Designation: E 1758 – 95ε1

Standard Test Method for Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography[1]

This standard is issued under the fixed designation E 1758; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

ε[1] NOTE—Paragraphs 11.4 and 11.7 were updated editorially in October 1996.

INTRODUCTION

The carbohydrates making up a major portion of biomass samples are polysaccharides constructed primarily of glucose, xylose, arabinose, galactose, and mannose subunits. The polysaccharides present in a biomass sample can be hydrolyzed to their component sugar monomers by sulfuric acid in a two-stage hydrolysis process. These monosaccharides can then be quantitated by ion-moderated partition HPLC.

1. Scope

1.1 This test method covers the determination of carbohydrates present in a biomass sample, expressed as the percent, by mass, of each sugar on a 105°C dried mass basis.

1.2 Sample materials suitable for this procedure include hard and soft woods, herbaceous materials (such as switchgrass and sericea), agricultural residues (such as corn stover, wheat straw, and bagasse), wastepaper (such as office waste, boxboard, and newsprint), acid or alkaline-pretreated biomass (washed free of any residual acid or alkali), and the solid fraction of fermentation residues. All results are reported relative to the 105°C oven-dried mass of the sample.

1.3 The options for the types of samples to be analyzed in this test method are as follows:

1.3.1 *As Received Samples*:

1.3.1.1 *Air Dried ($\%T_{ad}$)*—The percent, by mass, of total solids of the air-dried sample.

1.3.1.2 *45°C Dried ($\%T_{45}$)*—The percent, by mass, of total solids of the 45°C dried sample.

1.3.1.3 *Freeze Dried ($\%T_{fd}$)*—The percent, by mass, of total solids of the freeze dried sample.

1.3.2 *Extractives-Free Sample ($\%T_{ext}$)*—The percent, by mass, of total solids of the extracted sample determined at 105°C.

1.4 The values stated in SI units are to be regarded as the standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* Specific precautionary statements are given in Note 2 and Note 4.

2. Referenced Documents

2.1 *ASTM Standards:*

D 1193 Specification for Reagent Water[2]

E 1690 Test Method for the Determination of Ethanol Extractives in Biomass[3]

E 1721 Test Method for the Determination of Acid-Insoluble Residue in Biomass[3]

E 1756 Test Method for the Determination of Total Solids in Biomass[3]

E 1757 Practice for Preparation of Biomass for Compositional Analysis[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *as received biomass*—the biomass material as it is received in its field or process collected state.

3.1.2 *oven-dried mass*—the moisture-free mass of a biomass sample dried at 105°C as described in Test Method E 1756.

3.1.3 *prepared biomass*—material that has been treated according to Practice E 1757 in order to raise the total solids content above 85 %, by mass, based on an oven-dried solids mass.

3.2 *Abbreviations*—Abbreviations of standards used in the procedure, and definitions of terms used in the calculations are as follows:

3.2.1 C_1—known concentration of sugar recovery standard before hydrolysis, in mg/mL.

3.2.2 C_2—concentration of sugar recovery standard detected by HPLC after hydrolysis, in mg/mL.

[1] This test method is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.05 on Biomass Conversion.

Current edition approved Oct. 10, 1995. Published December 1995.

[2] *Annual Book of ASTM Standards*, Vol 11.01.

[3] *Annual Book of ASTM Standards*, Vol 11.05.

3.2.3 C_{corr}—concentration of sugar in hydrolyzed sample corrected for hydrolysis, in mg/mL.

3.2.4 C_{spl}—concentration of sugar in hydrolyzed sample detected by HPLC, in mg/mL.

3.2.5 *CVS (calibration verification standard)*—standards used in determining the quality of the calibration curve as well as the quality of the standard reagents used in preparing the calibration standards.

3.2.6 m_1—initial mass of sample, in mg.

3.2.7 *% extractives*—the percentage, by mass, of extractives in the extracted sample as described in Test Method E 1690.

3.2.8 $\%R_{srs}$—percent recovery of sugar recovery standard, as determined in 13.2.

3.2.9 $\%sugar_{extractives\text{-}free}$—the percentage, by mass, of sugar on an extractives-free 105°C dry weight basis, as determined in 13.6.1.

3.2.10 $\%\ sugar_{whole\ sample}$—the corrected mass percent sugar value on an extractives-free basis corrected to an as received (whole sample) 105°C dry mass basis.

