Corn Harvest Strategies for Combined Starch and Cellulosic Bioprocessing to Ethanol

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ABSTRACT

Conventional harvest and ethanol conversion strategies for corn (*Zea mays* L.) grain and corn stover involve multiple trips across the field and separate bioprocessing of the grain and stover. The objective of this study was to compare bioethanol yield between a traditional source separated corn harvest coupled with conventional separate starch and cellulosic bioprocessing streams, and novel whole-plant harvest strategies coupled with a whole-plant (starch plus cellulosic) bioprocessing platform. Composition analysis showed immature cut whole-plant fractions whether fresh processed (ImF) or ensiled (ImS), and mature cut whole-plant fraction (MWP) had higher glucan (56.0 ± 8.0% kg kg^{-1} dry biomass), lower xylan (13.3 ± 2.7%), arabinan (2.9 ± 0.6%) and acid-insoluble lignin (11.8 ± 2.6%) contents than mature cut source separated fractions of stover (MSep-S) and cob (MSep-C). Averaged across locations MWP corn had significantly higher bioethanol yield on a land area basis (6446 ± 974 L ha^{-1}) than the other harvest strategies. There was no difference in ethanol yield on a land area basis between ImF (5679 ± 1046 L ha^{-1}) or ImS (5294 ± 1052 L ha^{-1}) indicating that conventional ensiling is a viable feedstock storage method for bioethanol production in future biorefineries. The results suggest that whole-plant corn harvesting coupled with whole-plant bioconversion to ethanol is a viable alternative to the convention of separate grain and stover harvesting and bioprocessing.

Corn grain is the primary bioethanol feedstock resource in the United States, with more than 30% of domestic corn grain (about 91.5 million Mg) currently being used to produce ethanol (NCGA, 2009). United States Energy Policy (Congress, 2007) established a production target of 136 billion liters of bioethanol by the year 2022, of which about 58% (79 billion liters) is expected to come from cellulosic feedstock. Corn stover has been identified as the most abundant agricultural crop residue available in the Midwest and it is expected to be a primary source of bioethanol cellulosic feedstock with the production of about 200 million Mg per year (Atchinson and Hettenhaus, 2004; Kadam and McMillan, 2003; Sokhansanj et al., 2002; Wilhelm et al., 2004).

Recently, whole plant harvesting has been proposed as a viable harvest strategy for corn bioenergy feedstock (Shinners et al., 2003; Shinners et al., 2007). This one-pass harvest strategy offers several advantages: enables an earlier harvest, which accommodates the shorter Northern growing season and allows an opportunity for growing a winter annual cover crop; improves harvest efficiency by capturing more of the total plant biomass; and reduces harvest costs by 26% compared with traditional separate harvesting for the grain and stover components (Shinners et al., 2003, 2007). Different biorefinery processes involved in converting whole plant feedstock to bioethanol are reported in the literature (Garlock et al., 2009; Shao et al., 2010). These processes include pretreatment such as ammonia fiber expansion (AFEX), enzyme hydrolysis, and fermentation (using engineered yeast for glucose-xylose cofermentation). The AFEX pretreatment is a themochemical process, in which liquid ammonia is added to biomass in a pressurized vessel at high temperature (80–160°C) and high pressure (4.48 MPa) for a residence time of 5 to 30 min, followed by sudden pressure release (Teymouri et al., 2004; Alizadeh et al., 2005; Murnen et al., 2007; Lau et al., 2008). The AFEX process decrystallizes lignocellulose structure by removing hemicellulose and reducing lignin content (Kumar et al., 2009). Work by Teymouri et al. (2004) demonstrated that AFEX treated biomass samples produced 2.2 times more ethanol than unpretreated samples. Compared to acid pretreatment, AFEX produces less inhibitory degradation products (Chundawat et al., 2010) and eliminates the need for subsequent solution wash and pH adjustment before fermentation. Residual ammonia left in the pretreated biomass is used as a N source for the downstream microbial fermentation process (Teymouri et al., 2004; Wyman et al., 2005; Lau et al., 2008). After AFEX pretreatment and enzymatic hydrolysis, sugar monomers such as glucose and xylose are released and can be fermented to ethanol under anaerobic conditions by engineered S. *cerevisiae* 424A (LNH-ST) (Sedlak and Ho, 2004). Glucose is derived mainly from cellulose, and xylose and arabinose are derived mainly from hemicellulose (Wallace et al., 2005).

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Abbreviations: AFEX, ammonia fiber expansion; ImF, immature harvest whole-plant fraction, bioprocessed fresh; ImS, immature harvest whole-plant fraction, bioprocessed after ensiling; MSep-C, mature harvest source separated corn cob fraction; MSep-G, mature harvest source separated corn grain fraction; MSep-S, mature harvest source separated corn stover fraction; MWP, mature harvest whole-plant fraction.

Table 1. Location, planting, and yield information.

