425 μm, and 590 μm < x ≤ 850 μm) was determined at an enzyme concentration of 15 filter paper units and the results are presented in Figs. 1 and 2. Two phases of the rate are observed during the course of the enzymatic hydrolysis reaction for all particle size ranges. Approximately 70% of the total glucose (obtained in 72 h) is produced within the first 8 h of the hydrolysis reaction. In this first phase the rate is very rapid and the reaction proceeds in a logarithmic fashion and in the second phase the rate declines into zero order kinetics *(19)*.

This observation of two phase kinetics occurs possibly because the easily hydrolysable amorphous form of the cellulose is rapidly converted to glucose (phase 1), followed by the conversion of more recalcitrant crystalline form (phase 2) during the hydrolysis reaction by the enzyme *(8)*. There are a number of other factors that could explain the further slow down in reaction kinetics. One of these is likely because of the nonlinearly varying surface area (Langmuir-type isotherm relationship) for enzyme adsorption with the extent of conversion *(20,21)*. The surface area available on the substrate for the enzyme adsorption is large at the initial stages of the reaction, and it decreases as more cellulose is converted to glucose. The

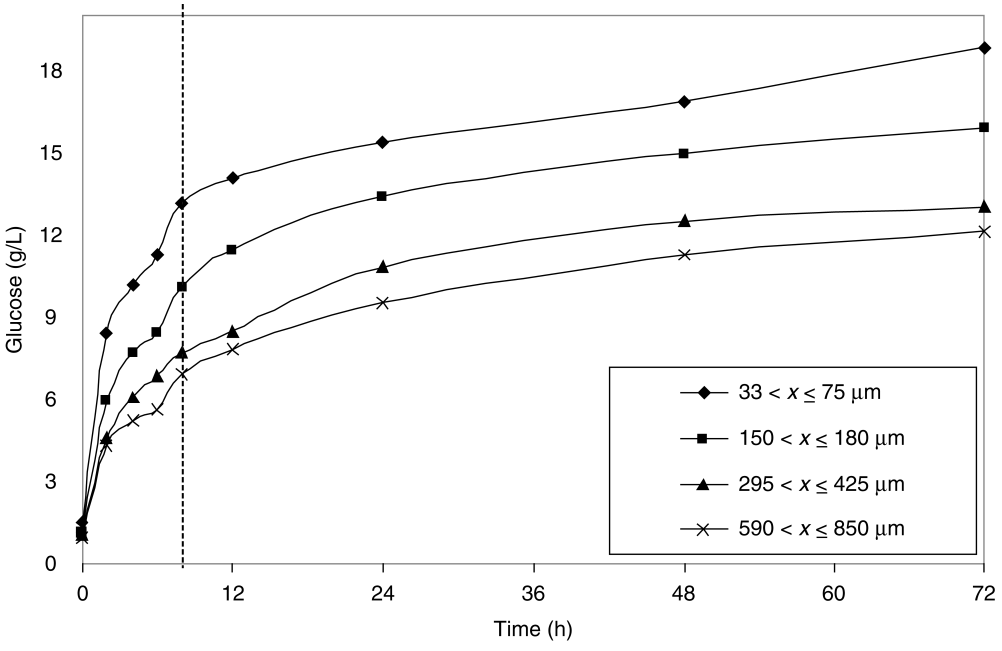

Fig. 2. The effect of initial particle size on glucose production with 13% initial solids concentration (glucose ± 0.15 g/L).

drop in the reaction rate of the hydrolysis after the first 8 h may also be because of product inhibition *(11,19)* or the enzyme deactivation by various factors such as thermal deactivation, mechanical deactivation, irreversible binding to lignin, and so on *(22)*. Eriksson et al. *(23)* proposed that this "inactivation" was caused either by the enzyme binding to cellulose at unproductive sites or failure to release from the substrate after catalytically processing a cellulose chain.

