

SUGARCANE BAGASSE: HOW EASY IS IT TO MEASURE ITS CONSTITUENTS?

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Abstract

Given the present international interest in cellulosic biomass as a feedstock for intermediate chemicals and second generation biofuels, the characterisation of the feedstock is a critical component of all current research. Established methods exist for characterising materials for use in pulp and paper products (e.g. TAPPI and ASTM methods), but are not necessarily the best methods for defining the suitability of the material as a biomass feedstock. This paper, in addition to describing the standard methods of analysis, highlights proposed biomass analytical procedures for cellulose, hemicellulose and lignin and, where possible, relates them to bagasse.

Keywords: cellulosic biomass, analysis, sugarcane bagasse, cellulose, hemicellulose, lignin, biofuels

Introduction

Utilisation of lignocellulosic materials such as residues from forestry or agricultural sources (including bagasse) has the potential to provide intermediate building block chemicals and second generation biofuels without negative competition for land. It has been estimated that utilisation of these residues could provide about 50-80 EJ/year of energy, (E=exa=10¹⁸) representing 10-20% of the current world energy demand (Okkerse and van Bekkum, 1999; Lange, 2007). Lignocellulosic materials do not contain readily accessible monosaccharides and chemicals but rather polymers which need to be hydrolysed to release the desired compounds. Characterisation of the composition of lignocellulosic materials is critical in providing direction for continued power, fuels and other product research. Established methods for characterisation were originally based on gravimetric or colorimetric tests. The appropriateness of these tests as methods for characterising a particular biomass residue as a raw material feedstock for further processing has been questioned (Martens, 2000; Martens and Loeffelmann, 2002). For example, the TAPPI (Technical Association of the Pulp and Paper Industry, http://www.tappi.org/s_tappi/index.asp) methods have been developed for characterising wood for use in the pulp and paper industry. It has been shown that these methods are not necessarily applicable for other lignocellulosic residues, especially when dealing with herbaceous and grass feedstocks such as bagasse (Hatfield and Fukushima, 2005). This review highlights the analytical procedures that can be used to characterise lignocellulosic biomass with particular emphasis, where possible, on bagasse.

Components of lignocellulosic materials

Plant biomass (lignocellulose) is the fibrous material that forms the structural framework of the plant cell wall. The important components that need to be considered when characterising these materials are:

- Carbohydrates. The two major polysaccharides that contain carbohydrates are:

Cellulose is the most abundant constituent and is a homo-polysaccharide composed entirely of β -1,4-glucosidic linked glucose monomers. It may have a degree of polymerisation (number of glucose units bound together) in excess of 10 000 (Figure 1). The linear structure of the cellulose chain enables the formation of inter- and intramolecular hydrogen bonds. This results in the aggregation of about 36 glucose chains into crystalline fibrils (Ding and Himmel, 2006). Approximately 50-90% of the total cellulose is crystalline, depending on the biomass source (Jacobsen and Wyman, 2000). The combination of the structure and intermolecular hydrogen bonding gives cellulose a high tensile strength, a resistance against microbial attack and makes it insoluble in most solvents.

Hemicellulose is a heterogeneous polysaccharide composed of D-xylose, D-glucose, D-mannose, D-galactose, L-arabinose, D-glucuronic acid and 4-O-methyl-D-glucuronic acid. The specific composition varies among different plants. It has a low degree of polymerisation (typically below 200), often contains side chains and is typically acetylated (Sun *et al.*, 2004). Classification is according to the main sugar in the polymer backbone, e.g. xylan (β -1,4-linked xylose) or mannan (β -1,4-linked mannose). Bagasse hemicellulose is composed of a backbone of xylose, branched with glucose and arabinose units (Sun *et al.*, 2004) (Figure 1).

