



Designation: D 5247 – 92

Standard Test Method for Determining the Aerobic Biodegradability of Degradable Plastics by Specific Microorganisms¹

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1. Scope

1.1 This test method describes the procedures required to carry out a pure-culture study for evaluating the biodegradation of degradable plastics in submerged culture under aerobic conditions. Degradation will be evaluated by weight loss, tensile strength loss, percent-elongation loss and changes in molecular-weight distribution.

1.2 *This standard does not purport to address all of the safety problems, 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.*

2. Referenced Documents

2.1 ASTM Standards:

D 638 Test Method for Tensile Properties of Plastics²

D 882 Test Methods for Tensile Properties of Thin Plastic Sheeting²

D 3536 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by Liquid Exclusion Chromatography (Gel Permeation Chromatography—GPC)³

G 22 Practice for Determining Resistance of Plastics to Bacteria⁴

3. Terminology

3.1 Definitions of Terms Specific to This Standard:

3.1.1 *degradable plastic*—a plastic designed to undergo a significant change in its chemical structure under specific environmental conditions resulting in a loss of some properties that may vary as measured by standard test methods appropriate to the plastic and the application in a period of time that determines its classification.

3.1.2 *residual plastic*—the remaining plastic material in the culture medium after incubation with shaking.

3.1.3 *uninoculated control*—a plastic that is chemically disinfected and incubated with shaking in the same manner as test samples but without exposure to microorganisms.

3.1.4 *zero control*—a plastic without disinfection or cultural incubation; the starting material.

4. Summary of Test Method

4.1 The test method described herein follows the method developed by Lee et al.⁵ and consists of the following steps:

4.1.1 Cut degradable plastic samples into appropriate lengths suitable for tensile strength determinations as specified in Test Methods D 882 and a suitable size for a 250-mL Erlenmeyer shake-flask.

4.1.2 Plastic strips are chemically disinfected, aseptically dried, and initial weights determined.

4.1.3 Disinfected plastic strips are aseptically added to the appropriate growth medium, incubated with shaking for 24 h (125 r/min; orbital shaker) at the appropriate temperature for the specific microorganism being used. If not contaminated (contaminated medium becomes cloudy) the medium is then inoculated with a pure culture.

4.1.4 Inoculated and uninoculated-control flasks are incubated with shaking for specified period of time. Each film is evaluated with a minimum of four replicates with and without each microorganism.

4.1.5 Residual plastic strips are washed in 70 % ethanol for 30 min and dried.

4.1.6 Biodegradation is determined by weight loss, tensile-strength loss, percent-elongation loss, and average-molecular-weight distribution changes.

4.1.7 Significant differences between the inoculated flask and its corresponding uninoculated-control film are determined by performing an analysis of variance ($p < 0.05$).

5. Significance and Use

5.1 Several states in the United States and many European countries have required the use of degradable plastics in disposable products. As a result, several regulatory agencies

¹ This test method is under the jurisdiction of ASTM Committee D-20 on Plastics and is the direct responsibility of Subcommittee D20.96 on Environmentally Degradable Plastics.

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² Annual Book of ASTM Standards, Vol 08.01.

³ Annual Book of ASTM Standards, Vol 08.02.

⁴ Annual Book of ASTM Standards, Vol 08.03.

⁵ Lee, B., Pometto III, A. L., Fratzke, A., and Bailey Jr., T. B., "Biodegradation of Degradable Plastic Polyethylene by *Phanerochaete* and *Streptomyces*," *Applied Environmental Microbiology* 57, 1991, pp. 678–685.

have the need to evaluate degradable products for compliance with state and local regulations. In today's market there are three main actions by which degradable plastics are designed to degrade: (1) biological, (2) chemical, or (3) photo degradation, or combination thereof. The final degradation of all degradable plastic materials, whether they be initially degraded by photo- or chemical-degradation, will be by microorganisms. This test method provides an indirect way to evaluate the biodegradability of degradable plastics before and after exposure to the environment or artificially accelerated tests. Furthermore, if the degradable plastic consists of more than one component (for example, starch and polyethylene) the biodegradation of each component needs to be evaluated.

5.2 This test method describes a procedure that can be used to indicate the aerobic biodegradability of degradable plastics. The microorganisms described in the following test method are lignin degraders that are common to forest litter and composting sites (Lee et al., 1991).⁵ However, the test is not limited to these described microbes.