It is observed that for smaller particle sizes the rate of release of glucose is higher. An amount of 50 and 55% more glucose is produced for the size range 33 µm < $x \leq$ 75 µm than for the size range 590 µm < $x \leq$ 850 µm for an equivalent initial solids concentration, of 10 and 13%, respectively, in 72 h. Smaller particles have larger surface area per unit volume and, therefore, more cellulose may be accessible for the enzyme to reach and at a faster rate. Another possibility is that smaller particles may have been exposed to more mechanical grinding at the surface, resulting in a reduction of crystallinity and an increase in amorphous nature at the surface *(24,25)*. Peters et al. *(15)* found no significant difference in the extent of sugar produced and the rate of cellulose conversion for the cellulosic substrate Avicel PH 102, as particle size range varies between 38 and 75 µm. Because Avicel is a crystalline structured cellulose and the authors found no significant difference in the rate between different particle sizes, this may

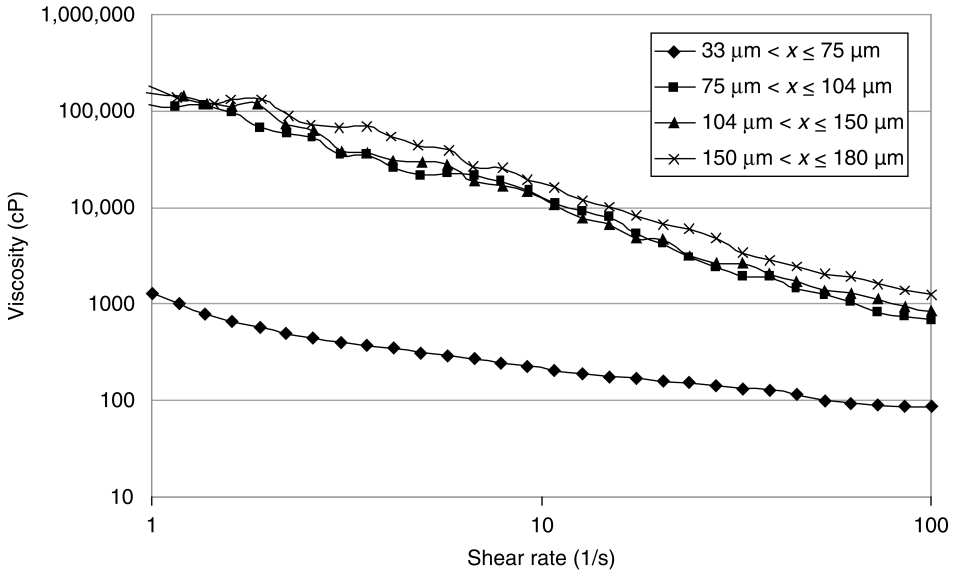

Fig. 3. Viscosity and glucose vs time (13% initial solids concentration, 33 μm < x ≤ 75 μm, 10.8/s).

indicate that the latter of the two possible explanations is the more likely reasoning for the increased rate.

Supporting the fact that 70% of the overall cellulose conversion in 72 h is obtained in the first 8 h of the hydrolysis reaction, a significant drop in slurry viscosity is observed within the first 8 h of the hydrolysis reaction. Figure 3 shows as an example the 13% initial solids concentration slurry with the size range 33 μm < x ≤ 75 μm at a shear rate of 10.8/s. The trend is similar for 10% initial solids (not shown). The drop in viscosity is because of a combination of the decrease in solids concentration and the fragmentation of the cellulose particles *(15)*. As the hydrolysis reaction proceeds, the particles break down into smaller particles and eventually the undissolved cellulose particles are converted into dissolved glucose.

Slurry viscosity is generally shear thinning but becomes less associated with shear above a certain applied shear rate. In the cases studied here, this shear rate is near 85/s and is independent of the particle size for slurries before the initiation of the hydrolysis reaction (Fig. 4). The same effect also appears to be independent of the time of the hydrolysis reaction (Fig. 5), with a steadying of the viscosity above a shear near 40/s. The viscosity actually appears to increase slightly with increase in shear above this point, but the apparent effect is likely because of better stirring in the viscometer cup at the higher rotation rate of the impeller. The shear thinning nature of the material is explained by Ebeling et al. *(26)* who reported that the cellulose microcrystal orientation is dependent on the shear rate. At a shear rate more than a certain value, the microcrystals align horizontally along the