	Branch		Ingham		Huron		Menominee	
	2008	2009	2008	2009	2008	2009	2008	2009
Hybrid	DKC5779	34F29	DKC5263	35F40	DKC5779	DKC 5044	GLH3663	38H08
RM†, d	107	108	102	107	107	100	86	92
Planting date	26 Apr.	9 May	5 May	6 May	6 May	23 May	8 May	15 May
Silage yield‡, Mg ha^{-1}	17.7	17.7	15.9	20.6	22.4	19.7	13.2	16.8
Grain yield§, Mg ha^{-1}	10.3	10.6	8.8	11.2	12.1	9.6	6.3	8.2
Location	Coldwater, MI		East Lansing, MI		Bad Axe, MI		Daggett, MI	
Soil	Oshtemo sandy loam irrigation	Fox sandy loam	Capac loam		Kilmanagh loam		Onaway sandy loam	
Soil pH	6.7	5.8	6.3	7.0	6.6	6.8	7.4	7.6

† RM: relative maturity is predicted relative maturity based on the moisture percentage of the grain at harvest based on Minnesota Relative Maturity Rating System.

‡ Silage yield was dry biomass obtained by green biomass subtracting moisture content.

§ Grain yield was at 15.5% moisture.

Garlock et al. (2009) analyzed the AFEX pretreatment process using corn stover husk, stem, leaf, and cob components and theoretically calculated ethanol yield at 912 L ha^{-1} assuming 30% of the in-field corn stover was harvested. The research of Shao et al. (2010) focused on AFEX effects of corn stover and whole plant hydrolysis and subsequent fermentation, and reported 28.4 and 29.8 g L^{-1} ethanol concentration (23.3 and 22.8 kg ethanol kg^{-1} biomass) at 6% glucan loading. However, to date there is little information available comparing biofuel yields of various whole-plant corn harvest methods.

The purpose of this study was to compare bioethanol yield between a traditional source separated corn harvest coupled with conventional separate starch and cellulosic bioprocessing streams, and novel whole-plant harvest strategies coupled with a whole-plant (starch plus cellulosic) bioprocessing platform.

MATERIALS AND METHODS

Field Experiment Design. Corn was grown on four farms (Table 1) located in Branch (Southern Michigan, Zone 1, 41°59'06" N, 85°02'50" W), Ingham (Southern-middle Michigan, Zone 2, 42°38'08" N, 84°13'31" W), Huron (Northern-middle, Zone 3, 43°50'10" N, 82°59'45" W) and Menominee (Northern Michigan, Zone 4, 45°26'60" N, 87°35'34" W) counties in 2008 and 2009. In each location, there were four replicate plots (3.05 by 5.18 m) with 0.76 m row spacing. The hybrids planted at these four locations were selected based on suitability for the specific latitude and are shown in Table 1. Harvest timing included: immature harvest at typical corn silage harvest stage (mid R5 stage) and harvest at full maturity when typical corn grain harvest occurs (post R6 stage) (Mueller et al., 1991). The whole plant harvested in the R5 stage was chopped in the field using a conventional corn forage harvester and then split into fresh (fraction ImF) and ensiled (fraction ImS) fractions. The ensiling process was accomplished by storing samples in vacuum sealed plastic bags for 1 mo followed by drying and grinding through a 3-mm sieve. The ensiling process is used to preserve and store silage biomass, which would facilitate a year-round feedstock supply to the ethanol biorefinery. During ensiling, anaerobic fermentation produces lactic and acetic acid and these acids preserve the feedstock from further decomposition (Cherney et al., 2004). The mature cut source-separated components of corn harvested at the typical corn grain harvest timing consisted of the following fractions: grain (MSep-G), stover (MSep-S), and cob (MSep-C). Finally, the nonseparated, mature cut whole-plant corn harvested at the typical corn grain harvest timing was labeled as fraction MWP. Average daily temperature and total monthly precipitation during the corn growing season in 2008 and 2009 can be found in Table 2.

Corn grain and silage yields (Table 1) were determined from field plots at the four locations referenced above (Michigan State University, 2008, 2009). A 6% biomass loss was assumed

Table 2. Monthly average temperature and total precipitation in 2008 and 2009 at the four experiment locations in Michigan.

	Branch			Ingham			Huron			Menominee		
Month	2008	2009	Norm†	2008	2009	Norm	2008	2009	Norm	2008	2009	Norm
	°C											
May	13.0	14.8	14.6	12.7	14.2	14.0	11.4	12.9	12.9	9.7	10.9	12.2
June	22.3	20.1	19.7	20.6	19.1	19.1	19.3	17.1	18.2	17.3	16.6	17.2
July	21.3	19.5	21.8	21.9	19.1	21.4	20.7	18.4	20.8	19.3	17.4	19.7
Aug	20.8	20.6	20.6	20.7	20.2	20.4	18.8	18.9	19.6	17.9	17.8	18.7
Sept	18.1	17.3	16.5	18.4	17.1	16.0	16.2	16.2	15.6	14.7	15.8	13.8
Average	19.1	18.4	18.6	18.9	17.9	18.2	17.3	16.7	17.4	15.8	15.7	16.3
	precipitation, mm											
May	69	103	74	30	72	69	46	59	73	105	127	80
June	95	114	87	113	126	81	103	101	74	30	39	88
July	95	31	87	96	61	76	67	87	78	86	20	94
Aug	25	95	91	17	105	87	59	62	93	51	89	94
Sept	200	20	89	207	24	89	115	35	98	61	42	89
Total	484	362	426	462	388	402	389	344	417	333	317	444

† Norm = averages calculated over 30 yr period (1971–2000).

during the ensiling process (ImF) (Johnson et al., 2003). The cob (MSep-C) and stover (MSep-S) yields were derived from corn grain and silage yields using an average cob/grain ratio of 0.17 (Buyanovsky and Wagner, 1986). The corn stover samples (MSep-S) consisted of the whole corn plant excluding corn grain and cob. In the source separated stover treatment (MSep-S) we assumed 30% of the total plant stover was available for machine harvest leaving the remaining 70% of the stover in the field to maintain soil organic carbon content.

