Available online at www.sciencedirect.com

ScienceDirect

http://www.elsevier.com/locate/biombioe

High dry matter whole-plant corn as a biomass feedstock

David E. Cook [a], Kevin J. Shinners [a,*], Paul J. Weimer [b], Richard E. Muck [b]

[a] Department of Biological Systems Engineering, University of Wisconsin, 460 Henry Mall, Madison, WI 53706, United States

[b] US Dairy Forage Research Center, USDA-ARS, Madison, WI, United States

ARTICLE INFO

Article history:
Received 22 December 2012
Received in revised form
7 February 2014
Accepted 14 February 2014
Available online xxx

Keywords:
Harvest
Storage
Corn
Pretreatment
Losses
Stover

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 350–840 g kg^{-1} whole-plant dry matter (DM) using a forage harvester and then ensiled in pilot-scale silos. Ambient pretreatment during storage was investigated using both dilute acid and lime. Both pretreated and control whole-plant silages were very well conserved during anaerobic storage with DM losses generally less than 40 g kg^{-1}. Hydrodynamic separation of the grain and stover fractions after storage was found to be more effective at fractionating starch and fiber than conventional dry grain harvest, and both fractions had desirable composition. The effects of pretreatment on the silage were very pronounced at 30 and 100 g (kg DM)$^{-1}$ sulfuric acid loading with less than 100 g (kg DM)$^{-1}$ of the hemicellulose still bound in the cell wall at DM contents greater than 500 g kg^{-1}. The whole-plant harvest and storage system was shown to be a viable alternative to conventional corn grain and stover systems for producing feedstocks for biochemical conversion.

1. Introduction

A new corn harvest and storage system is proposed which intends to lower the cost of both the starch and cellulose factions destined for ethanol production. This "single-pass; whole-plant" system eliminates many non-value added operations and simplifies the traditional fractionated systems into these major operations: whole-plant harvest with a forage harvester; anaerobic storage; co-transport of grain and stover; and grain and stover separation at the biorefinery. A recent study suggests that whole-plant (starch plus cellulosic) bioprocessing could also eliminate the need for separation of the grain and stover fractions, further reducing costs [1]. The whole-plant method attains these desirable goals: single-pass harvest; low soil contamination; weight limiting transport; size-reduction at harvest; reduced system energy inputs; and high yields. Due to the harvest of a moist crop, the grain and stover must be stored anaerobically and conserved in a non-neutral pH environment created by fermentation or chemical application.

Grain and stover are currently harvested well after crop maturity to reduce drying required for stable aerobic storage.

* *Corresponding author*. Tel.: +1 608 263 0756.
E-mail address: kjshinne@wisc.edu (K.J. Shinners).
http://dx.doi.org/10.1016/j.biombioe.2014.02.026
0961-9534/

However, field drying is costly in terms of weather risks; delayed harvest timeliness; and pre-conversion rehydration. Additionally, the grain fraction is often finish dried using fossil fuel, compromising its energy balance. Finally, drying of cellulose microfibrils results in the irreversible shrinking of the pore space, reducing the accessible surface area and conversion efficiency [2]. 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.

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 pilot-scale. Compositional analysis was used to quantify the effectiveness of the proposed system on feedstock conservation and potential for biochemical conversion.

2. Materials and methods

2.1. Substrate

Whole-plant corn was harvested from plots in 2009 and 2010 near Arlington, WI (43.30; 89.35). In 2009, DeKalb 6169, a 111-day comparative relative maturity (CRM) corn, was planted on 6-May and in 2010, Dekalb 57-79, a 107-day CRM corn, was planted on 27-May. Harvesting was done with a pull-type forage harvester. 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 characteristics between the different TLC, so in 2010 only the 19 mm TLC was used. Composition results from 2009 were pooled, one replicate from each TLC were combined to result in three replicates. From each harvest or treatment where TLC was varied, three sub-samples were taken for particle-size analysis using ASABE Standard S424.1 [3] and kernel damage assessment [4]. In 2010 hand shelled grain served as control to compare against the ensiled control grain.

