Designation: D 5988 – 03

Standard Test Method for Determining Aerobic Biodegradation in Soil of Plastic Materials or Residual Plastic Materials After Composting[1]

This standard is issued under the fixed designation D 5988; 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 determination of the degree and rate of aerobic biodegradation of synthetic plastic materials (including formulation additives that may be biodegradable) in contact with soil, or a mixture of soil and mature compost, under laboratory conditions.

1.2 This test method is designed to rate the biodegradability of plastic materials relative to a standard in an aerobic environment.

1.3 This test method is designed to be applicable to all plastic materials that are not inhibitory to the bacteria and fungi present in soil and compost.

1.4 The values stated in SI units are to be regarded as the 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.* A specific hazard statement is given in Section 8.

1.6 This ASTM test method is equivalent to ISO 17556:2003.

2. Referenced Documents

2.1 *ASTM Standards:* [2]

D 425 Test Method for Centrifuge Moisture Equivalent of Soils

D 618 Practice for Conditioning Plastics and Electrical Insulating Materials for Testing

D 883 Terminology Relating to Plastics

D 1193 Specification for Reagent Water

D 1293 Test Methods for pH of Water

D 1898 Practice for Sampling of Plastics

D 2980 Test Method for Volume Weights, Water-Holding Capacity, and Air Capacity of Water-Saturated Peat Materials

D 2989 Test Method for Acidity-Alkalinity of Halogenated Organic Solvents and Their Admixtures

D 4129 Test Method for Total and Organic Carbon in Water by High-Temperature Oxidation and Coulometric Detection

D 4972 Test Method for pH of Soils

D 5338 Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions

D 5511 Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High-Solids Anaerobic-Digestion Conditions

2.2 *APHA-AWWA-WPCF Standards:*[3]

2540 D Total Suspended Solids Dried at 103°–105°C

2540 G Total, Fixed, and Volatile Solids in Solids and Semi-Solid Samples

2.3 *ISO Standard:*

ISO 17556:2003 Plastics—Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in Soil by Measuring the Oxygen Demand in a Respirometer or the Amount of Carbon Dioxide Evolved

3. Terminology

3.1 *Definitions*—Definitions of terms applicable to this test method appear in Terminology D 883.

4. Summary of Test Method

4.1 The test method described consists of the selection of plastic material or compost containing residual plastic material after composting for the determination of aerobic biodegradability, obtaining soil as a matrix and source of inoculum, exposing the plastic materials or the compost containing residual plastic material to the soil, measuring the carbon dioxide evolved by the microorganisms as a function of time, and assessing the degree of biodegradability.

4.2 The CO_2 production measured for a material, expressed as a fraction of the measured or calculated carbon content, is

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

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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] Standard Methods for the Examination of Water and Wastewater, 17th Edition, 1989, American Public Health Association (APHA), 1015 Fifteenth Street NW, Washington, DC 20005.

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

reported with respect to time, from which the degree of biodegradability is assessed.

4.3 Alternatively, the consumption of oxygen, or biochemical oxygen demand (BOD), can be determined, for example, 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) either automatically or manually. The level of biodegradation expressed in percent is determined by comparing the BOD with the theoretical oxygen demand (ThOD). The influence of possible nitrification processes on the BOD has to be considered.

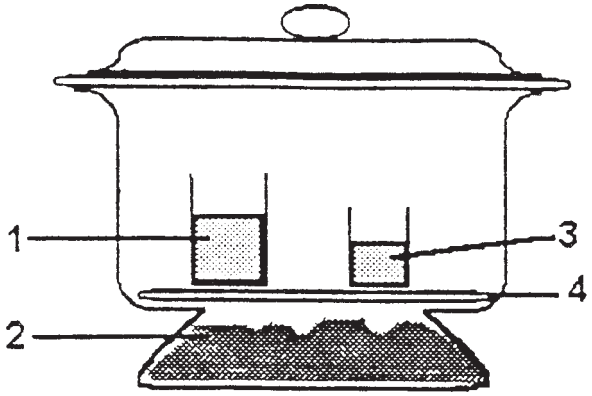
5. Significance and Use

5.1 The degree and rate of aerobic biodegradability of a plastic material in the environment determines the extent to which and time period over which plastic may be mineralized. Disposal is becoming a major issue with the increasing use of plastics, and the results of this test method may permit an estimation of the degree of biodegradability and the time period over which plastics will remain in an aerobic soil environment. This test method determines the degree of aerobic biodegradation by measuring evolved carbon dioxide as a function of time that the plastic is exposed to soil.

