2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

An ASABE Meeting Presentation
Paper Number: 1110797

WHOLE-PLANT CORN AS BIOMASS FEEDSTOCK: HARVEST, STORAGE AND PRETREATMENT

David E. Cook – Graduate Research Assistant
Kevin J. Shinners – Professor of Agricultural Engineering
Department of Biological Systems Engineering
University of Wisconsin
Paul J. Weimer – Research Microbiologist
Richard E. Muck – Research Engineer
US Dairy Forage Research Center – USDA-ARS
Madison, WI

Written for presentation at the
2011 ASABE Annual International Meeting
Sponsored by ASABE
Galt House
Louisville, Kentucky
August 7 – 10, 2011

Abstract. *This research investigated the harvest, ambient pre-treatment, and storage of whole-plant corn as an alternative to conventional systems where corn grain and stover are fractionated at harvest. Harvesting the whole-plant, both grain and most of the above ground stover, after physiological maturity can reduce the intense logistics challenges typically associated with corn harvest and expand the harvest window. To determine the feasibility of the proposed system, corn was harvested at 65 to 16% whole-plant moisture (w.b.) using a forage harvester and then ensiled in lab scale silos. Ambient pretreatment during storage was investigated using dilute acid and lime. Both pretreated and control whole-plant silages were well conserved during anaerobic storage with DM losses generally less than 4%. Hydrodynamic separation of the grain and stover fractions after storage was found to be more effective at fractionating starch and fiber than dry grain harvest, and both fractions had desirable composition. Pretreatment was effective at degrading the hemicellulose in the cell wall, with up to 93% removal. It also significantly enhanced enzymatic degradability and subsequent fermentation to ethanol, increasing the cellulose conversion efficiency by 19, 11, and 4 percentage units for sulfuric acid pretreatments of 100, 30, and 10 g(kg DM)-1, respectively. The whole-plant harvest and storage system shows promise as a viable alternative to conventional corn grain and stover systems for producing feedstocks for biochemical conversion.*

Keywords. *Biomass; corn stover; costs, economics.*

Mention of trade names in this manuscript are made solely to provide specific information and do not imply endorsement of the product or service by the University of Wisconsin-Madison.

WHOLE-PLANT CORN AS BIOMASS FEEDSTOCK: HARVEST, STORAGE AND PRETREATMENT

The authors are **David E. Cook and Kevin J. Shinners**, Department of Biological Systems Engineering, University of Wisconsin; and **Paul J. Weimer and Richard E. Muck**, US Dairy Forage Research Center, USDA-ARS. **Corresponding author:** Kevin J. Shinners, 460 Henry Mall; Madison, WI 53706; 608-263-0756; kjshinne@wisc.edu

ABSTRACT

This research investigated the harvest, ambient pre-treatment, and storage of whole-plant corn as an alternative to conventional systems where corn grain and stover are fractionated at harvest. Harvesting the whole-plant, both grain and most of the above ground stover, after physiological maturity can reduce the intense logistics challenges typically associated with corn harvest and expand the harvest window. To determine the feasibility of the proposed system, corn was harvested at 65 to 16% whole-plant moisture (w.b.) using a forage harvester and then ensiled in lab scale silos. Ambient pretreatment during storage was investigated using dilute acid and lime. Both pretreated and control whole-plant silages were well conserved during anaerobic storage with DM losses generally less than 4%. Hydrodynamic separation of the grain and stover fractions after storage was found to be more effective at fractionating starch and fiber than dry grain harvest, and both fractions had desirable composition. Pretreatment was effective at degrading the hemicellulose in the cell wall, with up to 93% removal. It also significantly enhanced enzymatic degradability and subsequent fermentation to ethanol, increasing the cellulose conversion efficiency by 19, 11, and 4 percentage units for sulfuric acid pretreatments of 100, 30, and 10 g(kg DM)$^{-1}$, respectively. The whole-plant harvest and storage system shows promise as a viable alternative to conventional corn grain and stover systems for producing feedstocks for biochemical conversion.

INTRODUCTION

The current harvest and logistics system for corn grain for ethanol production after the crop is near maturity is as follows: combine, transport, dry, store, transport, rehydrate in an acidic environment, wet milling and separation, saccharification, fermentation, distillation, and dehydration. The generally considered method for corn stover to ethanol production is as follows after grain harvest: field dry the stover, windrow, bale, transport, store, transport, rehydrate, pretreat, saccharification and fermentation, distillation, and dehydration. Both systems rely on drying the feedstock for satisfactory aerobic storage followed by a rehydration. This drying is costly in terms of weather risks, energy inputs, and harvest timeliness. Drying of cellulose microfibrils results in the irreversible shrinking of the pore space, reducing the accessible surface area (Esteghlalian et al., 2001). Additionally, the rehydration adds to the water requirements of biorefineries.

