Comparison of Corn Stover Cell Wall Polysaccharide Degradability by Rumen Microbes and a Cellulosic Ethanol Conversion Process

H.G. Jung[1,2]* and R. Bernardo[2]
[1]USDA-ARS Plant Science Research Unit, St. Paul, MN
[2]Dept. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul

INTRODUCTION

The prospect of global warming due to CO_2 emissions from fossil fuels and increased dependence on foreign sources of energy have led to large-scale research on the development of renewable, low-carbon emission energy systems. The U.S. Renewable Fuels Standards (RFS2) call for production of 36 billion gallons of bio-based transportation fuels by 2022, with corn grain-derived ethanol limited to 15 billion gallons and 16 billion gallons required to come from cellulosic biomass. Among the challenges faced by cellulosic biofuels is the need for biomass sources that have greater conversion efficiency of the cell wall polysaccharides to fermentable sugars.

Identification of germplasm with greater biofuel potential requires the use of screening systems to predict performance in industrial conversion processes. Because production of fermentable sugars from cell wall polysaccharides is generally expected to occur (at least partially) by enzymatic processes, rumen digestibility has been assumed to be a useful model for predicting performance in biofuel conversion processes. In vitro rumen dry matter digestibility and in vitro fermentation gas production by rumen inoculums have been used to screen biomass germplasm for conversion potential (Anderson et al., 2010; Weimer et al., 2005). However, when these in vitro rumen systems have been compared to a dilute acid/high temperature pretreatment followed by enzymatic saccharification and ethanol fermentation by yeast, the rumen systems have been only moderately successful in predicting conversion results. This is not surprising given that rumen microorganisms ferment soluble sugars, proteins, and other organic constituents of biomass in addition to the cell wall polysaccharides whereas yeast can only utilize hexose sugars. We attempted to avoid these confounding effects by examining only the actual breakdown of the cell wall polysaccharides by both processes.

The objectives of this study were to:

1) directly compare cell wall polysaccharide degradation by in vitro incubation with a rumen mixed-culture inoculum vs. a dilute acid/high temperature pretreatment followed by enzymatic hydrolysis conversion process, and

2) evaluate relationships of biomass lignification with cell wall degradability by these two degradative processes.

PROTOCOL

A set of 153 corn stover samples were used in the current study. Samples were drawn from a larger study involving 223 recombinant inbred line test crosses grown at four locations in Minnesota (Lewis et al., 2010; Lorenzana et al., 2010). Stover was collected at grain physiological maturity, dried at 60ºC, and ground to pass a 1-mm screen in a cyclone-type mill. The selected samples served as the calibration set for near-infrared reflectance spectroscopy (NIRS) prediction equation development for cell wall components and conversion potential. To provide a range in composition, 70 stover samples were selected based on NIRS spectral diversity, 80 samples were selected by stratified-random sampling based on the experimental design, and two random samples were included for each of the inbred parents of the population. All selected samples were analyzed by wet-chemistry for cell wall polysaccharide sugar components and Klason lignin concentration (Theander et al., 1995), esterified *p*-coumarates and ferulates (Jung and Shalita-Jones, 1990), etherified ferulates (Iiyama et al., 1990), in vitro rumen cell wall polysaccharide degradability (Jung and Buxton, 1994), and dilute acid/high temperature pretreatment followed by enzymatic hydrolysis (Dien et al., 2006). Corn stover samples were incubated with a rumen fluid inoculum at 39ºC for 72 h, subsequently frozen and lyophilized, and analyzed for residual cell wall polysaccharide sugar components. For the conversion test stover samples were autoclaved at 121ºC for 1 h with 1.5% (wt/wt) sulfuric acid, followed by pH adjustment to 4.5 and addition of enzymes (cellulase and β-glucosidase), and incubation at 45ºC for 72 h. Conversion test samples were then frozen, lyophilized, and analyzed for residual cell wall polysaccharide sugar components. All analyses were done in duplicate. One stover sample was lost during sample analyses.

Total cell wall polysaccharides were calculated as the sum of the sugar components (glucose, xylose, arabinose, galactose, mannose, and uronic acids) and cell wall concentration included lignin and hydroxycinnamates plus polysaccharides. Degradation of cell wall polysaccharides and component sugars were determined based on initial concentrations minus residual amounts after the rumen and conversion tests. In vitro rumen degradability was corrected for cell wall polysaccharides contributed by the rumen inoculum. The rumen and conversion results were compared with a paired *t*-test. Pearson correlations were calculated between the rumen and conversion tests, and correlations were determined for each degradation process with lignin and hydroxycinnamates.

