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Farm-scale anaerobic storage and aerobic stability of high dry matter perennial grasses as biomass feedstocks

*Shane D. Williams, Kevin J. Shinners**

Department of Biological Systems Engineering, University of Wisconsin, 460 Henry Mall, Madison, WI 53706, USA

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ABSTRACT

Research was conducted to determine the feasibility of using a chopped harvest and anaerobic storage system to conserve mature, high dry matter (DM) switchgrass and reed canarygrass intended as cellulosic biomass feedstocks. The grasses were anaerobically stored in farm-scale silo bags for over 220 days. Switchgrass DM content was either 459 or 566 g kg^{-1} and reed canarygrass DM content was 525 g kg^{-1}. Average storage losses were 27 and 22 g kg^{-1} of DM for switchgrass and reed canarygrass, respectively. Additional DM loss after two- and seven-day aerobic exposure was 16 and 23 g kg^{-1} or 11 and 19 g kg^{-1} for switchgrass and reed canarygrass, respectively. On-harvester inoculation with a combination of homofermentative (*Pediococcus pentosaceus*) and heterofermentative (*Lactobacillus buchneri*) bacterium increased the production of both lactic and acetic acid during storage and in some situations produced lower yeast and mold populations during aerobic exposure. Inoculation improved aerobic stability in reed canarygrass and the high DM switchgrass. Fermentation products were less than 25 g kg^{-1} for both grasses. Average recovery of cellulose and hemicellulose was 97% of initial mass. Anaerobic storage of chopped, inoculated, high DM, mature perennial grasses was shown to be a viable cellulosic biomass feedstock logistics system.

1. Introduction

Perennial grasses intended as cellulosic biomass feedstocks are typically stored aerobically as dry bales. When dry bales will be made, the mass fraction of water must be less than 20% to reduce the risk of detrimental biological activity in the stored bales [1,2]. Although perennial grasses like switchgrass (SWG) and reed canarygrass (RCG) dry more readily than typical forage crops [1], field drying of perennial grasses harvested late in the season can still be difficult because of high yields and poor ambient conditions at harvest [1]. Field 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 and reduces the accessible surface area resulting in a feedstock that is more resistant to enzymatic degradation [3]. Alternatively, harvesting moist perennial grasses by chopping and preserving by ensiling can reduce field wilting time and associated weather risks; produce a size-reduced flowable material at harvest; achieve greater productivity than baled systems; reduce negative consequences of cell wall hornification; and

* *Corresponding author*. Tel.: +1 608 263 0756.
E-mail address: kjshinne@wisc.edu (K.J. Shinners).
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reduce biorefinery water requirements. Direct-cut harvest may also be possible with this system, which would further improve timeliness, reduce weather risk and decrease chances for soil contamination. The above listed attributes must more than offset the added cost of transporting material with low-bulk density and high-moisture, both of which reduces the mass of dry matter (DM) shipped per truckload.

By size-reducing and processing grasses in the field with existing forage harvesting equipment and sending a uniform product to the storage site or biorefinery, the complexities associated with processing dry bales are eliminated [4]. Moist feedstocks can be harvested over a longer period and the machinery can be used to harvest many other crops, so the greater fixed costs of high capacity forage harvesting equipment can be diluted across more land area and time compared to dry bale equipment [5–7].

When the reduction in value from storage losses of outdoor stored bales is considered, the total cost of bales stored outdoors may be more than other more capital intensive systems [5–7]. Greater material degradation and DM loss occurs as the bales are stored longer. However, feedstocks will be needed during months outside of their seasonal availability; so long storage periods will be required [8]. DM losses of SWG ranged from 50 to 130 g kg^{-1} for bales stored outdoors over 12 months in Pennsylvania [9]. Dry bales of SWG and RCG stored outdoors for 9–11 months averaged 75, 87 and 149 g kg^{-1} DM loss for bales wrapped with net wrap, plastic twine, and sisal twine, respectively [1].

Storing biomass feedstocks anaerobically as silage is one way to ensure feedstock conservation for an extended period of time [1,2,10]. Average DM losses of 11 g kg^{-1} were achieved in ensiled bales of SWG and RCG between 530 and 660 g kg^{-1} DM content [1]. Low DM losses (between 10 and 50 g kg^{-1}) for ensiled, moist corn stover have been achieved at both lab- and farm-scale [2,10–12]. The addition of biological amendments to corn stover before ensiling improved conservation [11]. In addition to conserving feedstock value during storage, care needs to be taken to minimize aerobic degradation once the feedstock is removed from storage. Little research has been conducted on the aerobic stability of ensiled biomass feedstocks, however aerobic spoilage during feed-out has been known to represent up to 30–40% of total animal feed DM [13].