2.2. Experimental design

In 2009 a 4 × 2 replicated experimental design was used to investigate the effect of plant DM content (i.e. harvest date) and acid pretreatment (0 or 100 g sulfuric acid (kg DM)$^{-1}$). Harvest dates and DM contents were Oct. 9 (350 g kg^{-1}); Oct. 27 (440 g kg^{-1}); Nov. 12 (600 g kg^{-1}); and Dec. 14 (660 g kg^{-1}). In 2010 a 3 × 4 experimental design was used to investigate the effect of plant DM content and pretreatment. Harvest dates and DM contents (whole plant and grain, respectively) were Sept. 28 (550 and 710 g kg^{-1}); Oct. 7 (660 and 810 g kg^{-1}); and Oct. 19 (840 and 840 g kg^{-1}). Pretreatments were 0, 10, or 30 g sulfuric acid (kg DM)$^{-1}$ and the fourth pretreatment was 10 g calcium hydroxide (kg DM)$^{-1}$. In both years, all treatments were replicated three times at each harvest date.

2.3. Pilot-scale silos

Prior to treatment and storage, the substrate was sub-sampled and analyzed for DM content using a microwave oven according to ASABE Standard S358.2 [3] so that amendments, if any, were applied on a DM basis. From each replicate pilot-scale silo, one sub-sample was taken for later separation of the grain and stover fractions and two sub-samples each were taken for determination of DM content and constituent analysis. Constituent and DM sub-samples were dried at 60 °C for 72 h in a forced air oven [3]. All substrates were homogenized in a Hobart model 1401 mixer and pretreated, if applicable. Pretreatment amendments of dry pulverized calcium hydroxide (98% $Ca(OH)_2$ – Standard Hydrated Lime – Mississippi Lime Co., St. Louis, MO) or 18M liquid sulfuric acid (Sigma–Aldrich St. Loius, MO) were applied by top dressing over the course of the two min the substrate was in the mixer. After mixing, 4.3 kg OM of the substrate either with and without pretreatment was then placed into 19 l plastic containers, compacted using a hydraulic cylinder to a target density of 225 kg OM (m)$^{-3}$, sealed, and then weighed to the nearest 0.01 kg. The container had a locking lid with a neoprene gasket to tightly seal the container. The containers were filled to the top so the locking lid maintained achieved density. Gas was manually released one week after filling by partially opening the lid. The containers then remained sealed during the remainder of the storage period. The silos were stored indoors at approximately 20 °C for 120 days in 2009 and 60 days in 2010.

2.4. Fraction separation

In 2009, sub-samples used for fractionating the grain and stover were taken prior to pretreatment and dried in a forced air oven at 60 °C for 72 h. The sub-samples were separated by hand into grain or stover fractions for later constituent analysis. In 2010, the fractionation sub-samples (~200 g DM) taken prior to pretreatment were fractionated on the basis of differences in specific gravity, using a previously developed hydrodynamic technique using a single flotation step [5]. All material that floated was considered the “stover fraction” and all material that sank was considered the “grain fraction”. A sample of the water was taken to evaluate any dissociated solids and solubles.

2.5. Removal procedure

Each pilot-scale silo and its contents were first weighed to the nearest 0.01 kg, then the contents were removed and homogenized prior to sub-sampling. Two sub-samples from each silo were taken for DM content determination at 60 °C for 72 h [3]. In 2009, a sub-sample of about 300 g DM was frozen at −20 °C for fractionation and subsequent constituent analysis of the grain and stover fractions at a later date. Fractions were separated using hydrodynamic method described above. 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.

