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EFFECT OF STOVER FRACTION ON GLUCOSE PRODUCTION USING ENZYMATIC HYDROLYSIS

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ABSTRACT. *Corn stover was fractionated into three fractions: cobs, stalks, and leaves and husks. The fractions were dried and ground through a 2 mm screen. Samples of the three fractions and whole corn stover with and without NaOH pretreatment were subjected to enzymatic hydrolysis in order to determine the effect of fractionation on glucose production. The average amounts of glucose released after 60 h of hydrolysis from pretreated cobs, leaves and husks, stalks, and whole stover were 0.50, 0.36, 0.28, and 0.36 g/g dry biomass, respectively. The average amounts of glucose released after 60 h of hydrolysis from nonpretreated cobs, leaves and husks, stalks, and whole stover were 0.32, 0.23, 0.17, and 0.20 g/g dry biomass, respectively. Pretreatment resulted in an average increase of 60% in glucose production for all fractions and whole stover. The effect of stover fraction type on glucose production was significant with and without pretreatment. By collecting the fractions of the corn stover with the highest glucose potential (all the cobs and 74% of the leaves and husks) and leaving the remaining fraction (26% of the leaves and husks, and all the stalks) in the field for erosion control, the glucose potential of the collected biomass would increase by 21%. This could represent a decrease of up to 17% in the cost of ethanol production. This indicates that fractionation and collection of the biomass with the highest glucose potential may produce a higher quality feedstock for glucose production.*

Keywords. *Biomass, Cellulase, Fractionation, Glucose.*

Currently, 5.7 billion liters (1.5 billion gallons) of ethanol are added to gasoline in the U.S. each year to improve vehicle performance and reduce air pollution. Numerous researchers have investigated the production of ethanol from various lignocellulosic materials, including corn stover (Sun and Cheng, 2002). Corn stover, the aboveground residue left behind after harvesting corn for grain, includes cobs, stalks, husks, and leaves that are either partially tilled into the soil or remain undisturbed in the case of no−till farming. The dry weight of stover is considered to be equal to the weight of the harvested grain (Tyner and Buttum, 1978). The U.S. corn harvest between 1996 and 2000 averaged 242 Mt on 29 Mha. Perlack and Turhollow (2002) assumed that 2.5 t/ha was collected. They noted that this was a conservative estimate that would control soil erosion, maintain soil productivity, and maintain soil carbon levels.

There are several advantages to using corn stover as an ethanol feedstock, including its wide availability, adequate cellulose content, and that it can be collected and stored using conventional equipment (Sokhansanj et al., 2002). However, before widespread use of corn stover as an ethanol feedstock can be fully realized, the effect of fractionation into cobs, leaves, husks, and stalks needs to be investigated. In this way, the benefits of collecting the stover fractions with the highest potential sugar yield and leaving in the field the fraction with the lowest potential sugar yield can be estimated. The suitability of individual corn stover fractions for ethanol production has not been investigated, although fractionating wheat at harvest has been investigated (Hoskinson et al., 2001). Hoskinson et al. (2001) determined that a combine could be used to fractionate wheat into a higher value feedstock for use in biorefineries.

The primary objective of this work was to investigate the quantity of glucose released from the enzyme hydrolysis of pretreated and nonpretreated corn stover fractions (stalks, cobs, and leaves and husks) and whole corn stover.

MATERIALS AND METHODS

Corn stover (Dekalb 626) was sorted into individual fractions of stalks, cobs, and leaves and husks by hand after 10 months of storage. Leaves and husks were combined into one sample due to the difficulty associated with identifying one from the other. After separating the individual fractions, the material was dried at 45°C (final moisture content less than 2% w.b.). Approximately 600 g of each stover type was ground through a 2 mm circular screen, run through a Boerner divider to ensure uniformity, and stored in double plastic bags for future testing. A fourth sample was prepared by mixing the fractions back into a whole corn stover sample, where whole stover contained 16% cobs, 41% husks and leaves, and 43% stalks, based on to the percentages found in the field by Montross et al. (2002). Half of each sample type was

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subjected to NaOH pretreatment as described below, while the second half remained nonpretreated, resulting in eight sample types.