3.2.11 $\%T_{45}$—percentage, by mass, of total solids of the sample prepared by drying at 45°C, as described by Practice E 1757.

3.2.12 $\%T_{105}$—percentage, by mass, of total solids in the sample, dried at 105°C, as determined by Test Method E 1756.

3.2.13 $\%T_{ad}$—percentage, by mass, of total solids of the air-dried sample determined at 105°C as described by Test Method E 1756.

3.2.14 $\%T_{ext}$—percentage, by mass, of total solids of the extracted sample determined at 105°C as described by Test Method E 1756.

3.2.15 $\%T_{fd}$—percentage, by mass, of total solids of the sample prepared by freeze drying, as described by Test Method E 1756.

3.2.16 $\%T_{prep}$—percentage, by mass, of total solids of the sample prepared by freeze drying, $\%\ T_{fd}$, or by drying at 45°C, $\%\ T_{45}$, as determined by Practice E 1757.

3.2.17 *SRS (sugar recovery standards)*—standards used to determine sugar recoveries after hydrolysis.

3.2.18 V_F—volume of filtrate, 87.0 mL.

4. Significance and Use

4.1 The percentage, by mass, of sugar content is used in conjunction with other assays to determine the total composition of biomass samples.

5. Interferences

5.1 Samples with high protein content may result in the percentage, by mass, of sugar values being biased low, as a consequence of protein binding with some monosaccharides.

5.2 Test specimens not suitable for analysis by this procedure include alkaline and acid-pretreated biomass samples that have not been washed. Unwashed pretreated biomass samples containing free acid or alkali may change visibly on heating.

6. Apparatus

6.1 *Analytical Balance*, readable to 0.1 mg.

6.2 *Autoclave*, capable of maintaining 121 ± 3°C.

6.3 *Convection Ovens*, temperature control to 45 ± 3 and 105 ± 3°C.

6.4 *Desiccator*, using anhydrous calcium sulfate.

6.5 *Guard Columns*, cartridges appropriate for the column used.

NOTE 1—Deashing guard column cartridges from BioRad,[4] of the ionic form H^+/CO_3^-, are an option when using an HPX-87P[4] column, or equivalent. These cartridges are effective in eliminating baseline ramping.

6.6 *Hewlett Packard*[5] *Model 1090 HPLC*, or equivalent, with refractive index detector.

6.7 *HPLC Columns*, *BioRad* HPX-87C[4] or HPX-87P,[4] or both, or equivalent.

6.8 *Water Bath*, set at 30 ± 1°C.

7. Reagents and Materials

7.1 *Chemicals*:

7.1.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.1.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type 1 of Specification D 1193.

7.1.3 *Calcium Carbonate*.

7.1.4 *High-Purity Sugars (98 % +, By Mass)*—Two sets of glucose, xylose, galactose, arabinose, and mannose, meeting the requirements described above, dried at 45°C. The sugars are used in preparing calibration standards, calibration verification standards (CVS), and sugar recovery standards (SRS). The sugars used in preparing the calibration standards should be from a source (manufacturer or lot) other than that used in preparing the CVS. Either set of sugars may be used for preparing the SRS solutions used in determining sugar recoveries after hydrolysis.

7.1.5 *Sulfuric Acid Solution (72 % w/w or 12.00 ± 0.02 M)*—Slowly add 665 mL of concentrated sulfuric acid (H_2SO_4) to 300 mL of water while cooling in an ice bath and stirring. Allow to come to room temperature. Adjust the relative density to 1.6389 ± 0.0012 at 15.6°C/15.6°C.

7.2 *Materials*:

7.2.1 *Autosampler Vials*, with crimp top seals to fit.

7.2.2 *Disposable Syringes*, 3 mL.

7.2.3 *Disposable Syringe Filters*, nylon, 0.2 µm.

7.2.4 *Glass Serum Bottles*, crimp top style, 125 mL, with rubber stoppers and aluminum seals to fit.

[4] BioRad Aminex®, HPX-87C and Aminex® HPX-87P, available from BioRad, Main Office, 3300 Regatta Boulevard, Richmond, CA 94804 has been found suitable for this purpose.

[5] Available from Hewlett-Packard, HP Analytical Direct, 2850 Centerville Road, Wilmington, DE 19808.

[6] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmaceutical Convention, Inc. (USPC), Rockville, MD.