A new system is proposed here which intends to lower the cost of both the starch and cellulose factions of the corn plant destined for ethanol production. This “whole-plant silage” system simplifies these fractionated systems into the following single-pass system: whole-plant harvest with a forage harvester, transport, anaerobic storage, transport, grain and stover fractionation, pretreatment, saccharification followed by fermentation, distillation, and

dehydration. This method of harvest attains all of the desirable goals of cellulosic biomass harvesting: a single-pass harvest for low soil contamination, weight limiting transport density, a size-reduced flowable material from the field, reduced system energy inputs, and high yields. The costs attributed to the grain harvest and storage are reduced by eliminating grain drying, while the harvesting and storage costs for stover and grain are similar to the costs for grain only harvest and storage (Cook and Shinners, 2011). Due to the harvest of a moist crop, the grain and stover must be stored anaerobically and conserved by fermentation to reserve crop value. Grain losses during storage in this fashion are a primary concern, as the grain comprises most of the value of the crop

Transportation of the combined grain and stover fractions is advantageous over a fractionated crop transport because of the balancing of weight and volume limitations of each; grain is significantly weight limited, while stover is significantly volume limited. By balancing these two, the monetary and energy costs of biomass densification can be avoided, while still achieving the objective of a legal weight load.

Moist, bulk storage offers the opportunity for "on-farm" pretreatment of biomass. This ambient condition pretreatment could also be utilized at regional aggregation facilities as well. Sulfuric acid has been shown to readily hydrolyze the hemicellulose sugar arabinose. By degrading this sugar, the cell wall matrix begins to degrade significantly (Digman et al., 2007). Acid pretreatment of perennial grasses was effective at both preserving substrate and degrading the cell wall matrix to enhance cellulose hydrolysis in subsequent steps (Digman et al., 2007). Further the residual sulfuric acid present in the substrate at removal from storage could be used to further degrade the cell wall matrix by additional thermal processing (Digman et al., 2008). Calcium hydroxide has the effect of swelling cellulose fibers, delignification, and solubization of xylan, thereby improving cellulose accessibility.

Our primary objective was to investigate the feasibility of co-harvest and storage of both grain and stover to achieve an overall lower cost system for both fractions. Toward this goal we investigated the effect of whole-plant moisture content and ambient pretreatment on conservation at the laboratory scale. Compositional analysis and enzymatic availability were used to quantify the effectiveness of the proposed system on feedstock conservation and subsequent biochemical conversion.

MATERIALS AND METHODS

SUBSTRATE

Whole-plant corn was harvested from plots in 2009 and 2010 at the University of Wisconsin Arlington Agricultural Research Station located near Arlington, WI. In 2009, DeKalb 6169, a 111-day comparative relative maturity (CRM) corn, was planted on May 6th and first harvest occurred on Oct. 8, 2009. In 2010, Dekalb 57-79, a 107-day CRM corn, was planted on May 27th and first harvest occurred on Sept. 28, 2010. Harvesting was done with a New Holland 900 experimental pull-type forage harvester (PTFH) which was configured to allow the crop to bypass the kernel processor. In 2009, three different theoretical-length-of-cut (TLC) were utilized: 19, 25, and 38 mm. The results from 2009 indicated no significant differences in storage losses or fermentation acid production between the different TLC treatments, so in 2010

only the 19 mm TLC was used. Composition results from 2009 are pooled results, one replicate from each TLC were combined to result in three replicates for each harvest date and pretreatment. No matter the harvest conditions, it was observed that after processing through the PTFH, the grain was fully removed from the cob at harvest. From each harvest or treatment where TLC was varied, three sub-samples were taken for particle-size analysis using ASABE Standard S424.1 (ASABE, 2007) and kernel damage assessment. The kernel damage assessment follows the methods described in Shinners et al. (2000).

During the 2010 harvests, from the same field, several ears of corn were hand harvested, shelled, and the grain dried in a 60°C forced air oven until they reached approximately 13.5% moisture content (MC). Five sub-samples of hand shelled grain from each harvest were dried for 72 h in a 60°C forced air oven for constituent analysis, and another five sub-samples were used for quantifying starch digestibility. This dry shell corn was a control to compare against the grain from the ensiled control grain samples for starch, starch digestibility, grain fiber, and soluble carbohydrates assays.

EXPERIMENTAL DESIGN

The 2009 a 4 x 2 replicated experimental design was used to investigate the effect of plant moisture and acid pretreatment (0 or 100 g sulfuric acid (kg DM)$^{-1}$). Harvest dates and wet basis (w.b.) moisture contents were 09-Oct (65%); 27-Oct (56%); 12-Nov (40%); and 14-Dec (34%). In 2010 a 3 x 4 experimental design was used to investigate the effect of plant moisture and pretreatment. Harvest dates and w.b. moisture contents (whole plant / grain) were 28-Sept (45% / 29%); 07-Oct (34% / 19%); and 19-Oct (16% / 16%). Pretreatments applied to the crop were 0, 10, or 30 g sulfuric acid (kg DM)$^{-1}$ and the forth pretreatment was 10 g calcium hydroxide (kg DM)$^{-1}$. Each treatment was replicated three times per harvest date.

LAB-SCALE SILOS

After harvest and prior to treatment and storage, the substrate was sub-sampled and analyzed for dry matter (DM) content using a microwave oven according to ASABE Standard S358.2 (ASABE, 2008) so that amendments, if any, could be applied on a DM basis.

From each replicate lab-scale silo, one sub-sample was taken for separation of the grain and stover fractions and two sub-samples were then taken for moisture content and two for constituent analysis. Moisture and constituent sub-samples were dried at 60°C for 72 hours in a forced air oven following ASABE Standard S358.2 (ASABE, 2008). All substrates were homogenized with a commercial kitchen mixer and pretreated, if applicable. Pretreatment amendments were applied by top dressing over the course of the two minutes the substrate was in the mixer. After mixing for two minutes, the chopped whole-plant corn (either with and without pretreatment) was then transferred and packed into 19 l plastic bucket containers, sealed for storage and stored indoors at approximately 20°C for 120 days in 2009 and 60 days in 2010. These lab-scale silos were filled with 4.3 kg organic matter (OM) and compacted using a hydraulic cylinder and platen to a target density of 225 kg OM(m)$^{-3}$. The lab -scale silos were sealed by a snap-on lid, with a neoprene gasket in the rim of the lid. After the filling and sealing was complete, the silo was weighed to the nearest 0.01 kg.