Table 1. Variability in cell wall concentration and composition of the corn stover.

Trait	Mean	SE	Minimum	Maximum
		g kg^{-1} dry matter		
Cell wall	815	34	727	889
Glucose	344	17	297	384
Xylose	201	12	171	225
Arabinose	27	3	21	35
Galactose	11	1	7	16
Mannose	12	1	10	18
Uronic acids	27	2	22	32
Klason lignin	166	11	139	193
p-Coumarate esters	18.56	1.93	13.20	23.04
Ferulate esters	3.25	0.24	2.63	3.87
Ferulate ethers	4.93	0.93	2.96	7.11

References

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Table 2. Cell wall polysaccharide degradability of corn stover by in vitro rumen incubation and dilute acid/high temperature/enzymatic conversion processes, and the correlation between the processes.

Trait	Process	Mean	SE	Minimum	Maximum	r
			g kg^{-1}			
Cell wall polysaccharides†	Rumen	569	36	475	663	0.64***
	Conversion	649	34	529	751	
Glucose†	Rumen	583	37	502	679	0.70***
	Conversion	518	44	350	639	
Xylose†	Rumen	517	46	375	638	0.18*
	Conversion	849	18	796	900	
Arabinose†	Rumen	650	42	520	733	0.43***
	Conversion	879	20	816	948	
Galactose†	Rumen	647	33	563	743	0.08
	Conversion	882	95	696	1000	
Mannose†	Rumen	820	26	719	867	0.08
	Conversion	-8	11	-38	20	
Uronic acids†	Rumen	615	59	459	723	0.37***
	Conversion	753	30	676	829	

*, *** P < 0.05 and 0.001, respectively.
† Results for rumen and conversion tests differ significantly (P < 0.001).

Table 3. Correlations of cell wall polysaccharide degradability by in vitro rumen incubation and the dilute acid/high temperature/enzymatic conversion process with corn stover lignification.

Trait†	Polysaccharide	Glucose	Xylose	Arabinose	Galactose	Mannose	Uronics
			In Vitro Rumen Degradability				
Klason lignin	-0.41***	-0.51***	-0.16*	-0.32***	-0.29***	-0.25**	-0.40***
PCA ester	-0.53***	-0.57***	-0.38***	-0.39***	-0.28***	-0.18*	-0.40***
FA ester	NS‡	NS	NS	NS	NS	NS	NS
FA ether	NS	NS	-0.26**	-0.18*	NS	-0.19*	NS
			Conversion Process Degradability				
Klason lignin	-0.39***	-0.45***	NS	-0.29***	NS	0.23**	-0.45***
PCA ester	-0.62***	-0.65***	-0.31***	-0.45***	NS	NS	-0.33***
FA ester	NS	NS	NS	NS	NS	-0.24**	0.24**
FA ether	NS	NS	NS	NS	0.50***	-0.20*	NS

*, **, *** P < 0.05, 0.01, and 0.001, respectively.
† PCA, *p*-coumarate; FA, ferulate.
‡ NS, non-significant (P < 0.05).

Summary

- All measures of cell wall polysaccharide degradability differed between the in vitro rumen and conversion processes, but degradability of most cell wall polysaccharide sugar components was correlated between the two processes.
- With the exception of cell wall glucose and mannose degradability, the conversion process resulted in greater degradation than did rumen microbes.
- The data suggest that the conversion process was enzyme limited (amount and/or type) for degradation of cellulose (glucose) and mannan.
- Degradability of polysaccharides besides cellulose and mannan was due to acid hydrolysis in the conversion process, rather than enzymatic, which explains the limited correlations between processes for the degradability of hemicellulosic and pectic polysaccharides.
- Lignin and *p*-coumarate esters were negatively correlated with rumen degradability of all cell wall polysaccharide components; however, these two measures of lignification were also correlated (r = 0.52, P < 0.001). In contrast, degradability of xylose and galactose in the conversion process were not correlated with lignin, and mannose degradability was actually positively correlated with lignin.
- Ferulate ether cross linking of lignin-to-arabinoxylan was negatively correlated with rumen degradability of xylose and arabinose, but not for the conversion process.

Conclusions

- Cellulose degradability by the conversion process is reasonably well predicted by in vitro rumen incubation.
- In vitro rumen degradability of hemicellulosic and pectic polysaccharides is a poor predictor of the degradability of these polysaccharides by the conversion process because the conversion process proceeds by a different mechanism (acid hydrolysis). This is an issue because these polysaccharides represent 45% of total corn stover polysaccharides.
- Measures of lignification are poor predictors of cell wall polysaccharide degradability for the conversion process.