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Comparison of Sealing and Open Conditions for Long Term Storage of Corn Stover Using Low-Moisture Anhydrous Ammonia Pretreatment Method

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Abstract. *As a promising material for bioethanol production, corn stover has been studied under various pretreatment methods prior to production of bioethanol. However, the storage of pretreated corn stover is still challenged by both weather conditions and the physical properties of its own. The objective of this experiment is to evaluate the effect of low-moisture anhydrous ammonia (LMAA) pretreatment method on biomass quality during long periods of storage. In this study, corn stover was contacted with various ammonia loadings (0, 0.1, and 0.2 g/g DM biomass) and moisture content (20 wt.%, 40 wt.%, and 60 wt.%) from 1 day to 90 days both in sealed and open containers. As a result, the mass loss in sealed container increased with time; however, the mass loss in open container was affected by the conditions of the environment. In terms of the carbohydrate, no significant reduction was observed in either sealed or open containers.*

Keywords. *LMAA, corn stover, bioethanol, storage, dry matter loss, mold growth*

Introduction

Bioethanol, a promising replacement of fossil fuel, has been studies in various ways. Typically, bioethanol can be produced by food crops, such as corn and sugarcane, or lignocellulosic biomass, which is non edible plant and energy crops (Nagarajan et al., 2013). With the aim of producing 36 billion gallons of ethanol per year by 2022, 16 billion gallons was supposed to come from cellulosic biomass (Schnoor, 2011).

Corn stover, mainly comprised of the stalks and leaves, has the great potential to serve as the biofuel feedstock. According to the estimation of Kadam (2003), 80-100 million dry tonnes/year of corn stover could be collected, among which 80% is available for ethanol production (Kadam and McMillan, 2003). Currently, the potential of the conversion of corn stover to biofuel is targeted to be 90 gal/ton in the near future (DOE-EERE 2009). However, the sturdy structure of lignocellulosic biomass increases the difficulty in bioethanol production.

Generally speaking, lignocellulosic biomass is composed of three parts: cellulose, hemicellulose and lignin. As is known, cellulose is a linear polymer of glucose; hemicellulose is a branched polymer containing xylose, arabinose, mannose, and some other polysaccharides. In terms of lignin, it is a highly disordered polymer which serves as the protection since cellulose is embedded in the matrix of lignin and hemicellulose (Menon and Rao, 2012). In order to open the structure and expose cellulose within lignocellulosic biomass, a pretreatment process before hydrolysis and fermentation is critical.

Pretreatment processes have been developed by numerous studies. One of the base reagents adopted by researchers is ammonia. Ammonia fiber expansion (AFEX) uses concentrated ammonia to break down the inner structure of lignocellulosic biomass for the enzymatic hydrolysis and fermentation process (Lau et al., 2010); Soaking in aqueous ammonia (SAA) for pretreatment is proved to have the ability of retaining the hemicellulose at low temperature and increasing the fermentation yield (Kim and Lee, 2005); And the low-moisture anhydrous ammonia (LMAA) process is developed to minimize the water and ammonia input for bioethanol production (Yoo et al., 2011).

In terms of corn stover storage, two common approaches are applied: dry storage and wet storage (Cui et al., 2012). Dry product, which refers to 20 - 25% moisture content in raw corn stover, is typically harvested and packaged in round bales (Shinners et al., 2007), but the high drying cost and high dry matter losses during storage are the remaining problems (Richard, 2010). On the other side, wet storage, also named ensilage, is a method of preserving biomass at high moisture content (> 45%) (Cui et al., 2012). It could minimize the loss of nutrients and reduce the drying cost (Weinberg and Ashbell, 2003), but it still has the problem of mold growth, which may be hazardous to downstream operations (Essien et al., 2005). In order to produce bioethanol, higher effective preservation of carbohydrates during storage is required.

In this study, a low moisture anhydrous ammonia (LMAA) pretreatment process is applied before corn stover storage since ammonia could result in higher efficiency in ethanol production (Ko et al., 2009) as well as impeding mold growth. The objective of this research is to evaluate the effects of the LMAA pretreatment process on biomass quality (changes in carbohydrates, ash, and mass losses) during long periods from 1 day to 3 months. In addition, growth of fungi or other microorganisms will be monitored in these days.