Composition Analysis. Composition analyses of all lignocellulosic corn fractions (ImF, ImS, MSep-S, MSep-C, and MWP) was conducted before AFEX pretreatment, including polysaccharides (glucan, xylan, and arabinan), acid-insoluble lignin and ash, following standard methods outlined by the National Renewable Energy Laboratory standard protocols LAP 002, 003, and 005(NREL, 2004). In brief, ash content of dry biomass was obtained by burning and then baking in a 575°C muffle oven. For polysaccharide content determination, 3 mL 72% (w/w) H_2SO_4 was added into 100 mL autoclavable high-pressure tube (Chemglass Life Science Inc., Vineland, NJ) with 0.3 g corn fraction; after continuous shaking at 200 rpm for 1 h at 30°C, 84 mL distilled water was added to dilute acid until 4% and the high-pressure tube was autoclaved at 121°C for 1 h. After cooling down to room temperature, 1 mL supernatant was removed and passed through a 0.22 μM syringe filter with a 33 mm diam. (Millipore Inc., Billerica, MA) and stored under –20°C until analysis. The remaining suspension was passed through a weighted 0.22 μM membrane filter, which was dried in desiccators for 2 d at room temperature. The acid insoluble lignin content was calculated by the difference between the two weights of the membrane filter before and after filtration.

The polysaccharide glucan was defined as the glucose content in the corn fraction from starch, cellulose, and hemicelluloses. Similarly, xylan and arabinan were the xylose and arabinose content originating mainly from hemicelluloses (Gaspar et al., 2007; Hendriks and Zeeman, 2009). Thus, the polysaccharide content was calculated as follows:

$$\%\text{sugar} = \frac{C \times V \times 100}{R \times W \times (1 - m\%)} \qquad [1]$$

where %sugar (% kg kg^{-1} dry biomass) was the glucan, xylan, or arabinan content in dry biomass, C (kg L^{-1}) was the glucose, xylose, or arabinose concentration after acid hydrolysis, V (L) was the total volume of acid hydrolysis (0.087 L), R was the stoichiometric ratio of molecular weight glucose/glucan (1.11 kg kg^{-1}), xylose/xylan (1.136 kg kg^{-1}) or arabinose/arabinan (1.136 kg kg^{-1}), W (kg) and m% were the weight and moisture of the biomass added into acid hydrolysis.

Ethanol from Corn Grain Fermentation. The gravimetric method was applied to ground corn grain to produce ethanol, following Lemuz et al. (2009) and Singh and Graeber (2004). In brief, 25 g ground corn grain and 75 mL deionized H_2O were mixed with 27 μL α-amylase (Sigma-Aldrich Inc., Allentown, PA) and incubated in 90°C for 90 min with occasional shaking; when the slurry was cooled down to 60°C, the solution pH was adjusted to 4.1 to 4.5 with HCl (EMD Chemicals Inc. Gibbstown, NJ). Then, 20 μL glucoamylase (Sigma-Aldrich Inc., Allentown, PA), 5 mL 1% yeast inoculum (active dry yeast, Fleischmann's yeast, Fenton, MO) in 99 mL 0.1% autoclaved peptone water (DOT Scientific Inc., Burton, MI) and 4 mmol $(NH_4)_2SO_4$ (EM Science, Gibbstown, NJ) were added in the slurry, and the slurry was incubated at 60°C for 2 h. The fermentation of the slurry was then conducted at 32°C with continuous shaking at 150 rpm for 72 h. The difference between the total weight of slurry before and after fermentation was considered as the mass of CO_2 released during fermentation.

Ethanol from Corn Lignocellulosic Fractions Fermentation. Corn lignocellulosic fractions, including ImF, ImS, MSep-S, MSep-C, and MWP, were pretreated by AFEX (Balan et al., 2009). In this study, AFEX was conducted at 90°C, and the ratio (w/w) of water and dry biomass was 60%; a 1:1 (w/w) ratio of anhydrous ammonia and biomass was used at a 5 min residence time (Teymouri et al., 2004, 2005). Then, about 0.45 g dried AFEX treated biomass was added in 15 mL 0.05 M citric acid solution at pH 5.0; cellulase (Accelerase, Genencor International, Rochester, NY) at 15 FPU g^{-1} glucan (31.3 mg protein g^{-1} glucan) and β-glucosidase (Novozyme 188, Sigma, St. Louis, MO) at 64 p-NPGU g^{-1} glucan (41.3 mg protein g^{-1} glucan) were added, and 40 mg L^{-1} tetracycline was added to inhibit bacteria growth. The enzyme hydrolysis was conducted at 50°C for 96 h with continuing shaking at 150 rpm, and 1 mL supernatant was taken and passed through 0.22 μm syringe filter for sugar conversion analysis. Another 10 mL supernatant was transferred into another vial with engineered *S. cerevisiae* 424A (LNH-ST) to convert glucose and xylose to ethanol. The initial optical density of fermentation solution was about 0.5 (DU Series 720 UV/Vise spectrophotometer, Beckman Coutler Inc., Fullerton, CA) at 600 nm (1-cm light length) and fermentation was performed at pH 5.0 in a 30°C incubator at 150 rpm continuing shaking for 96 h. After fermentation, 1 mL filtered supernatant was taken for sugar and ethanol analysis.

