Designation: D5988 – 12

Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil[1]

This standard is issued under the fixed designation D5988; 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 under laboratory conditions of the degree and rate of aerobic biodegradation of plastic materials, including formulation additives, in contact with soil.

1.2 This test method is designed to measure the biodegradability of plastic materials relative to a reference material 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.

1.4 Claims of performance shall be limited to the numerical result obtained in the test and not be used for unqualified "biodegradable" claims. Reports shall clearly state the percentage of net gaseous carbon generation for both the test and reference samples at the completion of the test. Results shall not be extrapolated beyond the actual duration of the test.

1.5 The values stated in SI units are to be regarded as the standard.

1.6 *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.

1.7 This ASTM test method is equivalent to ISO 17556.

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D425 Test Method for Centrifuge Moisture Equivalent of Soils

D618 Practice for Conditioning Plastics for Testing

D883 Terminology Relating to Plastics

D1193 Specification for Reagent Water

D1293 Test Methods for pH of Water

D2980 Test Method for Volume Mass, Moisture-Holding Capacity, and Porocity of Saturated Peat Materials

D2989 Test Method for Acidity-Alkalinity of Halogenated Organic Solvents and Their Admixtures

D4129 Test Method for Total and Organic Carbon in Water by High Temperature Oxidation and by Coulometric Detection

D4972 Test Method for pH of Soils

D5338 Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions, Incorporating Thermophilic Temperatures

D5511 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 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 D883.

4. Summary of Test Method

4.1 The test method described consists of the selection of plastic material for the determination of aerobic biodegradability, obtaining soil as a matrix and source of inoculum, exposing the plastic material to the soil, measuring

[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 and Biobased Products.

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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**

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

4.3 Alternatively, it is possible to determine the consumption of oxygen, or biochemical oxygen demand (BOD), 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). In using this alternative approach, however, the influence of possible nitrification processes on the BOD must be considered.

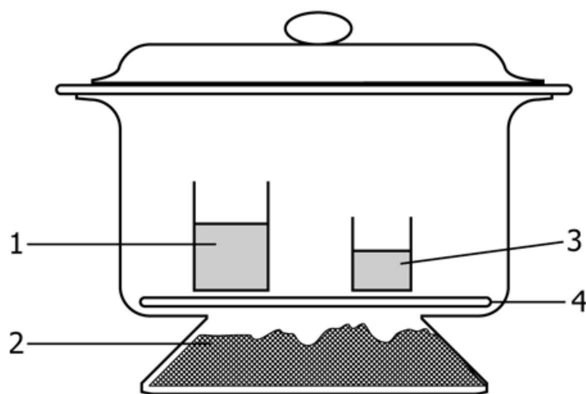
5. Significance and Use

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 materials are mineralized by soil microorganisms. Disposal is becoming a major issue with the increasing use of plastics, and the results of this test method 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.

6. Apparatus

6.1 *Soil-Contact Incubation Apparatus* (see Fig. 1; biometer flasks are also appropriate). Ensure that all glassware is thoroughly cleaned and, in particular, free from organic or toxic matter.



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 vessels with approximately 2 to 4-L of internal volume with air-tight seal, such as 150-mm desiccators. Provide three vessels for soil only (known as "blanks" or "controls," these vessels show the background activity of the soil), three vessels for a positive reference material (these vessels show the viability of the soil microbial community), three vessels per test material, and three vessels as technical controls. The technical controls contain only the absorbing solution and no soil. The ambient air which fills the headspace of all the vessels introduces carbon dioxide into the system. The technical controls allow accounting for and subtracting this introduced carbon dioxide. Additionally, the technical controls indicate the air-tightness of the vessel system by showing possible infiltration of carbon dioxide into the sealed vessel.

6.1.2 *Beakers,* sets of 150-mL 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. The support must be made from a material that will not absorb carbon dioxide.

6.1.4 *Darkened Chamber or Cabinet,* which allows selection of a temperature between 20°C to 28°C, and allows maintaining the selected temperature at ±2°C.

6.2 *Analytical Equipment:*

6.2.1 *Analytical Instrument,* 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, it is acceptable to use a flow-through apparatus or manometric apparatus as described in ISO 17566.

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] It is acceptable to use other grades, 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

[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.

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, it is acceptable to use potassium hydroxide solution (KOH, 0.5 N), prepared by dissolving 28 g of anhydrous KOH/L of distilled water and proceeding in the same way as for the $Ba(OH)_2$.

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 Use natural, fertile soil collected from the surface layers of fields and forests. Make a laboratory mixture of equal parts (by weight) of soil samples obtained from at least three diverse locations (for example, an agricultural field, a forest, and a pasture or meadow). Taking soil from multiple and diverse locations will maximize biodiversity. It is advisable to avoid soils that have been exposed to pollutants that cause significant perturbations of the microbial population. The soils are preferably used fresh from the field to assure active microbiota. Air-dried or frozen soils must be reactivated before use in this test. It is preferable to use fertile soil classified as "sandy loam" in accordance with USDA classification, or "silty sand" in accordance with the German DIN classification.

