Title: Preliminary Straw Characterization of Seven Biofuels Crops in the Pacific and Inland Northwest

PIs: Kristy Ott-Borrelli and Bill Pan

Introduction

As interest in alternative fuel increases, researchers and producers must consider potential multiple uses of biofuel feedstock. Plant residues high in cellulosic fiber compounds offer many benefits as fuel products and value-added by-products. Because plant biomass is a sustainable bioresource that can provide transportation fuels from renewable materials, research characterization of lignin, hemicellulose, and cellulose of various crop residues and their derivative products is encouraged (Zhang, 2008). Bioconversion of these products however, is difficult and expensive (Anderson and Akin, 2008; Emsley, 2008; Pauly and Keegstra, 2008). Some research has examined the possibility of altering these compounds in order to modify them for improved production (Li et al., 2008). In addition to biofuel production, fiber-rich crops are potentially useful for cloth and papermaking, and forage crops for ruminant livestock (Li et al., 2008). Lal (2007) recommended management practices that return carbon-rich compounds back to the soil in order to sequester carbon, improve soil health and nutrient cycling and mitigate global climate change. Crops with more than one use can stimulate both the economic and ecological benefits of a production system by potentially providing a producer with variable options and valuable by-products.

Current research for cellulosic ethanol production has focused mainly on corn-stover (*Zea maize*), wood, and hay residues from wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), triticale (*Triticosecale rimpaui*), rice (*Oryza sativa*), sugar cane (*Saccharum officinarum*), and switchgrass (*Panicum virgatum*) (Chen et al., 2007; Pauly and Keegstra, 2008). In the Pacific and Inland Northwest, researchers are also considering the production of crops such as canola (*Brassica napus*), camelina (*Camelina sativa*), and flax (*Linum narbonense*) in addition to weed species like *Arundo donax* and giant goat grass (*Aegilops* sp.) for oilseed and cellulosic fuels. The purpose of this research was to provide a preliminary assessment of the fiber content and carbon to nitrogen ratio (C:N) of seven plant species which can assist to determine their usefulness for biofuel production, rotational crops, soil amendments, and other by-products. Understanding the biochemical make up of these products can allow producers and researchers realize the potential uses and overcome difficult production barriers.

Materials and Methods

Subsamples of seven biofuel crop residues (camelina, flax, canola, wheat, giant goat grass, giant goatgrass wheat hybrid, and *Arundo*) were collected from researchers at Washington State University (Table 1). Camelina and canola are in the Brassicacea family, flax is in the Linaceae family and all others are grass species in the family Poaceae. Statistical analyses were performed between fiber contents of crop types but not between C:N. Data analysis for differences between treatments within a crop type (such as soil type and location) was performed but only average results based on crop type are presented in Table 2. Any notable trends between treatments are summarized in the results and discussion/conclusion sections.

Table 1. Location and description of plant samples used for fiber analyses.

Crop /Variety	**Location**	**Sample description**	**Plant parts sampled**
Camelina			
Calena	Paterson , WA	4 varieties from two different soil types;	Straw and
Ligena	Paterson , WA	sandy soil	inner membranes
Blaine Creek	Paterson , WA		of seed pods
Suneson	Paterson , WA		
Calena	Paterson , WA	Loam soil	
Ligena	Paterson , WA		
Blaine Creek	Paterson , WA		
Suneson	Paterson , WA		
Unknown	Prosser, WA	Camelina2; Roza plots	Whole plant
Flax			
Omega	Paterson , WA	Variety ‘McGregor’ only from Othello	Whole plant
Bethune	Paterson , WA		
Pembria	Paterson , WA		
Omega	Othello, WA		
Bethune	Othello, WA		
Pembria	Othello, WA		
McGregor	Othello, WA		
Canola			
High	Prosser, WA	Line source experiment for various	Stems and inner
Intermediate	Prosser, WA	irrigation rates; 12 total samples	membranes of
Low	Prosser, WA	(4 from each treatment) were collected	seed pods; leaves
Wheat			
Sample 1	Prosser, WA	From Roza plots; adjacent to Camelina2	Whole plant
Sample 2	Whitman Co.	Taken from roadside	Whole plant
Goat grass	Whitman Co.	Taken from roadside	Whole plant
Goat grass hybrid	Whitman Co.	Taken from roadside; unknown generation	Whole plant
Arundo donax	Prosser, WA	Mid-season, whole plant from 5 plots	Stem and leaves