Materials and Methods

Biomass

Corn stover, freshly harvested and delivered in bales, was obtained from central Iowa, USA, 2013. It was air-dried before baling and receiving by the lab. Then, the corn stover was ground through a 2-mm screen using a grinder (Wiley Model 4), and stored at room temperature. After that, deionized water was mixed with corn stover to achieve the target moisture contents (20 wt.%, 40 wt.%, and 60 wt.%). Moisturized corn stover was placed overnight at ambient temperature to reach equilibrium.

Low-moisture anhydrous ammonia (LMAA) pretreatment process

Before contacting with anhydrous ammonia, moisture content of treated corn stover was determined and recorded using the moisture tester. Then the corn stover was placed into the ammoniation reactor, to contact with various loadings of anhydrous ammonia (0.1 g/g DM biomass and 0.2 g/g DM biomass), tightly closed the valve of the reactor for 30 minutes after reaching the target pressure. After that, the ammoniated corn stover was transferred into several heavy-duty Ziploc plastic bags and open containers, thoroughly mixed and weighed. Sealed containers (Fig. 1) and open containers (Fig. 2) were placed at ambient temperature for 0h, 6h, 1d, 5d, 12d, 30d, 60d, and 90d.

Compositional analysis

Once the duration time was achieved, pretreated samples were weighed, and surplus ammonia was evaporated in the fume hood. Then the compositional analysis was followed by the NREL LAP procedure (Sluiter et al., 2011). The monosaccharides were analyzed by high performance liquid chromatography (HPLC) installed with a Bio-Rad Aminex HPX-87P column (Aminex HPX-87P, Bio-Rad Laboratories, Hercules, CA, USA) and a refractive index detector (Varian 356-LC, Varian, Inc., CA, USA). The content of acid soluble lignin (ASL) was determined by UV-Visible spectrophotometer (UV-2100 Spectrophotometer, Unico, United Product & Instruments, Inc., Dayton, NY, USA). All samples were analyzed in duplicate.

Mold growth observationThe observation experiment was conducted both in sealed container samples and open containers. Mold growth was monitored everyday during the whole experimental period by obseving the changes in color and shape.

Experimental design

In this study, two independent variables were designed to investigate the storage effect: ammonia loading, and moisture content. Each has three levels. Moreover, full factorial design was used as shown in Table 1.

Results and Discussion

Mold growth

Mold growth in both sealed containers and open containers was observed during 90 days storage of corn stover. The first appearance of mold was found in treatment 3 after 16 days (Fig.

3). One day later, the mold growth was observed in treatment 2. However, there was no other mold appearance in other treatments until 90 days.

The results indicate that high moisture content was the main reason of the mold growth since the moisture content in treatment 2 and 3 were 40 wt.% and 60 wt.%, respectively. Moreover, under the protection of ammonia, pretreated corn stover could be well preserved without mold growth. This was due to the anti-microbial characteristic of ammonia (Rideal, 1895). Even though ammonia is not currently listed as the disinfectant by the Environmental Protection Agency (EPA), its effect in killing microbes and molds has been proved (Tajkarimi et al., 2008).

Storage mass losses

Mass loss during storage was measured in the changes of entire treatment. Results for sealed container (Table 3) and open container (Table 4) were quite different. And Table 5 showed the moisture content (% dry basis) and dry matter at t=0 h and t=90 d.

For sealed container treatments, as time increased, mass loss also increased. Their relationship could be seen from Fig. 4. Moreover, under the same moisture content, treatments with 0.2 g/g DM biomass ammonia loadings tend to have higher mass losses.

However, as for open container treatments, the mass changes during 90 days were highly dependent on the ambient temperature and humidity; the relationship between time and mass losses was not as straightforward as sealed container treatments. What's more, under the same ammonia loading, treatments with 60 wt.% moisture content lost more mass than the other two levels; but there was no obvious difference in mass losses among three levels of ammonia loading under the same moisture content.

Ash content

Ash content was measured following the NREL standard lab procedure (Sluiter et al., 2008). Distribution of ash content in the sealed container treatments was shown in Fig. 5. As can be seen from the graph, higher moisture content under the same ammonia loading tended to have higher ash content; and treatment 3 (60 wt.% moisture content with no ammonia loading) has the highest ash percentage, a similar trend was also found in open container treatments. The results indicated that anhydrous ammonia may not have significant effect in retaining the ash.