5.3 This test method is also appropriate for the evaluation of degradable plastics that have undergone specific chemical, thermal, or photo degradation, or combination thereof, as specified by ASTM procedures. Research has demonstrated that the initiation of biodegradation of degradable plastic (starch/polyethylene with pro-oxidant blends) requires chemical-degradation pretreatment of 4 to 8 days 70°C heat treatment or photodegradation pretreatment of 2 to 4 week exposure to ultraviolet light (365 nm) (Lee et al., 1991).⁵ Therefore, a pretreatment of the degradable plastic by heat, light or other appropriate treatment could be a component for evaluating the biodegradability of the different materials.

5.4 A pure-culture method provides a test that is reproducible in almost any laboratory. The use of an uninoculated control allows for the separation of degradable plastic changes due to incubation condition from those that are the results of biological activity.

5.5 Plastics not significantly degraded in this test may degrade at acceptable rates in a natural environment under mixed microbial conditions. Degradation of these unique polymers may require some biological capabilities not possessed by the pure cultures used in this test but which are commonly available in a natural environment. The test cultures recommended by this test method are organisms widely found in the environment. However, observation of significant degradation rates when using pure cultures that are not ubiquitously present in the natural environment would not necessarily indicate that the plastic will biodegrade at an acceptable rate in a natural environment.

6. Apparatus

6.1 *Glassware*—Glass 250-mL Erlenmeyer flask and culture tubes with sterilizable biological closures.

6.2 *Plastic Ware*—Sterilized petri dishes, autoclavable 2-L beakers, and specimen jars with lids.

6.3 *Incubator*—Incubator orbital shaker which operates at a temperature range of room temperature to 65°C.

6.4 *Universal Tester*—Tensile-strength and percent-elongation measurements with a 2-kg load cell.

6.5 *High-Temperature Gel-Permeation Chromatograph*—Determining changes in average-molecular-weight distribution.

6.6 *Analytical Balance*, capable of measurement to 0.1 mg.

6.7 *Autoclave*—Sterilization of disinfection solutions and culture medium.

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 reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society, where 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 lessening the accuracy of the determination.

7.2 *Purity of Water*—Unless otherwise indicated, references to water shall be understood to mean deionized water.

7.3 *Streptomyces Medium*.

7.3.1 This medium is a carbon- and nitrogen-free solution to be used whenever the medium for *Streptomyces* calls for a buffered mineral salts solution (pH 7.1). Prepare this medium by dissolving in 1 L of water the designated amounts of the following reagents:

Sodium phosphate, dibasic (Na_2HPO_4)	5.03 g
Potassium phosphate, monobasic (KH_2PO_4)	1.98 g
Magnesium sulfate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$)	0.20 g
Sodium chloride (NaCl)	0.20 g
Calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$)	0.05 g

7.3.2 Prepare bacterial trace-elements solution separately, and store refrigerated. One mL of trace-element solution is added to 1 L of mineral-salts solution.

Cupric sulfate ($\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$)	6.1 g
Iron sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$)	1.1 g
Manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$)	7.9 g
Zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	1.5 g

7.3.3 To buffered-mineral salts solution add 0.6 % (w/v) yeast extract, hereafter referred to as 0.6 % yeast-extract medium.

7.4 *Fungal Medium*—Prepare a solution of 3 % (w/v) malt extract in water and adjust the pH to 4.5, hereafter referred to as 0.3 % malt-extract medium.

7.5 Add 100 mL of medium to a 250-mL flask, fitted with a cotton plug. Wrap the flask cotton plug and neck with aluminum foil to reduce evaporation and then sterilize by autoclaving.

7.5.1 *Medium Sterilization*—Sterilization will be at 121°C (250°F) for 20 min.

8. Microbial Inoculum

8.1 *Bacterial-Cell Suspension*:

8.1.1 Use the following test organisms, or a suitable bacterium as agreed upon among parties concerned:

⁶ "Reagent Chemicals, American Chemical Society Specifications," Am. Chemical Soc., Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see "Reagent Chemicals and Standards," by Joseph Rosin, D. Van Nostrand Co., Inc., New York, NY, and the "United States Pharmacopeia."