



Designation: E3181 – 24

Standard Practice for Determination of the Converted Fraction of Starch and Cellulosic Content From a Fuel Ethanol Production Facility¹

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1. Scope*

1.1 This practice provides criteria for the sampling, testing, and calculation methodologies used for the quantification of the converted fraction of starch and cellulosic content. Furthermore, this practice covers procedures for the management of the standard error associated with the sampling and testing of before conversion and after conversion samples from a fuel ethanol production facility.

1.1.1 This practice can be used to determine the volume of renewable fuel produced from the simultaneous conversion of starch and cellulosic material eligible for generating D3 RINs under the United States (U.S.) Renewable Fuel Standard (RFS).

1.2 This practice covers the collection and testing of heterogeneous material, including, but not limited to: corn, sorghum, wheat, mash, beer, whole stillage, dried distillers grains with solubles (DDGS), and dried distillers grains.

1.3 This practice is intended to be used in renewable fuel production facilities designed to produce renewable alcohols. Use of this practice in any other type of process has not been reviewed.

1.4 This practice can be utilized using either manual or automatic sampling techniques, so long as the criteria of this practice are followed.

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.*

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.*

¹ This practice 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. Referenced Documents

2.1 ASTM Standards:²

E122 Practice for Calculating Sample Size to Estimate, With Specified Precision, the Average for a Characteristic of a Lot or Process

E1705 Terminology Relating to Bioenergy and Industrial Chemicals from Biomass

E1755 Test Method for Ash in Biomass

E1757 Practice for Preparation of Biomass for Compositional Analysis

E2586 Practice for Calculating and Using Basic Statistics

2.2 AOAC Standard:³

AOAC 942.05 Ash of Animal Feed

2.3 Other Publications:

CFR 40 Code of Federal Regulations (CFR) Part 80 Subpart M Renewable Fuel Standard⁴

EPA-420-B-19-022 Guidance on Qualifying an Analytical Method for Determining the Cellulosic Converted Fraction of Corn Kernel Fiber Co-Processed with Starch⁵

EPA-HQ-OAR-2012-0401; FRL-9910-40-OAR (July, 2014) Additional Detail on the Calculation of the Cellulosic Converted Fraction, and Attribution of Batch RINs for D-code Dependent Feedstocks⁵

3. Terminology

3.1 Definitions of Terms:

3.1.1 For definitions of terms used in this standard, refer to Terminology E1705.

NOTE 1—The user is advised that the definitions used by various industries, marketers, regulatory bodies, and other ASTM Committees can differ from those in this standard. When using this standard, it is critical to use terminology from Committee E48.

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.

³ Available from AOAC International, 2275 Research Blvd., Suite 300, Rockville, MD 20850-3250, <http://www.aoac.org>.

⁴ Available from the U.S. Government Printing Office, Superintendent of Documents, 732 N. Capitol St. N.W. Mail Stop: SDE Washington, DC 20401.

⁵ Available from the United States Environmental Protection Agency (EPA), 1200 Pennsylvania Avenue, N.W., Washington, DE 20460, www.epa.gov.

*A Summary of Changes section appears at the end of this standard

3.1.2 *amyloglucosidase*, *n*—an enzyme that specifically catalyzes the hydrolysis of α -D-glucosidic bonds successively from the non-reducing ends of oligo- and polysaccharides with the release of β -D-glucose.

3.1.3 *ash*, *n*—inorganic residue remaining after combustion.

3.1.3.1 *Discussion*—Test Method E1755 and AOAC 942.05 are acceptable prescribed methods.

3.1.4 *backset*, *n*—a recycled liquid stream from distillation that also contains some level of dissolved and suspended solids used as makeup water in a fermentation facility.

3.1.5 *beer*, *n*—the mash after it has undergone fermentation and has been deemed complete by virtue of the carbohydrates being converted by enzymes and fermenting organisms.

3.1.6 *cellulose*, *n*—a straight chain glucan with $\beta(1\rightarrow4)$ linkages.

3.1.6.1 *Discussion*—The material can be amorphous or crystalline.

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

3.1.8 *cellulosic feedstock*, *n*—any feedstock composed predominately of cellulose, hemicellulose, or lignin.

3.1.8.1 *Discussion*—The EPA allows renewable fuel produced from cellulosic feedstocks with an average adjusted cellulosic content of 75 % (on a dry mass basis) to be eligible for the generation of the D3 RIN code.

3.1.8.2 *Discussion*—For example, in a facility producing ethanol from corn, the EPA considers the corn kernel fiber to be a cellulosic feedstock.

3.1.9 *converted fraction (CF)*, *n*—a unitless average mass fraction representing the portion of the feedstock converted to either cellulosic or non-cellulosic fuel.