FRACTION SEPARATION

In 2009, sub-samples used for fractionating the grain and stover were taken prior to pretreatment and storage and dried in a forced air oven at 60°C for 72 hours following ASABE Standard S358.2 (ASABE, 2008). The sub-samples were then separated by hand into two grain fractions (damaged and undamaged) and stover. Constituent analysis was conducted on the separated stover and undamaged grain fractions.

In 2010, the fractionation sub-samples (~200g DM) taken prior to pretreatment and storage, were fractionated at harvested moisture into a "grain fraction" and a "stover fraction" on the basis of differences in specific gravity, using a previously developed hydrodynamic fractionation technique (Savoie et al., 2004). The sample was placed into water and underwent a single floatation step. All material that floated was considered the "stover fraction" and all material that sank was considered the "grain fraction". The water was decanted off the grain through wire cloth with an opening size of 0.086 mm; all material left on the screen was included as part of the stover fraction. A sample of the water was taken to evaluate any solids and solubles that dissociated into the liquid fraction.

REMOVAL PROCEDURE

The mass of each lab-scale silo and its contents were weighed to the nearest 0.01 kg at the time of removal from storage. The contents of each silo were then removed and homogenized by hand prior to sub-sampling. Two sub-samples from each silo were taken for moisture content determination in a forced-air oven at 60°C for 72 hours (ASABE, 2008). In 2009, a subsample of about 300g DM was taken and frozen for constituent analysis of the grain and stover fractions. The remainder of the silage was size-reduced in a hammermill with a 32 mm screen, sub-sampled into plastic bags, and frozen for constituent analysis of the whole-plant silage. Another sub-sample of about 300g DM was taken for hydrodynamic separation using the method described above.

In 2010 the removal technique was modified to reduce sampling error in a heterogeneous material that was too easily fractionated by mechanical handling. Upon removal from storage, a sub-sample was taken to evaluate moisture content, and the remainder was hydrodynamically separated, using the method described above. Total approximate mass of each of the three fractions; grain, stover, and liquid (typically 2-3 kg DM; 1-2 kg DM; and 40-50 kg, respectively) was measured to the nearest 0.01 kg and each fraction was analyzed separately. In this way more accurate sub-sampling was enabled, and total analysis could be inferred by summation of the three fractions. From each of these fractions, two sub-samples were taken for moisture content and a sub-sample was taken and frozen for further preparation and analysis. From the grain fraction, two additional sub-samples (about 1 kg DM) were taken and stored frozen for starch digestibility assay.

COMPOSITIONAL ASSAYS

Post-storage sub-samples were frozen prior to preparation for constituent analysis. All of these samples were assayed for fermentation products and pH. Then the samples were titrated to neutral pH using 10M sodium hydroxide or 24N sulfuric acid, and the suspension was subsequently frozen and freeze-dried. The dry pre- and post-storage samples were then ground in a Wiley mill through a 1 mm screen. Finally, the grain samples had a portion further sub-

sampled that would be evaluated for starch content. This starch assay sub-sample was ground in a vortex mill (Udy Corporation, Fort Collins, CO) through a 1 mm screen.

Post-storage samples to be assayed for fermentation products and titrated were first mixed in a stomacher (Tekmar, Stomacher Lab Blender 400). From the suspension a 1.5 mL sub-sample was drawn and centrifuged to have a cell-free supernatant to analyze for fermentation products. The prepared supernatant was measured by high performance liquid chromatography (HPLC, Varian Model 410, Varian Inc., Palo Alto, CA) with a refractive index detector, for the following fermentation products: lactate, acetate, butyrate, proprionate, ethanol, succinate, 1,2 -propanediol, and 2,3 -butanediol. Samples were injected (100uL) onto an organic acid column (HPX-87H, Bio Rad Labratories Inc., Hercules, CA) and eluted with 0.015 N H_2SO_4 in 0.0034 M EDTA free acid at 0.7 ml(min)$^{-1}$ at 45˚C.

Wet chemistry constituent analysis was done by Dairyland Labs (Arcadia, WI) of prepared samples for ash-corrected neutral detergent fiber (NDF), ash-corrected acid-detergent fiber (ADF), ash-corrected acid detergent lignin (ADL), water-soluble carbohydrates (WSC), ash, and crude protein (CP). Fiber analysis was done using the crucible method (Goering and Van Soest, 1970), and WSC assay was done using the phenol-sulfuric method (Dubois et al., 1956). Dairyland Labs also conducted sulfur analysis using inductively coupled plasma mass spectrometry. A slightly modified starch digestibility assay was also conducted. The typical method for *in vitro* starch digestibility (IVSD) used by Dairyland Labs involves grinding the dry sample through a 4 mm Wiley mill and conducting a rumen fluid digestion assay. Due to the acid pretreatment of the samples and acid hydrolysis of the starch that would occur during drying, the frozen samples were ground through a 4 mm Wiley. This was followed by the rumen fluid digestion assay. Crude protein was assayed in some water samples, but no nitrogen was found in any of the samples assayed, so further testing was discontinued.

