0273-2289/05/125/0027/$30.00

Hydrolysis Characteristics of Tissue Fractions Resulting From Mechanical Separation of Corn Stover

JASON A. BOOTSMA AND BRENT H. SHANKS*

Department of Chemical Engineering, 2114 Sweeney Hall, Iowa State University, Ames, IA 50011, E-mail: bshanks@iastate.edu

Received July 14, 2004; Revised October 11, 2004; Accepted November 24, 2004

Abstract

Corn stover has potential as a resource for both fiber and chemical needs if separation strategies can be developed to deal with its heterogeneity. Relative hydrolysis characteristics were assessed for pith (sclerenchyma and parenchyma) and fiber (collenchyma) tissue fractions derived from mechanical separation of corn stover to determine whether classification by tissue type resulted in fractions with different hydrolysis response. The physical characteristics of the tissue fractions were analyzed. The hydrolysis behavior of the fractions was evaluated under both acidic and basic conditions. The results from the hydrolysis experiments are compared with previously reported compositional analysis for the tissue fractions.

Index Entries: Corn stover; hydrolysis; pith; fiber; cellulose; hemicellulose; mechanical separation.

Introduction

Corn stover continues to receive a great deal of attention as a potential source of valuable coproducts from corn production. Because it is high volume, low cost, and renewable, stover has desirable attributes as a feedstock for energy, chemicals, and materials. Although most corn stover is currently left in the field, collection strategies with improved economics are being developed and demonstrated that hold promise for delivering stover economically to processing sites *(1)*. Therefore, the limiting factor in the utilization of corn stover resides primarily with conversion technologies. Despite nearly a century of research into the utilization of corn stover in paper manufacturing, building materials, and ethanol and energy

*Author to whom all correspondence and reprint requests should be addressed.

production, no economically viable utilization strategy has yet been demonstrated.

The most active area of research in corn stover utilization is its hydrolysis into monomeric sugars, which can be subsequently converted into a number of possible chemicals including ethanol. Quite a number of different approaches have been explored relative to the production of monomeric sugars from stover including concentrated-acid hydrolysis *(2)*, dilute-acid hydrolysis *(3–5)*, and enzymatic hydrolysis *(5–11)*. In the case of enzymatic hydrolysis, a pretreatment step is required such as steam pretreatment *(6,12,13)*, ammonia fiber explosion *(14–16)*, lime pretreatment *(7,17)*, alkaline pretreatment *(8,18,19)*, dilute-acid pretreatment *(9,10,20)*, and alkaline peroxide pretreatment *(11)*. Many of these methods, as well as combinations of the methods, show promise; however, the economics have not been sufficiently compelling to lead to industrial use.

The challenge of hydrolyzing corn stover can be viewed from both the molecular and cellular level. At the molecular level, stover is a lignocellulosic biomass that is composed of cellulose interwoven with hemicellulose and coated with lignin. Lignin, which is a complex phenolic polymer, creates a barrier to the hydrolysis of the hemicellulose and cellulose. Cellulose, which forms relatively stiff fibrils, is a linear polymer of glucose units, and hemicellulose is a branched polymer of a variety of pentoses and hexoses that surrounds the cellulose fibrils. Owing to its structure, hemicellulose is more susceptible to hydrolysis than cellulose.

Corn stover also has a heterogeneous structure at the cellular level. Mature corn stover can be classified as consisting of two main types of tissue: fiber and pith. The pith fraction, which comprises 40 to 50% of the stalk by weight and about 75% by volume *(21)*, consists of the remains of the parenchyma and collenchyma cells from the growing plant. This tissue is a relatively soft, spongy, amorphous material. Parenchyma cells are thin-walled metabolically active cells whose function in stems is mostly storage. Collenchyma cells are thick walled; however, they are not lignified, which allows them to stretch as the stalk elongates. These cells provide structural support and are typically arranged in layers or bundles. The fiber fraction is the result of the sclerenchyma cells from the growing plant. These cells have very thick walls, with generally over half of the cell volume within the wall *(21)*, and are morphologically long and narrow. Sclerenchyma cells are typically dead at maturity and have the function of providing mechanical support to the plant.

The hydrolysis methods previously discussed are largely driven by molecular-level considerations. For example, a goal of many pretreatment procedures is to solubilize the lignin and hydrolyze the hemicellulose, leaving primarily cellulose for the subsequent enzymatic hydrolysis step. The heterogeneity of the distinct types of tissue lends them to a potential alternative processing approach. The differences in the functions of the types of tissue create tissue fractions that can be mechanically separated. Grinding corn stover yields morphologically distinct fractions, with the fiber portion having a high-aspect-ratio rodlike shape and the pith portion having irregular low-aspect-ratio shapes. A simple mechanical separation can then exploit the morphologic differences between the two types of tissue. Once the fiber and pith portions are separated, the fiber portion could have potential application in traditional fiber utilization whereas the pith fraction could be hydrolyzed to its constituent monomeric sugars. This mechanical separation according to type of tissue could be an attractive processing option if the pith fraction hydrolyzes more readily than the fiber portion and, therefore, the overall stover.

