



National Institute of Standards & Technology

Report of Investigation

Reference Material 8493

Monterey Pine Whole Biomass Feedstock

*A Joint Material of the
International Energy Agency (IEA) Biomass Annex, National Renewable Energy
Laboratory (NREL), and NIST*

This Reference Material (RM) is intended primarily for use in evaluating analytical methods for the determination of summative composition of lignocellulosic material. The RM can also be used for quality assurance when assigning values to in-house control materials. The whole softwood biomass material is derived from Monterey pine (*Pinus radiata*). A unit of the RM consists of five single-use Mylar bags of whole biomass, each containing approximately 10 g of material.

Reference Mass Fraction Values: The reference values for water extractives, ethanol extractives, sucrose, glucan, xylan, arabinan, galactan, mannan, total lignin, acid-insoluble residue, acid-soluble lignin, nitrogen (for calculation of protein), acetyl, extractives-free ash, and whole ash are listed in Table 1 and are reported as mass fractions on a dry-mass basis [1]. These reference values are derived from results reported in an interlaboratory comparison exercise organized by the National Renewable Energy Laboratory (NREL, Golden, CO) and NIST. The participating laboratories used their established analytical protocols. A NIST reference value is a noncertified value that is the best estimate of the true value based on available data; however, the value does not meet the NIST criteria for certification and is provided with associated uncertainties that may reflect only measurement reproducibility, may not include all sources of uncertainty, and/or may reflect a lack of sufficient statistical agreement among multiple analytical methods [2].

Expiration of Reference Values: **RM 8493** is valid, within the measurement uncertainty specified, until **01 June 2020**, provided the RM is handled and stored in accordance with the instructions given in this Report of Investigation (see "Instructions for Storage and Use"). This report is nullified if the RM is damaged, contaminated, or otherwise modified.

Maintenance of RM: NIST will monitor this RM over the period of its validity. If substantive technical changes occur that affect the value assignment before the expiration of this report, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Overall direction and coordination of the technical measurements leading to the original value assignments of this RM were performed by F.A. Agblevor, Virginia Polytechnic Institute and State University, Blacksburg, VA (formerly of NREL) [3–5]. Direction and coordination of the technical measurements leading to the recharacterization of this RM were performed by D.W. Templeton of the NREL National Bioenergy Center and K.E. Sharpless of the NIST Analytical Chemistry Division.

Statistical consultation and analysis were performed by S.B. Schiller and J.H. Yen of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this RM were coordinated through the NIST Measurement Services Division.

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INSTRUCTIONS FOR STORAGE AND USE

The material should be stored at controlled room temperature (20 °C to 25 °C), in an unopened packet, until needed. Prior to removal of a test portion for analysis, the contents of a packet of material should be mixed thoroughly. For reference values to be valid, test portions for extraction equal to or greater than 1 g and test portions for hydrolysis equal to or greater than 300 mg should be used. Test portions should be analyzed as received and results converted to a dry-mass basis by determining moisture content on a separate test portion. Moisture content should be determined by drying in a forced-air oven at 105 °C. The value assignments do not apply to contents of previously opened and stored packets as the stability of measurands in opened packets has not been investigated.

PREPARATION AND ANALYSIS⁽¹⁾

Material Acquisition and Preparation: In 1991, as part of the International Energy Agency (IEA) Voluntary Standards Activity Group’s work, four biomass samples were selected for use as test materials for a worldwide interlaboratory comparison of the Uppsala Method for biomass analysis [6]. The material for production of RM 8493 was supplied by P. Dare, Wood Technology Division, Forest Research Institute, Rotorua, New Zealand. The material was obtained from fifty 18-year-old Monterey pine trees taken at random, (but excluding gross abnormalities), from clear-felling in Compartment 1039, Kaingaroa Forest, North Island, New Zealand. The wood samples were taken from the top log (18 annual growth rings to a commercial end diameter, typically 100 mm to 150 mm and 3 rings to 4 rings). The logs were debarked in a commercial mechanical roundwood debarker, sawn, and chipped in a small (100 hp) commercial chipper. The chips were screened on a commercial perforated shaking screen and 40/+10 mm screen-size chips were collected for drying. The chips were dried batch-wise at a nominal temperature of 50 °C in a forced draught. The moisture content of the dried wood chips was about 7 % (mass fraction).

The dried wood chips were shipped to NIST where they were hammer-milled to a coarse fraction and then Wiley-milled until all the wood particles passed through a 1 mm (16 mesh) screen. During the milling operation, the cutting blades were continuously water-cooled to prevent overheating of the material. The milled samples were then sieved and the fraction of material between 190 µm and 850 µm (74 and 20 mesh) was collected. The bulk material was irradiated with ⁶⁰Co to an absorbed dose of 25 kGy to 46 kGy (Neutron Products, Inc., Dickerson, MD). The irradiated samples were then packaged in 10 g quantities in Mylar bags.