Ash content was also analyzed in the difference between sealed containers and open containers (Table 6). As time increased, the ash content decreased; and the corresponding p-value for time was larger than 0.0001, which indicated that there was little evidence of difference between ash content in either sealed containers or open containers.

Lignin content

As can be seen in Fig. 6, lignin content in sealed containers was higher in the first three treatments (without ammonia), and there was no significant evidence of difference between the two levels of ammonia loading in lignin content (p-value=0.0816).

The lignin content in open containers didn't resulted in significant difference among nine treatments (p-value=0.4647); however, under the same moisture content, the lignin content decreased rapidly with higher ammonia loading, which could be seen from Fig. 7.

Those results were the evidence that anhydrous ammonia has the potential to remove lignin, which could help to increase the accessibility of enzyme in hydrolyzing. Similar reduction in lignin content was also reported by other researchers (Lau et al., 2010).

Sugar content

Sugar content analysis focused on glucan content and xylan content in this study.

As for glucan content (Table 7), the overall trend in both sealed containers and open containers was decreasing over time. In sealed containers, there was no significant decline in glucan content among the nine treatments since p-value =0.6714; in open containers, the reduction trend was not obvious either (p-value=0.4468). The reason for this insignificant change was because the effect of the low-moisture anhydrous ammonia pretreatment was to break down the lignin-carbohydrate-compounds (LCC) for higher enzymatic hydrolysis rate; it didn't affect the glucan content in biomass.

In terms of xylan content (Table 8), the reduction in sealed containers under the same ammonia loading was observed, as shown in Fig. 8, but with the same moisture content, higher ammonia loading tended to retain more xylan. In open containers, no obvious reduction was observed. By analyzing the difference between two sealing conditions, even though xylan content in open containers was higher than in sealed containers, the difference was not obvious (p-value=0.4978). This could also be explained by the pretreatment effect; LMAA didn't affect the xylan content significantly in biomass.

Conclusions

LMAA pretreated corn stover could be well preserved up to 90 days without mold growth and reduction in carbohydrates. Compared between two sealing conditions (sealed containers and open containers), the effect of lignin removal was more obvious in sealed containers, but the sugar contents in both conditions were nearly the same. As for ammonia loading, mass losses in 0.2- was higher than 0.1 g/g DM biomass, however, no other significant differences were found in terms of ash content, lignin content and sugar contents. For future work, more attention could be focused on the interaction effect of time and temperature during long-term storage.

Tables and figures

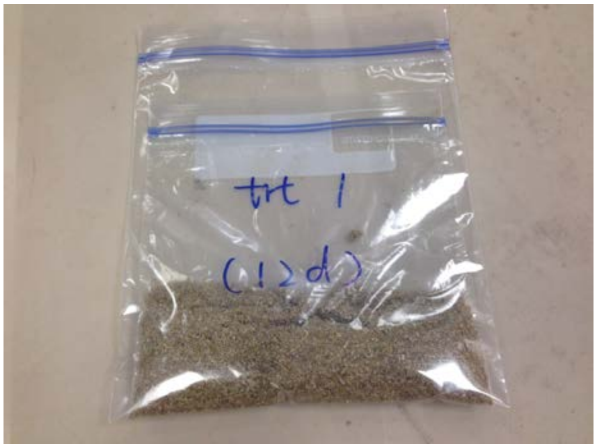

Figure 1. Sealed container.

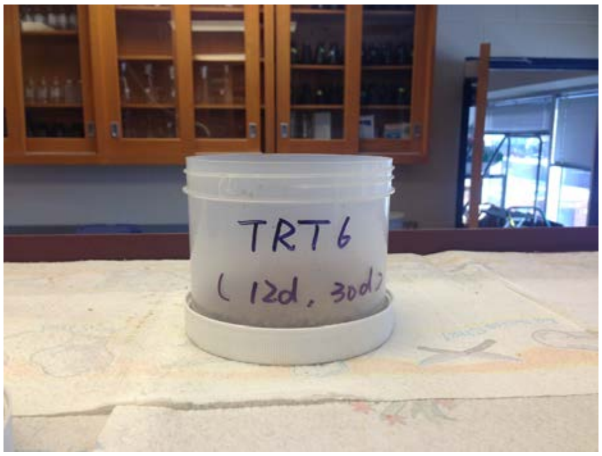

Figure 2. Open container.