Presented here are the results concerning the relative efficacy of hydrolysis for the two types of tissue to determine whether mechanical separation can be used to improve the subsequent processing of corn stover. The hydrolysis experiments were performed under both acidic and alkaline conditions to evaluate the relative hydrolysis behavior of the tissue fractions that result from mechanical separation. Hemicellulose is readily hydrolyzed under acidic conditions, whereas alkaline hydrolysis more readily targets the lignin. Saddler et al. *(12)* compared the results of ammonia-recycled percolation with those of dilute-acid hydrolysis. They found that both treatments solublized about 45% of the stover. Whereas the ammonia-recycled percolation process removed about 85% of the lignin and about 60% of the hemicellulose, the dilute-acid treatment removed only 33% of the lignin and nearly 95% of the hemicellulose. Using both types of hydrolysis approaches would provide additional information about the structural and chemical differences of the two tissue fractions.

The experiments were designed to determine the hydrolysis characteristics of these tissue fractions rather than to optimize the hydrolysis reaction. The results from the hydrolysis reactions with the tissue fractions are discussed within the context of evaluating the usefulness of physical separation prior to hydrolysis.

Materials and Methods

Chemicals and Corn Stover

Sulfuric acid and sodium hydroxide were from Fisher (Fairview, NJ). The corn stover fractions, which were harvested from the 2001 growth year near Harlan, IA, were provided by the former BioMass Agri-Products of Harlan. The stover was washed and chopped at its facility. The mechanical approach used to separate the tissue fractions was similar to that used in pulping processes. The chopped corn stover was slurried with water and the slurry was then fed through a Tornado underwater pulper for size reduction. To separate the fibers further, the pulped material was sent through a rotor-stator refiner. The slurry was then dewatered with a screw press and dried in a tubular flash drier. The dried material was separated using air classification with selective removal of the lighter pith fraction. Shown in Fig. 1 is the dried material prior to the air classification step.

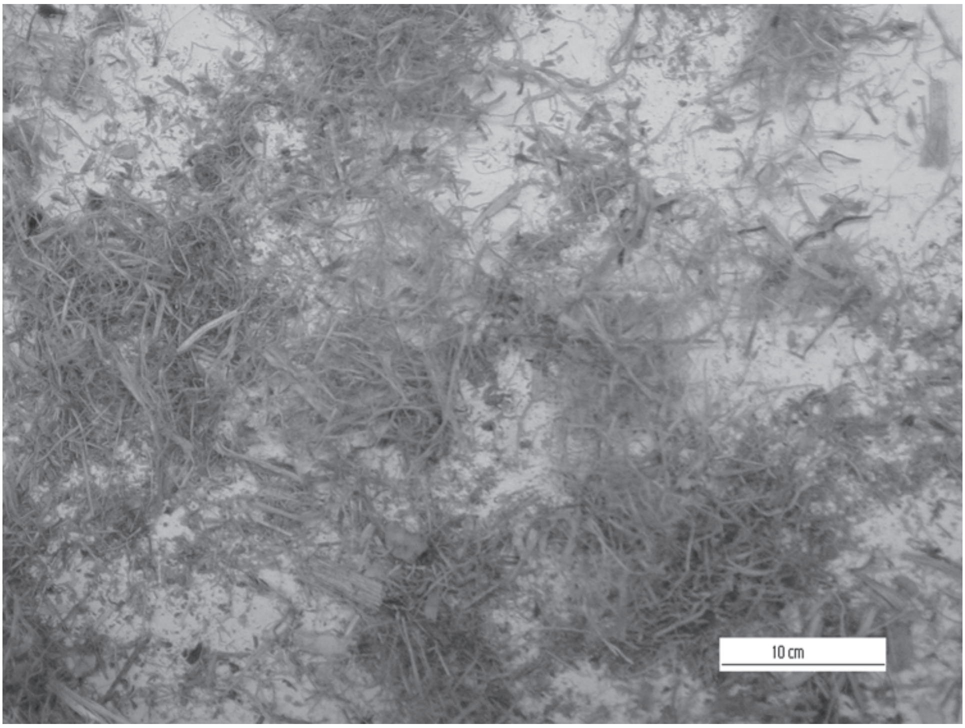

Fig. 1. Ground corn stover prior to air classification.

