Designation: D6731 – 01 (Reapproved 2011)

Standard Test Method for Determining the Aerobic, Aquatic Biodegradability of Lubricants or Lubricant Components in a Closed Respirometer[1]

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 and health practices and determine the applicability of regulatory limitations prior to use.* Specific hazards are given in Section 10.

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, Petroleum Products, 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 4259:1992(E) Petroleum Products–Determination and Application of Precision Data in Relation to Methods of Test
ISO 6107-2:1997 Water Quality–Vocabulary–Part 2
ISO 8192:1986 Water Quality–Test for Inhibition of Oxygen Consumption by Activated Sludge
ISO 9408:1999 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:1992 Ready Biodegradability-Manometric Respirometry

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 6107-2:1997.

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

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

[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.

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 test 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 incubation of an inoculum in the presence of the test material which is done prior to the initiation of the test and 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 is not expected to change the overall 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 (ThO_2) 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 to 100 mg/L, providing a theoretical oxygen demand of at least 50 mg O_2/L but no more than 200 mg O_2/L. The ThO_2 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 CO_2 (carbon dioxide) is absorbed in an alkaline trap solution (for example, 10 *M* NaOH or KOH) or other CO_2-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 (ThO_2) 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. Significance and Use

5.1 Results from this test method suggest the degree of aerobic, aquatic biodegradation of a lubricant or lubricant component. The rate and extent of oxygen consumption is measured upon exposure of the test material to an inoculum within the confines of a controlled laboratory setting. Test materials which 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 method, low results do not necessarily mean that the test material is not biodegradable under environmental conditions, but indicate that further testing is necessary to establish biodegradability.

5.3 If the pH value at the end of the test is outside the range from 6 to 8 and if the percentage degradation of the test

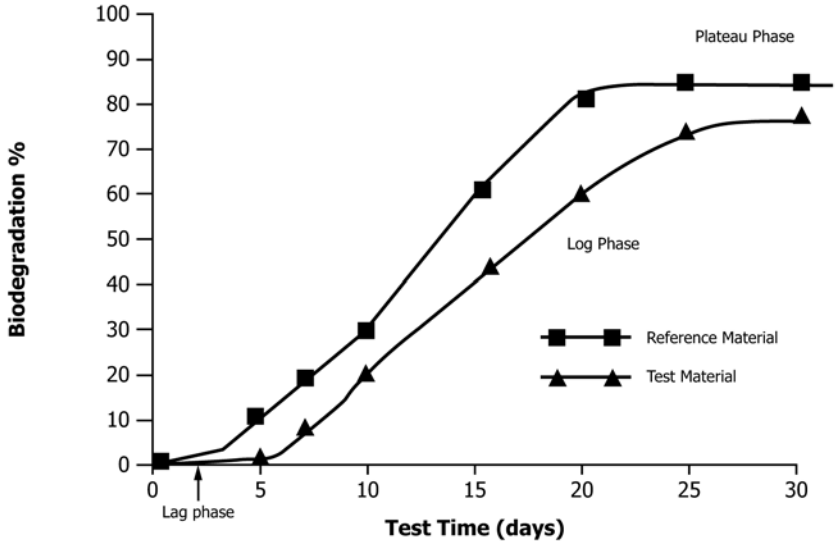

FIG. 1 Respirometric Test–Biodegradation Curve

material is less than 50 %, it is advisable to repeat the test with a lower concentration of the test material or a higher concentration of the buffer solution, or both.

5.4 A reference or control material known to biodegrade under the conditions of this test method is necessary in order to verify the activity of the inoculum. The test must be regarded as invalid and shall be repeated using a fresh inoculum if the reference material does not demonstrate biodegradation to the extent of >60 % of the ThO_2 within 28 days.

5.5 Information on the toxicity of the test material to the inoculum may be useful in the interpretation of low biodegradation results. Toxicity of the test material to the inoculum may be evaluated by testing the test material in combination with the reference material in inhibition control systems. If an inhibition control is included, the test material is assumed to be inhibiting if the degradation percentage of the reference material is lower than 40 % (ISO 8192:1986). In this case, it is advisable to repeat the test with lower concentrations of the test material.

5.6 Total oxygen utilization in the blank at the end of the test exceeding 60 mg O_2/L invalidates the test.

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

5.8 The behaviors of complex mixtures are not always consistent with the individual properties of the components. Test results for individual lubricant components may be suggestive of whether a mixture containing these components (that is, fully formulated lubricants) is biodegradable, but such information should be used judiciously.

6. Apparatus

6.1 *Closed Respirometer:*

6.1.1 The principle of a closed respirometer is given in Fig. 2. When testing volatile compounds, the apparatus used shall be appropriate or adapted to this particular purpose in accordance with the manufacturer's instructions. Exercise care that the closed respirometer apparatus is well sealed to prevent any loss (for example, leakage) of volatile compounds from the system or of oxygen into the system.

6.1.2 The test mixture is stirred by a magnetic stirrer in the test flask, which is filled with sufficient volume to minimize headspace and prevent delay of O_2 and CO_2 diffusion through the air-water phases. This volume is dependent on the selected flask size, and is normally specified by the manufacturer of the respirometer. If biodegradation takes place, the microorganisms consume oxygen and produce carbon dioxide. Oxygen from the headspace is then dissolved in the liquid to reestablish chemical equilibrium. The carbon dioxide produced by the microorganisms diffuses into the headspace where it is trapped in an absorbent solution or material and the total pressure in the flask then decreases.