In 2010 the removal technique was modified to reduce sampling error of the heterogeneous material that was too easily fractionated by mechanical handling. Upon removal, a sub-sample was taken to evaluate DM content, and the

Please cite this article in press as: Cook DE, et al., High dry matter whole-plant corn as a biomass feedstock, Biomass and Bioenergy (2014), http://dx.doi.org/10.1016/j.biombioe.2014.02.026

remainder separated using the hydrodynamic method described above. Total 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. From each of these fractions, two sub-samples were taken for DM content and a sub-sample was taken and frozen for further preparation and analysis.

2.6. Compositional assays

Post-storage sub-samples were frozen prior to preparation for constituent analysis. All post-storage sub-samples were assayed for fermentation products and pH (see below). Then the samples were titrated to neutral pH using 10 M sodium hydroxide or 24 N 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 with distilled water 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 typical fermentation products. Samples were injected (100 μL) 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. Crotonic acid was used as internal standard.

Constituent analysis was performed using wet chemistry by Dairyland Labs (Arcadia, WI) for neutral detergent fiber (NDF), acid-detergent fiber (ADF), acid detergent lignin (ADL), water-soluble carbohydrates (WSC), ash, crude protein (CP), and *in vitro* starch digestibility (IVSD) for the grain fraction. Fiber analysis was done using the crucible method [6] and WSC assay was done using the phenol-sulfuric method [7]. Cellulose content was estimated by the difference between ADF and ADL. Hemicellulose was estimated by the difference between NDF and ADF. Since all fiber assays were ash-corrected; they were non-sequential.

2.7. Statistical analysis

Experiments were analyzed using analysis of variance, using a randomized block design with replication. Statistical difference was assessed using Fischer's least-square difference (LSD) at with a probability of 0.95.

3. Results

3.1. Storage losses

In 2009, there were significant differences in losses between treatments only for material harvested at 350 g kg^{-1} DM content (Table 1). The maximum allowable loss to achieve an economic advantage over fractionated grain and stover harvest systems was found to be 160 g (kg OM)$^{-1}$ using the model described in Ref. [8]. Our goal was to achieve losses of less than 50 g (kg OM)$^{-1}$. In all treatments, except the control at 350 g kg^{-1}, OM losses were less than 30 g (kg OM)$^{-1}$, 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 1). This was consistent with other anaerobic storage studies [9,10]. 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 the 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. Only the lime treatment from the first harvest of the year exceeded the 50 g (kg OM)$^{-1}$ storage loss benchmark.

3.2. Post-storage fermentation products

In 2009, the control treatment produced primarily lactate, acetate, and ethanol, with greater quantities at higher

Table 1 – Storage losses of whole-plant silage (grain plus stover) in g per kg initial OM for 2009 and 2010 harvests and treatments.

2009					
	9-Oct	27-Oct.	12-Nov.	14-Dec.	Average by treatment[b]
DM content (g kg^{-1})	350	440	600	660	
Control	57b	23a	24a	10a	28a
Acid – 100 g (kg DM)$^{-1}$	5a	28a	13a	27a	18a
LSD[a]	37	21	18	19	12
Average by date[c]	31A	25A	19A	19A	LSD[d] = 26
2010					
	28-Sept.	7-Oct.	19-Oct.		Average by treatment[b]
DM Content (g kg^{-1})	550	660	840		
Control	43a	32ab	17b		31ab
Acid – 10 g (kg DM)$^{-1}$	34a	28a	9ab		24a
Acid – 30 (kg DM)$^{-1}$	27a	18a	11ab		19a
Lime – 10 (kg DM)$^{-1}$	75b	46b	8a		43b
LSD[a]	31	15	8		12
Average by date[c]	45C	31B	11A		LSD[d] = 12

[a] Least significant difference. Means within columns with different letters (a,b) are significantly different at 95% confidence.
[b] Data was pooled by 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 (A,B,C) are significantly different at 95% confidence.

Please cite this article in press as: Cook DE, et al., High dry matter whole-plant corn as a biomass feedstock, Biomass and Bioenergy (2014), http://dx.doi.org/10.1016/j.biombioe.2014.02.026

Table 2 – 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 100 g·(kg DM)$^{-1}$.