Homogeneity Assessment: About 27 kg of the fraction of material between 190 µm and 850 µm (74 and 20 mesh) was thoroughly homogenized in a large cone blender for 45 min. Four samples of the homogenized material were sent to NREL where they were tested for uniformity by pyrolysis mass spectrometry. Aliquots (3 × 30 mg) were drawn from each of the four samples and pyrolyzed at 600 °C. The pyrolyzates were analyzed using a molecular beam mass spectrometer [7]. Multivariate analysis techniques [8] were used to analyze the pyrolysis mass spectral data, and the relative variation in concentration of carbohydrate and lignin pyrolysis products was estimated for the four samples. No significant differences were detected in the concentrations of the cell wall components of the four samples. The material was therefore determined to be adequately uniform for polymeric constituent analysis.

Analytical Approach for Determination of Composition: Value assignment of the concentrations of the summative composition of lignocellulosic material was based on the analytical methods and parameters provided in Table 2. Laboratories used their established analytical methods, analyzed single test portions from each of three packets of material and a two-stage H₂SO₄ hydrolysis method similar to that described by NREL [9].

⁽¹⁾Certain commercial equipment, instrumentation, or materials are identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the NIST, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

Value Assignment: The medians of the laboratories' means were used to calculate assigned values. Values of zero that were reported were included in these calculations. The expanded uncertainty, U , is calculated as ku_c , where u_c incorporates the observed difference between the individual laboratory results, consistent with the ISO Guide [10] and its Supplement [11], and k is a coverage factor corresponding to approximately 95 % confidence for each analyte.

Table 1. Reference Mass Fraction Values^(a) for Constituents in RM 8493

	Mass Fraction (%)	Expanded Uncertainty (%)	Coverage Factor, k
Water Extractives	3.68	0.70	2.20
95 % Ethanol Extractives (after water extraction)	1.44	0.36	2.20
Sucrose ^(b)	0.030	0.035	2.20
Whole Ash	0.270	0.079	2.18
Extractives-Free Ash	0.17	0.12	2.26
Glucan	43.7	0.66	2.18
Xylan	5.94	0.49	2.18
Arabinan	1.09	0.79	2.18
Galactan	1.89	0.31	2.20
Mannan	10.31	0.83	2.18
Structural Sugars	62.8	3.1	2.18
Total Lignin ^(c)	28.2	1.3	2.18
Acid-Insoluble Residue	25.6	1.1	2.20
Acid-Soluble Lignin	1.4	1.2	2.20
Acetyl	1.40	0.49	2.23
Nitrogen	0.160	0.062	2.31
Total Component Closure ^(d)	100.2	1.2	2.26

^(a) Each value is the median of the mean results provided by the collaborating laboratories and is reported on a dry-mass basis.

^(b) The expanded uncertainty provided is symmetrical, but the lower bound for the mass fraction is zero.

^(c) The value for total lignin is the median of individual values for total lignin reported by the collaborating laboratories and not the mathematical summation of the median values for acid-insoluble residue and acid-soluble lignin.

^(d) Theoretically the total component closure is bounded by 0 % and 100 %. This is calculated as the sum of individual components that can each be over- or under-estimated.

Table 2. Analytical Methods and Parameters Used for Characterization of RM 8493^(a)

Extraction method:	Soxhlet (6), automated solvent extraction (4), Soxtec (1), not reported (1)
Extract concentration method:	Rotovap (1), Turbovap (3), hotplate or oven (2), Soxtec (1), drying in crucible (1), not reported (4)
Acid-soluble lignin ^(b) :	Absorptivity of 12 L g ⁻¹ cm ⁻¹ (5), absorptivity not reported (4), no wavelength or absorptivity reported (3)
Detection of free sugars in water extract:	Liquid chromatography (8), immobilized enzyme assay (3), not reported (1)
Sugar separation column type:	Lead ion (7), amino (3), anion exchange (2), not reported (1)
Sugar detection:	Refractive index (10), pulsed amperometric (2), evaporative light scattering detection (1)
Nitrogen:	Combustion (4), electron affinity (1), Kjeldahl (1), not reported (3)
Acetic acid separation column type:	Hydrogen ion (6), anion exchange (1), not reported (4)

^(a) The value in parentheses represents the number of labs reporting their use of each method or column.

^(b) All labs reporting information used a wavelength of 240 nm.

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