Biomass feedstocks must be transported off the farm and transportation costs can be reduced when the dry mass transported is maximized. From this standpoint, conserving biomass feedstocks by anaerobic storage at 500–700 g kg^{-1} DM content was shown to be economically desirable [7]. However, there is little published research concerning the on-farm conservation of chopped, mature SWG or RCG intended as a biomass feedstock at these DM contents. Research concerning conservation of perennial grasses intended as a biomass feedstock by ensiling has been limited, with most work using SWG or RCG at DM contents less than 500 g kg^{-1} [14–17].

The objectives of this research were to quantify the anaerobic storage characteristics of high DM content, mature SWG and RCG intended as biomass feedstocks; to conduct the research at the farm-scale; to investigate a biological amendment to improve conservation and aerobic stability; and to quantify the aerobic stability of the perennial grass feedstocks at removal from storage.

2. Materials and methods

2.1. Harvest

The two perennial grasses were used (RCG – *Phalaris arundinacea* – Palaton variety and SWG – *Panicum virgatum* L. – Shawnee variety) which were established in 2005 [1]. Two DM contents were targeted at harvest: approximately 600 and 500 g kg^{-1} (high and low DM content). The RCG and SWG were cut and swathed using a John Deere model 4990 windrower on August 31 and September 18, 2010, respectively. The grasses required one day of field wilting to achieve the target DM contents. After field wilting, the crop was harvested the day after cutting with a John Deere model 7800 self-propelled forage harvester (SPFH) equipped with a windrow pick-up. A heavy rainstorm prevented the harvest of the high DM RCG. The harvester theoretical-length-of-cut (TLC) was 12 mm. Crop yield averaged 7.4 and 8.5 Mg DM ha^{-1} for the RCG and SWG, respectively. Both grasses were mature at harvest and were in the seed ripening stage [18].

In addition to the two DM content treatments, a biological amendment was investigated. Biotal 500 (Lallmand Animal Nutrition Biotal 500 containing *Lactobacillus buchneri* 40788 (LB) and *Pediococcus pentosaceus* 12455 (PP)) was applied at harvest. The bacterial inoculants were applied using an on-harvester Dohrmann model DE-1000 inoculant applicator. The applicator was set to deliver approximately 100,000 cfu/g PP and 400,000 cfu/g LB. Untreated control treatments were also harvested. Random chopped samples of all treatments were taken for later analysis of particle-size following ASABE Standard S424.1 [19].

2.2. Storage and removal

The silo bags for this research were made using a modified Ag Bag model CT-5 bagger [20]. Harvested material was transported to the storage location, and randomly collected subsamples of about 2 kg were placed into polypropylene mesh bags measuring 53 cm by 80 cm with 10 mm mesh (McMaster-Carr part no. 9883T53). Before placing these replicate subsample parcels into the silo bag to quantify storage characteristics, four subsamples were collected from each parcel. Two subsamples were oven dried for moisture content determination at 103 °C for 24 h and two subsamples dried at 60 °C for 72 h for constituent determination, following ASABE Standard S358.2 [21]. Wireless temperature data loggers (Onset model UA-001-08) were placed in every other parcel to monitor temperature at a sampling rate of four times per day. Before the subsample parcels were placed in the silo bag, they were weighed to the nearest 0.005 kg. Six replicate parcels were used in each treatment.

Two silo bags were made, one each for the RCG and SWG. The RCG silo bag contained only the low DM content material with LB + PP treated material and the untreated control. The SWG bag had these two treatments at the two targeted DM contents. In either bag, each of the treatments were split into thirds and placed in three replicate locations in the silo bag. Sacrificial material was placed in the beginning and end of the bag and between treatments to reduce edge effects.

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Table 1 – Switchgrass and reed canarygrass DM content and DM loss in subsample parcels during anaerobic storage and additional DM loss after two or seven days of aerobic exposure.

Crop type and silo bag no.	Treatment	DM content g kg^{-1}		DM losses g kg^{-1}		
		Stored	Removed	Storage	Aerobic exposure duration	
					2-day	7-day
Switchgrass						
Bag 1[a]	Control	445a	450a	34	24	13a
	LB + PP[c]	472b	477b	23	16	24ab
	Control	596d	589d	26	10	38b
	LB + PP	536c	543c	23	15	17ab
	LSD[d]	**25**	**23**	**21**	**18**	**22**
	Control	521	520	30	17	26
	LB + PP	504	510	23	16	21
	LSD[e]	**17**	**16**	**15**	**12**	**15**
	Low DM	459a	464a	29	20	19
	High DM	566b	566b	25	13	28
	LSD[f]	**17**	**16**	**15**	**12**	**15**
Reed Canarygrass						
Bag 2[b]	Control	529	530	26	15	31
	LB + PP	522	534	17	6	6
	LSD[d]	**19**	**11**	**31**	**17**	**34**