High Performance Liquid Chromatography. Samples from acid hydrolysis, enzymatic hydrolysis and fermentation were analyzed using a Shimadzu LC2010 (Shimadzu Scientific Instruments, Columbia, MD) with a Waters 410 differential refractometer detector (Waters Corporations, Milford, MA), equipped with an Aminex HPX-87H column and a Bio-Rad De-ashing Cartridge guard column (Hercules, CA). The mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 mL min^{-1} at 50°C and injection volume was 20 μL with a 20 min run. Standard solutions of glucose, xylose, arabinose, and ethanol were also run for calibration. Other monomer sugars, such as galactose and mannose, were <2% (Huang et al., 2009) and were not measured in this study.

Statistical Analysis. Differences in compositions (ash, acid-insoluble lignin, and sugar content), ethanol production, and ethanol yield on a land area basis were evaluated using a mixed model, in which corn fraction was set as a fixed factor while year and location as random factors. The analyses were conducted using PROC MIXED in SAS 9.1.3 (SAS Institute, 2006). The statistical model was as follows:

$$y_{ijk} = \mu + \text{fr}_i + \text{location}_j + \text{year}_k + e_{ijk} \qquad [2]$$

where y_{ijk} was response variable, including composition (ash or acid-insoluble lignin or monosaccharide), ethanol production (% kg kg^{-1} dry biomass) or ethanol yield (L ha^{-1}) at location_j and corn fraction fr_i in year_k, e_{ijk} was the experiment error. The significant differences of the response variables between fractions were tested at $\alpha = 0.05$ with Tukey adjustment.

Table 3. Average compositions (% kg kg^{-1} dry mass) of corn fractions in Michigan in 2008 and 2009.

Fraction†	Ash	Acid-insoluble lignin‡	Glucan‡	Xylan	Arabinan‡
ImF	2.9b	12.3b	53.9b	14.6c	3.0b
ImS	2.7b	10.7bc	55.6ab	13.4cd	2.7b
MSep-S	4.1a	18.0a	38.8c	23.0b	4.0a
MSep-C	1.4d	18.6a	33.9d	30.8a	4.0a
MWP	2.2c	10.0c	58.4a	11.9d	2.8b
			Estimates of random factors		
year	0	0.00002	0.000505	1.8×10^{-7}	2.59×10^{-7}
location	0.000017	0	0.000285	0.000015	0
e	0.00004	0.001007	0.003745	0.000704	0.000037

† Corn fractions: ImF, whole-plant fraction harvested at R5 stage with fresh dry; ImS, whole-plant fraction at R5 stage with ensiled postharvest treatment; MSep-S, collected stover at maturity; MSep-C, collected cob at maturity; MWP, whole-plant fraction at maturity.

‡ Different letters (a, b, c, and d) after the values mean significant difference among fractions ($p < 0.05$).

To better visualize the relationships among compositions of corn fractions under different harvest methods, biplot was graphed using SigmaPlot 10.0 (SYSTAT Software, 2007) based on principle component analysis (PCA) of glucan, xylan, arabinan, acid-insoluble lignin, and ash content in corn fractions. Principal component analysis was conduct by PROC PRINCOMP in SAS 9.1.3 (SAS Institute, 2006).

Multiple linear regression was used to build a prediction model for ethanol production from sugar concentrations in hydrolysate using PROC GLM in SAS.

RESULTS AND DISCUSSION

Compared with normal 30-yr (1971–2000) precipitation patterns (Table 2), 2008 had less precipitation during August at all four locations and more precipitation in September at the Branch, Ingham, and Huron locations. In 2009, daily temperature was about 2°C lower than the normal temperature in July; precipitation was lower than the 30-yr norm during July at Branch and Menominee and during September at all four locations. No significant correlation was found between temperature, precipitation, and whole plant yield in this study and yields were considered consistent with regional norms.

Composition of Corn Fractions. Corn fraction composition analysis was conducted by acid hydrolysis (Table 3). Corn grain was included in whole plant fractions, which contributes significant amounts of glucan (42.8 ± 11.0%) while there was little starch in MSep-S and MSep-C source separated fractions. The average glucan content in all three whole-plant samples ImF, ImS, and MWP (56.0 ± 8.0% kg kg^{-1} dry biomass) was about 1.5 times as that in MSep-S (38.8 ± 3.7%) and MSep-C (33.9 ± 3.5%) fractions. There was no significant difference of glucan content between ImF and ImS fractions, while MWP had significant higher glucan content than ImF (Table 3). This result was due to increased grain content as the corn matured from stage R5 to R6. Conversely, xylan and arabinan content in MSep-C (30.8 ± 3.0% or 3.9 ± 0.4%) and MSep-S (23.0 ± 2.6% or 3.9 ± 0.6%) was 2.1 to 2.5 or 1.6 to 1.9 times higher than those in ImF, ImS, and MWP (average values 13.3 ± 2.7% or 2.9 ± 0.6%). There was more acid-insoluble lignin content in ImF than MWP while there was no difference between ImS and ImF. The fraction MSep-S had higher glucan and lower xlyan contents than MSep-C, but MSep-S and MSep-C had no difference in arabinan and acid-insoluble lignin contents. The ash content in the stover fraction MSep-S (4.1%) was higher than all other corn fractions (1.4–2.9%), and the ash content in MSep-C was lower than the other fractions. These component ranges were comparable with the data reported by Garlock et al. (2009) and Shao et al. (2010).