Harvest Date	DM content (g kg^{-1})	pH	Lactate	Acetate	Ethanol	Butyrate	Total
			g (kg OM)$^{-1}$				
Control treatment							
9-Oct.	350	3.9a	40.4c	27.7c	8.1bc	1.1a	83.6d
27-Oct.	440	3.9a	33.2b	11.3b	6.6b	1.1a	58.1c
12-Nov.	600	4.1b	21.9a	7.7ab	4.3ab	0.6a	41.2b
14-Dec.	660	4.3c	16.8a	4.9a	2.0a	0.8a	29.2a
LSD[a]		0.1	6.6	3.9	3.0	1.2	7.1
Average by date		4.1B	28.1B	12.9A	5.2B	0.9B	53.0B
Acid treatment							
Average by date[b]		1.3A	1.4A	17.2B	0.3A	–A	21.9A

[a] Least significant difference. Control treatment means within columns with different letters (a,b,c) are significantly different at 95% confidence. Averages by date within column with different letters (A, B) are significantly different at 95% confidence.
[b] Harvest date had no significant effect on the fermentation products produced in the acid treated material.

moisture levels (Table 2). The primary fermentation products were lactic and acetic acid for the untreated and acid treated silages, respectively. The exogenous acid immediately dropped the pH in the acid treated material, reducing the time available for lactic acid producing microorganisms to flourish. Acetic acid can be released by cleaving the ester linkage of the acetyl groups from acetylated xylans, and was 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 Ref. [9].

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 (Table 3). 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, likely due to the lower buffering capacity of the grain (Table 3). Fermentation products that were extracted 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 min. 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 solubilizations of 28%, 40%, and 50%, respectively.

3.3. *Material composition*

In 2009, approximately 52% of the whole-plant corn organic matter (OM) harvested was grain and 48% was stover, while in

Table 3 – Post-storage fermentation products averaged across three 2010 harvest dates for whole-plant silage (grain plus stover); and hydrodynamically separated stover and grain fractions after 60 days in storage for control with no pretreatment and pretreatment with sulfuric acid or lime.

Treatment	pH	Lactate	Acetate	Ethanol	Butyrate	Total
		g (kg OM)$^{-1}$				
Whole-plant						
Control	6.6c	8.1d	2.1b	7.7b	1.8b	21.4c
Acid – 10 g (kg DM)$^{-1}$	5.1b	1.7b	1.5a	9.9c	0.0a	13.3b
Acid – 30 g (kg DM)$^{-1}$	2.5a	0.0a	5.1c	0.9a	0.0a	6.2a
Lime – 10 g (kg DM)$^{-1}$	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
Separated grain fraction						
Control	4.8c	2.7d	0.7a	2.5c	0.5b	7.0c
Acid – 10 g (kg DM)$^{-1}$	4.1b	0.6b	0.5a	3.0d	0.0a	4.2b
Acid – 30 g (kg DM)$^{-1}$	2.2a	0.0a	2.0b	0.2a	0.0a	2.3a
Lime – 10 g (kg DM)$^{-1}$	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
Separated stover fraction						
Control	5.6c	7.8d	1.9a	6.0c	1.4b	18.9c
Acid – 10 g (kg DM)$^{-1}$	3.9b	1.6b	1.5a	7.3d	0.0a	10.4b
Acid – 30 g (kg DM)$^{-1}$	2.4a	0.0a	4.2b	0.6a	0.0a	5.2a
Lime – 10 g (kg DM)$^{-1}$	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 95% confidence.

Please cite this article in press as: Cook DE, et al., High dry matter whole-plant corn as a biomass feedstock, Biomass and Bioenergy (2014), http://dx.doi.org/10.1016/j.biombioe.2014.02.026

Table 4 – Composition of whole-plant silage (grain plus stover) and separated stover and grain fractions before and after anaerobic storage for silage with no pretreatment averaged across all harvests in that year.