5.2 Soil is an extremely species-rich source of inoculum for evaluation of the biodegradability of plastics in the environment. When maintained appropriately with regard to moisture content and oxygen availability, the biological activity is quite considerable, although lower than other biologically active environments, such as activated sewage-sludge or compost. Soil is also the application target for composted materials, and therefore the biodegradability of such materials should be evaluated in the soil environment after the materials have been composted. A mixture of soil and mature compost containing composted plastic material (as obtained after performing Test Method D 5338) is therefore also an appropriate matrix for evaluation of the biodegradability of plastics.

6. Apparatus

6.1 *Soil-Contact Incubation Apparatus* (see Fig. 1; biometer flasks are also appropriate):



NOTE 1—(1) Barium hydroxide solution or potassium hydroxide solution, (2) soil, (3) water, and (4) perforated plate.

FIG. 1 Soil-Contact Incubation Apparatus

6.1.1 *Vessels*, a set of approximately 2 to 4-L internal volume that can be sealed air-tight, such as 150-mm desiccators. For testing a plastic material in soil: three vessels for soil only controls, three for a positive control material, and three per test material. For testing a compost containing residual plastic material: three for soil only controls, three for a positive control material in soil, three for the compost-soil control, and three per compost containing test material (optional: three for the compost containing the positive reference from the previous composting test). In either case, three vessels may also be included as technical controls, containing only the absorbing solution and no soil.

6.1.2 *Beakers*, sets of 150 and 100-mL, equal in number to the soil incubation vessels.

6.1.3 *Perforated Plates or Other Support*, a set to hold the beakers above the soil inside each vessel.

6.1.4 *Darkened Chamber or Cabinet*, in which the temperature is maintained at 21 ± 2°C.

6.2 *Analytical Equipment*:

6.2.1 *Analytical Equipment*, to measure the total carbon content of the test specimen.

6.2.2 *Analytical Balance*, to weigh the test specimen.

6.2.3 *Burette*, 100 mL.

6.2.4 *Bench-Top Centrifuge*, for moisture-holding capacity (MHC) determination.

6.2.5 *Oven*, set to 104 ± 1°C for moisture determinations.

6.2.6 *Muffle Furnace*, set to 550°C for ash determinations.

6.2.7 *pH Meter*.

6.3 Alternatively, a flow-through apparatus or manometric apparatus as described in ISO 17566 may be used.

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 such specifications are available.[4] 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 *Ammonium phosphate*, ($(NH_4)_2HPO_4$), 4.72 g/L.

7.3 *Barium Hydroxide Solution* (0.025 N), prepared by dissolving 4.0 g anhydrous $Ba(OH)_2$/L of distilled water. Filter free of solid material, confirm normality by titration with standard acid, and store sealed as a clear solution to prevent absorption of CO_2 from the air. It is recommended that 5 to 20 L be prepared at a time when running a series of tests. When using $Ba(OH)_2$, however, care must be taken that a film of $BaCO_3$ does not form on the surface of the solution in the beaker, which would inhibit CO_2 diffusion into the absorbing medium. Alternatively, potassium hydroxide solution (KOH, 0.5 N) could be used and is prepared by dissolving 28 g of

[4] *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.

anhydrous KOH/L of distilled water and proceeding in the same way as for the KOH.

7.4 *Hydrochloric acid*, 0.05 N HCl when using 0.025 N $Ba(OH)_2$, or 0.25 N HCl when using 0.5 N KOH.

8. Hazards

8.1 This test method includes the use of hazardous chemicals. Avoid contact with chemicals and follow the manufacturer's instructions and material safety data sheets.