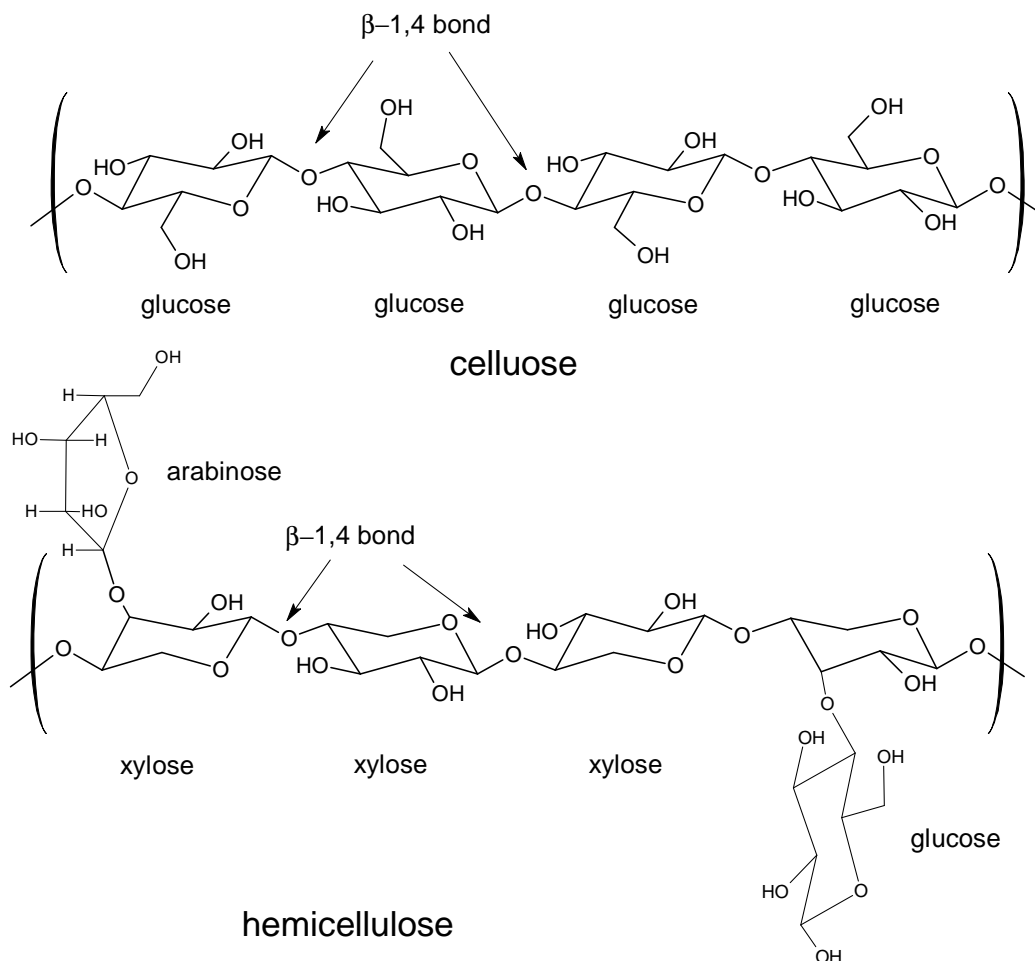


Figure 1. Simplified structure of cellulose (upper) and bagasse hemicellulose (lower).

- **Lignin** is a three dimensional polymer of three different phenyl-propane precursor monomers: *p*-coumaryl, coniferyl and sinapyl alcohols (Amen-Chen *et al.*, 2001). They are joined together by aryl-aryl, alkyl-aryl and alkyl-alkyl ether bonds. This polymer is imbedded in the cellulose/hemicellulose structure acting as a 'glue-like' material. It helps impart rigidity and offers further protection to the biomass against microbial and chemical attack (Figure 2).