3.1.10 *distillers dry grain, DDG*, *n*—the dried whole stillage; typically sold as an animal feed component.

3.1.11 *distillers dried grains with solubles, DDGS*, *n*—the dried whole stillage with solubles; typically sold as an animal feed component.

3.1.12 *greenhouse gas, GHG*, *n*—gases that can trap heat in the atmosphere.

3.1.12.1 *Discussion*—Greenhouse gases include: carbon dioxide, methane, nitrous oxide, and fluorinated gases.

3.1.13 *glucan*, *n*—refers to any polymer of glucose, regardless of structure.

3.1.14 *hemicellulose*, *n*—a polymeric, branched carbohydrate with mixed 5 and 6 carbon monomeric sugars, typically with a xylose or mannose backbone.

3.1.14.1 *Discussion*—For the purpose of this practice, five carbon sugars are derived from hemicellulose. Six carbon sugars are derived from either starch, hemicellulose, or cellulose.

3.1.15 *lignin*, *n*—a complex organic polymer with aromatic components.

3.1.15.1 *Discussion*—Non-carbohydrate found in cellulosic feedstock as defined by Committee E48.

3.1.16 *makeup water*, *n*—any liquid stream added to the process to prepare the mash slurry in a production facility.

3.1.17 *mash*, *n*—a mixture or slurry of water (including recycled water streams) and ground grain.

3.1.18 *monosaccharide*, *n*—any of the class of sugars that cannot be hydrolyzed to give a simpler sugar.

3.1.18.1 *Discussion*—Glucose is an example of a simple sugar.

3.1.19 *oligosaccharide*, *n*—a carbohydrate whose molecules are composed of a relatively small number of monosaccharide units.

3.1.19.1 *Discussion*—Typically containing less than 12 monosaccharide units.

3.1.20 *polysaccharide*, *n*—a carbohydrate whose molecules consist of a long chain of sugar residues bonded together.

3.1.20.1 *Discussion*—Examples of polysaccharides are starch, cellulose and glycogen.

3.1.21 *predominantly cellulosic, adj*—as defined by EPA RFS documentation, feedstock that has an average adjusted cellulosic content of 75 %, measured on a dry mass basis; furthermore, this “adjusted cellulosic content” is the percent of organic (non-ash) material that is cellulose, hemicellulose, or lignin.

3.1.21.1 *Discussion*—The EPA regulations are found in CFR 40, Part 80 and EPA-HQ-OAR-2012-0401; FRL-9910-40-OAR.

3.1.22 *Renewable Fuel Standard (RFS)*, *n*—program authorized under the Energy Policy Act of 2005 and expanded under the Energy Independence and Security Act of 2007; it requires an increasing amount of renewable fuel to be blended into the U.S. liquid fuel supply each year through the year 2022.

3.1.23 *resistant starch*, *n*—starch that cannot be readily monomerized by commercially available α amylase and glucoamylase; this type of starch can be caused by several different factors: physical inaccessibility, molecular structure configuration, cross linkage, and lipid binding.

3.1.23.1 *Discussion*—When resistant starch is suspected to be present, it is important that the starch measurement technique used be designed to access resistant starch. Many starch procedures have options that include, for example, dimethyl sulfoxide (DMSO) or potassium hydroxide (KOH) to ensure complete access and hydrolysis of any resistant starch present.

3.1.24 *RINs*, *n*—as defined by EPA RFS documentation, renewable identification numbers (RINs) are unique numbers generated to represent a volume of renewable fuel used for compliance to the RFS program.

3.1.24.1 *D3 RINs*, *n*—as defined by EPA RFS documentation, the RIN code assigned to gallons of renewable fuel produced from any cellulose, hemicellulose, or lignin that has lifecycle greenhouse gas emissions that are at least 60 % less than the baseline lifecycle greenhouse gas emissions.

3.1.24.2 *D6 RINs*, *n*—as defined by EPA RFS documentation, the RIN code assigned to gallons of renewable fuel produced from renewable biomass and has lifecycle greenhouse gas emissions that are at least 75 % less than

baseline lifecycle greenhouse gas emissions, unless the fuel is exempt from this requirement.

3.1.25 *starch, n*—a polysaccharide consisting of glucose monomers joined in α 1,4 or α 1,6 linkages.

3.1.25.1 *Discussion*—The simplest form of starch is the linear polymer amylose, while amylopectin is the branched form.

3.1.25.2 *Discussion*—For the purposes of this practice, starch shall include the soluble C6 glucan mono- and oligosaccharides. Since the EPA definition for “cellulosic content” is specific to the “sum of cellulose, hemicellulose, and lignin in cellulosic feedstock,” the soluble C6 glucan mono- and oligosaccharides shall be included with the starch total (CFR 40, Part 80). Since the only difference between starch and cellulose is the orientation of the 1, 4 linkage, to determine the starch content of a sample that contains both starch and cellulose, an assay that utilizes amyloglucosidase is necessary.