9. Soil

9.1 The soil can be a laboratory mixture of equal parts (by weight) of sand, topsoil, and composted manure or a natural soil sample. The soil should not be handled in any way that would inhibit the activity of the soil microorganisms. In the case of a natural soil, it is advisable to avoid soils that have been exposed to pollutants that may cause a significant perturbation of the microbial population. The source of the soil must be reported (see 14.1.1). The test soil may also be a mixture of a natural soil and a mature compost, as obtained at the end of Test Method D 5338. A ratio of 1 g compost to 25 g soil corresponds to a typical application of approximately 120 tons of compost per hectare of agricultural land, assuming 20 cm of soil depth and a bulk density of 1.5 Mg m^{-3}.

9.2 The soil is sieved to less than 2-mm particle size, and obvious plant material, stones, or other inert materials should be removed. The soil is then stored in a sealed container at 4 ± 1°C for a maximum of one month.

9.3 The soil is analyzed for MHC by Test Method D 425, Test Method D 2980, or another analogous test method for MHC or field capacity.

9.4 The pH of the soil is determined on a 5:1 (distilled water:soil) slurry using a glass combination electrode calibrated with standard buffers, following the guidelines given in Test Methods D 1293. The pH must fall between 6.0 and 8.0. (Soil with a pH above 8.0 may retain more of the CO_2 evolved by the microorganisms than a neutral soil, and soil with a pH below 6.0 may have an atypical microbial population.) Alternatively, the soil pH may be determined by Test Method D 4972.

9.5 The moisture (total solids—dry solids) and ash (total solids—volatile solids) contents of the soil are determined in accordance with APHA-AWWA-WPCF 2540 D and G, respectively.

10. Test Specimen

10.1 Test specimens should be of known weight and have sufficient carbon content to yield carbon dioxide that can be measured accurately by the trapping procedure described in this test method (see 11.1 and 11.4). The carbon content of the test material may be determined by calculation or elemental analysis, according to Test Method D 4129.

10.2 Test specimens may be in the form of films, pieces, fragments, powders, or formed articles, or in aqueous solution, and they should be in accordance with Practice D 618. Any test specimens in the form of powders should be characterized as to particle size distribution by sieve analysis.

10.3 Test specimens can be added directly to the soil matrix or, alternatively, after being submitted to a composting test (Test Method D 5338). In the latter case, a homogenous and representative sample of the compost containing the residual plastics is used.

11. Procedure

11.1 Place between 100 and 500 g of soil in the bottom of the vessel.

11.2 Amend the soil with nitrogen to give a C:N of between 10:1 and 20:1 (by weight) to the added carbon in the test specimen by adding the appropriate volume of ammonium phosphate solution. Add the same amount of nitrogen to the soil blanks as to those that will receive a test material or positive control material.

11.3 Add distilled water, prepared in accordance with Specification D 1193, to bring the moisture content to 80 to 100 % of the MHC of the soil (if the MHC is determined in accordance with Test Method D 425; if in accordance with Test Method D 2989, then 50 to 70 % MHC is appropriate).

11.4 Add the test specimen or positive control to the soil (approximately 200 mg to 1000 mg carbon for 500 g soil), and mix the soil thoroughly. In the case of compost with plastic materials coming from Test Method D 5338 or Test Method D 5511, the amount of compost should be 20 to 50 g added to 500 g of soil.

11.5 Record the weight of the vessel and lid (with the necessary amount of stopcock grease to seal air-tight) with amended soil.

11.6 Place 100 mL of 0.025 N barium hydroxide solution in a 150-mL beaker (or 20 mL of 0.5 N KOH in a 100-mL beaker) and 50 mL of distilled water in a 100-mL beaker on the perforated plate inside the vessel; seal the vessel and place it in the dark chamber or cabinet at 21 ± 2°C.

11.7 *Carbon Dioxide Analysis*:

11.7.1 The carbon dioxide produced in each vessel reacts with $Ba(OH)_2$ and is precipitated as barium carbonate ($BaCO_3$). The amount of carbon dioxide produced is determined by titrating the remaining barium hydroxide with 0.05 N hydrochloric acid to a phenolphthalein end-point or by automatic titrator. Because of the static incubation, the barium carbonate builds up on the surface of the liquid and must be broken up periodically by shaking the vessel gently to ensure continued absorption of the evolved carbon dioxide. (This problem can be avoided by using KOH instead of $Ba(OH)_2$, which does not form a precipitate.)

