



Designation: D6731 – 22

Standard Test Method for Determining the Aerobic, Aquatic Biodegradability of Lubricants or Lubricant Components in a Closed Respirometer¹

This standard is issued under the fixed designation D6731; 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 a procedure for determining the degree of biodegradability of lubricants or their components in an aerobic aqueous medium on exposure to an inoculum under controlled laboratory conditions. This test method is an ultimate biodegradation test that measures oxygen demand in a closed respirometer.

1.2 This test method is suitable for evaluating the biodegradation of volatile as well as nonvolatile lubricants or lubricant components.

1.3 This test method is applicable to lubricants and lubricant components which are not toxic and not inhibitory to the test microorganisms at the test concentration.

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 given 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.*

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 2001. Last previous edition approved in 2018 as D6731 – 18. DOI: 10.1520/D6731-22.

2. Referenced Documents

2.1 ASTM Standards:²

- D1129 Terminology Relating to Water
- 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
- D6384 Terminology Relating to Biodegradability and Ecotoxicity of Lubricants
- E943 Terminology Relating to Biological Effects and Environmental Fate

2.2 ISO Standards:³

- ISO 4259–1,2 Petroleum products—Determination and application of precision data in relation to methods of test
- ISO 6107–2 Water quality—Vocabulary—Part 2
- ISO 8192 Water quality—Test for inhibition of oxygen consumption by activated sludge for carbonaceous and ammonium oxidation
- ISO 9408 Water quality—Evaluation of ultimate aerobic biodegradability of organic compounds in aqueous medium by determination of oxygen demand in a closed respirometer

2.3 OECD Standards:⁴

- OECD 301F Ready Biodegradability-Manometric Respirometry

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 Available from Organisation for Economic Cooperation and Development (OECD), 2 rue André Pascal, F-75775, Paris Cedex 16, France, <http://www.oecd.org>.

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

2.4 APHA Standards:⁵

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 appear in the Compilation of ASTM Standard Definitions and the following terminology standards: D1129, D4175, D6384, E943, and ISO 6107-2.

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*—(a) taking place in the presence of oxygen; (b) living or active in the presence of oxygen.

3.1.4 *biochemical oxygen demand (BOD), n*—the mass concentration of dissolved oxygen consumed under specified conditions by the biological oxidation of organic or inorganic matter, or both.

3.1.4.1 *Discussion*—BOD determination is performed using empirical tests employing standardized laboratory procedures. These tests measure oxygen utilization during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) in water.

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

3.1.5.1 *Discussion*—Biodegradation is only one mechanism by which materials are removed, transformed, or both, in the environment.

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

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

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

3.1.8.1 *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 extent of biodegradation of the test material.

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

3.1.10 *sludge, n*—a water-formed sedimentary deposit.

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

4. Summary of Test Method

4.1 Biodegradation of a lubricant or the component(s) of a lubricant is determined by measuring the oxygen consumed when the lubricant or component is exposed to microorganisms under controlled aerobic aquatic conditions. This value is then compared to the theoretical amount of oxygen (ThO₂) which is required to oxidize all of the elements (that is, carbon, hydrogen, nitrogen, and so forth) in the test material. This test method mixes the test material (lubricant or component) with aerobic microorganisms in a closed respirometer containing a defined aquatic medium and measures the biodegradation of the test material by following the decrease in oxygen in the respirometer.

4.2 The test material is the sole source of carbon and energy in the medium. A reference material known to biodegrade, such as low erucic acid rapeseed oil (LEAR or canola oil) is run alongside the test material to confirm that the inoculum is viable and capable of biodegrading suitable materials under the test conditions. The test material or reference material concentration is normally 50 mg/L to 100 mg/L, providing a theoretical oxygen demand of at least 50 mg O₂/L but no more than 200 mg O₂/L. The ThO₂ of the test and reference materials will be determined from measured elemental compositional analysis and will be calculated as in 13.1.

4.3 The inoculated medium is stirred in a closed flask and the consumption of oxygen is determined either by measuring the amount of oxygen required to maintain a constant gas volume in the respirometer flask, or by measuring the change in volume or pressure (or a combination of the two) in the apparatus.

4.4 Evolved CO₂ (carbon dioxide) is absorbed in an alkaline trap solution (for example, 10 M NaOH or KOH) or other CO₂-absorbing system suspended within the test vessel, typically in the headspace of the test vessel.

4.5 Biodegradation is followed over a specified period by determining the consumption of oxygen. The amount of oxygen utilized in oxidation of the test and reference material is corrected for oxygen uptake by the inoculum in the blank controls and is expressed as a percentage of the theoretical oxygen demand (ThO₂) calculated from the empirical formula of the material. Evaluation of the biodegradability of the test material is made on the basis of these data. Normally the test duration is 28 days; however, the test may be terminated if oxygen consumption has plateaued. The test may be extended as long as the systems' integrity is maintained and the inoculum in the blank systems is viable. The duration of the test will be dependent on the length of time required for the rate of test material biodegradation to achieve a plateau. A graphical illustration of the test results for a biodegradable material is presented in Fig. 1.

⁵ From Standard Methods for the Examination of Water and Wastewater, latest edition. Available from the American Public Health Assoc., 1015 18th St., NW, Washington, DC 20036.