Cellulose content was estimated in the samples by the difference between ash-corrected acid-detergent fiber and ash-corrected acid detergent lignin. Hemicellulose was estimated in the samples by the difference between ash-corrected neutral detergent fiber and ash-corrected acid detergent fiber. All fiber assays were ash-corrected; therefore they were non-sequential assays.

ETHANOL YIELDS BY SSF

Ethanol and pentose sugar yields were determined using a modified simultaneous saccharification and fermentation (SSF) method (Dowe and McMillan, 2001). Yeast, *Saccharomyces cerevisiae* strain D5a, was grown overnight in a sterile culture of 10 g(l)$^{-1}$ yeast extract, 20 g(l)$^{-1}$ peptone, and 50 g(l)$^{-1}$ glucose at 35^{o}C while shaking at 250 rpm. The yeast cells were harvested by centrifugation (10,000 x g for 20 min) and were resuspended in a sterile solution of 200 mL water and 0.2 g of peptone.

A sterile inoculation solution was made from 1.842 L H_2O, 30 g peptone, 40 g yeast extract, and 100 mL of a citrate buffer (1 M sodium citrate added to 1 M citric acid until pH reaches 4.8). To this solution, the yeast suspension, Celluclast 1.5L (Novozymes, Bagsvared, Denmark) at 5 FPU(g)$^{-1}$ dry stover, Novozyme 188 beta-glucosidase (Novozymes, Bagsvared, Denmark) at 40 IU(g)$^{-1}$ cellulose, and 8 mL of a sterile tetracycline solution (10 g(L)$^{-1}$) were added.

A 1 g DM aliquot of the freeze-dried stover was added to a 30 ml media bottle and then autoclaved. To the substrate 10 mL of the inoculation solution was added, resulting in a solids loading of 10%. Fermentation was conducted for 72 h at 35°C with gentle shaking (150 rpm). After 72 h had elapsed, the bottles were put in ice and sampled for analysis by the HPLC described above. The samples were prepared and assayed using the method described in the post-fermentation products section. To account for assay inefficiency due to contamination, any lactate or acetate that was produced during SSF was converted to potential ethanol yield on a one mole lactate or acetate to one mole ethanol basis.

Cellulose conversion efficiency (η) was estimated on the basis of percent of theoretical ethanol production from cellulose. The ethanol produced was divided by 0.569 times the sample cellulose content, 0.569 being the theoretical yield of ethanol production from glucose on a mass basis.

$$\eta = \left(\frac{E_p}{(C)\ 0.569}\right)$$

where: E_p ethanol produced (g)

C cellulose in the sample (g)

RESULTS

PRE-STORAGE SUBSTRATE COMPOSITION

The composition and moisture content of the pre-storage substrate is summarized in table 1. In 2009, approximately 52% of the whole-plant corn organic matter (OM) harvested was grain and 48% was stover, while in 2010, approximately 60% of the whole-plant corn OM harvested was grain and 40% was stover.

In 2010, the grain was fractionated in two ways, by hand shelling and hydrodynamic separation of the grain from the whole-plant substrate. The compositional analysis showed that hydrodynamic separation of the chopped corn was more effective at concentrating the starch in the grain fraction than hand shelling corn (table 1). The reason for this is likely that during the hydrodynamic separation, many corn seed coats were observed to be removed in the water and remained with the stover fraction, while a small amount of stover resided with the grain fraction. The hydrodynamic fractionation process resulted in some soluble carbohydrates going into the liquid fractionation so the WSC content of the fractioned grain was less than that of the shell corn. Early harvest maturity resulted in greater WSC content in the whole-plant silage at the time of harvest. For harvests first through last harvests, the whole-plant WSC were 44, 39, and 35 g $(kg\ OM)^{-1}$, respectively. These differences were all found to be significant at $P<0.05$.

Table 1. Pre-storage composition of whole-plant corn (grain plus stover) and separated grain and stover fractions harvested in 2009 and 2010 and shell corn harvested in 2010, averaged across all experiments conducted in each year.

Year	Fraction	Starch	WSC	Cellulose	Hemicellulose	ADL	Ash
		g (kg OM)$^{-1}$					
2009	Grain[a]	721	26	-[e]	-[e]	-[e]	17
"	Stover[a]	37	53	430	276	67	58
"	Whole-plant	367	38	223	171	35	38
2010	Shell Corn [b]	709b	33a	8a	43a	12a	12a
"	Grain [b, c]	722a	24b	8a	35a	13a	11a
	LSD[b, d]	9[d]	4[d]	5[d]	11[d]	4[d]	2[d]
"	Stover[c]	17	33	444	297	77	53
"	Whole-plant	414	39	179	155	45	30

[a] Grain and stover fraction hand separated from the whole-plant mass.
[b] Statistical analysis was only conducted on averages between shell corn and fractionated grain.
[c] Grain and stover separated hydrodynamically from whole-plant mass.
[d] Least significant difference. Means within columns with different letters are statistically different at $P < 0.05$.
[e] Fiber analysis was not quantified for the grain fraction in 2009.

STORAGE LOSSES

Following storage, losses of DM were evaluated by the difference in the DM content post- and pre- storage, expressed as the percent reduction of initial mass. Total dry mass was estimated by weighing the WM of the silo contents and sub-sampling for DM content. The DM losses were converted to OM losses by adjusting for the ash content found post-storage.

