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Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions[1]

This standard is issued under the fixed designation D 5526; 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 anaerobic biodegradation of plastic materials in an accelerated-landfill test environment. This test method is also designed to produce mixtures of household waste and plastic materials after different degrees of decomposition under conditions that resemble landfill conditions. The test materials are mixed with pretreated household waste and exposed to a methanogenic inoculum derived from anaerobic digesters operating only on pretreated household waste. The anaerobic decomposition occurs under dry (more than 30 % total solids) and static nonmixed conditions. The mixtures obtained after this test method can be used to assess the environmental and health risks of plastic materials that are degraded in a landfill.

1.2 This test method is designed to yield a percentage of conversion of carbon in the sample to carbon in the gaseous form under conditions that resemble landfill conditions. This test method may not simulate all conditions found in landfills, especially biologically inactive landfills. This test method more closely resembles those types of landfills in which the gas generated is recovered or even actively promoted, or both, for example, by inoculation (codeposition of anaerobic sewage sludge and anaerobic leachate recirculation), moisture control in the landfill (leachate recirculation), and temperature control (short-term injection of oxygen and heating of recirculated leachate) **(1-7)**.[2]

1.3 This test method is designed to produce partially degraded mixtures of municipal solid waste and plastics that can be used to assess the ecotoxicological risks associated with the anaerobic degradation of plastics after various stages of anaerobic biodegradation in a landfill.

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.* Specific hazards statements are given in Section 8.

NOTE 1—There is no similar or equivalent ISO standard.

2. Referenced Documents

2.1 *ASTM Standards:*

D 618 Practice for Conditioning Plastics and Electrical Insulating Materials for Testing[3]
D 883 Terminology Relating to Plastics[3]
D 1293 Test Methods for pH of Water[4]
D 1888 Test Methods for Particulate and Dissolved Matter, Solids, or Residue in Water[5]
D 2908 Practice for Measuring Volatile Organic Matter in Water by Aqueous-Injection Gas Chromatography[6]
D 3590 Test Method for Total Kjeldahl Nitrogen in Water[4]
D 4129 Test Method for Total and Organic Carbon in Water by High-Temperature Oxidation and Coulometric Detection[6]
E 260 Practice for Packed Column Gas Chromatography[7]
E 355 Practice for Gas Chromatography Terms and Relationships[7]

2.2 *APHA-AWWA-WPCF Standards:*[8]

2540D Total Suspended Solids Dried at 103°–105°C
2540E Fixed and Volatile Solids Ignited at 550°C
212 Nitrogen Ammonia

3. Terminology

3.1 *Definitions*—For definitions of terms used in this test method see Terminology D 883.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *methanogenic inoculum*—anaerobically digested organic waste containing a high concentration of anaerobic methane-producing microorganisms.

4. Summary of Test Method

4.1 This test method described consists of the following: (*1*) selecting and analyzing material for testing; (*2*) obtaining a

[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] The boldface numbers in parentheses refer to the list of references at the end of this standard.

[3] *Annual Book of ASTM Standards*, Vol 08.01.

[4] *Annual Book of ASTM Standards*, Vol 11.01.

[5] *Discontinued*—See *1991 Annual Book of ASTM Standards*, Vol 11.01.

[6] *Annual Book of ASTM Standards*, Vol 11.02.

[7] *Annual Book of ASTM Standards*, Vol 14.01.

[8] *Standard Methods for the Examination of Water and Wastewater*, 17th ed., 1989, available from American Public Health Association, 1740 Broadway, New York, NY 10018.

pretreated municipal-solid-waste fraction and a concentrated anaerobic inoculum from an anaerobic digester; (*3*) exposing the material to an anaerobic static batch fermentation at more than 30 % solids; (*4*) measuring total carbon in the gas (CO_2 and CH_4) evolved as a function of time; (*5*) removing the specimens for cleaning (optional), conditioning, testing, and reporting; (*6*) assessing the degree of biodegradability; and (*7*) assessing the degree of biodegradability under less than optimum conditions.

4.2 The percentage of biodegradability is obtained by determining the percent of conversion of carbon from the test material to carbon in the gaseous phase (CH_4 and CO_2). This percentage of biodegradability will not include the amount of carbon from the test substance that is converted to cell biomass and that is not, in turn, metabolized to CO_2 and CH_4.