[a] Stored on September 8, 2010 and removed after 222 days.
[b] Stored on August 31, 2010 and removed after 230 days.
[c] On-harvester application of approximately 400,000 cfu/g *Lactobacillus buchneri* (LB) and 100,000 cfu/g *Pediococcus pentosaceus* (PP).
[d] Least significant difference. Mean values followed by different letters in the same column are significantly different at 95% confidence.
[e] Data pooled by treatment and analyzed using two-way analysis of variance.
[f] Data pooled by DM content and analyzed using two-way analysis of variance.

The silo bags were opened and material removed on April 18, 2011 after 230 and 222 days in storage for the RCG and SWG, respectively. As the silo bags were deconstructed at the end of the storage period, the subsample parcels were recovered; the parcels weighed to the nearest 0.005 kg; the contents homogenized; and two subsamples taken for moisture determination and one for compositional analyses. Because the material had undergone fermentation, both moisture and compositional samples were dried at 60 °C for 72 h following ASABE Standard S358.2 [21]. Another subsample was collected and refrigerated for later analysis of mold and yeast populations by Rock River Labs (Watertown, WI). A final subsample was taken and frozen for later analysis of pH and fermentation products by Rock River Labs. Constituent analysis consisted of ash corrected neutral detergent fiber (NDF); ash corrected acid detergent fiber (ADF); and ash corrected lignin (ADL); using the crucible method [22]. Hemicellulose was estimated by difference between NDF and ADF; and cellulose was estimated by difference between ADF and ADL. Fermentation products were quantified using high performance liquid chromatography.

2.3. *Aerobic stability*

As the material was removed, portions of each treatment were loaded into 200 liters plastic barrels. A total of four barrels were filled for each treatment. Roughly one third of the barrel was filled with material, and then a subsample parcel (described previously) containing roughly 2 kg DM of ensiled material was weighed, equipped with a temperature data logger, placed in the barrel and covered with more parent material. After two-thirds of the barrel was full, another subsample parcel with temperature data logger was placed into the barrel. The subsample parcels were approximately 10 and 45 cm from the material surface for the shallow and deep positions, respectively. The barrel was then filled level full and left uncovered. The barrels were loosely filled and material was not compacted creating a final DM density of 80–90 kg m^{-3}. The barrels were stored indoors in a ventilated shed for two or seven days. Procedures for sampling from the subsample parcels after the aerobic exposure period were similar to that previously described.

Heating in the material during aerobic exposure was quantified by heating degree days accumulated over the aerobic exposure period:

$$\mathrm{HDD} = \sum\left(\frac{T_{max} + T_{min}}{2} - T_{ave}\right) \quad (1)$$

where:

HDD accumulated heating degree days, °C
T_{max} daily maximum temperature, °C
T_{min} daily minimum temperature, °C
T_{ave} daily average ambient temperature, °C

2.4. *Statistical analysis*

Significant differences between treatments in individual experiments were determined using single factor analysis of variance (ANOVA) based on the variability among subsample parcels (experimental unit) within the silo bags. A two-way ANOVA was used to block confounding effects when analyzing data across treatments. All statistical differences

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Table 2 – Fermentation products and pH for switchgrass and reed canarygrass in subsample parcels after anaerobic storage and two or seven days of aerobic exposure.