In 2009, there were significant differences in losses between treatments only for material harvested at 65% (w.b.) moisture (table 2). On the remaining three harvest dates there were no significant differences and losses were less than 3% of OM. However losses were numerically lower at each subsequent harvest date. The maximum allowable loss to achieve an economic advantage over fractionated grain and stover harvest systems was found to be 16% (Cook and Shinners, 2011) and our goal was to achieve less than 5% loss. In all treatments, except the control at 65% (w.b.) moisture, OM losses were less than 3%, indicating the moist anaerobic conditions conserved the substrate well and met the desired economic threshold.

Storage losses were statistically less at each subsequent harvest date for all treatments in 2010 (table 2). This was consistent with other anaerobic storage studies (Shinners et al., 2010; Williams and Shinners, 2011). The acid pretreatment resulted in numerically lower storage losses by immediately lowering the substrate pH and inhibiting microbial growth. The lime pretreatments in the first two harvests of 2010 had high OM losses that were a result of poor storage conditions due to a low loading of lime that was insufficient to cause a pH greater than 8. By keeping the pH near neutral, the silage pH never inhibited anaerobic microbial growth. Here, only the lime treatment from the first harvest of the year exceeded the 5% storage loss benchmark.

Table 2. Storage losses of whole-plant silage (grain plus stover) as percent of initial OM for 2009 and 2010 harvests and treatments.

Harvest Date (2009)	9-Oct	27-Oct.	12-Nov.	14-Dec.	Average by Treatment[b]
Moisture (% w.b.)	65	56	40	34	
Control	5.7b	2.3a	2.4a	1.0a	2.8a
Acid – 10%	0.5a	2.8a	1.3a	2.7a	1.8a
LSD[a]	3.7	2.1	1.8	1.9	1.2
Average by Date[c]	3.1a	2.5a	1.9a	1.9a	LSD[d] = 2.6
Harvest Date (2010)	28-Sept.	7-Oct.	19-Oct.		Average by Treatment[b]
Moisture (% w.b.)	45	34	16		
Control	4.3a	3.2ab	1.7b		3.1ab
Acid – 1%	3.4a	2.8a	0.9ab		2.4a
Acid – 3%	2.7a	1.8a	1.1ab		1.9a
Lime– 1%	7.5b	4.6b	0.8a		4.3b
LSD[a]	3.1	1.5	0.8		1.2
Average by Date[c]	4.5c	3.1b	1.1a		LSD[d] = 1.2

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.
[b] Data was pooled by treatment and analyzed using two-way analysis of variance.
[c] Data was pooled by harvest date and analyzed using two-way analysis of variance.
[d] Least significant difference for pooled data averaged across harvest date. Means within row with different letters are significantly different at $P < 0.05$.

POST-STORAGE FERMENTATION PRODUCTS

In 2009, the control treatment produced primarily lactate, acetate, and ethanol, with greater quantities at higher moisture levels (table 3). In untreated silages, lactic acid was the primary fermentation product, while in acid treated material acetic acid was the primary fermentation product. This occurs due to the exogenous acid immediately dropping the pH, reducing the time available for lactic acid producing microorganisms to flourish. Acetic acid can be created by cleaving the ester linkage of the acetyl groups from acylated xylans, and is likely the primary source of acetic acid production in the silages with high acid loading, rather than anaerobic fermentation. The pH for the control treatment, where sufficient moisture existed for fermentation to take place, generally stabilized near 4. This is similar to results with ensiled corn stover reported in Shinners et al. (2010). Butyrate was observed in very low levels.

In 2010, it was found that low levels of acid pretreatment (10 g sulfuric acid (kg substrate DM)$^{-1}$) resulted in greater levels of ethanol production and suppressed the production of lactic and acetic acid compared to the control (tables 4-5). The likely cause of this was the acid pretreatment effectively minimized bacterial growth, but not yeast growth. The fermentation products were greater in the stover fraction than the grain fraction. A likely cause of this is the lower buffering capacity of the grain. As fermentation products accumulate, the pH is brought down more quickly and further in the grain, ending further fermentation (tables 5).

Fermentation products dissociated into solution during hydrodynamic separation were variable and dependent on duration and agitation in the liquid. These variables were not strictly controlled, but were a function of the processing time for each silo, all of which were at least 15 minutes. An analysis of the fermentation products dissociated into the liquid fraction showed that 40% of total fermentation products dissociated into the liquid fraction during the fractionation procedure. The primary fermentation products lactate, acetate, and ethanol had average dissociations of 28%, 40%, and 50%, respectively. No statistical analysis was done due to its dependency on processing time, which was not a strictly controlled part of the experiment.

Table 3. Post-storage fermentation products for 2009 harvests of whole-plant silage (grain plus stover) after 120 days in storage for control with no pretreatment and pretreatment with sulfuric acid at a rate of 100g (kg DM)$^{-1}$.

Harvest Date	Moisture (% w.b.)	pH	Lactate	Acetate	Ethanol	Butyrate	Total
			g (kg OM)$^{-1}$				
			Control Treatment				
9-Oct.	65	3.9a	40.4c	27.7c	8.1bc	1.1a	83.6d
27-Oct.	56	3.9a	33.2b	11.3b	6.6b	1.1a	58.1c
12-Nov.	40	4.1ab	21.9a	7.7ab	4.3ab	0.6a	41.2b
14-Dec.	34	4.3b	16.8a	4.9a	2.0a	0.8a	29.2a
LSD[a]		0.1	6.6	3.9	3	1.2	7.1
Average by Date			28.1	12.9	5.2	0.9	53.0
			Acid Treatment				
Average by Date[b]		1.3	1.4	17.2	0.3	-	21.9

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.
[b] Harvest date had no significant effect on the fermentation products produced in the acid treated material.