Three replicates were run for each of the eight sample types in the following manner. Three grams of dry ground biomass were placed in 125 mL conical flasks with 95 mL of sodium acetate (0.05 M) buffer. The solutions were adjusted to a pH of 4.80 using sodium hydroxide or hydrochloric acid. Sodium azide (1 mL of 5.4 mM) was added to the flask to prevent the growth of microorganisms. Crude cellulase powder, generously provided by Alltech, Inc. (Nicholasville, Ky.), was added to each flask (0.75 g, 53 FPU/g).

A small amount of deionized water was added to ensure that the total flask volume was 100 mL. The samples were placed in a shaking incubator (New Brunswick Scientific, Edison, N.J.) at a temperature of 50˚C for 60 h. Samples (200 µL) were taken at time 0, 6, 12, 24, 36, 48, and 60 h, placed in a micro−centrifuge tube, boiled for 5 min to inactivate the cellulase (Mandels et al., 1976), and stored at 4˚C until analysis. Assays for glucose were conducted on each sample. Control samples were run in duplicate for whole stover and all three fractions to determine whether any of the increase in glucose concentration was due to sugars initially present in the sample. For each control, the procedure was exactly the same without the addition of cellulase. Student t−tests were used to determine the statistical differences between the glucose measurements.

Pretreatment

The samples were pretreated using a procedure similar to that of Varga et al. (2002). Samples were soaked in a 0.2 N NaOH solution (10% dry weight/volume) for 2 h. The samples were then washed with deionized water (at least 20 times more than the initial NaOH solution). The wet material was vacuum filtered and rinsed with deionized water through three layers of nylon window screen. A pestle was used to press excess water out of the material. The pretreated stover samples were stored in a plastic bag at 4˚C until used (approximately 3 days).

Glucose Assay

Glucose was measured using the assay from Russell and Baldwin (1978). Six standards were used with glucose concentrations between 0 and 25 mg/L. Samples of 150 µL were diluted with 1 mL of buffer (50 mL triethanolamine buffer (50 mM with pH 7.5), 40 mg $MgCl_2 \cdot 6H_2O$, 20 mg ATP, 20 mg NADP, 5 µl HK (9 U), and 2.5 µL G6PD (12.5 U)) and incubated for 30 min. The absorbance for each sample was read at 340 nm (Cary 300 Bio Spectrometer, Varian, Inc., Walnut Creek, Cal.) and compared to the standard curve to determine the amount of glucose.

Cellulase Assay

Cellulase activity was estimated as filter paper units (FPU) by the method of Mandels et al. (1976) with slight modification. One mL of 0.1 M phosphate buffer (pH 6.0) was added to 0.5 mL of enzyme suitably diluted in the same buffer and a 50 mg strip of Whatman No. 1 filter paper, which had been curled using a glass rod. The reaction mixture was incubated at 60˚C for 4 h, and then 3 mL of DNS reagent was added to stop the reaction. The glucose released was measured using the above method. Enzyme blanks, reagent blanks, and glucose standard solutions were prepared in the same way. Cellulase activity was expressed in terms of FPU/g of crude cellulase powder. All reagents were purchased from Sigma (St. Louis, Mo.), unless otherwise indicated.

Results and Discussion

Glucose Concentrations

The glucose levels in the control runs were not statistically different from zero ($\alpha = 0.05$), indicating that the glucose levels measured after enzyme hydrolysis were not influenced by the amount of glucose present in the original stover fractions and whole stover. The values varied between −0.5 and 0.6 g/L and were assumed to be within the accuracy of the glucose assay.

