Designation: D6139 – 22

Standard Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants or Their Components Using the Gledhill Shake Flask[1]

This standard is issued under the fixed designation D6139; 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.

1. Scope*

1.1 This test method covers the determination of the degree of aerobic aquatic biodegradation of fully formulated lubricants or their components on exposure to an inoculum under controlled laboratory conditions. This test method is an ultimate biodegradation test that measures carbon dioxide (CO_2) evolution.

1.2 This test method is intended to specifically address the difficulties associated with testing water insoluble materials and complex mixtures such as are found in many lubricants.

1.3 This test method is designed to be applicable to all non-volatile lubricants or lubricant components that are not toxic and not inhibitory at the test concentration to the organisms present in the inoculum.

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 are discussed in Section 10.

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]

D1193 Specification for Reagent Water

D1293 Test Methods for pH of Water

D4447 Guide for Disposal of Laboratory Chemicals and Samples

D5291 Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Petroleum Products and Lubricants

D5864 Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components

E943 Terminology Relating to Biological Effects and Environmental Fate

2.2 *ISO Standard:*[3]

4259:1992(E) Petroleum Products—Determination and application of precision data in relation to methods of test

2.3 *APHA Standards:*[4]

2540B Total Solids Dried at 103–105°C

9215 Heterotrophic Plate Count

3. Terminology

3.1 *Definitions:*

3.1.1 Definitions of terms applicable to this test method that are not described herein appear in the ASTM Online Dictionary of Engineering Science and Technology[5] or Terminology E943.

[1] This test method is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.12 on Environmental Standards for Lubricants.

Current edition approved April 1, 2022. Published April 2022. Originally approved in 1997. Last previous edition approved in 2018 as D6139 – 18. DOI: 10.1520/D6139-22.

[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 American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

[4] Methods from *Standard Methods for the Examination of Water and Wastewater*, latest edition. Available from the American Public Health Association (APHA), 800 I Street, NW, Washington, DC 20001.

[5] *ASTM Online Dictionary of Engineering Science and Technology* (Stock#DEFONLINE) is available on the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org.

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

3.1.2 *activated sludge, n*—the precipitated solid matter, consisting mainly of bacteria and other aquatic microorganisms, that is produced at a domestic wastewater treatment plant and is used primarily in secondary sewage treatment to microbially oxidize dissolved organic matter in the effluent.

3.1.3 *aerobic, adj*—(*1*) taking place in the presence of oxygen; (*2*) living or active in the presence of oxygen.

3.1.4 *biodegradation, n*—the process of chemical breakdown or transformation of a material caused by organisms or their enzymes.

3.1.4.1 *Discussion*—Biodegradation is only one mechanism by which materials are transformed in the environment.

3.1.5 *biomass, n*—biological material including any material other than fossil fuels which is or was a living organism or component or product of a living organism.

3.1.5.1 *Discussion*—In biology and environmental science, biomass is typically expressed as density of biological material per unit sample volume, area, or mass (g biomass / g (or / mL or / cm^2) sample); when used for products derived from organisms biomass is typically expressed in terms of mass (kg, MT, etc.) or volume (L, m^3, bbl, etc.).

3.1.5.2 *Discussion*—Products of living organisms include those materials produced directly by living organisms as metabolites (for example, ethanol, various carbohydrates and fatty acids), materials manufactured by processing living organisms (for example: pellets manufactured by shredding and pelletizing plant material) and materials produced by processing living organisms, their components or metabolites (for example, transesterified oil; also called biodiesel).

3.1.6 *blank, n—in biodegradability testing*, a test system containing all system components with the exception of the test material.

3.1.7 *inoculum, n*—spores, bacteria, single celled organisms, or other live materials, that are introduced into a test medium.

3.1.8 *lag phase, n*—the period of diminished physiological activity and cell division following the addition of microorganisms to a new culture medium.

3.1.9 *log phase, n*—the period of growth of microorganisms during which cells divide at a positive constant rate.

3.1.10 *mixed liquor, n*—in sewage treatment, the contents of an aeration tank including the activated sludge mixed with primary effluent or the raw wastewater and return sludge.

3.1.11 *pre-adaptation, n*—the pre-incubation of an inoculum in the presence of the test material under conditions similar to the test conditions.

3.1.11.1 *Discussion*—The aim of pre-adaptation is to improve the precision of the test method by decreasing variability in the rate of biodegradation produced by the inoculum. Pre-adaptation may mimic the natural processes which cause changes in the microbial population of the inoculum leading to a more rapid rate of biodegradation of the test material but not to a change in the final degree of biodegradation.

3.1.12 *pre-condition, n*—the pre-incubation of an inoculum under the conditions of the test in the absence of the test material.

3.1.13 *supernatant, n*—the liquid above settled solids.

3.1.14 *suspended solids (of activated sludge or other inoculum samples), n*—solids present in activated sludge or inoculum samples that are not removed by settling under specified conditions.

3.1.15 *theoretical CO_2, n*—the amount of CO_2 which could in theory be produced from the complete oxidation of all of the carbon in a material.

3.1.16 *ultimate biodegradation, n*—degradation achieved when a material is totally utilized by microorganisms resulting in the production of CO_2 (and possibly methane in the case of anaerobic biodegradation), water, inorganic compounds, and new microbial cellular constituents (biomass or secretions, or both).

4. Summary of Test Method

4.1 Biodegradation of a lubricant or the component(s) of a lubricant is estimated by collecting and measuring the CO_2 produced when the lubricant or component is exposed to microorganisms under controlled aerobic aquatic conditions. This value is then compared to the theoretical amount of CO_2 which could be generated if all of the carbon in the test material were converted to CO_2. Carbon dioxide is a product of aerobic microbial metabolism of carbon-containing materials and so is a direct measure of the test material's ultimate biodegradation. The evolved CO_2 is trapped in a $Ba(OH)_2$ or other alkaline solution and the amount of CO_2 absorbed is determined by titrating the remaining hydroxide in solution.

4.2 The carbon content of the test material is determined by Test Methods D5291 or another appropriate method and the theoretical CO_2 is calculated from that measurement. It is necessary to directly measure the carbon content of the test material instead of calculating this number, because of the complexity of the mixture of compounds present in lubricants.

4.3 Biodegradability is expressed as a percentage of theoretical CO_2 production.

5. Significance and Use

5.1 Results from this CO_2 evolution test method suggest, *within the confines of a controlled laboratory setting*, the degree of ultimate aerobic aquatic biodegradability of a lubricant or components of a lubricant. Test materials which achieve a high degree of biodegradation in this test method may be assumed to easily biodegrade in many aerobic aquatic environments. (See also Test Method D5864.)

5.2 Because of the stringency of this test method, a low yield of CO_2 does not necessarily mean that the test material is not biodegradable under environmental conditions, but indicates that further testing needs to be carried out in order to establish biodegradability.

5.3 Information on the toxicity of the test material to the inoculum may be useful in the interpretation of low biodegradation results.