Figure 3. Visible mold grown in sealed containers after 16 days of storage.

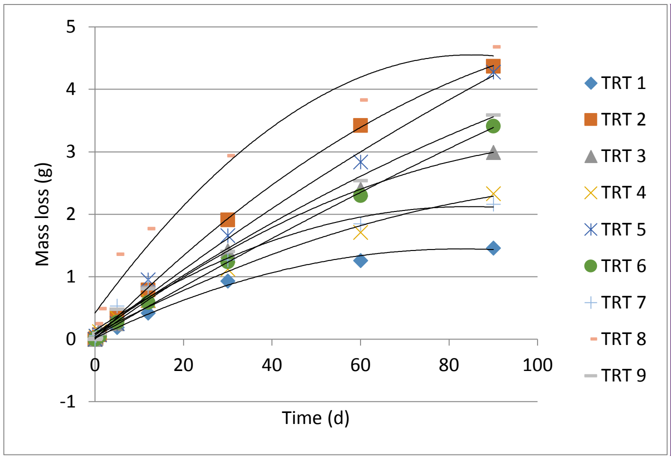

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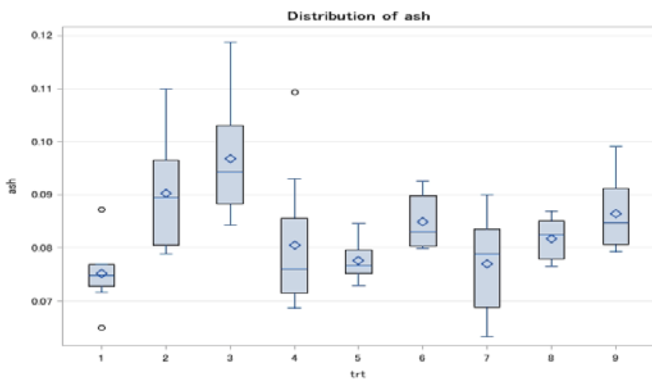
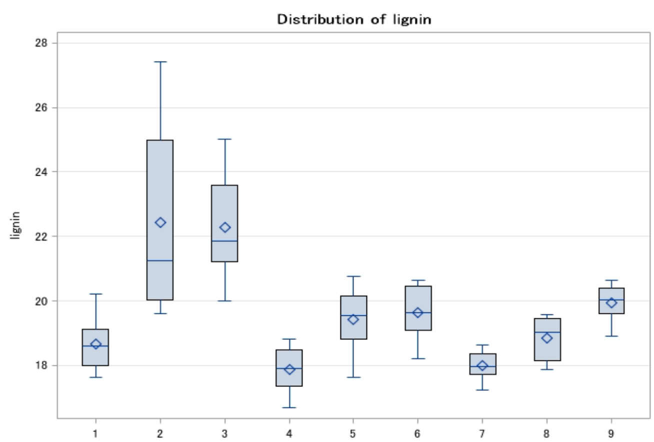
Figure 4. Relationship between mass changes (wet basis) and time in sealed containers.



S.D.: trt1=0.006; trt2=0.011; trt3=0.011; trt4=0.014; trt5=0.004; trt6=0.005; trt7=0.009; trt8=0.004; trt9=0.007.

Figure 5. Distribution of ash content in sealed containers.



S.D.: trt1=0.860; trt2=3.149; trt3=1.802; trt4=0.737; trt5=1.016; trt6=0.855; trt7=0.472; trt8=0.695; trt9=0.580.

Figure 6. Distribution of lignin content in sealed containers.