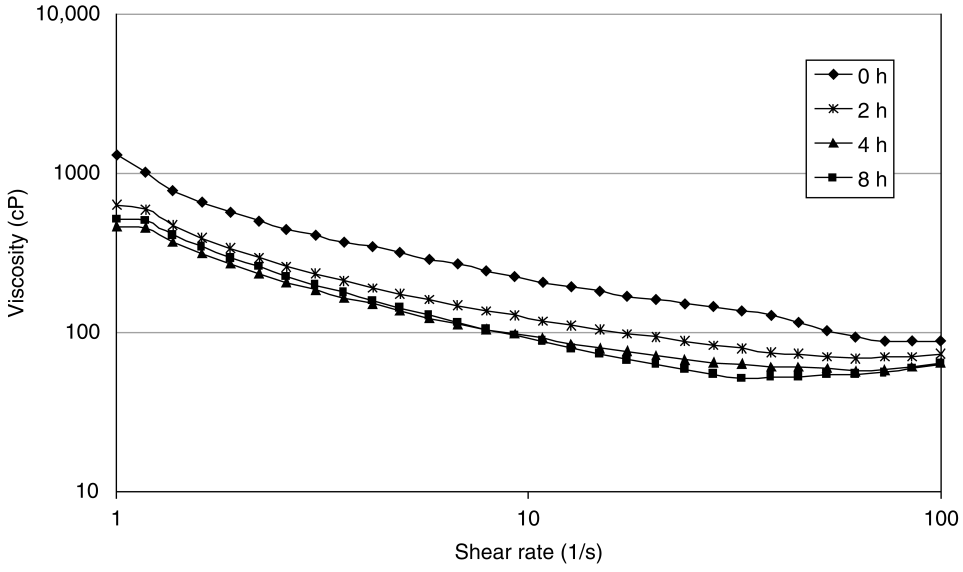

Fig. 4. Viscosity vs shear rate (t = 0 h, 13% initial solids concentration).

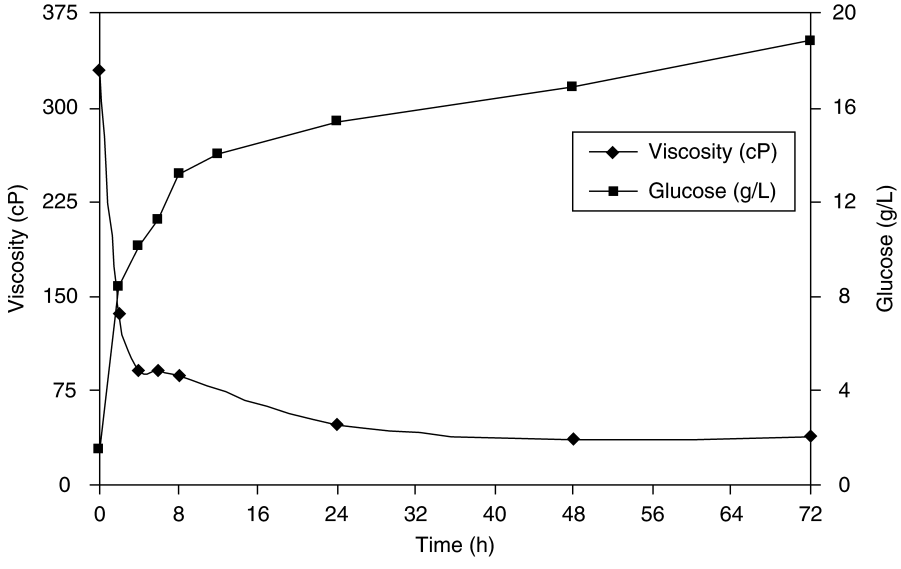

Fig. 5. Viscosity vs shear rate at different times during enzymatic hydrolysis (size range: 33 μm < x ≤ 75 μm, 13% initial solids concentration).

shear direction. At a certain degree of alignment, the resistance to the flow becomes approximately constant, and hence, viscosity stops changing. As a side note, the orientation phenomenon is completely reversible *(23)*.