3.1.26 *total solids, n*—the combined amount of suspended and dissolved matter contained in a sample.

3.1.27 *whole stillage, n*—the product resulting from the distillation of the beer after removing the main product.

3.2 Definitions of Terms Specific to This Standard:

3.2.1 *after conversion sample, n*—a sample obtained from the process after the conversion process of starch and cellulosic content is determined to be complete as defined by the producer’s reaction standards.

3.2.2 *before conversion sample, n*—a sample obtained from the process before any conversion has taken place.

3.2.2.1 *Discussion*—This includes converting starch and cellulosic content.

3.2.3 *certification run, n*—a group of converted fraction determinations to establish the converted fraction of starch and cellulosic content in a process that involves the simultaneous conversion of feedstocks that are predominantly cellulosic and feedstocks that are not predominantly cellulosic.

3.2.4 *feedstock, n*—any raw material used as a carbohydrate source for fermentation.

4. Significance and Use

4.1 In 2014, the U.S. EPA published the final rules adding renewable fuel pathways under the RFS Program. The rules qualified kernel fiber as a cellulosic feedstock meeting the 60 % greenhouse gas (GHG) reduction and qualifies for the generation of D3 RINs. These rules allow for two approaches for kernel fiber conversion (CFR 40, Part 80 and EPA-HQ-OAR-2012-0401; FRL-9910-40-OAR):

4.1.1 Producers of cellulosic fuels derived from conversion of feedstocks that are predominantly cellulosic, where “predominantly cellulosic” is defined as feedstock that has an average adjusted cellulosic content of 75 %, measured on a dry mass basis; furthermore, this “adjusted cellulosic content” is the percent of organic (non-ash) material that is cellulose, hemicellulose, or lignin (CFR 40, Part 80 and EPA-HQ-OAR-2012-0401; FRL-9910-40-OAR).

4.1.2 Producers of cellulosic fuels derived from the simultaneous conversion of feedstocks that are predominantly cel-

lulosic and feedstocks that are not predominantly cellulosic (CFR 40, Part 80 and EPA-HQ-OAR-2012-0401; FRL-9910-40-OAR).

4.2 Producers that wish to gain approval to the pathway that claims simultaneous conversion of feedstocks that are predominantly cellulosic and feedstocks that are not predominantly cellulosic are required to quantify the amount of renewable fuel that is derived specifically from cellulosic content and from starch. To accomplish this, the producer needs to quantify the amount of cellulosic content and starch present before the conversion process begins and after the conversion process is complete. These measurements of cellulosic content and starch content before and after conversion are used to calculate a converted fraction of each, which is then used to ratio the renewable fuel produced accordingly and assign those respective gallons the D6 or D3 RIN code (CFR 40, Part 80 and EPA-HQ-OAR-2012-0401; FRL-9910-40-OAR).

5. Test Method Requirements

5.1 Any test method used for the quantification of starch and cellulosic content and the related converted fraction of each shall be evaluated for both accuracy and precision.

5.1.1 Agreement of the test method for cellulosic content may be demonstrated by showing agreement of the test method for cellulosic content to a commercially available reference material⁶ with established and documented concentrations of cellulosic content. When the commercially available reference material is analyzed, the test method shall achieve a mean test value that falls within 20 % of the reported cellulosic content value (EPA-420-B-19-022) as documented with the commercially available reference material. Test values that fall within 20 % of the reported cellulosic content value are deemed reasonably accurate.

5.1.1.1 *Discussion*—Producers generating RINs with a D code of 3 or a D code of 7 using two or more different feedstocks (at least one of which does not have at least 75 % average adjusted cellulosic content) which are processed simultaneously through an in situ biochemical hydrolysis treatment will similarly have additional registration requirements to help ensure that cellulosic RINs are being generated accurately. At the time of registration, such a producer must submit: (1) the overall fuel yield, including supporting data demonstrating this yield and a discussion of the possible variability in overall fuel yield that could be expected between reporting periods; (2) the cellulosic converted fraction that will be used for generating RINs under § 80.1426(f)(3)(vi); including chemical analysis data (described in more detail below) supporting the calculated cellulosic converted fraction and a discussion of the possible variability that could be expected between reporting periods; and (3) a description of how the cellulosic converted fraction is determined and calculations showing how the data were used to determine the cellulosic converted fraction. Data used to calculate the cellulosic converted fraction by producers using in situ biochemical hydrolysis treatment who seek to

⁶ A possible source of reference materials is NIST (National Institute of Standards and Technology).