Plant samples were ground to 2mm with a Wiley mill and Acid Detergent Lignin (ADL) procedures for fiber analysis as described by Van Soest et al. (1991) were performed using an ANKOM 200 Fiber Analyzer (Ankom Technology, Macedon, NY). The ADL procedure utilizes H_2SO_4 to hydrolyze cell wall polysaccharides and leaves an insoluble lignin residue (Hatfield et al., 1994). Briefly, 0.5 g of straw residues in filter bags were heated in a neutral detergent for determination of Neutral Detergent Fiber (NDF) for 75 minutes, heated in an acid detergent for determination of Acid Detergent Fiber (ADF) for 60 minutes and soaked in cold 72% H_2SO_4 for 3 hours and washed with water until a neutral pH was obtained to determine ADL. After each wash, samples were soaked in acetone, dried at 60-65°C overnight, and weighed. After processing for ADL, samples were ashed in a muffle furnace and weighed to determine the ash value and the remaining lignin and cutin. Average percentages for each crop were determined mathematically as the difference between the washed sample and the initial sample and control as a proportion of the starting material. Detailed procedures can be found on the ANKOM Technologies website (http://www.ankom.com/00_products/product_a200.shtml).

The NDF wash removes soluble materials leaving predominantly hemicelluloses, cellulose and lignin, the ADF wash removes hemicellulose predominantly leaving cellulose and lignin and the ADL procedure removes celluloses predominantly leaving lignin and ash. Because this process does not necessarily remove all components with each wash, amounts of hemicellulose and cellulose are estimates. The %hemicellulose was estimated by subtracting %ADF from %NDF and %cellulose by subtracting %ADL from %ADF. Carbon to nitrogen ratios were determined using a dry combustion method (LECO Corp., St. Joseph, MI). Data were analyzed using the PROC GLM procedure in SAS (SAS Inst., Cary, NC). Results are summarized in Table 2.

Because plant samples came from various research projects, plant parts sampled for each crop type are variable (Table 1). The most notable difference is the unknown camelina variety taken from the Roza experiment plots in Prosser, WA. This sample was taken prior to harvest and had all plant parts intact including seed pods and seeds, leaves and straw. It has been designated as “Camelina2” and findings for this sample have been analyzed separately from the other camelina varieties from Paterson, WA. Diversity among plant parts of similar varieties could yield interesting findings warranting additional research.

Results

Table 2. Summary of fiber analyses and C:N for all crop types.

Crop type	**Ave %NDF**	**Ave %ADF**	**Ave %ADL**	**Ave %Lig/cut**	**Ave %hemi**	**Ave %cell**	**C:N**
Camelina	76.29**a**	60.17**a**	14.47**a**	14.36**a**	16.11**b**	45.71**ab**	109:1
Camelina2	70.37**a**	55.03**ab**	12.71**a**	12.55**a**	15.34**b**	42.33**abc**	46:1
Flax	70.37**a**	56.57**ab**	14.98**a**	14.90**a**	13.81**b**	41.59**bc**	72:1
Canola	76.65**a**	61.41**a**	13.00**a**	12.69**a**	15.24**b**	48.41**a**	117:1
Wheat	67.27**a**	40.27**d**	6.19**c**	5.50**b**	27.01**a**	34.08**de**	39:1
Goat grass	75.00**a**	45.58**cd**	7.72**bc**	5.89**b**	29.42**a**	37.87**dce**	67:1
Goat*wheat	75.61**a**	49.09**bc**	9.65**b**	7.49**b**	26.52**a**	39.45**dc**	89:1
Arundo	69.85**a**	40.76**d**	8.21**bc**	6.70**b**	29.09**a**	32.55**e**	25:1

NDF has hemicellulose + cellulose + lignin
ADF has cellulose + lignin
ADL has lignin + ash
Lignin/Cutin (no ash)

Basic Trends

Fiber

- Overall fiber trends were similar between Brassicacea and Linaceae vs. Poaceae species
- Lignin and cutin was significantly higher in the Brassicacea and Linaceae than Poaceae species
- Poaceae had higher hemicellulose than Brassicacea and Linaceae species
- Brassicacea and Linaceae had higher cellulose than Poaceae species

Specific fiber trends (statistically significant) - data not shown

- In general, camelina grown in sandy soil had higher fiber contents than when grown on loam soil
- Slight variations of flax fiber content occurred but were not consistently dependant on variety or location effects
- Significantly higher fiber in wheat from Whitman Co. than Prosser

C:N

- Canola > Camelina > Goat grass hybrid > Flax > Goat grass > Camelina2 > Wheat > Arundo