Principal component analysis was conducted on glucan, xylan, arabinan, acid-insoluble lignin, and ash contents in corn fractions under the different harvest strategies. The results (Fig. 1) showed that the first two components provided a good summary of the data, accounting for 78.2% of the total variance suggesting some compositions were closely associated. Compositions of glucan, xylan, arabinan, and acid-insoluble lignin in corn fractions were closely correlated with each other, ash had little correlation with the other components, and the contents of xylan, arabinan, and acid-insoluble lignin had a positive correlation with each other and they had a negative correlation with glucan content. For the same fraction (Fig. 1), compositions were quite similar. Apparently, MSep-S had a relative higher ash content and MSep-C had higher xylan content than the other treatments, while the three whole-plant corn samples (ImF, ImS, and MWP) had similar composition with relatively higher glucan content due to the presence of corn grain in the whole plant samples.

Corn Fractions and Ethanol Production. In the study, fermentation of the source separated grain fraction (MSep-G) produced 33.8 to 39.8% (kg kg^{-1} dry biomass) ethanol. The ethanol production from the lignocellulosic fractions (kg kg^{-1} dry biomass) was ImF 19.4 to 32.4%; ImS 16.1 to 30.8%; MWP 24.6 to 32.3%; MSep-S 7.3 to 18.4%; and MSep-C 11.5 to 22.2%.

After AFEX treatment, glucan conversion of lignocellulosic fractions ranged from 44.6 to 100%, xylan conversion was 35.3 to 100% and arabinan conversion was 25.1 to 84.6%. The MSep-S and MSep-C had significantly lower glucan and xylan conversion than whole plant fractions ($p < 0.05$). No difference in glucan and xylan conversion rates was detected among the whole-plant fractions. The MSep-S had the lowest glucan conversion efficiency (57.0 ± 11.4%) and MSep-C had the lowest xylan (52.0 ± 9.2%) and arabinan (43.8 ± 8.2%) conversion efficiencies. Ethanol production of MSep-S was lower than that of the MSep-C fraction ($p < 0.05$).

There were significant differences in ethanol yield among the lignocellulosic fractions (Fig. 2). Ethanol production from the whole plant fractions was greater than that from MSep-S and MSep-C due to the absence of a starch component in the stover and cob. Among the whole-plant corn samples, MWP ethanol production was greater than ImS and ImF and no difference was found between ImF and ImS. The stover fraction, MSep-S, had lower ethanol production than the cob fraction MSep-C. All glucose and about half of the xylose were consumed during

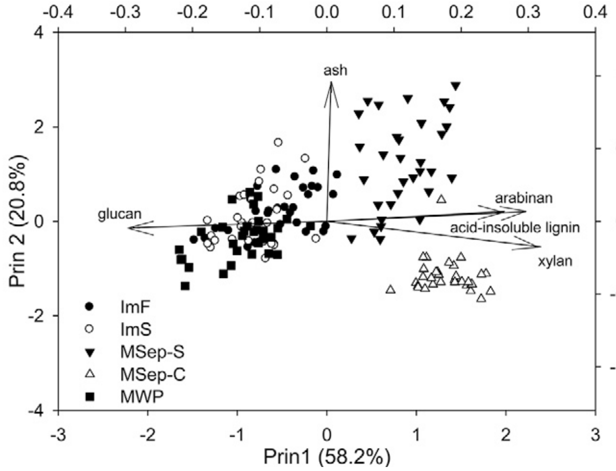

Fig. 1. Biplot of corn fraction compositions. Legend: ImF, whole-plant fraction harvested at R5 stage followed by fresh bioprocessing; ImS, whole-plant fraction harvested at R5 stage followed by ensiling before bioprocessing. MSep-S, corn stover harvested at plant maturity; MSep-C, corn cob harvested at plant maturity; MWP, whole-plant fraction harvested at plant maturity. The values in parentheses were cumulative values of principle components 1 and 2.

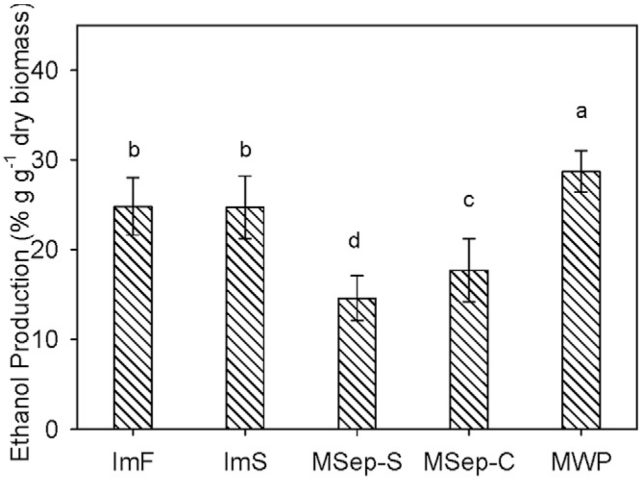

Fig. 2. Ethanol production (% kg kg^{-1} dry biomass) from corn lignocellulose fractions. Legend: ImF, immature harvest whole-plant fraction harvested at R5 stage and bioprocessed fresh; ImS, immature harvest whole-plant fraction harvested at R5 stage with ensiling postharvest treatment before bioprocessing; MSep-S, source separated corn stover harvested at maturity; MSep-C, source separated corn cob harvested at mature; MWP, mature harvest of the whole-plant fraction. Statistics were conducted among harvest strategies, different letters (a, b, c and d) indicated significant different at α = 0.05 (Tukey adjusted).