9.2 The sources of the soils must be reported (see 14.1.1). Record the sampling site, its location, the presence of plants or crops, the sampling date, the sampling depth, and, if possible, the history such as details of fertilizer and pesticide application.

9.3 Sieve the soil to less than 2-mm particle size, and remove obvious plant material, stones, or other inert materials. Store the soil in a sealed container at 4 ± 1°C for a maximum of one month.

9.4 Analyze the soil for MHC by Test Method D425, Test Method D2980, or another analogous test method for MHC or field capacity.

9.5 Determine the pH of the soil on a 5:1 (distilled water:soil) slurry using a glass combination electrode calibrated with standard buffers, following the guidelines given in Test Method D1293. Alternatively, it is acceptable to determine the soil pH by Test Method D4972. The pH must fall between 6.0 and 8.0. (Soil with a pH above 8.0 retains more of the CO_2 evolved by the microorganisms than a neutral soil, while a soil with a pH below 6.0 has the potential to contain an atypical microbial population.)

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

9.7 It is acceptable for the test matrix to be a mixture of natural soil, as described in 9.1, and mature compost, such as obtained at the end of Test Method D5338, at a ratio of 1 g compost to 25 g soil, which corresponds to a typical application in agriculture 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}).

10. Test Specimen

10.1 Test specimens shall be of known weight and have sufficient carbon content to yield enough carbon dioxide that can be measured accurately by the trapping procedure described in this test method (see 11.7 and 11.9). Determine the carbon content of the test material by calculation or elemental analysis, in accordance with Test Method D4129.

10.2 It is acceptable for test specimens to be in the form of films, pieces, fragments, powders, or formed articles, or in aqueous solution, in accordance with Practice D618. It is recommended, but not required, to characterize any test specimens in the form of powders as to particle size distribution by sieve analysis.

11. Procedure

11.1 The test shall be performed in triplicate for each of the technical control, soil blank, positive reference material, and test materials (triplicate for each test material).

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

11.3 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 reference material.

11.4 Add distilled water, prepared in accordance with Specification D1193, 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 D425; if in accordance with Test Method D2989, then 50 to 70 % of MHC is appropriate).

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

11.6 Add the test material or positive reference material to the soil (approximately 200 mg to 1000 mg carbon for 500 g soil), and mix thoroughly into the soil.

11.7 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.

11.8 Select a temperature between 20 to 28°C, and maintain that temperature at ±2°C.

11.9 *Carbon Dioxide Analysis:*

11.9.1 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 is avoided by using KOH instead of $Ba(OH)_2$, which does not form a precipitate.)

11.9.2 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. The recommended frequency is 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, weigh the vessel to monitor moisture loss from the soil and then allow it to sit open so that the air in the vessel is refreshed, before replacing 100 mL of fresh barium hydroxide and resealing the vessel. Allow the vessels to remain open a minimum of 15 min and a maximum of 1 hour. Add distilled or deionized water back periodically to the soil to maintain the initial weight of the vessel.

11.10 The cumulative carbon dioxide evolution reaches a plateau when all of the accessible carbon has been oxidized. Continue the incubation until no net carbon dioxide production is noted between consecutive measurements taken four weeks apart, from both the positive reference material and test material vessels. At the conclusion of the test, measure and record the pH and the moisture and ash contents of the soil. Extract any residual test material from the soil with an appropriate solvent and quantify (optional).

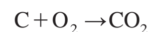
11.11 In the steps described in 11.7 and 11.9, it is acceptable to trap carbon dioxide by KOH and determine by titration the carbon dioxide produced.

11.12 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:*

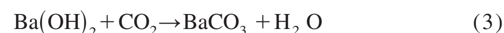
12.2.1 Correct for the carbon dioxide produced in the blank by subtracting titration from the test material titration.

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

where:

Z_n = calculated mL of HCl needed to titrate the CO_2 generated solely from the test material,
Z_b = mL HCl used to titrate the blank vessels with soil only, and
Z_t = mL HCl used to titrate the vessels containing the soil plus test material.

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 is determined by using the technical control as Z_b and the test material 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:

n 1 *and n*2 = number of replicate test material and blank 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:

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

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:

$$= \frac{B_t - B_{bt}}{C_T} \tag{8}$$

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 flasks containing soil only 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 the ratio of the specific biochemical oxygen demand to the theoretical oxygen demand (ThOD), in mg/g of test material, as shown below:

$$= \frac{BODs}{ThOD} \times 100 \tag{9}$$

13. Interpretation of Results and Validity Criteria

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

13.2 This test method includes the use of a reference material known to biodegrade (for example, starch or cellulose) in order to check the activity of the soil. If, after six months, limited biodegradation (<70 % theoretical CO_2 evolution) is observed for this reference material, the test must be regarded as invalid and must be repeated using fresh soil.

13.3 The amounts of carbon dioxide evolved from the blanks (or the BOD values for the alternative measurement of oxygen consumption) shall be within 20 % of the mean at the plateau phase or at the end of the test. If not, the test must be regarded as invalid and must be repeated using fresh soil.