	Starch	WSC	Cellulose	Hemicellulose	ADL	Ash	CP
	g (kg OM)$^{-1}$						
Before storage 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	
After storage 2009							
Grain	763	16	–[e]	–[e]	–[a]	16	74
Stover	39	35	419	243	66	53	69
Whole-plant	433	33	187	109	37	41	86
Before storage 2010							
Shelled Corn[b]	709b	33a	8a	43a	12a	12a	
Grain[b,c]	722a	24b	8a	35a	13a	11a	
LSD[b,d]	9	4	5	11	4	2	
Stover[c]	17	33	444	297	77	53	
Whole-plant	414	39	179	155	45	30	
After storage 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] Grain and stover fraction hand separated from the whole-plant mass.
[b] Statistical analysis was only conducted on averages between shelled 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 95% confidence.
[e] Fiber content was not quantified for the grain fraction in 2009.

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 by hydrodynamic separation of the grain from the whole-plant substrate. Hydrodynamic separation was more effective at concentrating the starch in the grain fraction than hand shelling (Table 4), and visual observation suggested that this was due to removal of seed coats during harvest and subsequent fractionation. The hydrodynamic fractionation process resulted in some soluble carbohydrates going into the liquid fractionation so the water soluble carbohydrates (WSC) content of the fractioned grain was less than that of the hand shelled corn.

After storage, 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 4). Acid pretreatment had the effect of decreasing hemicellulose by acid hydrolysis of cell wall components. The post-storage control grain fraction had numerically greater starch content compared to pre-storage values for the shelled corn, one indication that grain quality was well conserved during storage. In 2010, starch losses averaged across all moisture contents and treatments were 10 g kg^{-1} (Table 5). When this evidence is coupled with the low overall DM losses, generally 30 g kg^{-1} or less (Table 1), 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 100 g kg^{-1} acid loading, hemicellulose was decreased by greater than 85% for the last three harvest dates in 2009 [11]. In 2010 the 30 g kg^{-1} acid loading yielded hemicellulose degradation of greater than 35% on the last two harvest dates (Table 6). The 10 g kg^{-1} acid loading pretreatment produced greater degradation of hemicellulose than the control only at the greater DM contents.

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 silage (10–30 g kg^{-1} of DM) was extracted into the separation liquid. The liquid fraction was assayed for WSC, ash, sulfur, and fermentation products (Table 7). The constituents analyzed accounted for most of the DM in the liquid fraction. Further analysis revealed that starch or other minor fermentation products were diluted to the point that they were below 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. The amount of WSC in the liquid increased in the pretreated silage, likely as a result of acid hydrolysis of the hemicellulose.

4. Discussion

The whole-plant silage method produced desirable feedstock composition, specifically low ash, and high cellulose and hemicellulose contents (Table 4). Fermentation products, which may be inhibitory to some biochemical conversion processes, were extracted into the separation water (Table 7) so it is expected that during a prolonged hydration process fermentation products would be extracted, where they could

Please cite this article in press as: Cook DE, et al., High dry matter whole-plant corn as a biomass feedstock, Biomass and Bioenergy (2014), http://dx.doi.org/10.1016/j.biombioe.2014.02.026

Table 5 – Dry mass ratio of post-storage to pre-storage starch following pretreatment and anaerobic storage for sulfuric acid pretreated and control treatments for 2010 harvests.

Harvest date	28-Sept.	7-Oct.	19-Oct.	Average by treatment[b]
DM content (g kg^{-1})	550	660	840	
Control	1.06a	0.93a	1.17b	1.06b
Acid – 10 g (kg DM)$^{-1}$	1.12a	0.81b	0.97a	0.97ab
Acid – 30 g (kg DM)$^{-1}$.	0.95a	0.92a	0.97a	0.95a
Lime – 10 g (kg DM)$^{-1}$	1.01a	0.96a	1.01a	0.99ab
LSD[a]	0.28	0.08	0.11	0.09
Average by date[c]	1.04A	0.91B	1.03A	LSD[d] = 0.08

[a] Least significant difference. Means within columns with different letters (a,b) are significantly different at 95% confidence.
[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 (A,B) are significantly different at 95% confidence.