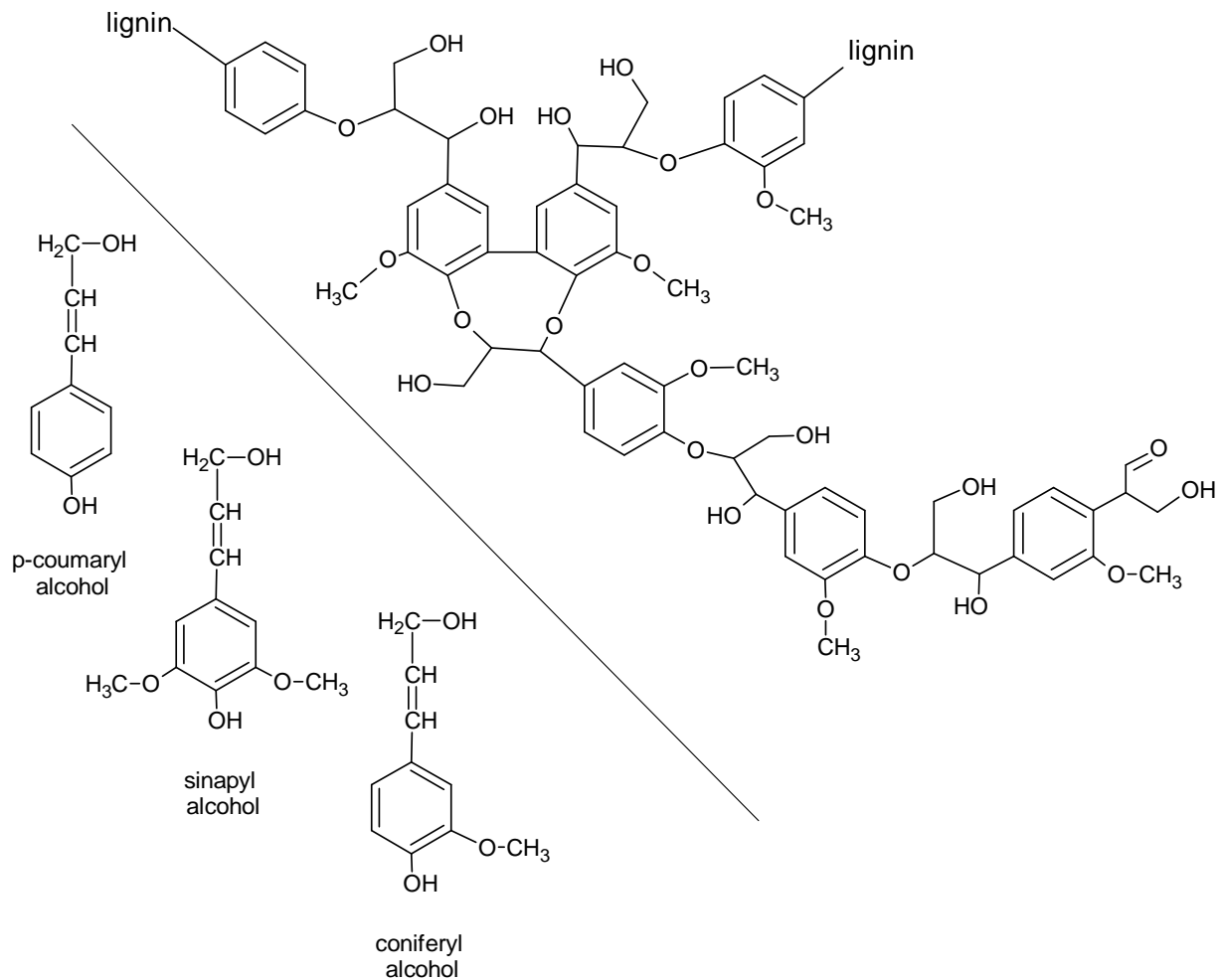


Figure 2. Phenyl-propane precursors (left) and a model lignin structure (right).

- **Ash** is typically the inorganic components of the biomass.
- **Protein** is typically a combination of protein and nitrogen containing compounds.
- **Extractables** are the non-structural material present in the biomass that can be easily extracted. Bagasse would contain traces of pol and soluble ash.
- **Moisture.**

The basic composition of bagasse has been reported by a number of authors (Table 1). A limited number of varietal studies have been reported but no comprehensive compositional analyses have been reported for South African varieties.

Table 1. Basic composition of bagasse.

Component (% dry mass)				Reference
Cellulose	Hemicellulose	Lignin	Ash	
37	28	21	Unreported	Bon (2007)
26-47	19-33	14-23	1-5	Paturau (1989)
38	33	22	3	Trickett and Neytzell-de Wilde (1982)

Analysis of lignocellulosic materials

A review of the literature shows that a wide range of methods are currently in use for characterising lignocellulosic materials. The procedures originate from a variety of sources including forestry, pulp, paper, animal forage and dietary fibre methods. Some of the important procedures are summarised in Table 2.