The average glucose concentrations for the various stover fractions as a function of hydrolysis time are shown in figure 1. The average amounts of glucose released after 60 h of hydrolysis from pretreated cobs, leaves and husks, stalks, and whole stover were 0.50, 0.36, 0.28, and 0.36 g/g dry biomass, respectively. The average amounts of glucose released after 60 h of hydrolysis from nonpretreated cobs, leaves and husks, stalks, and whole stover were 0.32, 0.23, 0.17, and 0.20 g/g dry biomass, respectively. After 60 h of hydrolysis, the effect of stover fraction and pretreatment was significant according to the analysis of variance ($P < 0.001$).

The glucose levels from the pretreated cobs, stalks, and leaves and husks increased by approximately 60% compared to the glucose levels from nonpretreated samples. However, pretreated whole stover increased by approximately 75% compared to nonpretreated whole stover. This was probably due to differences in combining the samples for producing the whole stover sample and errors associated with the assay. Interestingly, nonpretreated cobs produced more glucose than the pretreated stalks. As shown in figure 1, the pretreated stalks produced 21% less glucose than the pretreated whole stover. This data indicate that fractionation of corn stover during collection may be beneficial if high values of glucose production are desired.

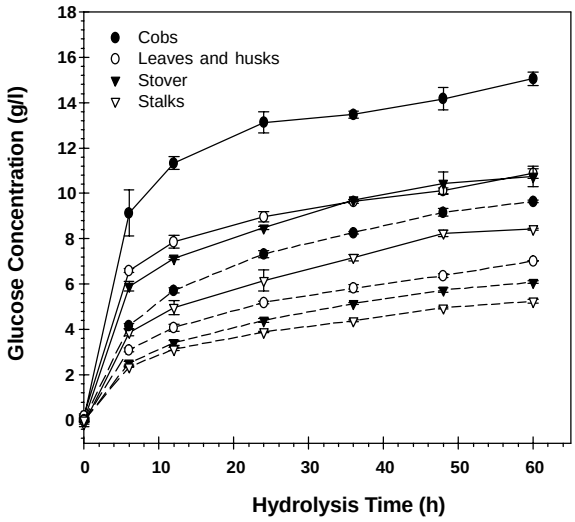

Figure 1. Average glucose concentration during enzyme hydrolysis of stover fractions and whole stover both pretreated (solid lines) and non-pretreated (dashed lines). Error bars represent standard error (*n* = 3).

In order to determine whether fractionation of the stover would result in an overall decrease in the amount of glucose produced, the actual glucose production from stover was compared to the glucose produced from the individual fractions combined in the appropriate proportions (16% cobs, 41% leaves and husks, and 43% stalks). The comparison between the actual stover glucose levels and the proportional combination of the glucose from the three fractions is shown in figure 2. Interestingly, the sum of the fractions overpredicts for the pretreated samples and underpredicts for the nonpretreated samples. Statistically speaking, the values at 60 h for the nonpretreated treatment are different ($p < 0.1$), while the values at 60 h for the pretreated treatment are not different ($p < 0.01$). However, in both cases, the values are quite similar (10.5 and 10.7 g/L for pretreated, and 6.7 and 6.1 g/L for nonpretreated). The variability due to crop conditions and field variability were not quantified and could have influenced the combination of material to create the whole stover. From a design perspective, the glucose values from the various fractions can be used to determine what the glucose production would be if the fractions were mixed prior to enzyme hydrolysis, such that the enzyme hydrolysis of 50% cobs and 50% leaves and husks would result in a glucose production approximately equal to 50% of the glucose produced by cobs and 50% of the glucose produced by leaves and husks. This information could prove useful when deciding what fractions should be collected to increase profitability.

Potential Economic Implications of Fractionation

The study by Aden et al. (2002) was used to estimate the potential economic benefit of selectively harvesting corn stover. The estimated plant production of ethanol was 374 L/t of corn stover (89.7 gal/ton), with an assumed ethanol selling price of \$0.283/L (\$1.07/gal) and a biomass feedstock cost of \$33/t. It was assumed that saccarification of the corn stover would result in 0.374 g of ethanol per g of corn stover (37.4%).