Table 6 – Ratio of the post-storage 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]
DM content (g kg^{-1})	550	660	840		
	Hemicellulose				Cellulose
Control	0.88a	0.93b	1.04c	0.96b	1.04a
Acid – 10 g (kg DM)$^{-1}$	0.97b	0.95b	0.85b	0.92b	1.01b
Acid – 30 g (kg DM)$^{-1}$	0.94ab	0.64a	0.48a	0.69a	1.05a
Lime – 10 g (kg DM)$^{-1}$	0.87a	0.94b	1.01c	0.94b	1.01b
LSD[a]	0.08	0.14	0.12	0.06	0.02

[a] Least significant difference. Means within columns with different letters are significantly different at 95% confidence.
[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.

potentially be recovered for other uses if economical separation technologies were applied.

The moisture content of the whole-plant silage provides benefits that are lost upon feedstock drying. Drying causes cell-wall hornification, resulting in a feedstock that is more resistant to enzymatic degradation [2]. Moist feedstock also brings along water required for some biochemical conversions, reducing biorefinery water needs. Although industrial drying of feedstocks has been proposed [12], this requires a greater energy input to the system that is avoided by using moist feedstocks. However, greater moisture content can reduce the quantity of organic matter on a truck, increasing its transport costs, a cost factor against which the advantages of moist systems need to be balanced [8].

If this system was employed where harvest took place over 10 weeks, with whole-plant DM ranging from 400 to 850 g kg^{-1}and no pretreatments used to further conserve the crop, losses greater than 50 g kg^{-1} would be uncommon (Table 1). Using a conservative assumption of 50 g kg^{-1} storage losses, the combined whole-plant corn for biomass system was found to reduce the feedstock cost by $55 (Mg DM)$^{-1}$, as compared to a conventional stover harvest system [8]. In a farm-scale evaluation, DM losses of 28 g kg^{-1} were found for whole-plant corn ensiled in a silo bag for 213 days at DM content of 650 g kg^{-1} [13].

Grain starch losses and conversion efficiency are important concerns with this system because the grain carries the majority of the crop value. Any impairment to the value of grain, by storage losses or a less advantageous ethanol process, would have to be assigned to the stover. Due to stover's relatively low value compared to grain, only modest grain value impairment can be tolerated. Conversely, any net benefit provided to the grain would have a marked effect on further cost reductions to the stover fraction. Given the low measured starch losses (Table 5), higher starch concentration in the grain (Table 4), and low overall silage losses (Table 1), these results gives strong evidence the starch fraction was very well conserved.

Acid pretreatment prior to storage was effective at degrading the stover hemicellulose (and enhanced enzymatic degradability and subsequent fermentation to ethanol – see Supplementary Section). Pretreatment with 100 g (kg DM)$^{-1}$ sulfuric acid was particularly effective at high DM contents with less than 100 g (kg DM)$^{-1}$ of the hemicellulose bound in the cell wall at DM contents greater than 500 g kg^{-1}. All acid pretreatments were effective at inhibiting anaerobic fermentation during storage as seen by the low levels of fermentation products produced (Tables 2 and 3). Hydrodynamic separation was chosen over a mechanical system to replicate the most likely industrial scale fractionation technique to be employed because it resulted

Table 7 – Components from substrate that were dissociated into the hydrodynamic separation liquid fraction, in g kg^{-1} of the component of the whole.

Year	Treatment	WSC	Ash	Sulfur	Total fermentation products
2009	Control	70a	140a	180a	410a
	Acid – 100 g (kg DM)$^{-1}$	140b	80a	330b	390a
	LSD[a]	50	80	80	90
2010	Control	40a	130a	230a	300a
	Acid – 10 g (kg DM)$^{-1}$	60a	230b	480b	460b
	Acid – 30″.	120b	220b	480b	440ab
	Lime – 10″.	130b	180b	220a	490b
	LSD[a]	30	50	70	150

[a] Least significant difference. Means within columns with different letters are significantly different at 95% confidence.