11.7.2 The barium hydroxide traps must be removed and titrated before their capacity is exceeded. Considering that a 150-mm desiccator vessel provides approximately 2000 cm^3 headspace, which under standard conditions contains approximately 18.7 mmol O_2, then 100 mL $Ba(OH)_2$ has the capacity to trap approximately 2.5 mmol CO_2. Therefore, assuming a respiratory quotient of 1.0, the O_2 content of the vessel headspace will never fall below approximately 18 % if the trap is changed before saturation is reached. The period of time will vary with soils and test materials and increases slowly as the carbon content of the soil is reduced (a recommended frequency of every 3 to 4 days for the first 2 to 3 weeks and every 1 to 3 weeks thereafter). At the time of removal of the traps, the vessel should be weighed to monitor moisture loss from the soil and allowed to sit open so that the air is refreshed before

replacing 100 mL of fresh barium hydroxide and resealing the vessel. The vessels should remain open a minimum of 15 min and a maximum of 1 h. Distilled or deionized water should be added back periodically to the soil to maintain the initial weight of the vessel.

11.7.3 The carbon dioxide evolution rate may reach a plateau when all of the accessible carbon has been oxidized. The test may be terminated at this point or earlier, at the discretion of the user. At the conclusion of the test, the pH and moisture and ash content of the soil should be measured and recorded. If possible, the residual test material may be extracted from the soil with an appropriate solvent and quantified (optional).

11.7.4 In the steps described in 11.7.1-11.7.3, the carbon dioxide produced could also be trapped by KOH and determined by titration.

11.8 Alternatively, for measuring the oxygen consumption, take the necessary readings on the manometers (for a manual system) or verify that the recorder of oxygen consumption functions properly (for an automatic respirometer).

12. Calculation

12.1 Determine, by calculation (if the chemical composition is well established) or by elemental analysis, the total organic carbon content of the test material. This allows the theoretical quantity of carbon dioxide evolution to be calculated as illustrated by the following:

$$\text{material} = w\ \%\ \text{carbon};\ w/100 \times \text{mg of material charged} = Y\ \text{mg carbon charged to vessel:} \quad (1)$$

$$C + O_2 \rightarrow CO_2$$

$$12\ \text{g C yields } 44\ \text{g } CO_2$$

$$Y\ \text{mg C yields } \frac{44 \times Y}{12}\ \text{mg } CO_2$$

12.2 *Amount of Net Carbon Dioxide Produced*:

12.2.1 Correct for the carbon dioxide produced in the blank test by subtracting titration from the test material titration.

$$Z_n = Z_b - Z_t \quad (2)$$

where:

Z_n = calculated mL of HCl needed to titrate the CO_2 generated solely from the test substance,
Z_b = mL HCl used to titrate the blank, and
Z_t = mL HCl used to titrate the test substance.

Then:

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 + H_2O \quad (3)$$

$$Ba(OH)_2 + 2\ HCl \rightarrow BaCl_2 + 2\ H_2O$$

$$\text{m moles of } CO_2 = \frac{\text{m moles HCl}}{2}$$

(The total CO_2 generated can be determined by using the technical control as Z_b and the test substance or blank as Z_t.)

12.2.2 *Correcting for Normality of HCl*:

$$\text{m moles } CO_2 = \frac{(0.05\ \text{N} \times \text{mL HCl})}{2} \quad (4)$$

$$\text{mg of } CO_2 = \frac{(0.05\ \text{N}) \times \text{mL HCl} \times 44}{2}$$

Hence, carbon dioxide evolved in mg is obtained by multiplying the HCl titration by 1.1.

12.3 The percentage of carbon dioxide evolved is calculated as shown below:

$$= \frac{\text{mg } CO_2 \text{ produced}}{\text{mg } CO_2 \text{ theoretical}} \times 100 \quad (5)$$

$$= \frac{1.1\ w\ \text{mL HCl} \times 12}{44 \times Y} \times 100$$

12.4 Calculate the standard error, s_e , of the percentage of biodegradation as follows:

$$s_e = \text{SQRT}((s^2_{\text{test}}/n1) + (s^2_{\text{blank}}/n2)) \times 100/C_i \quad (6)$$

where:

$n1$ *and* $n2$ = number of replicate test and control vessels, respectively,
s = standard deviation of the total gaseous carbon produced (mg), and
C_i = amount of carbon (mg) initially added to the vessel.