Hydrolysis Reactor

The hydrolysis experiments were conducted in a Parr 300-mL stainless steel batch reactor, which was equipped with a glass liner and an impeller-type mixer. The reactor temperature was controlled using a programmable logic controller (PLC) connected to both a heating jacket and an internal cooling loop. In the hydrolysis experiments, pith and fiber materials were individually slurried in a 1:30 weight ratio (stover:liquid).

Analytical Methods

The hydrolysis products were analyzed with a high-performance liquid chromatography (HPLC) system (Agilent 1050) using a Hewlett-Packard refractive index detector. The mobile phase was ultrapure water with a pump rate of 0.4 mL/min, and the column was a Supelcogel Pb (Supelco). The column temperature was set at 85°C. Data were collected and analyzed with Chemstation software.

The Branauer-Emmett-Teller (BET) surface area of the materials was determined using nitrogen adsorption at 77 K with an ASAP 2000 Surface Area and Porosity Analyzer (Micromeritics). Scanning electron microscopy (SEM) micrographs were obtained using a JEOL JSM-840 microscope operating at a vacuum of 10^{-6} torr. Samples were sputter coated with gold for analysis. The micrographs used were taken at ×200 magnification.

Acid Hydrolysis

From trials at several dilute-sulfuric acid concentrations, it was determined that 0.8 wt% was a reasonable condition for the dilute-acid hydrolysis experiments. The hydrolysis experiments were conducted over a temperature range of 140–180°C. Below 140°C, very little hydrolysis occurred for either material, and above 180°C, excessive sugar degradation made accurate mass balances difficult to obtain. These conditions were consistent with others reported in the literature *(9)*. For the acid hydrolysis experiments, the temperature was ramped from 140 to 180°C. The reactor temperature was increased in 10°C increments with the temperature change requiring about 5 min. The reactor was then allowed to maintain this temperature for 5 min before the sample was taken. This procedure was used throughout the specified temperature range.

Base Hydrolysis

Base-catalyzed hydrolysis is most often used as a pretreatment to remove lignin and swell the cellulosic strands. Alkali hydrolysis was used in our study to determine to what extent any lignification differences affected the response of the tissue fractions to hydrolysis. A 1 wt% sodium hydroxide solution was used for the experiments. Although the alkaline hydrolysis experiments were performed at a range of temperatures, the results did not exhibit strong temperature dependence. Therefore, the base hydrolysis experiments included here are for varying hydrolysis times at 140°C.

Results and Discussion

Shown in Fig. 2A,B are representative SEM micrographs for the separated pith and fiber fractions. As can be seen, the pith fraction consisted of irregularly sized and shaped particles, whereas the primary particles in the fiber fraction were more uniformly dimensioned. The fiber particles were found to exist individually or with several bundled together. Review of several SEM micrographs as well as visual inspection revealed that mechanical separation resulted in a fiber fraction with little pith cell content. By contrast, the pith fraction contained about 20% of particles that had fiber-type morphology and could be described as short fibers. Morphology and surface area of the corn stover fractions are important because hydrolysis is affected by mass transfer considerations *(22,23)*. SEM micrographs were used to estimate surface-to-volume ratios for the cell fractions. Although the fiber cells were bundled together into larger particles, as seen in Fig. 2B, the fiber cell bundles appear to have adequate porosity such that the surface-to-volume ratio of fiber particles was taken to be that of the individual fibers. With this assumption, the surface-to-volume ratio of the fiber fraction was found to fall within a range of 28:1 to 32:1 m^2/m^3. The pith fraction was significantly more heterogeneous than the fiber fraction and

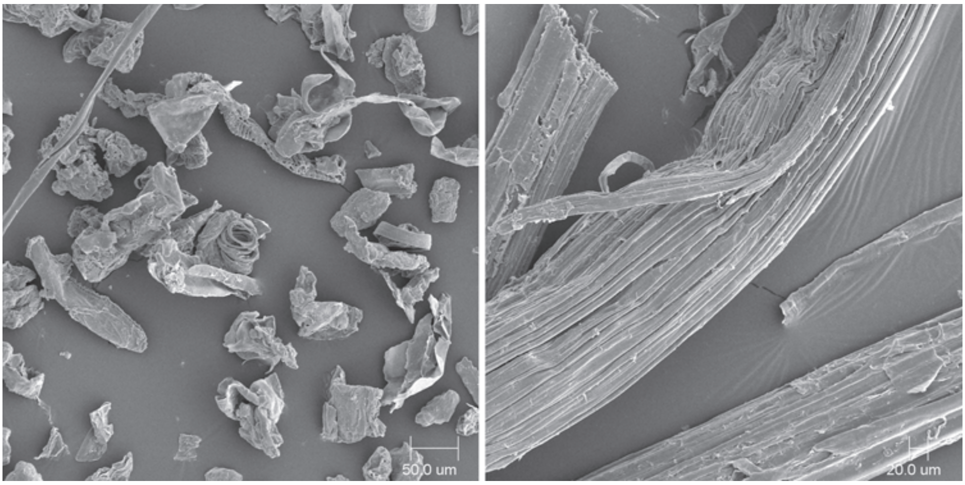

Fig. 2. SEM micrographs (×200) of mechanically separated tissue fractions: **(left)** pith; **(right)** fiber.

