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RESEARCH ARTICLE

Effects of protein and lignin on cellulose and xylan analyses of lignocellulosic biomass



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Abstract

Interactions of lignocellulosic components during fiber analysis were investigated using the highly adopted compositional analysis procedure from the National Renewable Energy Laboratory (NREL), USA. Synthetic feedstock samples were used to study the effects of lignin/protein, cellulose/protein, and xylan/protein interaction on carbohydrate analysis. Disregarding structural influence in the synthetic samples, lignin and protein components were the most significant ($P < 0.05$) factors on cellulose analysis. Measured xylan was consistent and unaffected by content variation throughout the synthetic analysis. Validation of the observed relationships from synthetic feedstocks was fulfilled using real lignocellulosic feedstocks: corn stover, poplar, and alfalfa, in which similar results have been obtained, excluding cellulose analysis of poplar under higher protein content and xylan analysis of alfalfa under higher protein content. The results elucidated that according to their protein and lignin contents of different lignocellulosic materials, accuracy of the NREL method on cellulose and xylan analyses could be improved by applying a stronger extraction step to replace water/ethanol extraction.

Keywords: lignocellulosic biomass, cellulose, xylan, lignin, protein

1. Introduction

Compositional fiber analysis has been extensively used to provide useful data on lignocellulosic materials. Fiber data are comprehensively utilized by the agricultural and paper pulping industries, and are becoming more adopted with emerging biobased products and technologies (Moxley and Zhang 2007). Traditionally, fiber has been analyzed to

measure lignin and carbohydrate contents in various plant materials, as well as to estimate nutritional value in animal feed and human food (Moxley and Zhang 2007; Sluiter *et al.* 2010). Within the last couple of decades, as more interest has been focused into fuels derived from plant materials, compositional analysis of feedstocks has helped scientists and engineers compare potential bioenergy feedstocks and measure efficiencies within conversion processes (Sluiter *et al.* 2010). Regardless of how the information is inferred upon, current biotechnology applications use compositional methods to characterize the lignocellulosic materials by describing its potential resource quality (Roberts and Rowland 1998).

Two compositional methods have emerged as protocols for analyzing components in lignocellulosic materials; fiber analysis, based upon acid hydrolysis approach to break down the structural carbohydrates into their monomeric

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forms, which is employed by the National Renewable Energy Laboratory (NREL), USA (Sluiter *et al.* 2008), and forage analysis, based upon solution extraction and developed by van Soest (1965). One of the main challenges experienced within these methods are the severe complexity of the plant cell wall linkages. Primary and secondary plant cell walls have evolved to become chemically and physically non-uniform as a defense mechanism, which makes the current approach of these two methods difficult to achieve homogeneous degradation of individual components. Another issue is that these analyses provide only empirical information and depend heavily on how the method runs (Templeton *et al.* 2010). A way to better understand the interactions between plant cell walls components are desirable and could lead to more accurate compositional results.

Research has expanded upon these compositional analysis methodologies to develop more accurate ways to quantify carbohydrates in lignocellulosic biomass. An example can be seen with a modified NREL method suggested by Moxley and Zhang (2007) to use milder acid concentrations to yield more accurate xylan concentrations. It has also been proposed to develop standard neutral detergent procedures when applying forage analysis, leading to more precise estimation of cellulose and hemicellulose portions (van Soest *et al.* 1991). Some studies have even developed models that try to correlate the two compositional methods together to provide quicker compositional data on certain biomass feedstocks (Wolfrum *et al.* 2009). However, most of this work has been focused on methodology employment with little emphasis on understanding true chemical interactions that play an important role in identifying structural carbohydrates.

With a focus on the fiber analysis procedure from NREL, this current work looked to delve into the chemical interactions of the plant cell wall components and their influence on carbohydrate analysis, specifically cellulose and xylan. For instance, to the author's knowledge, there has been no set limit as to how much protein can be present before carbohydrate analysis is negatively affected. Synthetic feedstock was created by chemical compounds, mimicking natural biomass, to understand how different components influenced cellulose and xylan analyses. Consequential analysis was performed on actual biomass feedstocks of corn stover, poplar and alfalfa to generally validate observations from the synthetic samples.

2. Materials and methods

2.1. Feedstocks

Commercial chemicals of peptone (Sigma, USA), cellulose powder about 20 microns (Sigma, USA), xylan from

beechwood (Sigma, USA), and alkali lignin (Sigma, USA) were used to create synthetic biomass samples. Real lignocellulosic feedstocks of corn stover, poplar, and alfalfa were used to verify the experimental results from synthetic biomass. Corn stover was harvested and collected in 2009 from a private farm in Muir, MI, USA. Poplar was donated from the Crop and Soil Sciences Department at Michigan State University (MSU), USA and were acquired from Michigan State University's Forest Biomass Innovation Center in Escanaba, MI, USA. Poplar hybrids were planted in 1998 at a uniform spacing of 8×8 feet and harvested in fall of 2009. Alfalfa sample was collected from the dairy farm at MSU and was harvested in 2011 at a private farm in Riverdale, MI. All feedstock samples were milled through a 2-mm screen using a Schutte Buffalo hammer mill (Model No. WA-6-H; Schutte Buffalo, USA). Samples were then collected and dried at 105°C for approximately 24 h. Their carbon, nitrogen, cellulose, xylan, and lignin contents were listed in Table 1.

2.2. Effects of xylan, cellulose, protein, and lignin on the concentrated acid analysis of synthetic feedstocks

The effects of lignin/protein, cellulose/protein, and xylan/protein on concentrated acid carbohydrate analysis were first evaluated by a completely randomized design (CRD). Considering the facts that contents of these components in real lignocellulosic materials are varied, in order to fix the amount of synthetic feedstock for analysis and avoid unrealistic compositions of the synthetic feedstock, protein content as the smallest component for most lignocellulosic materials was used as the base component, and ratios of different components to protein were then used by the CRD design. Two cellulose/protein ratios (2:1 and 6:1), three lignin/protein ratios (1:1, 3:1, and 5:1), and three xylan/protein ratios (1:1, 3:1, and 5:1) were used by the CRD to create a total of 18 experimental runs with triplicates (Table 2). The laboratory analytical procedure (LAP) of NREL was modified to take weight measurement accuracy into consideration.

Table 1 Characteristics of raw feedstocks¹⁾

	Alfalfa	Poplar	Corn stover
Carbon content (wt%)	41.7	47.9	45.4
Nitrogen content (wt%)	2.2	0.2	0.4
Protein content (wt%)	13.8	1.3	2.5
Cellulose (wt%)	23.5	33.0	32.1
Xylan (wt%)	10.7	13.0	23.0
Lignin (wt%)	21.4	23.2	16.7

¹⁾ The data are average of two replicates. The NREL (National Renewable Energy Laboratory) method with hot water extraction was used to analyze cellulose, xylan, and lignin (Sluiter *et al.* 2008).

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