Crop type and silo bag no.	Treatment	DM Content[a]	At removal from storage			pH	After 2-days aerobic exposure			pH	After 7-days aerobic exposure			pH
			Fermentation products … g kg^{-1}				Fermentation products … g·kg^{-1}				Fermentation products … g·kg^{-1}			
			Lactic acid	Acetic acid	Total		Lactic acid	Acetic acid	Total		Lactic acid	Acetic acid	Total	
Switchgrass														
Bag 1[a]	Control	Low	1.6a	6.0	7.6ab	5.2	2.5a	4.2a	6.7a	6.2	–	–	–	6.2
	LB + PP[c]		3.4a	15.2	18.6bc	5.0	3.6a	17.9b	21.5b	5.4	–	3.4	3.4	6.4
	Control	High	5.2a	–[g]	5.2a	4.8	–	–	–	5.7	1.3	–	1.3	7.7
	LB + PP		12.9b	9.5	22.4c	4.7	13.5b	8.0a	21.5b	5.2	–	–	–	5.8
	LSD[d]		**7.4**	**11.5**	**13.0**	**0.7**	**6.7**	**4.9**	**9.3**	**1.9**			**10.1**	**3.6**
	Control		3.4	3.0a	6.4a	5.0	1.3a	2.1a	3.4a	6.0	0.7	–	0.7	7.0
	LB + PP		8.2	12.4b	20.5b	4.9	8.6b	13.0b	21.5b	5.3	–	1.7	1.7	6.1
	LSD[e]		**5.1**	**7.9**	**8.6**	**0.5**	**4.0**	**3.1**	**5.8**	**1.2**			**4.0**	**2.2**
	Low DM		2.5a	10.6	13.1	5.1	3.1	11.1b	14.2	5.8	–	1.7	1.7	6.3
	High DM		9.1b	5.0	14.0	4.8	6.8	4.0a	10.8	5.5	0.7	–	0.7	6.8
	LSD[f]		**5.1**	**7.9**	**8.6**	**0.5**	**4.0**	**3.0**	**6.0**	**1.2**			**0.4**	**2.2**
Reed Canarygrass														
Bag 2[b]	Control		9.2	–	9.2a	4.4	6.7	–	6.7	4.9a	4.9	2.5	7.4	5.6
	LB + PP		10.9	9.0	22.1b	4.4	5.7	2.0	7.7	5.7b	11.0	4.3	15.3	5.1
	LSD[d]		**4.7**		**7.5**	**0.2**	**7.0**		**12.0**	**0.4**	**9.3**	**8.0**	**13.0**	**2.5**

[a] Stored on September 8, 2010 and removed after 222 days. Low and high DM contents were 459 and 566 g kg^{-1}, respectively.
[b] Stored on August 31, 2010 and removed after 230 days. Average DM content was 526 g kg^{-1}.
[c] On-harvester application of approximately 400,000 cfu/g *Lactobacillus buchneri* (LB) and 100,000 cfu/g *Pediococcus pentosaceus* (PP).
[d] Least significant difference. Mean values followed by different letters in the same column are significantly different at 95% confidence.
[e] Data pooled by treatment and analyzed using two-way analysis of variance.
[f] Data pooled by DM content and analyzed using two-way analysis of variance.
[g] Acid content was below detectable level of the assay.

were based on a least significant difference (LSD) with a probability of 0.95 [23].

3. Results

The geometric mean particle-size (GMPS) was 9 and 12 mm and the dry density of the chopped material in the silo bags was 128 and 173 kg m^{-3} for the RCG and SWG treatments, respectively.

Both grasses were well conserved during anaerobic storage with average storage losses of 24 g kg^{-1} of DM (Table 1). Additional DM losses after two- and seven-day aerobic exposure averaged 13 and 21 g kg^{-1} of DM, respectively. Amendment and DM content had no significant effect on storage losses when data was pooled and analyzed by treatment. Losses during the two aerobic exposure durations were generally not affected by the treatments considered. Losses of ryegrass harvested at DM contents similar to those used in this research and stored in lab-scale mini-silos ranged from 16 to 55 g kg^{-1} [24,25]. Ryegrass inoculated with LB + PP had slightly smaller DM losses than the control [25]. Losses of mature SWG and RCG bales wrapped in plastic film and ensiled at 510–660 g kg^{-1} DM ranged from 3 to 20 g kg^{-1} of DM [1]. Losses of mature SWG and RCG chopped and stored in silo bags at 330–410 g kg^{-1} DM ranged from 3 to 37 g kg^{-1} of DM [17].

The pH of SWG and RCG removed from storage averaged 4.9 and 4.4, respectively (Table 2). Low protein crops like SWG and RCG have less buffering capacity than high protein crops like alfalfa, which may be one reason for the relatively low pH despite the relatively small quantities of fermentation products produced [26]. Buffering capacity also declines with grass maturity [26] and both SWG and RCG were at the seed ripening maturity stage when harvested. Both SWG and RCG inoculated with LB + PP produced significantly more total fermentation products than untreated controls primarily because of greater acetic acid production. *L. buchneri* 40788 is known to produce acetic acid, so more acetic acid would be expected with inoculation. An end product of *P. pentosaceus* 12455 metabolism is lactic acid, so inoculation should have resulted in greater lactic acid production. Although inoculated grasses did have numerically greater lactic acid production, the means were not statistically different from the controls. After two days of aerobic exposure, the control SWG had a significantly less acid remaining than the inoculated material. After seven days of aerobic exposure, the acids from both SWG treatments had were almost completely degraded and volatilized, while RCG did maintain some acids. Acids produced during fermentation are one of the most abundant groups of compounds emitted from aerobically exposed silages and both lactic and acetic acid are particularly prone to volatilization [27]. Also, during aerobic deterioration proteins and amino acids are being broken down to ammonia, permitting the pH to rise above 7.0. After seven days of aerobic exposure, the high DM SWG had a pH of 7.7 (Table 2) but also the greatest losses and heating (Tables 1 and 5), so the greatest increase in pH was observed.