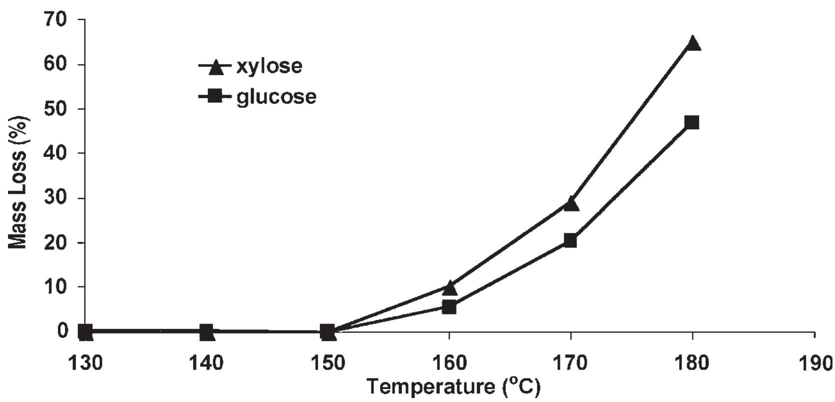

Fig. 3. Mass of glucose and xylose from degradation.

was found to have surface-to-volume ratios from 5:1 to 22:1 m^2/m^3. The surface area of the two fractions was determined using BET analysis with N_2 adsorption. The measured surface area of the pith fraction was 2.85 m^2/g and for the fiber fraction was 2.93 m^2/g, indicating that the fractions would be equally accessible to acid hydrolysis.

It has been demonstrated under similar acid hydrolysis conditions to those used in the current study that degradation of xylose and glucose will occur. To ensure closure of the mass balances for the hydrolysis experiments, the degradation rates of the free sugars were determined. Therefore, experiments were conducted with xylose and glucose under the same protocol as that used to hydrolyze the corn stover fractions to quantify their degradation rate under the hydrolysis conditions. Figure 3 presents the results from the degradation experiments. The quantitative values for glu-

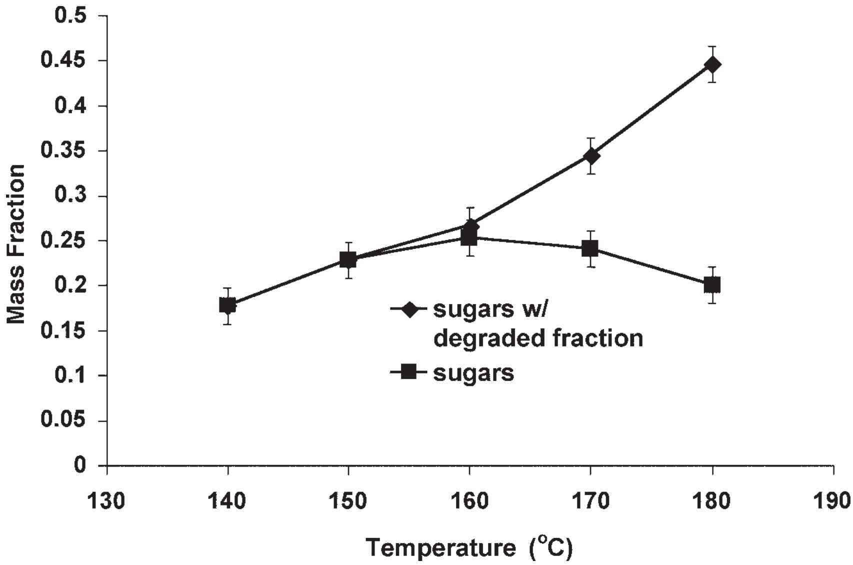

Fig. 4. Sugar released during acid hydrolysis with correction for sugar loss owing to degradation (two replicates).

cose degradation agreed closely with published results *(24)*. Comparable quantitative degradation rate data for xylose were not available, but consistent with prior work, the xylose degraded more rapidly and at a lower temperature than the glucose.