As highlighted by Nelson and Leming (1957), methods to determine cellulose are “*more or less*” empirical. Brauns (1952) stated “... *there is at present no method which can be applied for the quantitative determination of lignin,*” and “*For the present we must be satisfied with approximate determinations only.*” These comments are still appropriate in the 21st century (Martens, 2000) and highlight the difficulty of characterising and comparing lignocellulosic materials.

The plethora of methods and different results that can be reported has been partially addressed by the United States Department of Energy (US DOE). As part of the US DOE focus on renewable resources, the National Renewable Energy Laboratory (NREL) developed a set of Laboratory Analytical Procedures (LAPs) and calculation workbooks for their biomass programme (http://www.nrel.gov/biomass/analytical_procedures.html). Lignocellulosic material characterisation using this set of standardised procedures ensures that results will be comparable amongst researchers. The methods are based on a variety of sources including TAPPI, ASTM (American Society for Testing and Materials; now known as ASTM International) and those shown in Table 1. The basics of the procedures to be followed are described below.

Preparation of samples for compositional analysis (Anon, 2005b)

Biomass samples contain varying amounts of moisture which can change on standing. It is important that this be minimised and a dry (typically <10% moisture), homogenous sample be prepared for compositional analysis. Typically the sample can be allowed to air-dry, dried in an oven (<45°C to minimise other losses) or lyophilized (freeze-dried) if the sample is sensitive to heat. In the case of bagasse, residual sugar will ferment if allowed to air-dry; consequently drying in an oven at 45°C is recommended. Homogenisation is achieved by knifing and milling, and the sample is then sieved to collect the 20-80 mesh material. Material less than 20 mesh is used for the ash analysis. The presence of dirt in bagasse which may influence the uniformity of sampling has not been studied and would need to be reviewed.

Moisture and total solids (Anon, 2005c)

For meaningful results, all chemical analyses are reported on a dry weight basis. Thus the total solids and moisture result of the prepared material is required for use in all subsequent analytical calculations. A standard convection oven analysis at 105°C is used in the LAP to determine the moisture content and the total solids by difference.

Table 2. Procedures for characterising lignocellulosic biomass.

Method	Reference	Comments
Cellulose		
Monoethanolamine	Nelson and Leming (1957)	Gives total cellulose by a gravimetric method (lignin and the hemicellulose dissolved and cellulose filtered off and massed).
Sulphuric acid (H ₂ SO ₄)	Saeman <i>et al</i> (1944)	A 72% H ₂ SO ₄ hydrolysis for 3 hours @ room temperature, dilution to 4% and 1 hour @ 100°C. The released glucose was measured spectrophotometrically.
	Grohmann <i>et al</i> (1984)	Based on 64% H ₂ SO ₄ and 2 h hydrolysis @ room temperature. A 2-step dilution and heating was used for complete dissolution. Glucose was measured enzymatically.
Hemicellulose		
Trifluoroacetic acid (TFA)	Fengel and Wegener (1979)	Diluted TFA, otherwise similar to cellulose method.
Hydrochloric acid (HCl)	Moore and Johnson (1967)	Hydrolysis with 30% HCl, followed by conversion of pentosans to furfural and measured spectrophotometrically.
Total carbohydrate		
Trifluoroacetic acid (TFA)	Fengel and Wegener (1979)	Undiluted TFA used. The reaction times of the method modified for differing levels of lignin. All sugars hydrolysed and measured by HPLC.
Sulphuric acid (H ₂ SO ₄)	Foyle <i>et al</i> (2007)	Based on the Grohmann cellulose method with modification of hydrolysis times. Sugars measured by HPLC.
Lignin		
Sulphuric acid (H ₂ SO ₄)	Klason (1923)	Gravimetric method in which lignin is isolated as the insoluble material from acid hydrolysis. Has been modified many times over the years.
	Saeman <i>et al</i> (1954)	A gravimetric method based on the 74% H ₂ SO ₄ cellulose hydrolysis method.
	Grohmann <i>et al</i> (1984)	Insoluble material left after TFA hydrolysis
Acid detergent	Van Soest (1963)	Method developed for fibre and lignin content in forage samples.
Neutral detergent		
Nitrobenzene	Lapierre <i>et al</i> (1989)	Oxidation of the lignin using these chemicals gives different degradation products leading to structural analysis and characterisation. The acetyl bromide method is also known as the DFRC method.
Acidolysis		
Thioacidolysis		
Acetyl bromide	Lu and Ralph (1997)	
Permanganate	Tasman and Berzins (1957)	Based on oxidation of lignin by the addition of excess KMnO ₄ . Basis of the Kappa number used in the paper industry.
Thioglycolate	Hatfield and Fukushima (2005)	Relies on complete solubilisation of the lignin, followed by spectrophotometric measurement in solution.