Table 4. Post-storage fermentation products averaged across three harvest dates in 2010 for whole-plant silage (grain plus stover) after 60 days in storage for control with no pretreatment and pretreatment with sulfuric acid or lime.

	pH	Lactate	Acetate	Ethanol	Butyrate	Total
		g (kg OM)$^{-1}$				
Control	6.6c	8.1d	2.1b	7.7b	1.8b	21.4c
Acid – 1%	5.1b	1.7b	1.5a	9.9c	0.0a	13.3b
Acid – 3%	2.5a	0.0a	5.1c	0.9a	0.0a	6.2a
Lime – 1%	7.8d	4.6c	2.1b	7.6b	4.2c	20.7c
LSD[a]	0.3	0.9	0.5	1.4	0.6	2.7

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.

Table 5. Post-storage fermentation products averaged across three harvest dates in 2010 for grain and stover fractions hydrodynamically separated from the whole-plant silage after 60 days in storage for control with no pretreatment and pretreatment with sulfuric acid or lime.

Fraction	Treatment	pH	Lactate	Acetate	Ethanol	Butyrate	Total
			g (kg OM)$^{-1}$				
Grain	Control	4.8c	2.7d	0.7a	2.5c	0.5b	7.0c
"	Acid – 1%	4.1b	0.6b	0.5a	3.0d	0.0a	4.2b
"	Acid – 3%	2.2a	0.0a	2.0b	0.2a	0.0a	2.3a
"	Lime – 1%	5.8d	1.1c	0.3a	1.7b	0.8b	4.5b
"	LSD[a]	0.1	0.3	0.5	0.4	0.1	0.9
Stover	Control	5.6c	7.8d	1.9a	6.0c	1.4b	18.9c
"	Acid – 1%	3.9b	1.6b	1.5a	7.3d	0.0a	10.4b
"	Acid – 3%	2.4a	0.0a	4.2b	0.6a	0.0a	5.2a
"	Lime – 1%	6.8d	4.3c	2.2a	4.5b	4.0c	17.5c
"	LSD[a]	0.2	0.8	0.9	1.1	0.3	1.9

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.

POST-STORAGE COMPOSITION

Both the grain and stover fractions retained compositional characteristics that make them attractive for use as feed or fuel: low ash, high starch content in the grain, and high cellulose and hemicellulose components in the stover (table 6). Acid pretreatment had the effect of increasing WSC and decreasing hemicellulose, likely through acid hydrolysis of cell wall components. The post-storage control grain fraction had numerically greater starch and WSC content compared to pre-storage values for the shelled corn (table 1), one indication that grain quality was maintained during storage. In 2010, starch losses averaged across all moisture contents and treatments were 1%. When this evidence is coupled with the low overall dry matter losses, generally 3% or less (table 2), it may be inferred that starch losses were less than DM losses from the silage.

Degradation of hemicellulose in the cell wall by acid hydrolysis in the sulfuric acid pretreatment was very effective. At 10% acid loading hemicellulose was reduced by greater than 85% for the last three harvest dates in 2009 (Cook, 2011). In 2010 the 3% acid loading yielded hemicellulose degradation of greater than 35% on the last two harvest dates (table 7). The 1% acid loading pretreatment produced greater degradation of hemicellulose than the control only at the lowest crop moisture.

Table 6. Composition of whole-plant silage (grain plus stover) and separated stover and grain fractions after anaerobic storage for silage with no pretreatment averaged across all harvests in that year.

Year		Starch	WSC	Cellulose	Hemicellulose	ADL	Ash	CP
		g (kg OM)$^{-1}$						
2009	Grain	763	16	-[a]	-[a]	-[a]	16	74
	Stover	39	35	419	243	66	53	69
	Whole-plant	433	33	187	109	37	41	86
2010	Grain	731	19	-[a]	-[a]	13	14	64
	Stover	15	26	461	283	74	46	39
	Whole-plant	519	22	-[a]	-[a]	34	30	55

[a] Data not available.

Table 7. Ratio (%) of the post- to pre-storage content of hemicellulose or cellulose in the separated stover fraction for 2010 harvests.

Harvest Date	28-Sept.	7-Oct.	19-Oct.	Average[b]	Average[c]
Moisture (% w.b.)	45	34	16		
	Hemicellulose				Cellulose
Control	88a	93b	104c	96b	104a
Acid – 1%	97b	95b	85b	92b	101b
Acid – 3%	94ab	64a	48a	69a	105a
Lime – 1%	87a	94b	101c	94b	101b
LSD[a]	8	14	12	6	2

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.
[b] Hemicellulose data was pooled by date and analyzed using two-way analysis of variance.
[c] Cellulose ratio was not affected by harvest date, so data was pooled by date and analyzed using two-way analysis of variance.