Shown in Fig. 4 are the results from acid hydrolysis of the pith cell fraction in which the mass fraction of pith cells hydrolyzed is given as a function of temperature during the experimental run. The lower curve on the figure corresponds to the apparent mass fraction of the pith cells hydrolyzed as determined by the amount of sugars measured by HPLC. As demonstrated in Fig. 3, sugar degradation was simultaneously occurring with the generation of sugars by hydrolysis. Therefore, the hydrolysis curve was corrected for the amount of sugar degraded based on the pure sugar controls run under similar reaction conditions. For the correction, the degradation rates for the hexoses and pentoses were assumed to be characterized by those of glucose and xylose, respectively, and to be zero order in sugar concentration. By invoking these assumptions, the HPLC results were corrected for the sugar degradation rate to yield the actual hydrolysis rate. The top curve in Fig. 4 corresponds to the corrected hydrolysis rate.

No solubilized oligosaccharides were observed from HPLC analysis in the pith cell hydrolysate, which is consistent with previous results demonstrating that the concentration of oligomers is typically very low under comparable hydrolysis conditions *(25)*. For further validation, a secondary hydrolysis was performed using the method of Kim et al. *(26)* to check for the presence of oligomers. No change in sugar concentrations was detected in the solution following the secondary hydrolysis, so the oligomer content

Table 1
Representative Mass Balances for Pith and Fiber Fractions
Under 0.8 wt% Acid Hydrolysis at 180°C

	Pith	Fiber
Initial weight (g)	5.0	5.3
Mass after hydrolysis (g)	2.6	2.5
Mass of detected sugars (g)	1.04	1.20
Mass of degraded sugars (g)	1.27	1.46
Total difference (g)	0.09	0.14

of the hydrolysate had to be sufficiently low as to be under the detection limit of the HPLC analysis.

Table 1 gives a representative mass balance taken at the 180°C sample point. Included in Table 1 are the initial pith cell mass and the solid unhydrolyzed mass remaining at the conclusion of the experiment, as well as the measured quantity of free sugars and the amount of sugars that were lost to degradation as determined from the glucose and xylose degradation experiments. As can be seen, the closure of the mass balance is quite good when only the solids and free and degraded sugars are considered, indicating that the sugar degradation estimate used to find the actual hydrolysis rate was reasonable. The closure within 2 to 3% also would indicate that the lignin, which is present in corn stover at about 20 wt% *(27)*, must have been present on the solids fraction at the conclusion of the experiment. Therefore, any lignin that was solubilized during hydrolysis must primarily have been re-precipitated on the solids. This lignin precipitation behavior has been demonstrated previously in the hydrothermal treatment of corn stover *(28)*.

A comparison of the acid hydrolysis of the pith and fiber fractions is shown in Fig. 5, with the mass hydrolyzed given as a function of reaction temperature. Both curves were determined from the HPLC analysis of dissolved sugars corrected for sugar degradation, as already described. A mass balance for the fiber cell hydrolysis experiment corresponding to the 180°C sample point is given in Table 1. As with the pith cell hydrolysis, good mass balance closure was achieved for the fiber cell hydrolysis. From Fig. 5 it is clear that the acid hydrolysis behavior of the two corn stover cell fractions was indistinguishable.

The commonality of the acid hydrolysis results for the two corn stover fractions can also be seen when the individual sugar measurements are compared. Shown in Fig. 6 are the hexose and pentose mass fractions measured in solution over the course of the hydrolysis experiments for the pith and fiber fractions. The mass fraction represents the amount of free sugar measured relative to the initial mass of pith or fiber cells. The curves shown in Fig. 6 were not corrected for sugar degradation, as can be seen most clearly from the pentose curves. As expected, the pentoses were the first sugars to be detected because the hemicellulose fraction of the material is

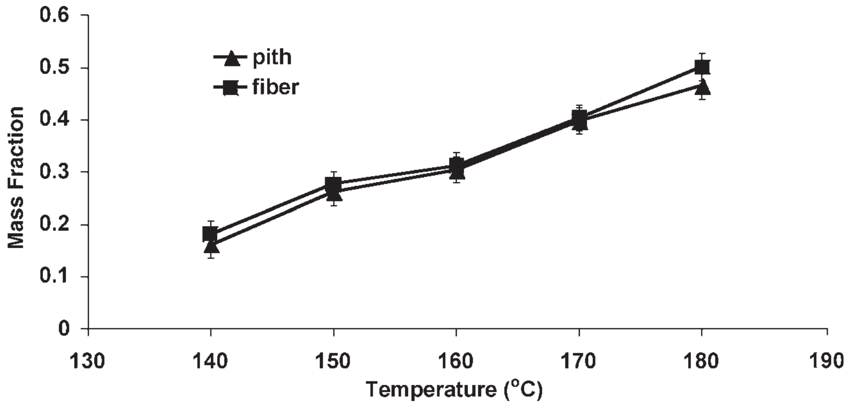

Fig. 5. Acid hydrolysis results for pith and fiber fractions (two replicates).