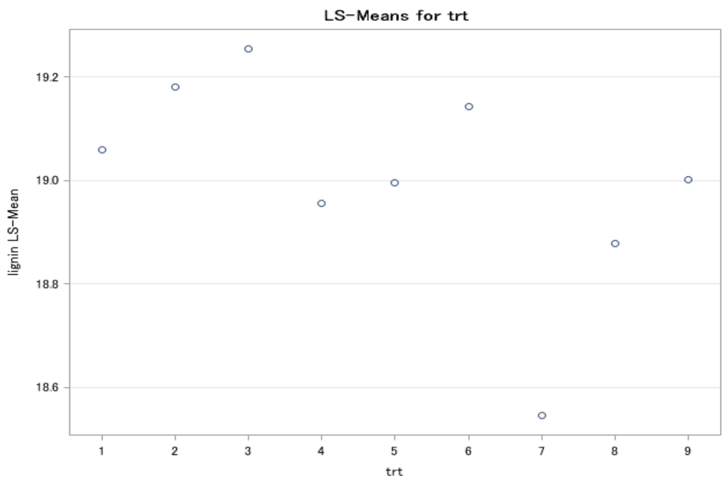


Figure 7. Least square means of lignin content in open containers.

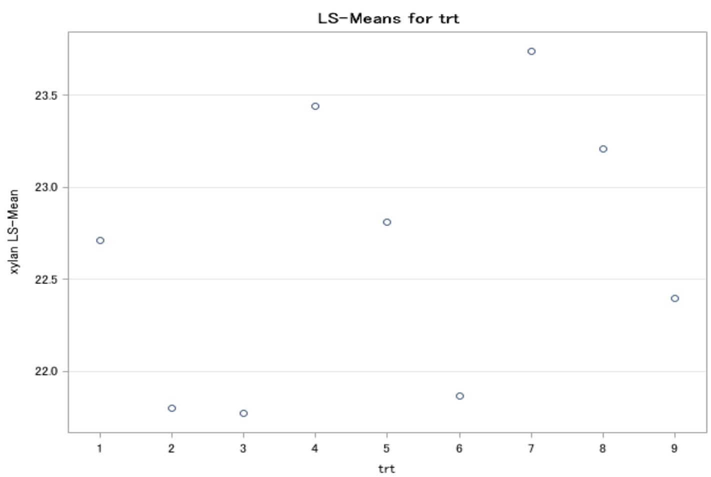


Figure 8. Least square means of xylan content in sealed containers.

Table 1. Experimental design.

Treatment	Ammonia Loading	M.C.
1	0	20%
2	0	45%
3	0	60%
4	0.1	20%
5	0.1	45%
6	0.1	60%
7	0.2	20%
8	0.2	45%
9	0.2	60%

Table 2. Regression analysis of the mass loss (Y) and time (X) of the seal containers.

	Regression Analysis	R^2
TRT 1	$Y = -0.0002X^2+0.0345X+0.0162$	0.9952
TRT 2	$Y = -0.0003X^2+0.0723X-0.0029$	0.9998
TRT 3	$Y = -0.0002X^2+0.0529X+0.0239$	0.9992
TRT 4	$Y = -0.0001X^2+0.0376X+0.0861$	0.9922
TRT 5	$Y = -9*10^{-5}X^2+0.0545X+0.0584$	0.9939
TRT 6	$Y = -4*10^{-5}X^2+0.0413X+0.0325$	0.999
TRT 7	$Y = -0.0003X^2+0.0469X+0.1411$	0.9784
TRT 8	$Y = -0.0006X^2+0.0973X+0.4176$	0.9683
TRT 9	$Y = -0.0001X^2+0.0488X+0.09$	0.9943

Table 3. Storage mass loss results for sealed containers.

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	0	0.01	0.04	0.19	0.42	0.93	1.26	1.46
TRT 2	0	0.01	0.07	0.39	0.79	1.91	3.42	4.37
TRT 3	0	*0.02*	0.15	0.25	0.62	1.42	2.4	2.99
TRT 4	0	0.04	0.12	0.34	0.62	1.12	1.71	2.33
TRT 5	0	0.05	0.07	0.25	0.95	1.66	2.84	4.28
TRT 6	0	0.02	0.05	0.27	0.59	1.24	2.30	3.41
TRT 7	0	0.06	0.16	0.53	0.85	1.27	1.85	2.16
TRT 8	0	0.25	0.49	1.36	1.77	2.94	3.83	4.68
TRT 9	0	0.02	0.09	0.48	0.81	1.41	2.54	3.59

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Table 4. Storage mass loss results for open containers.