Camelina

- Blaine Creek (125:1) > Suneson (120:1) > Calena (102:1) > Ligena (80:1) > Camelina2 (46:1)
- Sand soil (116:1) > Loam soil (94:1)

Flax

- Bethune (80:1) > McGregor (73:1) > Pembria (71:1) > Omega (64:1)
- Paterson (76:1) > Othello (68:1)

Canola

- High water (128:1) > Intermediate water (127:1) > Low water (92:1)

Wheat

- Wheat from Roza plots in Prosser (39:1)
- Subsample of Whitman Co. wheat was not sufficient to perform C:N analyses

Discussion/Conclusion

The complex chemical structure of lignin makes crops high in this compound difficult to process for biofuels and ruminant digestion. The type of lignin compound is going to determine the difficulty of extracting other useful compounds from plant tissues (Anderson and Akin, 2008; Akin, 2008; Zhang, 2008). Forage digestion research has produced knowledge on limitations of biodegradation of lignocellulose by rumen microorganisms, the same factors which will act as barriers to the production of these products for bioenergy (Akin, 2008). High lignin content in residues from canola, camelina and flax suggest these crops might be difficult to use in cellulosic ethanol production or as ruminant feed. However, further research on the nature and properties of specific lignin and cellulose compounds in these crops would be necessary to fully understand their potential uses and hindrances. Plant residues high in lignin may be ideal soil amendments as these recalcitrant materials can aid to sequester carbon and improve physical characteristics of soil (Fierer et al., 2003; Sylvia et al., 2005). Soil type and location appears to affect fiber content in camelina and wheat and management practices may be used to control high or low amounts of fiber compounds. These differences could also be attributed to variety and/or plant part sampled. For example C:N is highly variable between all camelina varieties. The low C:N found in Camelina2 suggest that the presence of seeds may increase the amount of nitrogen in the sample and lower the ratio. However, 'Ligena' camelina also had a low C:N compared to 'Blanie Creek', 'Suneson' and 'Calena' camelina and only straw was analyzed for all these varieties. Additionally, although irrigation rates did not affect the fiber content of canola, the C:N was greater for canola grown with high rather than low irrigation levels. Preliminary research on fiber content and C:N of biofuel crops reveals interesting trends that should be further investigated to assess the value of these crops for biofuel production, soil amendments, ruminant feed, rotational crops and by-products for value-added production. Specifically, variations in C:N and fiber content trends between plant samples may have resulted from comparing whole plant samples to straw samples, management practices, soil types or varieties. Additional research between similar sample materials would allow more apt conclusions.

Literature Cited

Anderson, W.F. and D.E. Akin. 2008. Structural and chemical properties of grass lignocelluloses related to conversion for biofuels. J. Ind. Microbiol. Biotechnol. 35:355-366.

ANKOM Technology. 2005. http://www.ankom.com/00_products/product_a200.shtml. Accessed 2008, December 17.

Akin, D.E. 2008. Plant cell wall aromatics: influence on degradation of biomass. Biofuels, Bioprod. Bioref. 2:288-303.

Chen, Y., R.R. Sharma-Shivappa, D. Keshwani, C. Chen. 2007. Potential of agricultural residues and hay for bioethanol production. Appl. Biochem. Biotechnol. 142:276-290.

Emsley, A.M. 2008. Cellulosic ethanol re-ignites the fire of cellulose degradation. Cellulose 15:187-192.

Fierer, N., J.P. Schimel, P.A. Holden. 2003. Variations in microbial community composition through two soil depth profiles. Soil biology and biochemistry 35:167-176.

Hatfield, R.D., H.J.G. Jung, J. Ralph, D.R. Buxton, and P.J. Weimer. 1994. A comparison of the insoluble residues produced by the Klason Lignin and acid detergent lignin procedures. J. Sci. Food Agric. 65:51-58.

Lal, R. 2007. Farming carbon: An editorial. Soil and Tillage Research 96:1-5.

Li, X., J.-K. Weng, C. Chapple. 2008. Improvement of biomass through lignin modification. The Plant Journal 54:569-581.

Pauly, M. and K. Keegstra. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. The Plant Journal 54:559-568.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, D.A. Zuberer. 2005. Principles and applications of soil microbiology. 2nd edition. Prentice Hall, Upper Saddle River, NJ.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74:3583-3597.

Zhang,Y.-H. Percival. 2008. Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. J. Ind. Microbiol. Biotechnol. 35:367-375.