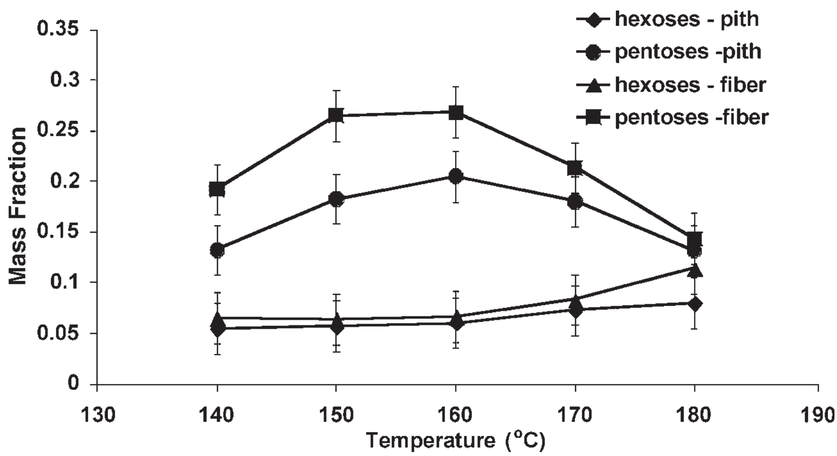

Fig. 6. Distribution of hexoses and pentoses released by acid hydrolysis of tissue fractions (two replicates).

easier to hydrolyze. Higher temperatures were required to hydrolyze the cellulose fraction to glucose, which is consistent with cellulose being more recalcitrant toward hydrolysis.

Because the goal of the current study was to compare the hydrolysis characteristics of the pith and fiber fractions, hydrolysis experiments were also performed under alkaline conditions. It has been demonstrated that alkali pretreatment in the hydrolysis of lignocellulose, given sufficiently high alkali concentration and reaction temperature, can solubilize most of the lignin and hemicellulose *(12)*. By contrast, the glycosidic bonds in cellulose are known to be stable under alkaline conditions *(12)*, so the cellulose will remain intact even after most of the lignin and hemicellulose are solubilized *(14)*. For the growing corn plant, the fiber fraction of corn stover is known to be slightly more lignified *(26)*. Therefore, the alkaline hydrolysis

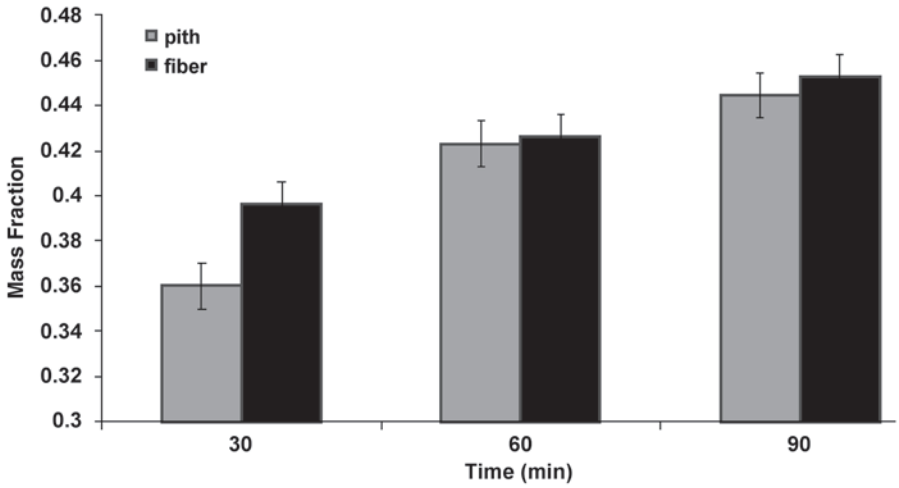

Fig. 7. Mass loss of pith and fiber fractions under alkaline conditions (two replicates).

experiments should provide some additional information on the hydrolysis characteristics of the two cell fractions.

Figure 7 presents the results for the alkaline hydrolysis in which the loss of mass of the solids was determined as a function of reaction time. Unlike in the acid hydrolysis experiments, mass balance closure was difficult to achieve under the alkaline hydrolysis, because the hydrolysis resulted in few monomers that could be detected with the HPLC method. Solubilized oligomers were detected but were not separated into molecular species, making quantification difficult. Therefore, the reported mass loss was determined exclusively by direct measurement of the mass of the solids portion left after hydrolysis. As can be seen from Fig. 7, the fiber portion had a more rapid initial mass loss under the alkaline conditions, but by 90 min both fractions had similar amounts of solubilized mass. Because the loss of mass in these reactions could be attributed to solubilization of lignin and hemicellulose, the overall mass losses for the cell fractions would be consistent with the two types of tissue having similar mass composition of the two polymeric types. The slight difference in time dependence of the mass could indicate a difference in the architecture and/or accessibility of the type of tissue to the alkaline catalyst.