5. Significance and Use

5.1 Decomposition of a plastic within a landfill involves biological processes that will affect the decomposition of other materials enclosed by, or in close proximity to, the plastic. Rapid degradation of the plastic may increase the economic feasibility of landfill-gas recovery, minimize the duration of after-care of the landfill, and make possible the recovery of the volume reduction of the waste due to biodegradation during the active life of the landfill. This procedure has been developed to permit determination of the anaerobic biodegradability of plastic products when placed in biologically active environments simulating landfill conditions.

5.2 The decomposition of plastic materials in a landfill is of limited importance, as few landfills are operated so as to be biologically active. However, as degradation occurs inevitably in a landfill, it is of immediate concern that the plastic materials do not produce toxic metabolites or end products under the various conditions that may occur in a landfill. The mixtures remaining after completion of the test method, containing fully or partially degraded plastic materials or extracts, can be submitted subsequently to ecotoxicity testing in order to assess the environmental hazards posed by the breakdown of plastics to varying degrees in landfills. This test method has been designed to assess biodegradation under optimum and less-than-optimum conditions.

5.3 *Limitations*—Because a wide variation exists in the construction and operation of landfills, and because regulatory requirements for landfills vary greatly, this procedure is not intended to simulate the environment of all landfills. However, it is expected to closely resemble the environment of a biologically active landfill. More specifically, the procedure is intended to create a standard laboratory environment that permits rapid and reproducible determination of the anaerobic biodegradability under accelerated landfill conditions, while at the same time producing reproducible mixtures of fully and partially decomposed household waste with plastic materials for ecotoxicological assessment.

6. Apparatus

6.1 *Pressure-Resistant Glass Vessels*—Twenty-seven, each with a volume of 4 to 6 L, which can be closed airtight and capable of withstanding an overpressure of two atmospheres. The lids of the reactors are equipped with an overpressure valve (to prevent the overpressure from becoming higher than 2 bars), a manometer that provides a rough indication of the overpressure, a septum that allows one to take gas samples and measure the exact overpressure, and, finally, a valve to release the overpressure (Fig. 1).

6.2 *Incubators*, sufficient to store the vessels in the dark at 35 ± 2°C for the duration of the test.

6.3 *Pressure Transducer*, connected to a syringe needle to measure the headspace pressure in the test vessel.

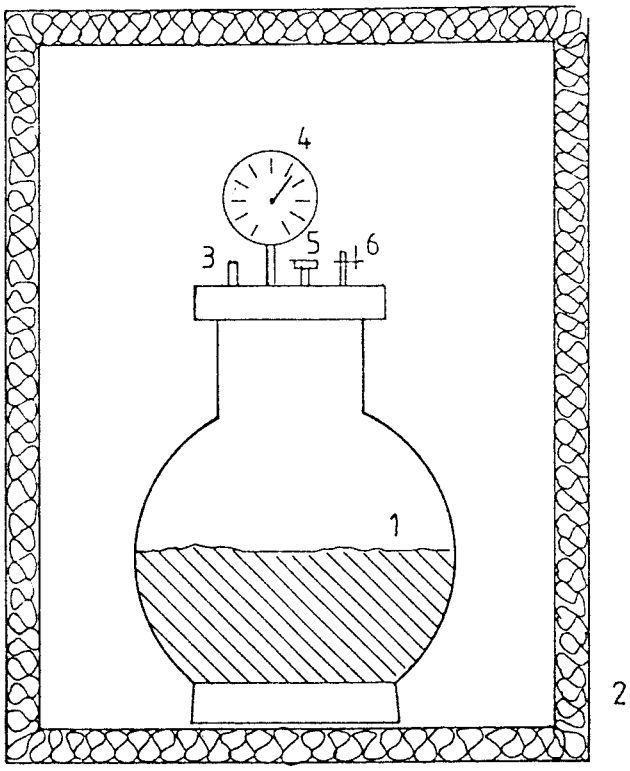
6.4 *Gas Chromatograph*, or other apparatus, equipped with a suitable detector and column(s) for measuring methane and carbon dioxide concentrations in the evolved gases.

6.5 *pH Meter*, precision balance (±0.1 g), analytical balance (±0.1 mg), thermometer, and barometer.

6.6 *Suitable Devices*, for determining volatile fatty acids by aqueous-injection chromatography, total Kjeldahl nitrogen, ammonia nitrogen, dry solids (105°C), and volatile solids (550°C) concentrations.