13.4 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 determination method used), MHC (and determination method used), ash content, C:N ratio, date of collection, storage conditions, handling, and potential acclimation to test material.

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

14.1.3 Form or particle size distribution (if determined for powder) of the plastic materials.

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

14.1.5 Percentages of theoretical aerobic biodegradation for each plastic material tested and the positive reference material.

14.1.6 Temperature range of the test.

14.1.7 pH of the soil, initially and finally.

14.2 Report the following optional data and information, if determined:

14.2.1 Residual weight of the test material.

14.2.2 Microorganisms per gram in the original soil.

14.2.3 Molecular weight of the plastic material.

14.2.4 Molecular weight of the residual polymeric material after the test.

15. Precision and Bias

15.1 Results for within-laboratory repeatability testing are presented in Table 1. These data represent three different determinations of the biodegradation of cellulose as a positive reference. The average biodegradation of cellulose after four months of soil contact in the dark at a constant temperature of 21°C (±2°C) was 89.4 %, with an average standard deviation of 2.4 %. Figure 2 represents a graphical view of the first run, in which a biodegradability of 87.2 % was obtained as the mean for the three replicates, with a standard deviation of 2.2 %.

16. Keywords

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

TABLE 1 Results from Within-Laboratory Testing for the Aerobic Biodegradability of Cellulose Positive Reference under Soil Contact Conditions

Run	Net CO_2 production (mg/g test item)	Biodegradation (%)	
		Average	Standard Deviation
Run 1	1359	87.2	2.2
Run 2	1469	94.3	2.4
Run 3	1322	86.6	2.5
Mean of three runs	1383	89.4	2.4

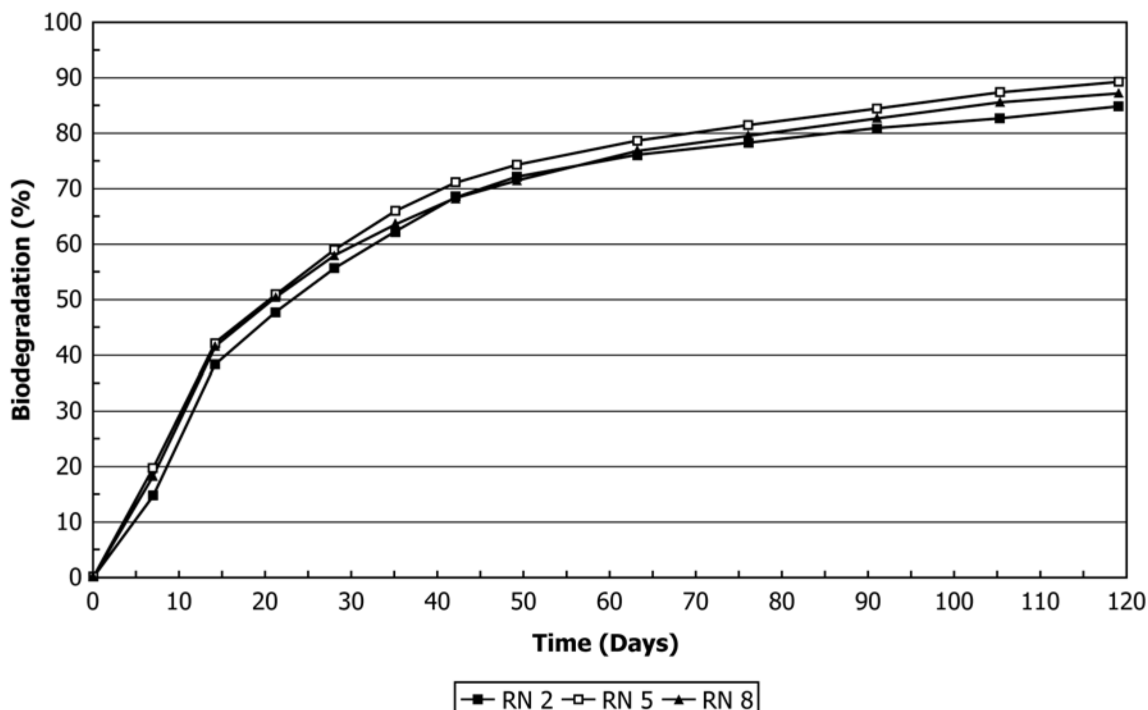


FIG. 2 Soil-Contact Incubation Apparatus

SUMMARY OF CHANGES

Committee D20 has identified the location of selected changes to this standard since the last issue (D5988 - 03) that may impact the use of this standard. (May 1, 2012)

(1) Added subsections 1.4, 9.7, 11.1, 11.8, 13.3, and 14.2.
(2) Corrected Section 2, Referenced Documents.
(3) Added to subsection 6.1: clarification of blank, reference material, test material, and technical control vessels.
(4) Changed and clarified temperature regime in subsection 6.1.4.
(5) Added to subsection 9.1: requirement for using soil from at least three diverse locations as inoculum.
(6) Switched order of subsections 11.5 and 11.6.
(7) Revised subsections 9.2, 11.10, 12.6, 13.2, 14.1.7, and 15.1.
(8) Deleted subsection old subsection 14.1.2.

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