POST-STORAGE LIQUID FRACTION

Analysis of the liquid fraction after hydrodynamic separation was done to assess the components leaving the substrate and going into solution or suspension. Overall, a small proportion of the whole (1 - 3% of DM) was dissociated into the separation liquid. The liquid fraction was assayed for WSC, ash, sulfur, and fermentation products (table 8). The constituents analyzed accounted for most of the DM in the liquid fraction. Further analysis for starch or other potential constituents was not attempted as the remaining components were diluted to the point that they were near the detectable limits of the assays. Almost half the fermentation products left the substrate during hydrodynamic separation, which may be an advantage if some fermentation products prove inhibitory to downstream processes.

ETHANOL YIELD

SSF results are yields of ethanol from *S. cerevesiae* D_5A, a yeast that converts glucose to ethanol, but does not utilize pentose sugars. Acid pretreatments were effective at increasing ethanol yields and cellulose conversion by hydrolyzing hemicellulose sugars and opening the cellulose up for enzymatic degradation (table 9). Increased acid loading improved the ethanol yield. Early harvest maturities had a significant impact on cellulose conversion efficiency. In 2009, the first harvest done at a whole-plant moisture content of 65% (w.b.) had a cellulose conversion efficiency 18 percentage units higher than the harvest conducted at 56% moisture content (table 10). The pretreatment by lime and sulfuric acid at 10g(kg DM)$^{-1}$ produced only a minor increase in cellulose conversion to 42% and 43%, respectively, from the control conversion efficiency of 38% (Cook, 2011).

Table 8. Components from substrate that were dissociated into the hydrodynamic separation liquid fraction, as a percent of the component of the whole.

Year	Treatment	WSC	Ash	Sulfur	Fermentation Products
2009	Control	7 a	14a	18 a	41a
	Acid – 10%	14 b	8a	33 b	39a
	LSD[a]	5	8	8	9
2010	Control	4 a	13 a	23 a	30 a
	Acid – 1%	6 a	23 b	48 b	46 b
	Acid – 3%	12 b	22 b	48 b	44 ab
	Lime – 1%	13 b	18 b	22 a	49 b
	LSD[a]	3	5	7	15

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.

Table 9. Comparison of pretreatment and no pretreatment prior to anaerobic storage and hydrodynamic fractionation on corn stover ethanol yield and percent of stover cellulose converted to ethanol by SSF.

Year	Treatment	Ethanol Yield ($l(Mg\ OM)^{-1}$)	Cellulose Conversion Efficiency (%)
2009[a]	Control	123b	41 b
	Acid – 10%	179a	60 a
	LSD[b]	7	2
2010[a]	Control	106c	32 c
	Acid – 1%	117b	36 b
	Acid – 3%	143a	43 a
	Lime – 1%	112bc	35 b
	LSD[b]	7	2

[a] Data averaged across all harvest dates and analyzed using two-way analysis of variance.
[b] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.

Table 10. Comparison of effects of harvest date in 2009 on percent of stover cellulose converted to ethanol by SSF following pretreatment, anaerobic storage, and hydrodynamic fractionation.

Harvest Date	9-Oct	27-Oct.	12-Nov.	14-Dec.	
Moisture (% w.b.)	65	56	40	34	Average by Treatment[b]
			% of theoretical ethanol yield		
Control	54 b	38 b	37b	36b	41b
Acid – 10%	76 a	58 a	55 a	52 a	60 a
LSD[a]	7	4	6	3	2
Average by date[c]	65 a	48 b	46 bc	44 c	LSD[d]=3

[a] Least significant difference. Means within columns with different letters are significantly different at $P < 0.05$.
[b] Data was pooled by treatment and analyzed using two-way analysis of variance.
[c] Data was pooled by harvest date and analyzed using two-way analysis of variance.
[d] Least significant difference for pooled data averaged across harvest date. Means within row with different letters are significantly different at $P < 0.05$.

DISCUSSION

The composition of the silage following storage has many characteristics desired for biochemical conversion, specifically low ash, high cellulose and hemicellulose contents (table 6). However, fermentation products are also present which may be inhibitory to some of the proposed biochemical processes. This may be partially overcome by their observed volatility and the propensity to dissociate in water (table 8). During a prolonged hydration process, it is expected most of the fermentation products would dissociate into the water, where they coiuld be recovered for other uses.

The moisture content of the whole-plant silage has a twofold advantage over dry feedstock. Drying the feedstock causes hornification of the biomass, resulting in a feedstock that is more resistant to enzymatic degradation. Secondly, moist feedstock brings along some of the water required for some biochemical conversions, resulting in a lower water requirement for the biorefinery. Although industrial drying of feedstocks has been proposed (Hess et al., 2009), this causes a much greater energy input to the system that moist feedstock systems are not saddled with. The greater moisture content can reduce the legal load of organic matter on a truck, increasing its transport costs, a cost factor that the advantages of moist systems need to be balanced against (Cook and Shinners, 2011).

The whole-plant silage of grain plus stover was well conserved during storage. The results of this work suggest that if this system was employed and harvest took place over 10 weeks, with whole-plant harvest moisture contents ranging from 60-15% and no pretreatments used to further conserve the crop, losses greater than 5% would be uncommon as long as anaerobic conditions are maintained (tables 2). Grain starch losses are an important concern with the proposed harvest system because the grain carries the majority of the value. Also of concern is the grain's ability to be converted to energy and the possible requirement of significant changes to an ethanol process that could be required to utilize corn grain originating from the silage system. Any impairment to the value of grain harvested, by storage losses or a less advantageous ethanol process, would have to be assigned to the stover. Due to its relatively low value compared to grain, only modest grain value impairment can be experienced in the system. Conversely, any net benefit this system provides to the grain would have a marked effect on further cost reductions to the stover fraction. To this end, starch losses were assessed following anaerobic storage. Given the low starch losses, higher starch concentration in the grain (tables 1 and 6), and low overall silage losses (table 2), these results gives strong evidence the starch fraction was very well conserved.