Please cite this article in press as: Cook DE, et al., High dry matter whole-plant corn as a biomass feedstock, Biomass and Bioenergy (2014), http://dx.doi.org/10.1016/j.biombioe.2014.02.026

in a very high degree of starch and fiber fractionation with minimal losses.

5. Conclusions

We investigated the harvest, ambient pretreatment, and anaerobic storage of high DM whole-plant corn as an alternative to conventional systems where corn grain and stover are fractionated at harvest. Conservation of both fractions was excellent over a wide range of DM contents, ambient pretreatment was effective at beginning cell wall degradation, and grain value was very well conserved. Our results clearly show that the whole-plant system can be a viable alternative to conventional corn grain and stover systems for producing feedstocks for biochemical conversion.

Acknowledgements

This research was partially sponsored by the University Of Wisconsin College Of Agriculture and Life Sciences; USDA-ARS US Dairy Forage Research through Specific Cooperative Research Agreement 58-3655-2-128; and by Archer-Daniels-Midland. This research could not have been completed without the assistance of the staff of the Arlington Agricultural Research Station.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biombioe.2014.02.026.

REFERENCES

[1] Gao J, Qian L, Thelen KD, Hao X, da Costa Sousa L, Lau MW, et al. Corn harvest strategies for combined starch and cellulosic bioprocessing to ethanol. Agron J 2011;103:844–50.
[2] Esteghlalian AR, Bilogesu M, Mansfield SD, Saddler JN. Do enzymatic hydrolyzability and simons' stain reflect the changes in the accessibility of lignocellulosic substrates to cellulase enzymes? Biotechnol Prog 2001;17(6):1049–54.
[3] Anon. ASABE Standard 424.1: method of determining and expressing particle-size of chopped forage materials by screening. St. Joseph, MI: ASABE; 2011.
[4] Shinners KJ, Jirovec AG, Shaver RD, Bal M. Processing whole-plant corn silage with crop processing rolls on a pull-type forage harvester. Appl Eng Agric 2000;16(4):323–31.
[5] Savoie P, Shinners KJ, Binversie BN. Hydrodynamic separation of grain and stover components in corn silage. Appl Biochem Biotech 2004;113:41–54.
[6] Goering HK, Van Soest PJ. Forage fiber analysis (apparatus, reagents, procedures and some applications); 1970. USDA agricultural handbook no. 379.
[7] Dubois M, Gilles KA, Hamilton PA. Colormetric method for determination of sugars and related substances. Anal Chem 1956;28(3):350–6.
[8] Cook DE, Shinners KJ. Economics of alternative corn stover logistics systems. ASABE paper No. 1111130. St. Joseph, MI: ASABE; 2011.
[9] Shinners KJ, Wepner AD, Muck RE, Weimer PJ. Aerobic and anaerobic storage of single-pass chopped corn stover. Bioenerg Res 2011;4(1):62–75.
[10] Williams SD, Shinners KJ. Farm-scale anaerobic storage and aerobic stability of high dry matter sorghum as biomass feedstock. Biomass Bioenergy 2012;46(11):309–16.
[11] Cook DE. Whole-plant harvest, storage, and pretreatment of corn grain and stover for biochemical conversion. Madison, WI: University of Wisconsin; 2011. Unpublished Master of Science thesis.
[12] Hess JR, Wright CT, Kenney KL. Cellulosic biomass feedstocks and logistics for ethanol production. Biofuels Bioprod Bioref 2007;1(3):181–90.
[13] Williams SD. Anaerobic storage and aerobic stability of moist biomass feedstocks. Madison, WI: University of Wisconsin; 2011. Unpublished Master of Science thesis.