Designation: E3417 – 24

Standard Test Method for Determination of Cellulose/Hemicellulose-Derived Glucan and Galactan Content in Solid Corn Biomass Samples[1]

This standard is issued under the fixed designation E3417; 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

The US biofuel industry generates ~15 billion gal of bioethanol annually. Most of this bioethanol is produced using processes that convert the starch content of corn biomass to glucose through enzymatic degradation and subsequently to ethanol via yeast fermentation. Starch [a branched polymer of α-(1-4)-D-glucose units] is the source of the majority of the glucose content present in corn. A tiny proportion of bioethanol is generated using processes that convert cellulosic feedstock to glucose before yeast fermentation. The emergence of in-situ corn kernel fiber (CKF) conversion processes that allow the simultaneous conversion of starch and cellulosic content in biomass presents an excellent opportunity for the sector. While starch measurement methodology has been well described in literature, the analytical methodology required to accurately measure cellulosic content in corn biomass has not been published to date despite the urgent requirement for the same to be used in the assignment of a small but valuable percentage of total ethanol produced during such a fermentation as cellulosic.

The procedure outlined herein seeks to address this need by modifying the assay described in the seminal 2021 work of Sluiter *et al*.[2]

1. Scope

1.1 This procedure can be used to quantify the cellulose/hemicellulose-derived glucan and galactan (CHDGG) content in corn biomass samples that also contain varying levels of starch-derived and yeast-derived glucan. The method has been shown to provide accurate values for samples with cellulose content up to 40 % *w/w*.[3]

1.2 *Units*—The values stated in SI units are to be regarded as the standard. No other units of 34 measurement are included in this standard.

1.3 *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.*

1.4 *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:*[4]

E1757 Practice for Preparation of Biomass for Compositional Analysis

E3181 Practice for Determination of the Converted Fraction of Starch and Cellulosic Content From a Fuel Ethanol Production Facility

3. Terminology

3.1 *Definitions:*

3.1.1 *biomass, n*—substance wholly comprised of living or recently living (non-fossil) material.

3.1.1.1 *Discussion*—This method is selective for corn biomass which is defined as the biomass from large kernels set in rows on a cob from a cereal plant.

[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] Sluiter, J. B. *et al*., "Direct Determination of Cellulosic Glucan Content in Starch-Containing Samples," *Cellulose*, 2021, https://doi.org/10.1007/s10570-020-03652-2.

[3] See Supporting data document - Determination of cellulosic carbohydrate content in corn biomass samples

[4] 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.

3.1.2 *calibration verification standard, CVS, n*—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.

3.1.2.1 *Discussion*—The concentration of the CVS should be set in the middle of the chosen calibration range and is used to ensure the calibration remains accurate throughout the entirety of the sample run. Additional CVS concentrations at the lower or higher end of the calibration curve can also be run to ensure accuracy of a sample's respective concentrations.

3.1.3 *cellulose, n*—a crystalline, straight chain glucan with β-(1→4) linkages.

3.1.4 *cellulose/hemicellulose-derived glucan and galactan, n*—carbohydrate content present in a sample comprising glucan and galactan, but specifically does not include glucan from starch or yeast.

3.1.4.1 *Discussion*—For the purpose of this test method, cellulose/hemicellulose-derived glucan and galactan is abbreviated as CHDGG.

3.1.5 *cellulosic content, n—as defined by EPA RFS documentation*, the sum of cellulose, hemicellulose, and lignin in cellulosic feedstock.

3.1.6 *cellulosic pellet, n*—solid fraction of sample remaining on quartz filter after precipitation and filtration following starch and yeast glucan removal.

3.1.7 *galactose, n*—six-carbon monosaccharide common in biomass.

3.1.8 *glucose, n*—six-carbon monosaccharide common in biomass.

3.1.9 *glycogen, n*—high molecular weight branched homopolymer found in yeast consisting of linear α-(1,4)-glucosyl chains with α-(1,6)-branch points.

3.1.10 *hemicellulose, n*—a polymeric, branched carbohydrate with mixed C5 and C6 monomeric sugars, typically with a xylose or mannose backbone.

3.1.11 *starch, n*—a polysaccharide consisting of glucose monomers joined in α 1,4 linkages; the simplest form of starch is the linear polymer amylose, while amylopectin is the branched form.

3.1.12 *yeast degrading cocktail, n*—an enzymatic cocktail for degradation of yeast glucan.

3.1.12.1 *Discussion*—The reagent should contain the necessary suite of hydrolytic activities to effect yeast glucan removal under the conditions specified in this standard, but should not exhibit contaminating activities that would degrade cellulosic or hemicellulosic derived glucan and galactan.

3.1.13 *yeast glucan, n*—any glucan from a yeast source.

3.1.13.1 *Discussion*—Includes compounds such as yeast β-glucan; a branched polymer of 1,3/1,6-β-D-glucose units, glycogen, and trehalose; an α-1,1-linked glucosyl disaccharide.

3.2 *Abbreviations:*

3.2.1 *CF*—Converted Fraction

3.2.2 *CHDGG*—Cellulose/Hemicellulose-Derived Glucan and Galactan

3.2.3 *CVS*—Calibration Verification Standard

3.2.4 *SRS*—Sugar Recovery Standard

3.2.5 *YDC*—Yeast Degrading Cocktail

4. Significance and Use

4.1 The procedure, in brief, consists of solubilization of biomass using cold caustic extraction, an enzymatic removal of starch and yeast glucan followed by precipitation of hemicellulosic material, and acid hydrolysis of the residual carbohydrate pellet before measurement of glucose and galactose derived from cellulose and hemicellulose.

4.2 The starch removal procedure is identical to that employed in the NREL assay[2] which was itself adapted from the Megazyme-published starch analysis procedure: RTS-NaOH, 2019.[5,6]

4.3 The cellulosic pellet hydrolysis and monosaccharide measurements are identical to those described in the NREL laboratory analytical procedure "Determination of Structural Carbohydrates and Lignin in Biomass," NREL/TP-5100-42618 and "Determination of Cellulosic Glucan Content in Starch Containing Feedstocks," NREL/TP-2800-76724.[7]

4.4 This test method references Practices E1757 and E3181.

4.5 This test method is intended for use in the measurement of the fermentable portion of cellulosic content in pre- and post-fermentation corn biomass specifically for the apportionment of a percentage of the total ethanol produced in a fermentation as cellulosic.

5. Apparatus

5.1 Analytical balance with precision to 0.1 mg.

5.2 Water bath capable of maintaining 50 °C ± 2 °C, 40 °C ± 2 °C, and 30 °C ± 2 °C.

5.3 HPLC system equipped with a refractive index detector, column oven capable of reaching 85 °C, and the following column: Concise Separations CarboSep CHO782, Lead Form column (or equivalent) with ionic form H^+/CO_3^- deashing guard column.

NOTE 1—The deashing guard column should be replaced every ~50 injections to protect the analytical column.

6. Reagents and Materials

6.1 *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

[5] McCleary, B. V., Charmier, L. M. J., and McKie, V. A., Megazyme, "Measurement of Starch: Critical Evaluation of Current Methodology," *Starch*, 1800146, No. 71, 2018, pp. 1-13, DOI: 10.1002/star.201800146.

[6] The Megazyme starch assay procedure referenced in this test method can be found at:
https://www.megazyme.com/documents/Booklet/K-TSTA-100A_DATA.pdf.

[7] All NREL biomass compositional analysis laboratory procedures referenced in this test method can be found at: https://www.nrel.gov/bioenergy/biomass-compositional-analysis.html.