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Table 3 – Initial and recovery of cellulose and hemicellulose for switchgrass and reed canarygrass in subsample parcels after anaerobic storage.

Crop type and silo bag no.	Treatment	DM Content[a]	Into storage … g kg^{-1}		Recovery[g]	
			Cellulose	Hemicellulose	Cellulose	Hemicellulose
Switchgrass						
Bag 1[a]	Control	Low	388a	288	0.94	1.10
	LB + PP[c]		380a	280	0.91	1.12
	Control	High	401ab	274	0.94	1.06
	LB + PP		424b	288	0.93	1.03
	LSD[d]		**23**	**18**	**0.10**	**0.16**
	Control		395	281	0.94	1.08
	LB + PP		402	284	0.92	1.08
	LSD[e]		**16**	**13**	**0.07**	**0.12**
	Low DM		384a	284	0.93	1.11
	High DM		413b	281	0.93	1.04
	LSD[f]		**16**	**13**	**0.07**	**0.12**
Reed Canarygrass						
Bag 2[b]	Control		364	302	0.98	0.93
	LB + PP		369	316	0.94	0.93
	LSD[d]		**7**	**26**	**0.05**	**0.21**

[a] Stored on September 8, 2010 and removed after 222 days. Low and high DM contents were 459 and 566 g kg^{-1}, respectively.
[b] Stored on August 31, 2010 and removed after 230 days. Average DM content was 526 g kg^{-1}.
[c] On-harvester application of approximately 400,000 cfu/g *Lactobacillus buchneri* (LB) and 100,000 cfu/g *Pediococcus pentosaceus* (PP).
[d] Least significant difference. Mean values followed by different letters in the same column are significantly different at 95% confidence.
[e] Data pooled by treatment and analyzed using two-way analysis of variance.
[f] Data pooled by DM content and analyzed using two-way analysis of variance.
[g] Recovery of constituent = (final/initial)*(1-DM loss).

The average ash content was 66 and 107 g kg^{-1} of DM for SWG and RCG, respectively. Standing SWG and RCG ash content typically ranges between 40–50 and 70–80 g kg^{-1}, respectively [28], so soil contamination at cutting and picking up likely accounts for these differences. The cellulose and hemicellulose content of SWG was slightly greater than that reported in other SWG ensiling research while RCG was similar to other publish values [1,28,29] (Table 3). The recovery

Table 4 – Mold and yeast populations in switchgrass and reed canarygrass subsample parcels after anaerobic storage and two or seven days of aerobic exposure.

Crop type and silo bag no.	Treatment	DM content[a]	Mold or yeast population … log (CFU/g)					
			At removal		After 2-days aerobic exposure		After 7-days aerobic exposure	
			Mold	Yeast	Mold	Yeast	Mold	Yeast
Switchgrass								
Bag 1[a]	Control	Low	6.3	6.3	7.3b	6.3 ab	6.0a	7.8b
	LB + PP[c]		5.2	4.5	5.3a	5.3a	6.5a	7.2 ab
	Control	High	5.0	6.0	6.5 ab	8.0b	6.4a	7.6b
	LB + PP		4.8	4.6	5.8a	5.7a	7.4b	6.5a
	LSD[d]		**3.2**	**2.1**	**1.3**	**2.1**	**0.8**	**1.1**
	Control		5.7	6.2b	6.9b	7.2b	6.2a	7.7b
	LB + PP		5.0	4.6a	5.6a	5.5a	7.0b	6.9a
	LSD[e]		**2.2**	**1.4**	**0.8**	**1.3**	**0.5**	**0.7**
	Low DM		5.8b	5.4	6.3	5.8	6.3	7.5
	High DM		4.9a	5.3	6.2	6.9	6.9	7.1
	LSD[f]		**2.2**	**1.4**	**0.8**	**1.3**	**0.5**	**0.7**
Reed Canarygrass								
Bag 2[b]	Control		6.7	6.9	3.0a	8.4b	6.6	7.3b
	LB + PP		5.7	5.8	7.2b	6.5a	6.0	5.0a
	LSD[d]		**1.9**	**3.5**	**1.1**	**0.8**	**1.6**	**1.9**

[a] Stored on September 8, 2010 and removed after 222 days. Low and high DM contents were 459 and 566 g kg^{-1}, respectively.
[b] Stored on August 31, 2010 and removed after 230 days. Average DM content was 526 g kg^{-1}.
[c] On-harvester application of approximately 400,000 cfu/g *Lactobacillus buchneri* (LB) and 100,000 cfu/g *Pediococcus pentosaceus* (PP).
[d] Least significant difference. Mean values followed by different letters in the same column are significantly different at 95% confidence.
[e] Data pooled by treatment and analyzed using two-way analysis of variance.
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Table 5 – Aerobic stability as quantified by heating degree days (°C) during two aerobic exposure durations.