7. Reagents and Materials

7.1 *Pretreated-Household Waste*, derived from mixed municipal solid waste or the organic fraction thereof, after homogenizing, screening over a screen with holes of a diameter of 40 to 80 mm, and aerobically stabilized over a period of 2 to 4 weeks by blowing air into the material and maintaining a dry-matter content of 50 ± 5 % and a temperature of 55 ± 10°C. (Optional: the pretreated household waste can be replaced by a similarly pretreated simulated solid waste.)



1 = Digester.
2 = Incubation chamber.
3 = Overpressure valve.
4 = Manometer.
5 = Septum.
6 = Valve.

FIG. 1 Setup of Accelerated Landfill

7.2 *Anaerobic Inoculum*, derived from a properly operating anaerobic digester with pretreated household waste as a sole substrate or a digester that treats predominantly household waste.

7.3 *Cellulose, Analytical-Grade*, for thin-layer chromatography as a positive control.[9]

7.4 *Polyethylene* (optional), as a negative control. It should be in the same form as that in which the sample is tested: film polyethylene for film samples, pellets of polyethylene in case the sample is in the form of pellets, etc.

8. Hazards

8.1 This procedure involves the use of inoculum and municipal solid waste containing biologically and possibly chemically active materials known to produce a variety of diseases. Avoid contact with these materials by wearing gloves and other appropriate protective equipment. Use good personal hygiene to minimize exposure.

8.2 The solid-waste mixture may contain sharp objects. Take extreme care when handling this mixture to avoid injury.

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

8.4 The methane produced during the procedure is explosive and flammable. Upon release of the biogas from the gas-collection system, take care in venting the biogas to the outside or to a hood.

9. Inoculum

9.1 The inoculum can be derived either from a laboratory-scale or full-scale continuous digester or batch digester, operating at 35°C and functioning with an organic fraction of household waste as the predominant substrate. In case the inoculum is derived from a continuous laboratory-scale or full-scale digester, the digester must be operating for a period of at least one month on the organic fraction of household waste, with a maximum retention time of 30 days under mesophilic conditions (35 ± 2°C). Gas production yields must be at least 15 mL at standard temperature and pressure of biogas/gram of dry solids in the digester and per day for at least 7 days. In case the inoculum is derived from a batch digester, the gas production rate must have exceeded 1 L/kg waste/day, and the methane concentration of the biogas being produced must be above 60 %.

9.2 The prepared inoculum should undergo a short mesophilic post-fermentation of approximately 7 days at the same dry-matter content as the digester from which it was derived. This means that the inoculum is not fed but is allowed to post-ferment anaerobically by itself. This is to ensure that large, easily biodegradable particles are degraded during this period and also to reduce the background level of degradation of the inoculum itself.

9.3 The biochemical characteristics of the inoculum shall be as follows:

9.3.1 *pH*—Between 7.5 and 8.5 (in accordance with Test Methods D 1293);

9.3.2 *Volatile Fatty Acids (VFA)*—Below 1 g/kg wet weight (in accordance with Practice D 2908); and

9.3.3 NH^{+}_{4}-*N*—Between 0.5 and 2 g/kg (in accordance with APHA Test 212 and Test Method D 3590).

9.4 Analyses are performed after dilution of the inoculum with distilled water on a ratio of distilled water to inoculum of 5 to 1 on a weight-over-weight basis.

10. Test Specimen

10.1 The test specimen should be of sufficient carbon content, analyzed in accordance with Test Method D 4129, to yield carbon dioxide and methane volumes that can be measured accurately by the trapping devices described. Add more test specimen when low biodegradability is expected, up to 100 g of dry matter of the test specimen.

10.2 The test specimen may be in the form of films, powder, pellets, or formed articles, or in the form of a dog bone and in accordance with Practice D 618. The test setup should be capable of handling articles that are 100 by 50 by 4 mm thick.

11. Procedure

11.1 *Preparation of the Mixtures*:

11.1.1 Determine the volatile solids, dry solids, and nitrogen content of the pretreated household waste and the inoculum in accordance with Test Methods D 1888, D 3590, and APHA 2540D and 2540E.

11.1.2 Determine the volatile solids, dry solids, and carbon content of all test substances in accordance with APHA 2540D and 2540E and Test Method D 4129.