6.1.3 This pressure drop is detected by a manometer, which produces a signal that results in the electrolytic generation of oxygen. When the original pressure is re-established, the signal is stopped and the quantity of electricity used is measured. The amount of electricity used is proportional to the amount of consumed oxygen. This is indicated on a plotter or a printer, or the data are collected using an appropriate software program.

6.2 *Water-Bath or Constant Temperature Room,* to comply with 11.2.

6.3 *Centrifuge.*

6.4 *pH-meter.*

6.5 *Analytical Balance,* capable of weighing to appropriate precision and accuracy (for example, ±0.0001 g).

7. Reagents and Materials

7.1 *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

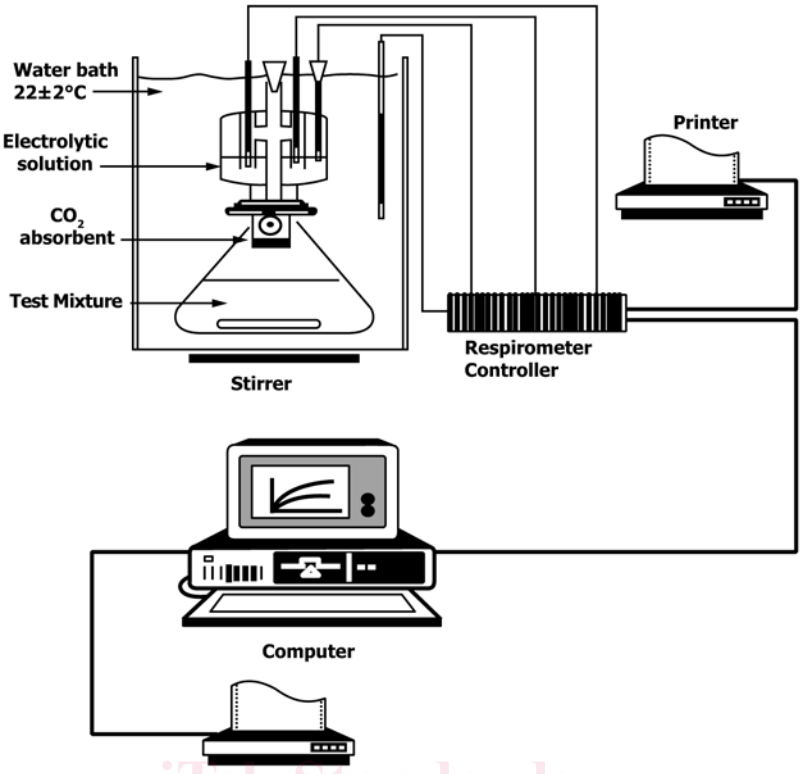

FIG. 2 Principle of a Closed Respirometer

such specifications are available.[6] Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without decreasing the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean reagent water as defined by Type II of Specification D1193.

7.3 Prepare the following stock solutions:

7.3.1 *Calcium Chloride Solution*—Dissolve 27.5 g of anhydrous calcium chloride ($CaCl_2$) or 36.4 g of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) in water and dilute to 1 L.

7.3.2 *Ferric Chloride Solution*—Dissolve 0.25 g of iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) in water and dilute to 1 L. Prepare this solution just before use or add a drop of concentrated hydrochloric acid (HCl) or 0.4 g/L of ethylenediaminetetraacetic acid (EDTA).

7.3.3 *Magnesium Sulfate Solution*—Dissolve 22.5 g of magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$) in water and dilute to 1 L.

7.3.4 *Phosphate Buffer Solution*—Dissolve 8.5 g of anhydrous potassium dihydrogen phosphate (KH_2PO_4), 21.75 g anhydrous potassium monohydrogen phosphate (K_2HPO_4), 33.4 g disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \cdot 2H_2O$), and 0.5 g ammonium chloride (NH_4Cl) in water and dilute to 1 L. Alternatively, 50.3 g of disodium hydrogen phosphate, heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) may be used in place of $Na_2HPO_4 \cdot 2H_2O$. The pH of this solution shall be about 7.4.

8. Inoculum Test Organisms

8.1 *Sources of the Inoculum*—Activated sewage-sludge from a sewage-treatment plant that treats principally domestic waste may be considered as an aerobic inoculum. An inoculum derived from soil or natural surface waters, or any combination of the three sources, may also be used in this test method. 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. The following provides several options for where and how to obtain an appropriate inoculum:

8.1.1 *Inoculum from Activated Sludge*—Activated sludge freshly sampled (that is, less than 24 h old) from a well-operated predominantly domestic sewage treatment plant (that is, one with no recent upsets and operating within its design parameters) may be used. This sewage treatment plant should receive minimal or no effluent from industry.

8.1.1.1 Using CO_2-free air, aerate sludge in the laboratory for 4 h. Depending on the number of test systems, sufficient volume of the mixed liquor is sampled and homogenized for 2 min at medium speed using a high-sheer/high-speed blender. Withdraw a sample for the determination of the dry weight of

[6] *Reagent Chemicals, American Chemical Society Specifications*, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see *Analar Standards for Laboratory Chemicals*, BDH Ltd., Poole, Dorset, U.K., and the *United States Pharmacopeia and National Formulary*, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.