The current results have suggested that despite the difference in cell function between the pith and fiber tissue fractions, the types of tissue when taken from the mature corn stover have similar hydrolysis characteristics. These results can be compared against reports that have attempted to quantify the molecular content of corn stover. Morrison et al. *(29)* conducted studies on the cell-wall composition of maize that compares the pith and rind tissues. The tissue that is referred to as rind in their study would be similar to the fiber tissue used in the current study. In that work, stalks of varying maturity were separated into outer rind and central pith por-

Table 2
Sugar Distribution Reported Previously for Cell Wall Matrix of Pith and Rind Tissues

	Glucose	Xylose	Arabinose	Galactose	Mannose
Pith (wt%)[a]	60.6	25.4	4.9	4.4	4.7
Rind (wt%)[a]	60.8	31.8	2.9	2.5	2.0
Pith (wt%)[b]	65.5	29.5	4.6	Trace	Trace
Fibrovascular bundles (wt%)[b]	67.5	29.5	3.0	Trace	Trace
Fiber/rind (wt%)[b]	69.0	26.8	4.6	Trace	Trace

[a]From ref. *29*.
[b]From ref. *30*.

tions. Shown in the first two rows of Table 2 are neutral sugar compositions for the two tissue fractions taken from the most mature stover used in their study. The neutral sugar compositions as determined by HPLC analysis of the acid-hydrolyzed starch-free residue were found to be very similar. The glucose content of both fractions, which correlates with the cellulose content, was nearly identical. Although the distribution of the four major neutral sugars that comprise the hemicellulose fraction were distributed differently, the total amount of sugars was the same.

In another study, Jones et al. *(30)* examined neutral sugar contents from mature corn stover that was separated into three segments: pith, fibrovascular bundles, and fiber from the rind. The pith tissue in their study would be similar to the pith tissue in the current study and the combination of the fibrovascular bundles and fiber from the rind correlates with the fiber tissue. Results from their study are given in the lower three rows of Table 2. In contrast to the other study, their results show a higher percentage of glucose in each tissue fraction. Their work included an attempt to quantify the hemicellulose and cellulose portions of the tissues by using alkaline extraction of hemicellulose from delignified tissues. Although they acknowledged that their method would overestimate the glucose content in the hemicellulose, and therefore underestimate the cellulose content, the results provide some insight into the composition of the tissues examined. They found that the fibrovascular bundles had the highest cellulose content (32.3 wt%), followed by pith (27.8 wt%), with the rind lowest (20.3 wt%).

Although these two studies gave slightly different results for the composition of neutral sugars in the pith and fiber tissue fractions as defined in the current study, the overall conclusion is that both fractions appear to have similar distributions of neutral sugars, suggesting that the cellulose and hemicellulose composition in each fraction is comparable. The current results were consistent with this conclusion because the overall mass hydrolyzed for the pith and fiber fractions was similar.

Because the amount of cellulose and hemicellulose present in the pith and fiber fractions are comparable, the other factor that could have affected the hydrolysis characteristics of the fractions was their respective lignification. Lignification of cell wall is widely regarded as the primary factor limiting acidic and enzymatic hydrolysis. This conclusion has been drawn from the correlation between degradability and lignin content. The degree of lignification between the pith and fiber tissue does differ slightly *(29)*, but for corn stover the lignin content in general increases as the plant matures *(31)*. Degradability studies based on mature tissue have suggested that there is another factor that affects the degradability of the tissues as they age, because lignin content was not predictive of degradability with tissue maturity *(32)*. Jung et al. *(33)* performed experiments in which corn stover was ball milled to assess whether substrate accessibility was a factor in degradation rates. However, they were unable to explain differences in polysaccharide degradation that remained even when accessibility limitations were removed, and they concluded that the most important factor limiting cell wall degradation is currently not being measured *(33)*.

Conclusion

Hydrolysis of corn stover is a promising source of monomeric sugars if an economic hydrolysis process could be developed. Mechanical separation of the pith and fiber tissue fractions provides an inexpensive approach to separation of corn stover by cellular function. However, hydrolysis experiments performed on the separated tissue fractions showed no significant difference in hydrolysis characteristics despite the significant difference in gross cell structure of the two tissue fractions. The comparable hydrolysis characteristics for the tissue types was likely related to the fact that mature corn stover was used in the study, because the lignin content in the stover increases with maturation. Because mature corn stover is the best target for a hydrolysis feedstock, the use of mechanical separation prior to hydrolysis does not appear to present an attractive alternative to the current hydrolysis work that emphasizes hydrolysis processing of the entire corn stover.