Crop type	DM content[a] g kg^{-1}	Treatment	Aerobic exposure duration			
			2 Days		7 Days	
			Container position[c]		Container position[c]	
			Shallow	Deep	Shallow	Deep
Switchgrass	444	Control	1	0	0	0
	472	LB + PP[b]	0	0	0	0
	596	Control	3	6	77	59
	536	LB + PP	0	0	4	11
Reed Canarygrass	536	Control	1	0	75	53
	545	LB + PP	7	2	11	16

[a] Dry matter (DM) content of subsample parcels at the beginning of the aerobic exposure.
[b] On-harvester application of approximately 400,000 cfu/g *Lactobacillus buchneri* (LB) and 100,000 cfu/g *Pediococcus pentosaceus* (PP).
[c] The subsample parcels were approximately 10 and 45 cm from the material surface for the shallow and deep positions, respectively.

of these cell wall polysaccharides after ensiling was very good (Table 3). Ensiling solubilized an average of 7% of SWG cellulose mass, but there were apparent gains in hemicellulose during storage. This is an unusual result because hemicellulose is generally considered more accessible to silage microorganisms than cellulose; however these results are not without precedence [30]. Recovery ratio of SWG lignin averaged 1.02 and there were no significant differences between treatments. Ensiling solubilized an average of 4% and 7% of the mass fraction of RCG cellulose and hemicellulose content, respectively. Cellulose and hemicellulose recovery of wrapped and ensiled bales of SWG and RCG at 510–660 g kg^{-1} DM ranged from 0.97 to 1.0 for both constituents [1].

Inoculation with LB + PP significantly reduced yeast colonization during storage in SWG, but not RCG (Table 4). After two days of aerobic exposure, inoculated SWG had statistically smaller mold and yeast populations and smaller yeast populations after seven day exposure. Yeast populations were significantly less with inoculated RCG after both exposure durations.

Shortly after the silo bags were sealed, the temperature increased quickly during the aerobic phase (Fig. 1). The temperature typically peaked within five to seven days during the aerobic ensiling phase. Once this phase was over, the temperatures gradually declined and stabilized for the remainder of the storage period. Temperature histories of this type indicate that stable conditions existed in the bag silos during the storage period [31].

Both grasses were relatively stable during the first two days of aerobic exposure (Table 5). The SWG ensiled at low DM content was more stable than the high DM SWG or RCG over the seven day exposure period. The material inoculated with LB + PP was more stable in those circumstances when heating did occur in the control treatment. Subsample parcels containing the control treatments of SWG and RCG that were located closer to the container surface heated more than those buried deeper in the material (Fig. 2).

4. Discussion

The removal rate of ensiled biomass feedstocks would be comparatively much faster than animal feed removed from a farm silo, so concerns about ensiling at high DM, the resulting weak fermentation, and potential for aerobic instability should be mitigated with grasses ensiled at high DM because the duration of aerobic exposure will be short. Since biomass feedstocks must be transported off the farm, transportation costs can be reduced when the dry mass transported is maximized. From this standpoint, conserving biomass feedstocks by anaerobic storage at 500–600 g kg^{-1} DM would be desirable provided storage conservation and aerobic stability can be achieved. In this research, SWG and RCG were well conserved by anaerobic storage even when DM was greater than 590 g kg^{-1}. Both SWG and RCG losses were generally below 35 g kg^{-1} of DM (Table 1). These losses are much less than those reported for dry bales stored outdoors [1,9]. Losses of DM during aerobic exposure were small, averaging 14 and 21 g kg^{-1} of DM during two- and seven-day exposure durations. It is likely that most feedstocks would be consumed in that time given the mass input requirements of large scale biorefineries. The temperature of low DM content SWG and RCG without inoculation increased rapidly after several days of aerobic exposure (Table 5, Fig. 2). On-harvester inoculation with a combination of homofermentative (*P. pentosaceus*) and