the fermentation process. The multi-linear model of ethanol production with glucose and xylose concentrations in hydrolysate ($R^2 = 0.86$) was as follows:

$$\text{EtOH\_Prod} = 0.48 \times glc + 0.29 \times xyl \qquad [3]$$

where EtOH_Prod (% kg kg^{-1} dry biomass) was ethanol production from the corn lignocellulosic fraction, *glc* (% kg kg^{-1} dry biomass), and *xyl* (% kg kg^{-1} dry biomass) were glucose and xylose concentrations released after enzymatic hydrolysis. Ethanol production was positively related with glucose and xylose concentrations. However, arabinose concentration was not significantly correlated with ethanol production. Theoretically, one molecule of glucose can be fermented to two molecules of ethanol giving a coefficient parameter of 0.51 (Garlock et al., 2009). The coefficient parameter of 0.48 for glucose to ethanol was reported by Ghose and Tyagi (1979). This prediction model (Eq. [3]) was consistent with hydrolysate data of corn stover, rice straw, and switchgrass (Lau and Dale, 2009; Zhong et al., 2009; Bals et al., 2010) where *S. cerevisiae* 424A was used to ferment ethanol.

Harvest Strategy Comparison. Corn stover left in the field is important for maintaining soil organic carbon levels and preventing soil erosion (Cruse and Herndl, 2009; Wilhelm et al., 2007). Loam, silt loam, and silt clay loam soils require 5.25 or 7.58 Mg ha^{-1} of stover for continuous corn cropping system with no conservation tillage or moldboard plow, and 7.90 or 12.50 Mg ha^{-1} stover for corn–soybean cropping system with no conservation tillage or moldboard plow just to maintain current soil organic carbon levels (Wilhelm et al., 2007). Thus, about 30% of stover could be removed as cellulosic ethanol feedstock in a continuous no-till corn system, which would increase total ethanol yield on a land area basis by 10.4% (472.7 L ha^{-1}) compared with harvesting and processing corn grain alone. Analogous to conventional corn silage cropping systems, manure applications, cover crops, or other management practices to augment soil carbon would likely be necessary for bioethanol whole-plant corn harvest strategies (Thelen et al., 2010).

Ethanol yield on a land area basis (Table 4) was estimated by multiplying ethanol production by biomass yield. Harvesting whole plant at maturity (MWP) had a higher ethanol yield (4431–8279 L ha^{-1}) than the source separated strategy (grain + cob + 30% stover) and green-cut whole-plant harvest

Table 4. Ethanol yield (L ha^{-1}) from four locations in Michigan in 2 yr as affected by harvest strategy.

	Branch		Ingham		Huron		Menominee		
Strategy†	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**Average‡**
ImF	6282ab	5544b	5940ab	5946b	7291a	5812ab	3829b	4788b	5679b
ImS	5745bc	4992c	5474bc	5732b	7659a	4990b	3929b	4844b	5294b
MSep	5382c	5721b	4896c	6269ab	6532a	5385b	3534b	4750b	5297b
MSep-G	4824	5109	4083	5374	5581	4489	2895	4135	
MSep-S	880	719	1242	1290	1560	1418	1166	1188	
MSep-C	294	396	441	508	483	471	290	259	
MWP	6595a	6228a	6240a	7573a	7780a	6521a	4774a	5861a	6446a

† Harvest strategy: ImF, immature cut whole-plant harvested at R5 stage with fresh processing; ImS, immature cut whole-plant harvest at R5 stage with ensiled post-harvest treatment; MSep, mature cut source separated harvest (grain MSep-G, stover MSep-S, and cob MSep-C fractions), and only 30% stover was included in harvest; MWP, mature cut whole-plant harvest; for whole-plant harvest strategies ImF, ImS, and MWP, 100% stover was included in harvest.

‡ Average values of harvest strategies from four locations, statistical analysis was conducted among strategies. The different letters (a, b and c) after values indicate significant difference ($p < 0.05$).

regardless of whether it was ensiled or not. Postharvest ensiling (ImS) did not decrease ethanol yield (5294 ± 1052 L ha^{-1}) significantly when compared with fresh processing of the green cut whole-plant fraction (ImF) (5679 ± 1046 L ha^{-1}), which indicates that ensiling can be considered as an easily adapted storage method for immature cut whole-plant harvesting before bioprocessing. The ethanol yield assuming 30% stover harvest following grain harvest was not significantly different than green cut whole plant fractions ImF and ImS.