11.1.3 Weigh and combine the components and adjust the dry matter content of the final mixtures with water to reach the desired dry-matter content for each vessel. Roughly weigh out 600 g on a dry-weight basis of pretreated household waste, and mix it with 100 g on a dry-weight basis of mesophilic anaerobic inoculum from a continuously operating digester or 150 g on a dry-weight basis of anaerobic inoculum from a batch digester. Add 60 to 100 g of dry matter of the test substance. Add water until the appropriate final dry matter content is reached. (In order to reach 60 % dry matter content in the mixture, water may have to be removed prior to combining the different components of the mixture. This can be accomplished by drying the pretreated household waste or centrifuging the anaerobic inoculum.) Mix the required amounts of pretreated household waste, inoculum, and test substance in a small container for 2 to 3 min. Introduce the mixture in the vessel, weigh the vessel with all of the contents, and close it airtight. Prepare the pressure vessels in the triplicate at each of the following dry matter contents: 35, 45, and 60 %, so nine vessels are necessary for each sample.

11.1.4 The blanks consist of 600 g dry matter of pretreated household waste and anaerobic inoculum at the respective total dry-matter contents. As references, thin-layer chromatography cellulose must be used as a positive control. The blank and reference are performed in triplicate at the three different dry-matter contents.

11.2 *Start-Up Procedure*—After all reactors are filled and closed, place them in incubators at 35 ± 2°C. Acclimate the reactors for approximately 1 h and release the pressure, which originates from the temperature increase, to the atmosphere.

[9] Avicel®, available from EM Chemicals, Inc., Hawthorne, NY, was used for development of this test method.

Incubate the reactors in the dark for a period of four months.

11.3 *Operating Procedure*:

11.3.1 Check the gas production (measured as a pressure increase) at least weekly. When the overpressure reaches more than 700 mbar, measure the pressure exactly with the pressure transducer and release to atmospheric pressure. Take care that the temperature decrease, due to the opening of the incubator or incubation room, is not more than 1°C during measurement of the overpressure.

11.3.2 Analyze the gas composition biweekly. Determine the methane and carbon dioxide concentration by using analytical devices suitable for the detection and quantification of these gases, such as a gas chromatograph with an appropriate detector, conforming to Practices E 260 and E 355. Pay special attention to the occurrence of leaks through the septum.

11.4 *End of the Test*:

11.4.1 The incubation time may be extended, depending on the activity of the inoculum, until no significant gas production in excess of the blank has been recorded during one week or until the positive reference has degraded for more than 70 %.

11.4.2 At the end of the test, analyze the dry matter, volatile fatty acids, and pH for each of the reactor mixtures, in accordance with APHA 2540E, Practice D 2908, and Test Methods D 1293.

11.4.3 Remove sufficient residual material from the vessel and submit to ecotoxicity testing, in accordance with appropriate standard test methods and practices (optional).

12. Calculation

12.1 By using the total carbon in the test specimen, calculate the maximum theoretical gas production (carbon dioxide plus methane) originating from the anaerobic biodegradation of the test specimen, based on the following biochemical transformations:

$$C + 2H_2 \rightarrow CH_4 \qquad (1)$$
$$C + O_2 \rightarrow CO_2$$

Each millimole (12 mg) of organic carbon from the test sample can be converted into 1 mmole of gaseous CH_4 or CO_2, or both. One millimole of gas produced occupies 22.4 mL at standard temperature and pressure (STP).

12.2 *Temperature and Pressure*—Measure the percentages of CH_4 and CO_2, and transform the gas volumes to STP. Also correct for vapor pressure and atmospheric pressure variation during the test. Calculate the amount of gaseous carbon. Determine the mean (of the three replicates) net gaseous carbon production by anaerobic biodegradation of the test substances by subtracting the mean gaseous carbon production of the control (three replicates) containing only the inoculum.

12.3 Calculate the percent of biodegradation for each dry-matter concentration by dividing the average net gaseous-carbon production of the test material by the original average amount of total carbon of the test compound and multiplying by 100.

$$\%\ \text{biodegradation} = \frac{\text{mean } C_g\ (\text{test}) - \text{mean } C_g\ (\text{blank})}{C_i} \times 100 \qquad (2)$$

where:

C_g = amount of gaseous carbon produced, g, and
C_i = amount of carbon in test compound added, g.

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

$$s_e = SQRT\,((s^2_{\text{test}}/n1) + (s^2_{\text{blank}}/n2)) \times 100/C_i \qquad (3)$$

where:

$n1$ and $n2$ = number of replicate test and blank digesters, respectively, and
s = standard deviation of the total gaseous carbon produced.

