Lecture-4 Characterization of biomass

The characterization of the conversion processes of lignocellulosic biomass to biofuels requires a large array of methods and analytical systems to extract the meaningful parameters necessary to describe the solid materials and the conversion liquors. The crucial point is also to develop robust, reliable and high-throughput methods that allow the analysis of large number of samples of various sizes (from mg to kg) and of high heterogeneity. The Walker lab has been developing new and cutting-edge methods ranging from single molecule analysis, microplate assays for the studies of cellulose's and cellulose cocktails, and non-destructive vibrational spectroscopy methods for biomass and bioprocesses analysis, among others. The Biofuel Research Laboratory has been equipped with analytical systems that meet these needs and expand the analytical capabilities for our group and the broader community involve in biofuel R&D. These systems include HPLCs for fluorescence, UV-VIS and Refractive Index (RI) detection, Liquid Chromatography coupled with a Mass Spectrometer (LC-MS) for metabolites characterization and quantification, UV-Vis and fluorescence plate readers, Fourier Transform Infrared and Near-Infrared spectrometers, gas chromatograph, UV-Vis spectrometers, automated protein purification system (FPLC). Some of these methods and instrumentation are described below.

Detailed and accurate characterization of biomass feedstock, intermediates, and products is a necessity for any biomass-to-biofuels conversion. Understanding how the individual biomass components and reaction products interact at each stage in the process is important for researchers.

With a large inventory of standard biomass samples as reference materials, NREL maintains a biomass feedstock composition and property database with the chemical, thermal, and mechanical properties of various biomass feedstock materials.

NREL's biomass characterization capabilities include:

Developing standard laboratory analytical procedures NREL wrote most of the standard biomass laboratory analytical procedures for characterization that are used throughout

the research community. At NREL, we develop new methods and tools to understand more about chemical composition of raw biomass feedstock and the solid, liquid, and slurry samples produced during conversion.

Performing real-time biomass analysis NREL combines multivariate analysis with near-infrared spectroscopy to provide real-time biomass analysis. Using this rapid analysis technique, researchers can analyze a wide range of physical and chemical characteristics of raw and processed biomass within minutes instead of days.

Investigating structural changes Researchers investigate structural changes in biomass from the plant tissue to the macromolecular scale using established and advanced imaging tools in the Biomass Surface Characterization Laboratory. NREL has developed methods to visualize the deconstruction of plant cell walls during biomass conversion. This provides an understanding, for example, of the behavior of lignin during the pretreatment process or of the interactions between enzymes and the biomass cell wall.

With such a wide range of biomass sources and production process variables, understanding the chemical composition of the material becomes an important issue.

Typical biomass components

(a) Cellulose

A polysaccharide in which D-glucose is linked uniformly by β-glucosidic bonds. Its molecular formula is $(C_6H_{12}O_6)_n$. The degree of polymerization, indicated by n, is broad, ranging from several thousand to several tens of thousands. Total hydrolysis of cellulose yields D-glucose (a monosaccharide), but partial hydrolysis yields a disaccharide (cellobiose) and polysaccharides in which n is in the order of 3 to 10. Cellulose has a crystalline structure and great resistance to acids and alkalis.

(b) Hemicellulose

A polysaccharide whose units are 5-carbon monosaccharides including D-xylose and D-arabinose, and 6-carbon monosaccharides including D-mannose, D-galactose, and D-glucose. The 5-carbon monosaccharides outnumber the 6-carbon monosaccharides, and the average molecular formula is $(C_5H_8O_4)$n. Because the degree of polymerization

n is 50 to 200, which is smaller than that of cellulose, it breaks down more easily than cellulose, and many hemicelluloses are soluble in alkaline solutions. A common hemicellulose is xylan, which consists of xylose with 1,4 bonds. Figure 2.3.1-c shows the structural formula of xylan. Other hemicelluloses include glucomannan, but all hemicelluloses vary in amounts depending on tree species and the part of the plant.

(c) Lignin

A compound whose constituent units, phenylpropane and its derivatives, are bonded 3-dimensionally. Its structure is complex and not yet fully understood. Figure 2.3.1-d shows a constituent unit. Its complex 3-dimensional structure is decomposed with difficulty by microorganisms and chemicals, and its function is therefore thought to be conferring mechanical strength and protection. Cellulose, hemicellulose, and lignin are universally found in many kinds of biomass, and are the most plentiful natural carbon resources on Earth.

(d) Starch

Like cellulose, starch is a polysaccharide whose constituent units are D-glucose, but they are linked by α-glycosidic bonds. Owing to the difference in the bond structures, cellulose is not water-soluble, while part of starch is soluble in hot water (amylose, with a molecular weight of about 10,000 to 50,000, accounting for 10%–20% of starch) and part is not soluble (amylopectin, with a molecular weight of about 50,000 to 100,000, accounting for 80%–90% of starch). Starch is found in seeds, tubers (roots), and stems, and has a very high value as food.

(e) Proteins

These are macromolecular compounds in which amino acids are polymerized to a high degree. Properties differ depending on the kinds and ratios of constituent amino acids, and the degree of polymerization. Proteins are not a primary component of biomass, and account for a lower proportion than do the previous three components.