CONCLUSIONS

This study provided detailed information on sugar contents and ethanol yield from different corn fractions and harvest strategies. Composition analyses showed no difference between ImF and ImS samples in glucan, xylan, arabinan, acid-insoluble lignin, and ash content. Glucan contents in whole plant fractions (MWP, ImF, and ImS) were higher than that in MSep-S and MSep-C source separated fractions. Ethanol production (% kg kg^{-1}) from MWP was higher than ImF and ImS. From a cropping systems perspective, the earlier harvest associated with an immature cut system facilitates integration of a cover crop, manure applications, or other strategies to augment carbon losses associated with the whole-plant harvest. Although 6% dry biomass loss was estimated during the ensiling process (Johnson et al., 2003), there was no significant decrease in ethanol yield from ImS compared to ImF. This result indicates that ensiling may be an effective postharvest storage method that would facilitate a year-round feedstock supply to the bioprocessor.

Ethanol yield on a land area basis was higher for MWP relative to the source separated strategy of harvesting with 30% stover and processing the grain, cob, and stover fractions separately (MSep). This result suggests that the efficiencies gained with the one-pass harvest and single bioprocessing stream associated with the whole-plant harvest strategy make it a viable alternative to the current proposed strategy of separating the starch (grain) and cellulosic (stover/cobs) feedstock.

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REFERENCES

Alizadeh, H., F. Teymouri, T.I. Gilbert, and B.E. Dale. 2005. Pretreatment of switchgrass by ammonia fiber explosion (AFEX). Appl. Biochem. Biotechnol. 121:1133–1141.

Atchinson, J.E., and J.R. Hettenhaus. 2004. Innovative methods for corn stover collecting, handling, storing and transporting. Subcontractor Rep. NREL/SR-510-33893. Natl. Renewable Energy Lab., Golden, CO.

Balan, V., L. da Costa Sousa, S.P.S. Chundawat, D. Marshall, L.N. Sharma, C.K. Chambliss, and B.E. Dale. 2009. Enzymatic digestibility and pretreatment degradation products of AFEX-treated hardwoods (*Populus nigra*). Biotechnol. Prog. 25:365–375.

Bals, B., C. Rogers, M. Jin, V. Balan, and B.E. Dale. 2010. Evaluation of ammonia fibre expansion (AFEX) pretreatment for enzymatic hydrolysis of switchgrass harvested in different seasons and locations. Biotechnol. Biofuels 3:1–12.

Buyanovsky, G., and G. Wagner. 1986. Post-harvest residue input to cropland. Plant Soil 93:57–65.

Cherney, D.J.R., J.H. Cherney, and W.J. Cox. 2004. Fermentation characteristics of corn forage ensiled in mini-silos. J. Dairy Sci. 87:4238–4246.

Chundawat, S.P.S., R. Vismeh, L.N. Sharma, J.F. Humpula, L. da Costa Sousa, C.K. Chambliss, A.D. Jones, V. Balan, and B.E. Dale. 2010. Multifaceted characterization of cell wall decomposition products formed during ammonia fiber expansion (AFEX) and diluted acid based pretreatments. Bioresour. Technol. 101:8429–8438.

Congress. 2007. Energy Independence and Security Act of 2007. 110th Congress, Washington, DC.

Cruse, R.M., and C.G. Herndl. 2009. Balancing corn stover harvest for biofuels with soil and water conservation. J. Soil Water Conserv. 64:286–291.

Garlock, R., S.P. Chundawat, V. Balan, and B. Dale. 2009. Optimizing harvest of corn stover fractions based on overall sugar yields following ammonia fiber expansion pretreatment and enzymatic hydrolysis. Biotechnol. Biofuels 2:29–42.

Gaspar, M., G. Kalman, and K. Reczey. 2007. Corn fiber as a raw material for hemicellulose and ethanol production. Process Biochem. 42:1135–1139.

Ghose, T.K., and R.D. Tyagi. 1979. Rapid ethanol fermentation of cellulose hydrolysate: II. Product and substrate inhibition and optimization of fermentor design. Biotechnol. Bioeng. 21:1401–1420.

Hendriks, A.T.W.M., and G. Zeeman. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresour. Technol. 100:10–18.

Huang, H.J., S. Ramaswamy, W. Al-Dajani, U. Tschirner, and R.A. Cairncross. 2009. Effect of biomass species and plant size on cellulosic ethanol: A comparative process and economic analysis. Biomass Bioenergy 33:234–246.

Johnson, L.M., J.H. Harrison, D. Davidson, W.C. Mahanna, and K. Shinners. 2003. Corn silage management: Effects of hybrid, maturity, inoculation, and mechanical processing on fermentation characteristics. J. Dairy Sci. 86:287–308.

Kadam, K.L., and J.D. McMillan. 2003. Availability of corn stover as a sustainable feedstock for bioethanol production. Bioresour. Technol. 88:17–25.

Kumar, P., D.M. Barrett, M.J. Delwiche, and P. Stroeve. 2009. Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. Ind. Eng. Chem. Res. 48:3713–3729.

Lau, M.W., and B.E. Dale. 2009. Cellulosic ethanol production from AFEX-treated corn stover using Saccharomyces cerevisiae 424A(LNH-ST). Proc. Natl. Acad. Sci. USA 106:1368–1373.

Lau, M.W., B.E. Dale, and V. Balan. 2008. Ethanolic fermentation of hydrolysates from ammonia fiber expansion (AFEX) treated corn stover and distillers grain without detoxification and external nutrient supplementation. Biotechnol. Bioeng. 99:529–539.