The effects of pretreatment on the silage were very pronounced for the 30 g(kg DM)$^{-1}$ and 100 g(kg DM)$^{-1}$ sulfuric acid pretreatments, particularly effective at lower moisture. At the high sulfuric acid loading, less than 10% of the hemicellulose remained bound in the cell wall at less than 50% moisture. All acid pretreatments were effective at inhibiting anaerobic fermentation during storage as seen by the low levels of fermentation products produced (tables 3 and 4). The pretreatment was also found to affect the grain fraction. The sulfuric acid would be expected to promote starch availability by a step similar to the steeping of corn in lactic acid and sulfuric acid prior to some milling processes; however, this was not found in this starch digestibility assay.

The hydrodynamic separation method was chosen over a mechanical system in an effort to replicate what was felt was the most likely industrial scale fractionation technique to be employed. The system seems to be the most logical choice as it results in a very high degree of starch and fiber fractionation. In fact, it was found to be more effective than a combine in the traditional dry grain system at separating the starch and fiber (72.2% starch of the hydrodynamic separated grain as compared to 70.9% for the shelled corn). The material needs to be rehydrated; therefore the water separation is plausible.

CONCLUSIONS

A new system to produce both corn grain and stover for biochemical conversion was proposed. Harvesting both grain and most of the above ground stover by chopping after physiological maturity can reduce the intense logistics challenges typically associated with corn harvest and expand the harvest window. Both the grain and stover fractions were well conserved by anaerobic fermentation across moistures from 65 to 16% (w.b.). Of particular importance is that the grain properties were well conserved. Following storage the stover and grain fractionated at least as well as dry grain harvest, and both fractions had desirable composition: low ash, high starch content in the grain, and high cellulose and hemicellulose components in the stover. Acid pretreatment prior to storage was effective at degrading the hemicellulose in the cell wall and enhanced enzymatic degradability and subsequent fermentation to ethanol. By all measures the storage of chopped whole-plant corn was successful so the proposed system warrants further investigation.

References

ASABE. 2007. Standard S424.1: Method of determining and expressing particle size of chopped forage materials by screening. ASABE St. Joseph, MI.

ASABE. 2008. Standard S358.2: Moisture measurement - forages. ASABE St. Joseph, MI

Cook, D.E. and K.J. Shinners. 2011. Economics of alternative corn stover logistics systems. ASABE Paper No. 1111130. ASABE St. Joseph, MI.

Digman, M. F., K.J. Shinners, B.S. Dien, R.D. Hatfield, X. Li and R.E. Muck. 2007. On-farm pretreatment technologies for improving enzymatic degredability of cellulose and hemicellulose present in perennial grass. ASABE Paper No. 071021. ASABE St. Joseph, MI.

Digman, M.F, K.J. Shinners, B.S. Dien, R.D. Hatfield, R.E. Muck and P.J. Weimer. 2008. Investigation of a two-stage process for increasing yields of ethanol from switchgrass. ASABE Paper No. 084954. ASABE St. Joseph, MI.

Digman, M. F., K.J. Shinners, R.E. Muck and B.S. Dien. 2010. Pilot-scale on-farm pretreatment of perennial grasses with dilute acid and alkali for fuel ethanol production. Transactions of the ASABE 53(3): 1007-1014.

Dowe, N. and J. McMillan, J. 2001. SSF experimental protocols: lignocellulosic biomass hydrolysis and fermentation. National Renewable Energy Laboratory, Golden, CO

Dubois, M., K. A. Gilles and P.A. Hamilton. 1956. Colormetric method for determination of sugars and related substances. Anal. Chem., 28(3): 350-356.

Esteghlalian, A. R., Bilogesu, M., Mansfield, S. D., and Saddler, J. N. 2001. Do enzymatic hydrolyzability and simons' stain reflect the changes in the accessibility of lignocellulosic substrates to cellulase enzymes? Biotechnology Progress, 17(6): 1049-1054.

Goering, H. K., and P.J. Van Soest. 1970. Forage fiber analysis. Agriculture Handbook 379, USDA-ARS, Washington, DC.

Hess, J. R., C.T. Wright. K.L. Kenney and E.M. Searcy. 2009. Uniform-format solid feedstock supply system. Idaho Falls, Idaho: Idaho National Laboratory. www.inl.gov/portal-files/solid-feedstock-supply-system.pdf

Savoie, P., K.J. Shinners and B.N. Binversie. 2004. Hydrodynamic separation of grain and stover components in corn silage. Applied Biochemistry and Biotechnology, 113:41-54.

Shinners, K.J., A.G. Jirovec, R.D. Shaver and M. Bal. 2000. Processing whole-plant corn silage with crop processing rolls on a pull-type forage harvester. Applied Engineering in Agriculture. 16(4):323-331.

Shinners, K. J., A.D. Wepner, R. E. Muck and P.J. Weimer. 2010. Aerobic and anaerobic storage of single-pass, chopped corn stover. Bioenergy Research, 4(1):61-75.

Williams, S. D. and K.J. Shinners. 2011. Anaerobic storage and aerobic stability of moist biomass feedstocks. ASABE Paper No.1111082. ASABE, St. Joseph, MI.