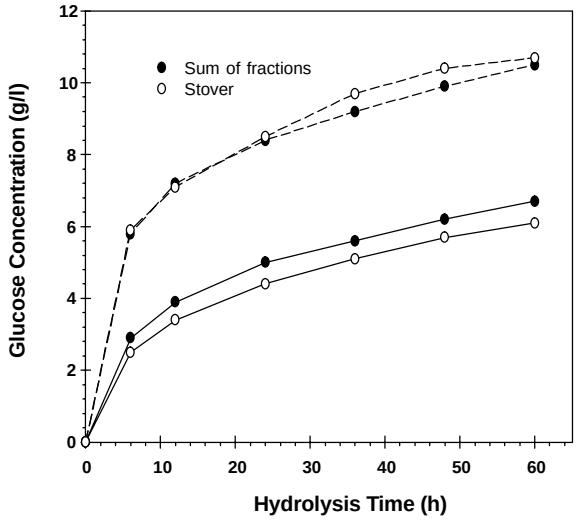

Figure 2. Comparison of the observed glucose levels from the enzyme hydrolysis of whole stover and the predicted glucose levels based on glucose levels from the enzyme hydrolysis of the three stover fractions (16% cobs, 41% leaves and husks, and 43% stalks). Dashed lines indicate pretreated stover.

Based on the stover collected in our study, 1.27, 1.66, and 4.98 t/ha of cobs, leaves and husks, and stalks were available, respectively (Montross et al., 2002). The average glucose concentration after enzymatic hydrolysis of pretreated corn stover from the field was 35.8%, which was similar to the value (37.4%) used by Aden et al. (2002). The difference between the average glucose concentration of the material used by Aden et al. (2002) and the value measured in our study could be due to corn variety, growing conditions, and quality changes during storage. Assuming that 2.5 t/ha of stover could be removed sustainably from the field (Perlack and Turhollow, 2002), the optimum collection of selectively harvested corn stover (SHCS) from our study would include all the cobs (1.27 t/ha) and 74% of the leaves and husks (1.23 t/ha). The resulting average glucose production would be 0.433 g of glucose per g of SHCS, an increase of approximately 21% compared to the yield from whole corn stover.

Hence, selected corn stover fractions would be worth 21% more, \$39.9/t for SHCS compared to \$33/t for whole stover. If the price of the SHCS was assumed to remain at \$33/t, then the increase in glucose production could result in a decrease in the final ethanol price. The 21% increase in glucose production due to the SHCS would result in an additional 78.6 liters of ethanol per tonne of biomass. The additional ethanol production would result in a final ethanol cost of \$0.234/L (\$0.885/gal), a 17% decrease compared to the base case given by Aden et al. (2002). These estimates are all based on the increase in glucose production, when in reality the xylose production from hemicellulose should also increase. Foley and Vander Hooven (1981) measured the amount of hemicellulose in cobs to be 36%, while Sloneker (1976) measured the amount of hemicellulose in stalks to be only 16%. This would indicate that collecting only cobs, leaves, and husks would increase the hemicellulose as well as the cellulose concentration of the feedstock.

Conclusions

Collection of specific fractions of corn stover (primarily cobs, husks, and leaves) would increase the glucose yields from saccarification of the fractionated stover, while the stover left behind in the fields (primarily stalks) should provide sufficient erosion control. Collection of cobs, leaves, and husks could be accomplished with minor modifications to current combines, considering that existing combines provide separation of corn kernels from cobs, leaves, and husks.

Based on the glucose yields in this study, the amount of glucose released from the stover would increase by approximately 21% by selectively collecting cobs and most of the leaves and husks (74%) and leaving the stalks and remainder of the leaves and husks in the field for erosion control and soil sustainability. Pretreatment improved the glucose release from each component by approximately 60%. The glucose potential appeared to be the same whether considering whole stover or the proportional summation of the glucose potential from the selectively harvested corn stover. Preliminary economic analysis indicates that the ethanol cost could be reduced by up to 17% if the glucose potential of the biomass feedstock was increased by selective collection.

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