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	0	1.37	2.14	0.33	4.02	-0.15	2.99	-0.37
TRT 2	0	3.44	5.6	1.4	11.13	-0.08	10.4	-0.07
TRT 3	0	5.31	7.7	3.32	17.72	-0.13	17.19	-0.08
TRT 4	0	2.14	1.44	0.35	3.93	-0.25	3.08	-0.07
TRT 5	0	5.09	4.96	0.77	9.76	-0.18	8.74	-0.07
TRT 6	0	4.5	8.4	2.63	17.98	-0.09	16.59	-0.04
TRT 7	0	2.83	1.6	0.32	4.43	-0.13	4.23	-0.24
TRT 8	0	5.77	5.36	1.03	10.63	-0.1	10.63	-0.04
TRT 9	0	6.8	7.64	2.45	17.8	-0.11	16.8	-0.05

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Table 5. Moisture content and dry matter at t=0h and t=90d.

SEALED CONTAINERS				
	Moisture content (dry basis)		*Dry matter (g)*	
	t=0h	*t=90d*	*t=0h*	*t=90d*
TRT 1	19.20%	12.16%	11.48	11.20
TRT 2	43.29%	36.21%	11.39	10.02
TRT 3	58.38%	41.87%	7.21	8.34
TRT 4	19.20%	11.49%	15.91	15.37
TRT 5	43.29%	32.59%	12.66	12.17
TRT 6	58.38%	48.50%	7.98	8.12
TRT 7	19.20%	9.41%	14.80	14.64
TRT 8	43.29%	28.00%	11.14	10.78
TRT 9	58.38%	45.83%	7.50	7.81
OPEN CONTAINERS				
	Moisture content (dry basis)		*Dry matter (g)*	
	t=0h	*t=90d*	*t=0h*	*t=90d*
TRT 1	19.20%	10.13%	12.14	12.15
TRT 2	43.29%	15.95%	17.05	16.53
TRT 3	58.38%	29.89%	12.49	9.00
TRT 4	19.20%	8.98%	12.38	12.55
TRT 5	43.29%	18.96%	17.04	17.27
TRT 6	58.38%	29.75%	12.65	9.69
TRT 7	19.20%	9.12%	24.31	23.50
TRT 8	43.29%	18.19%	17.48	16.53
TRT 9	58.38%	27.56%	12.58	9.72

Table 6. Differences (db %) in ash between sealed and open containers.*

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	-0.90(0.41)	1.35(0.91)	0.30(0.04)	0.74(0.27)	-1.77(1.57)	-1.95(1.90)	2.81(3.94)	-3.90(7.61)
TRT 2	0.44(0.09)	1.16(0.07)	2.33(2.70)	0.61(0.19)	1.57(1.23)	2.73(3.72)	2.42(2.92)	0.10(0.01)
TRT 3	0.31(0.05)	0.34(0.06)	1.92(1.84)	-0.26(0.03)	0.40(0.08)	1.97(1.95)	1.27(0.80)	-0.07(0.01)
TRT 4	0.82(0.33)	1.64(1.33)	2.56(3.27)	-0.65(0.21)	0.76(0.28)	1.07(0.57)	3.35(5.61)	-4.03(8.12)
TRT 5	0.91(0.42)	0.63(0.20)	1.92(1.84)	0.08(0.01)	0.47(0.11)	0.26(0.03)	0.71(0.25)	-2.79(3.88)
TRT 6	0.78(0.30)	0.57(0.16)	0.85(0.36)	0.75(0.28)	-0.53(0.14)	-0.39(0.07)	-0.58(0.16)	-3.88(7.52)
TRT 7	0.69(0.24)	1.18(0.69)	1.22(0.75)	1.23(0.58)	1.08(0.99)	1.41(0.52)	-1.03(5.99)	-3.46(0.40)
TRT 8	0.90(0.40)	0.23(0.03)	1.04(0.54)	0.21(0.02)	-0.06(0.01)	1.02(0.51)	-0.01(0.01)	-4.58(1.05)
TRT 9	0.60(0.17)	-0.13(0.01)	2.52(3.18)	1.11(0.62)	-0.66(0.21)	-0.06(0.01)	-0.66(0.21)	-3.52(0.62)

* Difference = Ash (sealed container) - Ash (open container). Values in parentheses are standard deviation.