Calculate the 95 % confidence limits as follows:

$$95\ \%\ CL = \%\ \text{biodegradation} \pm (t \times s_e) \qquad (4)$$

where:

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

13. Interpretation of Results

13.1 Information on the toxicity of the plastic material may be useful for interpreting inhibitory effects on the inoculum.

13.2 This test method includes the use of thin-layer chromatography cellulose as a positive control. If sufficient biodegradation (a minimum of 70 % for cellulose) is not observed within the duration of the test, the test must be regarded as invalid and should be repeated with fresh inoculum.

14. Report

14.1 Report the following data and information:

14.1.1 Information on the inoculum, including source, pH, volatile fatty acids (in milligram per kilogram wet weight), NH_4^+ -N (in gram per kilogram wet weight), percent dry solids, percent volatile solids, date of collection and use, storage time and conditions, handling, and potential acclimation to the test material.

14.1.2 Information on the pretreated household waste used to produce the inoculum and used as a substance. In case a simulated solid waste is used, report the composition of the mix. For both pretreated household waste and simulated solid waste, report the source, pH, type of pretreatment, NH_4^+ -N (in gram per kilogram), percent dry solids, percent volatile solids, date of collection, storage time and conditions, handling, and transportation.

14.1.3 Carbon content of the plastic material and the positive control and maximum theoretical gas production (carbon dioxide and methane) for each.

14.1.4 Record and display graphically the cumulative gas evolution over time.

14.1.5 Analysis of gas as percent methane and percent carbon dioxide for each reading at the end of the test, or each time the gas is released to the atmosphere during the course of the test. Concomitantly, report the barometric pressure and temperature in the incubator and in the gas-collection device.

14.1.6 Record the percent of carbon conversion, along with the form of plastic material, that is, sheet, powder, pellets, etc. Record specific information on the size, shape, volume, and thickness of the plastic materials and control substances tested.

14.1.7 Percent of biodegradation relative to cellulose.

14.1.8 Standard deviation and 95 % confidence interval for the percentage of biodegradation for each triplicate set.

14.1.9 In case biogas production has not reached a plateau for the vessels at 45 and 60 % dry matter, report total biogas production as percentage of total biogas production at 35 % dry matter.

14.1.10 Temperature range of the test.

14.1.11 Wet-weight loss (optional).

15. Precision and Bias

15.1 The precision and bias of the procedure presented in this test method for measuring the anaerobic biodegradation of plastic materials under accelerated landfill conditions is being determined.

15.2 Preliminary results at 40 % dry-matter content and 35°C are presented in Fig. 2. The curves in Fig. 2 represent the biogas production in litres from 1 kg of pretreated household waste plus 10 % mesophilic inoculum without sample (blank) and with 60 g of cellulose (plus positive control).

16. Keywords

16.1 accelerated landfill; anaerobic biodegradation; biodegradation; dry digestion; ecotoxicity; landfill; metabolites; plastics; test method

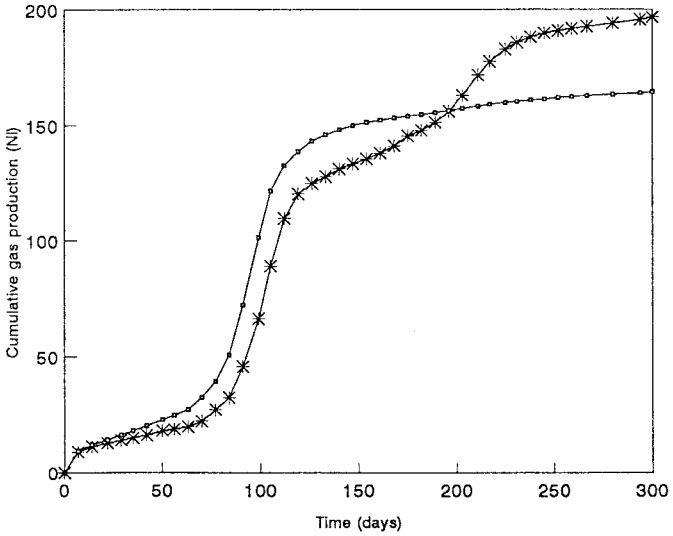


FIG. 2 Cumulative Biogas Production Over a Period of 300 Days for 1 kg of Pretreated Household Waste Plus 100 g of Mesophilic Anaerobic Inoculum Without Sample (Blank) and With 60 g of Cellulose (Plus Positive Control)

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