Designation: E1721 – 01 (Reapproved 2020)

Standard Test Method for Determination of Acid-Insoluble Residue in Biomass[1]

This standard is issued under the fixed designation E1721; 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.

INTRODUCTION

Biomass is composed largely of the following: cellulose, a polymer of glucose; hemicellulose, a complex polymer, the main chain of which consists of xylans or glucomannans; and lignin, a complex phenolic polymer. The lignin is mostly insoluble in mineral acids, unlike the other cell wall components of biomass. For this reason, lignin can be analyzed gravimetrically after hydrolyzing the cellulose and hemicellulose fractions with sulfuric acid.

1. Scope

1.1 This test method covers determination of the acid-insoluble residue of 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 and alkaline pretreated biomass, and the solid fraction of fermentation residues. All results are reported relative to the 105 °C oven-dried weight of the sample.

1.2 The residue collected contains the acid-insoluble lignin and any condensed proteins from the original sample. An independent nitrogen analysis would be required to determine the acid-insoluble lignin content separate from the condensed protein fraction and is outside the scope of this test method.

1.3 A portion of the lignin in some biomass samples will remain soluble during this procedure. The total lignin in a biomass sample includes both acid-soluble lignin and lignin in the acid insoluble residue.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.* Specific hazards statements are given in Section 8 and Note 2 *and Note 4.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

E1690 Test Method for Determination of Ethanol Extractives in Biomass

E1756 Test Method for Determination of Total Solids in Biomass

E1757 Practice for Preparation of Biomass for Compositional Analysis

3. Terminology

3.1 *Definitions:*

3.1.1 *acid-insoluble residue*—the solid residue, corrected for acid-insoluble ash, retained on a medium-porosity filter crucible after the primary 72 % and secondary 4 % H_2SO_4 hydrolysis described in this test method. This material is primarily acid-insoluble lignin and any condensed proteins.

3.1.2 *prepared biomass*—material that has been treated in accordance with Practice E1757 in order to raise the total solids content above 85 %, based on an oven-dried solids weight.

4. Significance and Use

4.1 The acid-insoluble residue content is used in conjunction with other assays to determine the total composition of biomass samples.

[1] This test method is under the jurisdiction of ASTM Committee E48 on Bioenergy and Industrial Chemicals from Biomass and is the direct responsibility of Subcommittee E48.05 on Biomass Conversion.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

5. Interferences

5.1 The results of acid-insoluble residue analysis are affected by the incomplete hydrolysis of biomass. The results will be biased high unless the sample is hydrolyzed completely. Take care to mix the acid/biomass slurry thoroughly during the concentrated acid hydrolysis.

5.2 The results of acid-insoluble residue analysis are affected by the timing of the acid digestion steps. The insoluble residue will dissolve slowly into solution in an irreproducible fashion. The timing within this test method must be followed closely.

6. Apparatus

6.1 *Analytical Balance,* readable to 0.1 mg.

6.2 *Convection Oven,* with a temperature control of 105 ± 3 °C.

6.3 *Muffle Furnace*—An electric furnace is recommended for igniting the sample. The furnace should be fitted with an indicating pyrometer or thermocouple so that the required temperature of 575 ± 25 °C can be maintained.

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

6.5 *Water Bath,* set at 30 ± 1 °C.

6.6 *Desiccator,* using anhydrous calcium sulfate.

7. Reagents and Materials

7.1 *Chemicals:*

7.1.1 *72 % H_2SO_4,* specific gravity 1.6389 ± 0.0012 at 15.6 °C/15.6 °C or 12.00 ± 0.02 M.

7.1.2 *Water,* 18 MΩ deionized.

7.2 *Materials:*

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

7.2.2 *Glass Filtering Crucible,* 50 mL, medium porosity, with a nominal maximum pore size of 10 µm.

7.2.3 *Vacuum Adapter for Crucibles.*

8. Hazards

8.1 Handle the sulfuric acid carefully.

8.2 Use caution when handling glass bottles after the autoclave step since they may become pressurized.

9. Sampling, Test Specimens, and Test Units

9.1 Test specimens suitable for analysis with this procedure are as follows:

9.1.1 Prepared biomass samples that have been treated in accordance with Practice E1757.

9.1.2 Extractives-free material prepared in accordance with Test Method E1690.

9.2 The test specimen shall consist of approximately 0.3 g of sample obtained in such a manner to ensure that it is representative of the entire lot of material being tested. Prepared biomass is used in this test, but the weight of the material must be corrected to 105 °C dry weight by using the percent total solids value determined in accordance with Test Method E1756, prior to calculating the acid-insoluble residue.

9.3 The samples for total solids determination should be weighed out at the same time as those for acid-insoluble residue determination. If this is performed later, it can introduce an error in the calculation because ground biomass can gain or lose moisture rapidly when exposed to the atmosphere.

10. Procedure

10.1 Label the crucibles needed for analysis individually, and ignite them at 575 ± 25 °C to achieve a constant weight of ±0.3 mg. Store the ignited crucibles in a desiccator until needed.

NOTE 1—In order to determine the absolute amounts of acid-insoluble residue and acid-insoluble ash, for quality control purposes, it is useful to weigh and record the ignited crucible to the nearest 0.1 mg.

10.2 Weigh a 0.3 ± 0.01-g sample to the nearest 0.1 mg, and place it in a test tube. Record the initial weight as W_1.

NOTE 2—**Warning:** 72 % sulfuric acid is very corrosive and should be handled only by trained personnel.

10.3 Add 3.00 ± 0.01 mL (4.92 ± 0.01 g) of 72 % H_2SO_4, and stir for 1 min or until mixed thoroughly.

10.4 Place the test tube in the water bath controlled to 30 ± 1 °C, and hydrolyze for 2 h.

NOTE 3—The hydrolysis time may be reduced to 1 h if the dried sample has been milled and sieved to pass through a 20-mesh sieve and be retained on a 80-mesh sieve.

10.5 Stir the sample every 15 min to ensure complete mixing and wetting.

10.6 Transfer the hydrolyzate to a glass bottle, and dilute to a 4 % acid concentration by adding 84.00 ± 0.04 mL water or by bringing the combined weight of sample, acid, and water up to 89.22 ± 0.04 g. Be careful to transfer all of the residual solids along with the hydrolysis liquor.

10.7 Stopper each of the bottles, and crimp the aluminum seals into place.

10.8 Set the autoclave to a liquid vent cycle to prevent loss of sample from the bottle in the event of a loose crimp seal. Autoclave the samples in their sealed bottles for 1 h at 121 ± 3 °C.

NOTE 4—**Warning:** Handle sealed bottles with caution after the autoclave step since they may become pressurized.

10.9 After completion of the autoclave cycle, allow the samples to cool for approximately 20 min at room temperature before removing the seals and stoppers.

10.10 Vacuum filter the hydrolysis solution through a previously ignited filtering crucible.

10.11 If a carbohydrate analysis or acid-soluble lignin analysis, or both, is desired, decant 15 to 25 mL of filtrate into a resealable container. If the aliquot is not used immediately for further analysis, store it in a refrigerator at 4 °C.

NOTE 5—Acid-soluble lignin should be analyzed within 24 h and preferably within 6 h of hydrolysis.

10.12 Use hot water to wash any particles clinging to the glass bottle into the crucible and to wash the filtered residue free of acid using vacuum filtration.