Table 7. Differences in glucan content (db %) in sealed and open containers.*

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	-7.73(2.98)	-0.74(0.28)	-1.92(1.85)	-3.81(7.28)	2.17(2.34)	2.04(2.08)	4.88(1.19)	-2.23(2.49)
TRT 2	-2.72(3.71)	0.40(0.08)	-3.07(4.73)	-2.46(3.03)	-1.83(1.67)	-5.30(1.41)	-3.15(4.97)	-1.09(0.59)
TRT 3	-2.33(2.73)	0.99(0.48)	-1.79(1.61)	-3.91(7.62)	-1.71(1.46)	-1.55(1.19)	-3.09(4.76)	-1.65(1.36)
TRT 4	-3.33(5.53)	-3.59(3.46)	-1.17(0.68)	-2.74(3.75)	0.58(0.17)	-1.06(0.56)	4.76(1.13)	-2.87(4.11)
TRT 5	-3.97(7.89)	-2.46(3.01)	-1.50(1.12)	-5.87(1.72)	0.44(0.09)	0.71(0.25)	-0.17(0.01)	-3.05(4.64)
TRT 6	-6.39(2.04)	-7.86(3.08)	-1.10(0.61)	-5.19(1.35)	0.64(0.20)	-0.72(0.26)	6.41(2.05)	-3.54(6.27)
TRT 7	-4.92(1.21)	1.14(0.65)	-0.48(0.12)	-1.45 (1.06)	-0.20(0.02)	-4.70(1.11)	5.95(1.77)	-2.85(4.07)
TRT 8	-5.58(1.56)	1.25(0.78)	1.89(1.78)	-4.00(0.81)	1.15(0.65)	1.96(1.92)	1.10 (0.61)	-1.17(0.68)
TRT 9	-6.84(2.34)	-0.33(0.05)	-1.22(0.75)	-4.34(9.43)	-0.93(0.44)	-1.12(0.63)	0.21(0.02)	-3.47(6.00)

* Difference = Glucan (sealed container) - Glucan (open container). Values in parentheses are standard deviation.

Table 8. Differences in xylan content (db %) in sealed and open containers.*

	0h	6h	1d	5d	12d	30d	60d	90d
TRT 1	2.24(2.52)	0.24(0.03)	1.33(0.88)	2.79(3.89)	-3.08(4.75)	1.62(1.31)	-1.72(1.48)	-1.10(0.61)
TRT 2	-1.23(0.76)	1.96(1.92)	5.28(1.39)	2.57(3.30)	-0.57(0.17)	4.60(1.06)	-1.64(1.35)	-7.03(2.47)
TRT 3	4.80(1.15)	-1.02(0.52)	0.89(0.39)	1.49(1.11)	-0.72(0.26)	2.69(3.61)	-1.86(1.73)	3.95(7.82)
TRT 4	1.54(1.18)	1.25(0.78)	0.54(0.15)	1.65(1.36)	-1.63(1.33)	3.87(7.48)	0.36(0.06)	-4.45(2.99)
TRT 5	4.24(8.98)	0.60(0.19)	1.49(1.12)	1.33(0.88)	-0.66(0.22)	4.04(8.15)	-2.10(2.19)	-5.38(1.45)
TRT 6	2.65(3.52)	2.96(4.38)	-0.17(0.01)	1.20(0.73)	-0.79(0.32)	4.28(9.14)	1.09(0.60)	-6.05(1.83)
TRT 7	1.25(0.78)	-4.04(8.18)	-0.07(0.02)	-0.42(0.08)	-0.34(0.06)	2.02(2.04)	-1.75(1.53)	-5.71(1.63)
TRT 8	2.19(2.41)	-1.27(0.81)	-1.75(1.54)	3.16(5.01)	0.06(0.01)	2.77(3.85)	-1.45(1.05)	-5.62(1.58)
TRT 9	2.21(2.44)	-1.74(1.52)	-0.08(0.03)	3.41(5.81)	2.88(4.16)	4.21(8.87)	-2.93(4.31)	-4.85(1.17)

* Difference = Xylan (sealed container) - Xylan (open container). Values in parentheses are standard deviation.

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