Extractables (Anon, 2007a)

It is necessary to remove non-structural material from the lignocellulosic materials prior to analysis to prevent interference with later analytical steps. Non-removal of non-structural sugars can inflate the structural sugar values and the acid insoluble lignin value. The LAP describes a typical two-step Soxhlet extraction using water (to remove inorganic material, non-structural sugars and nitrogenous material) followed by ethanol (to remove chlorophyll, waxes and other minor components).

Protein (Anon, 2005a)

Protein in biomass is difficult to measure directly. The total nitrogen can be measured by combustion or the Kjeldahl method, and the protein content estimated by applying an appropriate factor (typically 6.25) to the result. Bagasse naturally contains a low crude protein content. The LAP also describes how to determine the nitrogen factor for the particular biomass material being studied.

Structural carbohydrates (Anon, 2007b)

The LAP describes a procedure using a two-step hydrolysis to fractionate the biomass into more easily quantifiable forms. The extractive-free, dry sample is hydrolysed in 72% sulphuric acid for one hour at 30°C, followed by a dilution to 4% acid and hydrolysis at 121°C for a further hour. The polymeric carbohydrates are hydrolysed into the monomers. To correct for losses due to destruction of sugars during the dilute acid hydrolysis step, a sugar recovery standard is made including D-(+) glucose, D-(+) xylose, D-(+) galactose, L-(+) arabinose, and D-(+) mannose. The sugar concentrations in this standard are chosen to resemble the lignocellulosic biomass. This standard undergoes the same procedure as the diluted sample. After hydrolysis, the solution is neutralised and the individual sugar concentrations determined by high performance liquid chromatography (HPLC). As bagasse hemicellulose contains a xylan backbone containing acetyl groups, a determination of the acetyl content has to be made using HPLC to correct the hemicellulose result.

Lignin (Anon, 2007b)

Numerous methods have been developed over the years to measure the amount of lignin in a biomass sample quantitatively. Non-invasive spectroscopic techniques such as ultraviolet-, infrared-, near-infrared- and nuclear magnetic resonance spectroscopy have been studied. However, destructive techniques such as the acid detergent, Klason, permanganate and acetyl bromide methods are more commonly used. A drawback is that each of these methods can give different lignin values for the same type of sample. For grasses (including bagasse), values differed by a factor of nearly four times (Hatfield and Fukushima, 2005). In the NREL method, the lignin fractionates into acid insoluble material and acid soluble material during the hydrolysis procedure. The acid insoluble lignin is measured gravimetrically. This may also include ash and protein which must be taken into account in the calculations. Acid soluble lignin (at the end of the hydrolysis step) is quantified using a UV spectrophotometric method at a suggested wavelength of 240 nm for bagasse.

Conclusion

Standard wet chemical methods for the chemical characterisation of a biomass feedstock, such as bagasse, are labour intensive and time consuming. It is estimated that to characterise a feedstock using these methods will take the better part of a week of an experienced analyst's time. Thus these methods are not applicable in a commercial setting, because they cannot provide the analysis information in a time frame useful for process control. However, in a research environment, with the collection of a sufficiently large composited sample, these

methods can provide a compositional background that can be the basis for comparison of the effectiveness of pretreatment methods.

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