(f) Other components (organic and inorganic)

The amounts of the other organic components vary widely depending on specie, but there are also organic components with high value, such as glycerides (representative examples include rapeseed oil, palm oil, and other vegetable oils) and sucrose in sugarcane and sugar beet. Other examples are alkaloids, pigments, terpenes, and

waxes. Although these are found in small amounts, they have very high added value as pharmaceutical ingredients. Biomass comprises organic macromolecular compounds, but it also contains inorganic substances (ash) in trace amounts. The primary metal elements include Ca, K, P, Mg, Si, Al, Fe, and Na. Substances and their amounts differ according to the feedstock type.

Several of the industry standard tests for characterizing biomass are described below:

Total Solids

A way to determine the moisture content within the sample.

Ash Determination

The amount of inorganic or mineral material present in the sample.

Exhaustive Ethanol and Water Extractable:

The removal of non-structural material from the biomass sample to prevent interferences during other analyses, as well as free sugar determination.

Structural Carbohydrates:

The determination of glucose, xylose, galactose, arabinose and mannose concentrations in the sample; used to determine cellulose and hemicellulose concentrations in the biomass.

Acetyl Content:

Acetic acid concentration in the sample, may also include formic and levulinic acid content depending on the feedstock.

Lignin:

Determination of the structural plant material that does not contribute to the sugar content in the sample.

Starch Content:

Represents the readily available source of sugar within some feedstock.

Ethanol Content:

Analysis of fermentation broths using gas chromatography.

Bomb Calorimetry:

The determination of the sample’s calorific value.

Using FT-MIR, fundamental vibrations of complex organic samples can be analyzed and sample specific fingerprints can be obtained. FT-MIR exploits the mid-infrared region (4,000 - 400 wavenumber, cm-1). A wild range of samples can be analyzed, categorized and quantified by FT-MIR, ranging from microbes to proteins and biomass. Applied to lingo-cellulosic biomass conversion for sugar production, the prominent peaks within the spectra are at 1033, 1059, 1112, and 1163 cm-1 where 1033 and 1059 cm-1 correspond to C-O stretching vibration, 1112 cm-1 corresponds to asymmetric glucose ring stretch, and 1163 cm-1 corresponds to C-O-C asymmetric vibrations within cellulose. Another important peak pertinent to enzymatic hydrolysis of cellulose is the peak at 897 cm-1 corresponding to beta-glucosidic bonds of amorphous cellulose. Therefore a direct analysis of conversion efficiency can be performed using FT-IR. The most advanced FT-MIR can be coupled with automated platforms for high throughput analysis in a 96-well microplate format.

Fourier Transform Near Infrared spectrometer (FT-NIR)

Chemometric models associated with Near Infrared (NIR) spectral analysis lends themselves handily to the high throughput, off-line or on-line, monitoring and process control industry, where fast and inexpensive systems are needed to test, predict and make decisions about product quality, or real-time adjustments with online process monitoring. These methods have been developed for agricultural and industrial applications mainly to assess the quality of feeds. FT-NIR is based on the quantification of the vibration overtone intensities in the near-infrared region (12,000-4,000 wavenumber, cm-1). Fourier Transform Near Infrared spectroscopy (FT-NIR) and multivariate modeling are the core of new analytical methods that can be applied for the high-throughput, fast, online or offline analysis of biomass throughout the logistics of the harvest, storage, conversion and assessing chemical composition of biomass. Indeed, the new generations of FT-NIR spectrometers have gained in improved accuracy and reliability compared to diffusive NIR spectrometers (NIRS). The most recent advances

include the use of high quality optic fibers and process-resistant optic probes for the online and real-time monitoring of chemical and biochemical processes. The construction of robust chemometric models associated with the speed and accuracy of FT-NIR spectrometers (FT-NIRS) is used for compositional analysis of feedstock, and analytical monitoring of conversion of feedstocks to the final products, to ultimately develop quality analysis (QA) of the feedstocks and quality control (QC) of the conversion processes.

Fast Protein Liquid Chromatography (FPLC)

Fluorescently tagging functional molecules such as enzymes is key to develop single molecule applications and fluorescence based assays for example. However, it is not trivial as enzyme functions and activities rely upon their conformation, charges, hydrophobicity, all of which can be hindered by the addition of the signaling molecules. Applied to the study of any enzymes, fluorescent labeling has to ensure that the catalytic activity remain unchanged. We have developed an analytical and preparative method based on FPLC purification for the production of enzymes for which we can assess and sort by their degree of labeling and their catalytic activities ensuring optimal fluorescence and activities compared to the native ones. This polishing purification method allows reaching defined and homogenous biochemical reagents.

High performance Liquid Chromatography (HPLC)

An important aspect for the conversion of lignocellulosic biomass to biofuels is the saccharification step which consists in using enzymes to convert the cellulose polymer into sugars. Characterizing and quantifying these sugars is necessary as they will be later fermented into ethanol or other biofuels. HPLC systems based on appropriate separation columns are used to measure these sugars and to measure the specific activities of enzymes, engineered enzymes and cocktails of these. Other methods have been implemented by the Walker lab to measure other compounds and metabolites from lignocellulosic biomass pretreatment and fermentation such as organic acids and alcohols during anaerobic fermentation for hydrogen production.