12.5 Calculate the 95 % confidence limits (CL) as follows:

$$95\ \%\ \text{CL} = \%\ \text{biodegradation} \pm (t \times s_e) \quad (7)$$

where:

t = t-distribution value for 95 % probability with ($n1 + n2 - 2$) degrees of freedom, thus $t = 3 + 3 - 2 = 4$.

12.6 *Percentage of biodegradation from oxygen consumption values* Read the oxygen consumption value for each flask, using the method provided by the manufacturer for the appropriate type of respirometer. Calculate the specific biochemical oxygen demand (BODs) of the test compound as shown below:

$$= \frac{B_t - B_{bt}}{C_T} \quad (8)$$

where:

B_t = the BOD of the flasks containing test material at time t, in milligrams per kg (mg/kg) of the test soil,
B_{bt} = the BOD of the blank control at time t, in milligrams per kg (mg/kg) of the test soil, and
C_T = the concentration of the test material in the test flasks, in milligrams per kg (mg/kg) of the test soil.

Calculate the percentage of biodegradation as ratio of the specific biochemical oxygen demand to the theoretical oxygen demand (ThOD), in mg/g of test material, as shown below:

$$= \frac{BODs}{ThOD} \times 100 \quad (9)$$

13. Interpretation of Results

13.1 Information on toxicity of the plastic material may be useful in the interpretation of low results.

13.2 A reference or control substance known to biodegrade (for example, starch and cellulose) is necessary in order to check the activity of the soil. If, after six months, limited

biodegradation (<70 % theoretical CO_2 evolution) is observed for the reference, the test must be regarded as invalid and should be repeated using fresh soil.

13.3 The plateau level of carbon dioxide evolution in this test method and, optionally, the quantified residual test material will suggest the degree of biodegradability of the plastic material.

14. Report

14.1 Report the following data and information:

14.1.1 Information on the soil, including source, pH, percent moisture (and method used), MHC (and method used), ash content, C:N ratio, date of collection, storage conditions, handling, and potential acclimation to test material.

14.1.2 Information on the mature compost, including pH, percent moisture, ash content, and source compost process, as well as percent of biodegradation on the basis of C-evolution in the aerobic-controlled composting test (Test Method D 5338) or high-solids anaerobic-digestion test (Test Method D 5511).

14.1.3 Carbon content or theoretical oxygen demand of the plastic material.

14.1.4 Form or particle size distribution (if powder) of the plastic materials.

14.1.5 Cumulative average carbon dioxide evolution over time to plateau (or termination), reported and displayed graphically since lag-phase and slope (rate) are important.

14.1.6 Residual weight of the test material, if determined.

14.1.7 Percent of theoretical aerobic biodegradation for each plastic material tested and the positive control polymer.

14.1.8 Temperature range of the test.

14.1.9 pH of the soil, initially and finally.

14.1.10 Microorganisms per gram in the original soil (optional).

14.1.11 Molecular weight of the plastic material, if measured.

14.1.12 Molecular weight of the residual polymeric material after the test (optional).

15. Precision and Bias

15.1 Precision and bias statements for this test method cannot be made at this time. They will be developed during future round-robin testing.

16. Keywords

16.1 aerobic; biodegradation; degree (of biodegradation); mineralization; plastics; soil

SUMMARY OF CHANGES

This section identifies the location of selected changes to this test method. For the convenience of the user, Committee D20 has highlighted those changes that may impact the use of this test method. This section may also include descriptions of the changes or reasons for the changes, or both.

D 5988 - 03:

(*1*) Added paragraph 1.6.
(*2*) Added subsection 2.3 for ISO Standard reference.
(*3*) Added paragraph 4.3.
(*4*) Added paragraph 6.3.
(*5*) Added paragraph 11.8.
(*6*) Added paragraph 12.5.
(*7*) Revised paragraph 14.1.3.