



Designation: D5864 – 23

Standard Test Method for Determining Aerobic Aquatic Biodegradation of Lubricants or Their Components¹

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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 laboratory conditions.

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 lubricants that are not volatile and are not inhibitory at the test concentration to the organisms present in the inoculum.

1.4 *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.

1.5 *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

D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants

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

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 Standard:⁴

2540B Total Solids Dried at 103–105°C

9215 Heterotrophic Plate Count

3. Terminology

3.1 Definitions:

3.1.1 For definitions of terms used in this test method, refer to Terminology D4175.

3.1.2 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.

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 / cm²) sample); when used for products derived from

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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.

³ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.

⁴ From *Standard Methods for the Examination of Water and Wastewater*, latest edition. Available from the American Public Health Association, 1015 18th St., N.W., Washington, DC 20036.

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

organisms biomass is typically expressed in terms of mass (kg, MT, etc.) or volume (L, m³, 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*—a flask containing the test medium and the inoculum with no additional carbon source added.

3.1.7 *inoculum, n*—the viable microorganisms used to contaminate a sample, device, or surface, often expressed as to number and type.

3.1.8 *lag phase, n*—the period of physiological activity and diminished cell division following the addition of microorganisms to a new culture medium.⁶

3.1.9 *log phase, n*—the period of growth of microorganisms during which cells divide at a constant rate.⁶

3.1.10 *mixed liquor, n*—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 more rapid biodegradation of the test material but not to a change in the final degree of biodegradation.

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

3.1.13 *theoretical CO₂, n*—the amount of CO₂ which could in theory be produced from the complete oxidation of all of the carbon in a material.

3.1.14 *ultimate biodegradation, n*—degradation achieved when a material is totally utilized by microorganisms resulting in the production of CO₂ (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 measured by collecting and measuring the CO₂ 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 CO₂ which could be generated if all of the carbon in the test material were converted to CO₂. CO₂ is a product of aerobic microbial metabolism of carbon-containing substances and so is a direct

measure of the test substance's ultimate biodegradation. CO₂ production is quantified by trapping it in a Ba(OH)₂ solution and titrating the solution to calculate the amount of CO₂ absorbed.

4.2 The carbon content of the test substance is determined by Test Method D5291 or an equivalent method and the theoretical CO₂ is calculated from that measurement. It is necessary to directly measure the carbon content of the test substance 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 CO₂ production.

5. Significance and Use

5.1 Results from the test method suggest, within the confines of a controlled laboratory setting, the degree of aerobic aquatic biodegradation of a lubricant or components of a lubricant by measuring the evolved carbon dioxide upon exposure of the test material to an inoculum. The plateau level of CO₂ evolution in this test method will suggest the degree of biodegradability of the lubricant. Test substances that achieve a high degree of biodegradation in this test may be assumed to easily biodegrade in many aerobic aquatic environments.

5.2 Because of the stringency of this test, a low yield of CO₂ does not necessarily mean that the test substance is not biodegradable under environmental conditions, but indicates that further testing is necessary to establish biodegradability.

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

5.4 Activated sewage-sludge from a sewage-treatment plant that principally treats domestic waste is considered an acceptable active aerobic inoculum available over a wide geographical area in which to test a broad range of lubricants. An inoculum derived from soil or natural surface waters, or both, or any combination of the three sources, is also appropriate for this test method.

NOTE 1—Allowance for various and multiple inoculum sources provides access to a greater diversity of biochemical competency and potentially represents more accurately the capacity for biodegradation.

5.5 A reference or control substance known to biodegrade is necessary in order to verify the activity of the inoculum. The test must be regarded as invalid and should be repeated using a fresh inoculum if the reference does not demonstrate a biodegradation of >60 % of the theoretical CO₂ evolution within 28 days.

5.6 A total CO₂ evolution in the blank at the end of the test exceeding 75 mg CO₂ per 3 L of medium shall be considered as invalidating the test.

5.7 The water solubility or dispersibility of the lubricant or component may influence the results obtained and hence the procedure may be limited to comparing lubricants or components with similar solubilities.

5.8 The ratio of carbon incorporated into cellular material to carbon released as CO₂ will vary depending on the organic

⁶ Adapted from *McGraw-Hill Dictionary of Scientific and Technical Terms*, 4th ed., 1989.