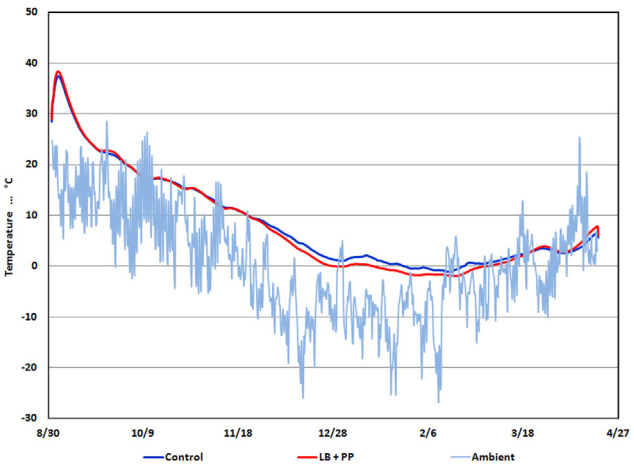

Fig. 1 – Temperature history of reed canarygrass with or without application of *Lactobacillus buchneri* (LB) and *Pediococcus pentosaceus* (PP), stored in silo bags at 520 g kg^{-1} DM.

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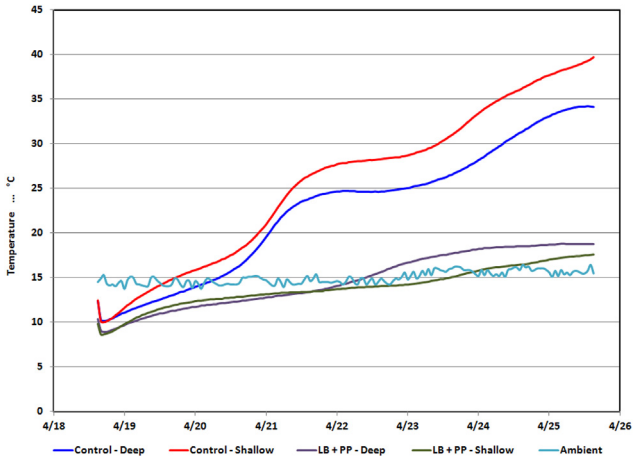

Fig. 2 – Temperature history of switchgrass with or without application of *Lactobacillus buchneri* (LB) and *Pediococcus pentosaceus* (PP), at 570 g kg^{-1} DM content in subsample parcels located 10 and 45 cm from the exposed surface (shallow and deep positions, respectively).

heterofermentative (*L. buchneri*) bacterium increased the production of both lactic and acetic acid during storage (Table 2), resulted in smaller yeast populations during aerobic exposure (Table 4) and improved aerobic stability so that little heating occurred in the inoculated grasses (Table 5, Fig. 2). Based on the positive results with inoculation, on-harvester application of the inoculant appeared successful.

Biorefineries desire feedstocks with very consistent properties. The DM content of material removed after many months of anaerobic storage was similar to the harvested DM (Table 1) and the spatial distribution within the silo bag was uniform [20]. Recovery of cell wall contents at storage was very good (Table 3). Moist biomass crops stored anaerobically in silo bags should produce a more desirable feedstock than outdoor stored bales because of lower losses and more consistent properties.

5. Conclusions

Anaerobic storage successfully conserved high DM switchgrass and reed canarygrass with losses during storage generally less than 35 g kg^{-1} of DM. On-harvester application of the bacterial inoculants improved the aerobic stability of the silages after removal from storage. Anaerobic storage of chopped, inoculated, high DM, mature perennial grasses was shown to be a viable cellulosic biomass feedstock logistics system.