References

1. Glassher, D., Hettenhaus, J. R., and Schechinger, T. M. (1998), in *Proceedings of BioEnergy 98*, Weichart, D., ed., Great Lakes Regional Biomass Energy Program, Madison, WI, pp. 1100–1110.
2. Goldstein, I. S., Pereira, H., et al. (1983), *Biotechnol. Bioeng.* **13,** 17–25.
3. McParland, J. J., Grethlein, H. E., et al. (1982), *Solar Energy* **28,** 55–63.
4. Bhandari, N., Macdonald, D. G., et al. (1984), *Biotechnol. Bioeng.* **26,** 320–327.
5. Chen, R., Wu, Z., et al. (1998), *Appl. Biochem. Biotechnol.* **70–72,** 37–50.
6. Belkacemi, K., Turcotte, G., et al. (2002), *Ind. Eng. Chem. Res.* **41,** 173–179.
7. Kaar, W. E. and Holtzapple, M. T. (2000), *Biomass and Bioenergy* **18,** 189–199.
8. Varga, E., Schimdt, A. S., et al. (2003), *Appl. Biochem. Biotechnol.* **104,** 37–50.
9. Esteghlalian, A., Hashimoto, A. G., et al. (1997), *Bioresour. Technol.* **59,** 129–136.
10. Um, B.-H., Karim, M. N., et al. (2003), *Appl. Biochem. Biotechnol.* **105–108,** 115–125.
11. Gould, J. M. (1984), *Biotechnol. Bioeng.* **26,** 46–52.

12. Saddler, J. N., Ramos, L. P., and Breuil, C. (1993), in *Bioconversion of Forest and Agricultural Plant Wastes*, Saddler, J. N., ed., C.A.B. International, Wallingford, UK, pp. 73–91.
13. Nevell, T. P. and Zeronian, S. H. (1985), *Cellulose Chemistry and Its Applications*, John Wiley & Sons, New York.
14. Iyer, P. V., Wu, Z.-W., et al. (1996), *Appl. Biochem. Biotechnol.* **57/58,** 121–132.
15. Holtzapple, M. T., Jun, J., Ashok, G., Patibandla, S. L., and Dale, B. E. (1991), *Appl. Biochem. Biotechnol.* **28/29,** 59–74.
16. Moniruzzaman, M., Dale, B. E., Hespell, R. B., and Bothast, R. J. (1997), *Appl. Biochem. Biotechnol.* **67,** 113–126.
17. Chang, V. S., Nagwani, M., and Holtzapple, M. T. (1998), *Appl. Biochem. Biotechnol.* **74,** 135–159.
18. Kim, T. H., Kim, J. S., et al. (2003), *Bioresour. Technol.* **90,** 39–47.
19. Hsu, T. A. (1996), in *Handbook on Bioethanol: Production and Utilization*, Wyman, C. E., ed., Taylor & Francis, Washington, DC, pp. 179–212.
20. Grohmann, K., Torget, R. W., et al. (1986), *Biotechnol. Bioeng. Symp.* **17,** 135–151.
21. Brett, C. and Waldron, K., eds. (1990), *Physiology and Biochemistry of Plant Cell Walls*, Unwin Hyman, London, UK.
22. Mosier, N. S., Hall, P., et al. (1999), *Adv. Biochem. Eng./Biotechnol.* **65,** 23–40.
23. Kim, S. B. and Lee, Y. Y. (2002), *Bioresour. Technol.* **83,** 165–171.
24. Bienkowski, P. R., Ladisch, M. R., et al. (1987), *Chem. Eng. Commun.* **51,** 179–192.
25. Lee, Y. Y., Iyer, P., et al. (1999), *Adv. Biochem. Eng./Biotechnol.* **65,** 93–115.
26. Kim, J. S., Lee, Y. Y., et al. (2001), *Appl. Biochem. Biotechnol.* **91–93,** 331–340.
27. Hames, B. R., Thomas, S. R., et al. (2003), *Appl. Biochem. Biotechnol.* **105–108,** 5–16.
28. Rubio, M., Tortosa, J. F., et al. (1998), *Biomass Bioenergy* **15,** 483–491.
29. Morrison, T. A., Jung, H. G., et al. (1998), *Crop Sci.* **38,** 455–460.
30. Jones, R. W., Krull, L. H., et al. (1979), *Cereal Chem.* **56,** 441, 442.
31. Hedin, P. A., Williams, W. P., and Buckley, P. M. (1998) *J. Agric. Food Chem.* **46,** 4754–4758.
32. Jung, H. G., Buxton, D. R., et al., eds. (1993), *Forage Cell Wall Structure and Digestibility*, American Society of Agronomy, Madison, WI.
33. Jung, H. G., Jorgensen, M. A., et al. (2000), *J. Sci. Food Agric.* **80,** 419–427.