It is well known that as the viscosity of the slurry increases, the power to agitate also increases significantly ($p = \mu_c N^2 D^3$) *(27)*. So, to reduce the power consumption while retaining a high solids loading, it is necessary

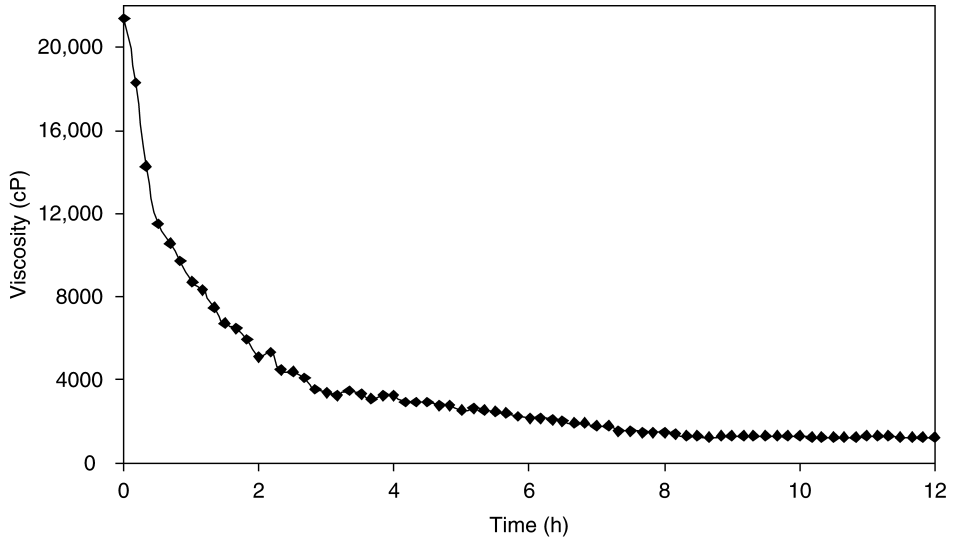

Fig. 6. Continuous viscosity vs time measurement (13% initial solids concentration, 150 μm < x ≤ 180 μm, 10/s).

to run the reactors at low rpm. For instance, the high solids bioreactor was operated at 7 rpm with 32% of initial insoluble solids by Hodge et al. *(28)*. Therefore, all rheological measurements of the sawdust slurries are measured at a low steady-state shear rate of 10.8/s, which is in the approximate shear rate range of a slow mixing vessel.

In order to better track the viscosity change during the initial stage of the enzymatic hydrolysis reaction, viscosity was measured at 10 min intervals during the first 12 h of the reaction for the size range 150 μm < x ≤ 180 μm. The applied shear rate was 10.8/s and the initial solids concentration was 13%. The viscosity data are shown in Fig. 6. From this figure it can be seen that the biggest drop in viscosity occurs in the first 3.5 h of the hydrolysis reaction, indicating the fastest reaction kinetics are actually occurring in this first 3.5-h period.

The effect of the initial particle size of the substrate on the slurry viscosity is studied for the size ranges 33 μm < x ≤ 75 μm, 75 μm < x ≤ 104 μm, 104 μm < x ≤ 150 μm, and 150 μm < x ≤ 180 μm for equivalent initial solids concentrations. Results for the case of 10% initial solids are presented in Fig. 7 at a shear rate of 10.8/s. As the particle size range decreases from 150 μm < x ≤ 180 μm to 33 μm < x ≤ 75 μm, a significant drop in viscosity occurs from 3000 to 61.4 cP. The reason for this significant difference in viscosity with varying particle size is related to the nature of the particle–particle interactions. Amorphous fibers on the surface of particles will affect slurry viscosity as they interact with neighboring particles. Larger fibers will become more entangled than smaller ones, leading to increased resistance to flow and