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REFERENCES

[1] Shinners KJ, Boettcher GC, Muck RE, Weimer PJ, Casler MD. Harvest and storage of two perennial grasses as biomass feedstocks. Trans ASABE 2010;53(2):359–70.
[2] Shinners KJ, Wepner AD, Muck RE, Weimer PJ. Aerobic and anaerobic storage of single-pass chopped corn stover. Bioenergy Res 2011;4(1):62–75.
[3] 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.
[4] Hess JR, Wright CT, Kenney KL. Cellulosic biomass feedstocks and logistics for ethanol production. Biofuels Bioprod Bioref 2007;1(3):181–90.
[5] Worley J, Cundiff J. Comparison of harvesting and transport issues when biomass crops are handled as hay versus silage. Bioresour Technol 1996;56(1):69–75.
[6] Shinners KJ, Binversie BN, Savoie P. Whole-plant corn harvesting for biomass: comparison of single-pass and multiple-pass harvest systems. St. Joseph, MI: ASABE; 2003.
[7] Cook DE, Shinners KJ. Economics of alternative corn stover logistics systems. ASABE Paper No. 1111130. St. Joseph, MI: ASABE; 2011.
[8] Rentizelas AA, Tolis AJ, Tatsiopoulos IP. Logistics issues of biomass: the storage problem and the multi-biomass supply chain. Renew Sustain Energy Rev; 2009:887–94.
[9] Sanderson MA, Egg RP, Wiselogel AE. Biomass losses during harvest and storage of switchgrass. Biomass Bioenergy 1997;12(2):107–14.
[10] Shinners KJ, Binversie BN, Muck RE, Weimer PJ. Comparison of wet and dry corn stover harvest and storage. Trans ASABE 2007;53(2):359–70.
[11] Ren H, Richard TL, Chen Z, Kuo M, Bian Y, Moore KJ. Ensiling corn stover: effect of feedstock preservation on particleboard performance. Biotechnol Prog 2006;22(1):78–85.
[12] Muck RE, Shinners KJ. Effect of inoculants on the ensiling of corn stover. ASABE Paper No. 061013. St. Joseph, MI: ASABE; 2006.
[13] Kung Jr L. The aerobic stability of silage. Second International Symposium on animal Production Under Grazing. Brazil: University of Vicosa; 2008. pp. 233–48.
[14] Burns JC, Fisher DS, Pond KR. Ensiling characteristics and utilization of switchgrass preserved as silage. Postharvest Biol Technol 1993;3(4):349–59.
[15] Cherney DJR, Alessi MA, Cherney JH. Influence of grass species and sample preparation on ensiling characteristics. Crop Sci 2006;46:256–63.
[16] Digman M, Shinners K, Muck R, Dien B. Full-scale on-farm pretreatment of perennial grasses with dilute acid for fuel ethanol production. Bioenergy Res 2010;3:335–41.
[17] Digman M, Shinners K, Muck R, Dien B. Pilot-scale on-farm pretreatment of perennial grasses with dilute acid and alkali for fuel ethanol production. Trans ASABE 2010;53(3):1007–14.
[18] Moore KJ, Moser LE, Vogel KP, Waller SS, Johnson BE, Pedersen JF. Describing and quantifying growth stages of perennial forage grasses. Paper 507. Agronomy – Faculty Publications; 1991., http://digitalcommons.unl.edu/agronomyfacpub/507.
[19] Anon. ASABE Standard 424.1: method of determining and expressing particle size of chopped forage materials by screening. St. Joseph, MI: ASABE; 2011.

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[20] Williams SD. Anaerobic storage and aerobic stability of moist biomass feedstocks. Unpublished Master of Science thesis. Madison, WI: Univeristy of Wisconsin; 2011.
[21] Anon. ASABE standard S358.2 moisture measurement – forages. St. Joseph, MI: ASABE; 2011.
[22] Goering HK, Van Soest PJ. Forage fiber analysis (apparatus, reagents, procedures and some applications. USDA Agricultural Handbook No. 379; 1970.
[23] Steel RGD, Torrie JH, Dickey DA. Principles and procedures of statistics: a biometrical approach. New York, NY: McGraw-Hill; 1996.
[24] Driehuis F, van Wikselaar PG, van Vuuren AM, Spoelstra SF. Effect of a bacterial inoculant on rate of fermentation and chemical composition of high dry matter grass silages. J Agric Sci 1997;128:323–9.
[25] Driehuis F, Oude Elferink SJWH, Van Wikselaar PG. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. Grass Forage Sci 2001;56(4):330–43.
[26] Buxton DR, O'Kiely P. Preharvest plant factors affecting ensiling. In: Buxton DR, Muck RE, Harrison JH, editors. Silage science and technology. Madison, WI: American Society of Agronomy; 2003.
[27] Mitloehner FM, Malkina IL, Green PG. Impacts of dairies and silage on air quality. California Alfalfa & Forage Symposium and Corn/Cereal Silage Mini-Symposium, http://alfalfa.ucdavis.edu/+symposium/proceedings/2010/10-25.pdf; 2010.
[28] Bakker RB, Elbersen HW. Managing ash content and quality of in herbaceous biomass: an analysis from plant to product. In: Paris, France: 14th European Biomass Conference; 2005. pp. 210–3.
[29] Dien BS, Jung HG, Vogel KP, Casler MD, Lamb JFS, Itena L, et al. Chemical composition and response to dilute-acid pretreatment and enzymatic saccharification of alfalfa, reed canarygrass, and switchgrass. Biomass Bioenergy 2006;30:880–91.
[30] Morrison IM. Influence of some chemical and biological additives on the fibre fraction of lucerne on ensilage in laboratory silos. J Agric Sci 1989;111:35–9.
[31] Pitt RE. Silage and hay production. NRAES Publication No. 5. Ithica, NY: Northeast Regional Agricultural Engineering Service; 1990.

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