Lemuz, C.R., B.S. Dien, V. Singh, J. McKinney, M.E. Tumbleson, and K.D. Rausch. 2009. Development of an ethanol yield procedure for dry-gring corn processing. Cereal Chem. 86(3):355–360.

Michigan State University. 2008. MSU-Extension-431. 2008 Michigan corn hybrids compared. Ext. Bull. E-43. Michigan State Univ., East Lansing.

Michigan State University. 2009. MSU-Extension-431. 2009 Michigan corn hybrids compared. Ext. Bull. E-431. Michigan State Univ., East Lansing.

Mueller, J.P., J.T. Green, and W.L. Kjelgaard. 1991. Corn silage harvest techniques (NCH-49). Available at http://www.extension.purdue.edu/extmedia/NCH/NCH-49.html (accessed 8 Dec. 2010; verified 14 Mar. 2011). National corn handbook. Coop. Ext. Serv., Purdue Univ., West Lafayette, IN.

Murnen, H.K., V. Balan, S.P.S. Chundawat, B. Bals, L. da Costa Sousa, and B.E. Dale. 2007. Optimization of ammonia fiber expansion (AFEX) pretreatment and enzymatic hydrolysis of Miscanthus x giganteus to fermentable sugars. Biotechnol. Prog. 23:846–850.

NCGA. 2009. 2009 World of corn report. Making the grade. Available at http://ncga.com/files/pdf/2009WOC.pdf (accessed 6 Dec. 2010; verified 14 Mar. 2011). Natl. Corn Growers Assoc., Chesterfield, MO.

NREL. 2004. Chemical Analysis and Testing (CAT) Standard Procedures. Natl. Renewable Energy Lab., Golden, CO.

SAS Institute. 2006. The SAS system for Windows. Release 9.1.3. SAS Inst., Cary, NC.

Sedlak, M., and N.W. Ho. 2004. Production of ethanol from cellulosic biomass hydrolysates using genetically engineered saccharomyces yeast capable of cofermenting glucose and xylose. Appl. Biochem. Biotechnol. 114:403–416.

Shao, Q., S.P. Chundawat, C. Krishnan, B. Bals, L. da Costa Sousa, K.D. Thelen, B. Dale, and V. Balan. 2010. Enzymatic digestibility and ethanol fermentability of AFEX-treated starch-rich lignocellulosics such as corn silage and whole corn plant. Biotechnol. Biofuels 3:12–21.

Shinners, K.J., G.S. Adsit, B.N. Binversie, M.F. Digman, R.E. Muck, and P.J. Weimer. 2007. Single-pass, split-stream harvest of corn grain and stover. Trans. ASABE 50:355–363.

Shinners, K.J., B.N. Binversie, and P. Savoie. 2003. Whole-plant corn harvesting for biomass: Comparison of single-pass and multi-pass harvest syetems. ASAE Pap. 036089. ASAE, St. Joseph, MI.

Singh, V., and V. Graeber. 2004. Effects of corn hybrid variability and planting location on dry grind ethanol production. Trans. ASABE 48:709–714.

Sokhansanj, S., A. Turhollow, J. Cushman, and J. Cundiff. 2002. Engineering aspects of collecting corn stover for bioenergy. Biomass Bioenergy 23:347–355.

SYSTAT Software. 2007. SigmaPlot exact graphs and data analysis. Release 10.0. SYSTAT Software, Chicago, IL.

Teymouri, F., L. Laureano-Perez, H. Alizadeh, and B.E. Dale. 2004. Ammonia fiber explosion treatment of corn stover. Appl. Biochem. Biotechnol. 115:951–963.

Teymouri, F., L. Laureano-Perez, H. Alizadeh, and B.E. Dale. 2005. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover. Bioresour. Technol. 96:2014–2018.

Thelen, K.D., B.E. Fronning, S.A. Kravchenko, D.H. Min, and G.P. Robertson. 2010. Integrating livestock manure with corn-soybean bioenergy cropping systems improves short-term carbon sequestration rates and net global warming potential. Biomass Bioenergy 34:960–966.

Wallace, R., K. Ibsen, A. McAloon, and W. Yee. 2005. Feasibility study for co-locating and integrating ethanol production plants from corn starch and lignocellulosic feedstocks. NREL/TP-510-37092 and USDA-ARS 1935-41000-055-00D. Natl. Tech. Information Serv., Springfield, VA.

Wilhelm, W.W., J.M.F. Johnson, J.L. Hatfield, W.B. Voorhees, and D.R. Linden. 2004. Crop and soil productivity response to corn residue removal: A literature review. Agron. J. 96:1–17.

Wilhelm, W.W., J.M.F. Johnson, D.L. Karlen, and D.T. Lightle. 2007. Corn stover to sustain soil organic carbon further constrains biomass supply. Agron. J. 99:1665–1667.

Wyman, C.E., B.E. Dale, R.T. Elander, M. Holtzapple, M.R. Ladisch, and Y.Y. Lee. 2005. Coordinated development of leading biomass pretreatment technologies. Bioresour. Technol. 96:1959–1966.

Zhong, C., M.W. Lau, V. Balan, B. Dale, and Y. Yuan. 2009. Optimization of enzymatic hydrolysis and ethanol fermentation from AFEX-treated rice straw. Appl. Microbiol. Biotechnol. 84:667–676.