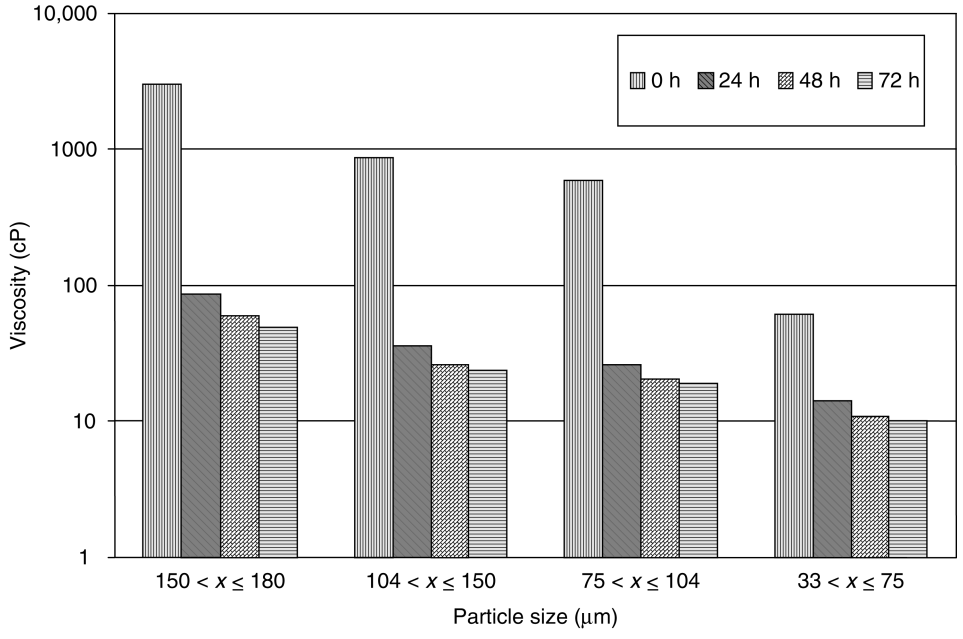

Fig. 7. Viscosity vs time of enzymatic hydrolysis for different particle size ranges (10% initial solids concentration, 10.8/s).

hence increased viscosity. Although just speculation, it is reasonable to guess that smaller particles have smaller surface fibers, which would explain the observed lower viscosity for smaller size particle slurries.

The changes of viscosity of the slurries were tracked for initial particle size ranges 33 μm < x ≤ 75 μm, 75 μm < x ≤ 104 μm, 104 μm < x ≤ 150 μm, and 150 μm < x ≤ 180 μm during the course of the hydrolysis reaction and results are presented for the 10% initial solids concentration case. The data at 24-h intervals are presented in Fig. 7 at a shear rate of 10.8/s. For the three larger size ranges (75 μm < x ≤ 104 μm, 104 μm < x ≤ 150 μm, and 150 μm < x ≤ 180 μm) 96% of the initial viscosity is reduced with in the first 24 h of the hydrolysis reaction, whereas only 76% of the initial viscosity is reduced for smaller size particles (33 μm < x ≤ 75 μm). Peters et al. *(15)* reported that the rate of cellulose fragmentation, which results in smaller fragments, is higher for larger size particles. Our results show that the smaller size particles result in lower viscosity. So it is expected that the higher the rate of cellulose fragmentation, the faster the drop in viscosity of the slurry. This explains the bigger viscosity drop in the first 24 h for larger size particles. Hence, the smaller substrate particle size results in lower viscosities of the biomass slurries and therefore lower power consumption and operating costs for the reactor operation at large scale. Also, the lower viscosity may allow for higher solids loading in the reactor.

Conclusions

These results show that the initial particle size of the biomass substrate has a significant impact on the rates of glucose released and on the viscosity of the biomass slurries. In enzyme hydrolysis, 50–55% more glucose was produced for the size range 33 μm < x ≤ 75 μm than that for the size range 590 μm < x ≤ 850 μm in 72 h of the reaction. Therefore, the minimum particle size range that can be obtained should be used for the maximum cellulose conversion. Further investigation is required to determine the minimum particle size range of substrate at which the reaction rate is no longer affected by the particle size. The viscosity of the sawdust slurry becomes approximately constant above an applied shear rate of 40/s. A correlation was observed between the glucose released and the viscosity reduction during the hydrolysis of cellulose, i.e., 70% of the overall glucose released in 72 h was obtained with in the first 8 h of the hydrolysis reaction and resulted in 70% drop in the viscosity of its initial value. Therefore, reducing particle size can be a way to lower the viscosity of the biomass slurries, to reduce operating costs, or allow for increased solids loading.

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