



# Standard Test Method for Determining the Anaerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge<sup>1</sup>

This standard is issued under the fixed designation D5210; 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 ( $\epsilon$ ) indicates an editorial change since the last revision or reapproval.

## 1. Scope

1.1 This test method determines the degree and rate of anaerobic biodegradation of synthetic plastic materials (including formulation additives) on exposure to anaerobic-digester municipal sewage sludge from a waste-water plant, under laboratory conditions.

1.2 This test method is designed to index plastic materials that are more or less biodegradable relative to a positive standard in an anaerobic environment.

1.3 This test method is applicable to all plastic materials that are not inhibitory to the microorganisms present in anaerobic sewage sludge.

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 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.* Specific hazards are given in Section 8.

## 2. Referenced Documents

2.1 *ASTM Standards:*<sup>2</sup>

[D883 Terminology Relating to Plastics](#)

[D1193 Specification for Reagent Water](#)

[D3593 Test Method for Molecular Weight Averages/ Distribution of Certain Polymers by Liquid Size-Exclusion Chromatography \(Gel Permeation Chromatography GPC\) Using Universal Calibration \(Withdrawn 1993\)](#)<sup>3</sup>

<sup>1</sup> 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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<sup>2</sup> For referenced ASTM standards, visit the ASTM website, [www.astm.org](http://www.astm.org), or contact ASTM Customer Service at [service@astm.org](mailto:service@astm.org). For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

<sup>3</sup> The last approved version of this historical standard is referenced on [www.astm.org](http://www.astm.org).

## 3. Terminology

3.1 *Definitions:*

3.1.1 Definitions of terms applying to this test method appear in Terminology [D883](#).

## 4. Summary of Test Method

4.1 This test method consists of selecting plastic material for testing, obtaining sludge from an anaerobic-digester at a waste-treatment plant, exposing the plastic material to the inoculum obtained from the sewage sludge, measuring total gas, carbon dioxide and methane (CO<sub>2</sub> and CH<sub>4</sub>), evolved as a function of time; soluble organic carbon (SOC), and residual polymer weight at the termination of the test, and assessing degree of biodegradability.

4.2 The percent of theoretical gas production based on measured or calculated carbon content is reported with respect to time from which the degree of biodegradability is assessed.

## 5. Significance and Use

5.1 The degree and rate of anaerobic biodegradability of a plastic material in this test method may be predictive of the time period required to eliminate that plastic from the environment depending on the similarities of the environments. With increasing use of plastics, disposal is a major issue. This test method may be useful to estimate the degree and persistence of plastics in biologically active anaerobic disposal sites. This test method determines the rate and degree of anaerobic biodegradation by measuring the evolved volume of carbon dioxide and methane, as a function of time of exposure to anaerobic-digester sludge.

5.2 Anaerobic sewer-digester sludge from treatment of clarifier sludge at a waste-water treatment plant that treats principally municipal waste is an acceptable active anaerobic environment (available over a wide geographical area) in which to test a broad range of plastic materials. This test method may be considered an accelerated test with respect to a typical anaerobic environment, such as landfill sites that plastics encounter in usual disposal methods because of the highly active microbial population of anaerobic-digester sludge.

## 6. Apparatus

6.1 Gas generated will be collected in either an inverted graduated cylinder submerged in water, water acidified to pH <3 with sulfuric acid, a syringe with a freely moving plunger, or other suitable devices for measuring gas volume such as a pressure transducer.

6.2 *Gas Chromatograph*, or other apparatus, equipped with a suitable detector and column(s), shall be used to quantify methane and carbon dioxide evolution using an analytical procedure specific for these gases.

6.3 *Incubator*, sufficient to store the test bottles at  $35 \pm 2^\circ\text{C}$  in the dark for the duration of the test.

6.4 *Medium Handling Apparatus*, suitable for maintaining anaerobic conditions during medium preparation and inoculation (See Fig. 1).

6.5 *Serum Bottles*, with sufficient capacity for the experiment, with butyl-rubber stoppers and crimp clamps to hold the rubber stoppers.

6.6 *Analytical Balance*, to weigh samples before and after test.

6.7 *Analytical Instrument*, to measure soluble organic carbon content of aqueous medium before and after test.

## 7. Reagents and Materials

7.1 Reagent grade chemicals shall be used in all tests.

7.2 *Purity of Water*—Purity of water unless indicated otherwise shall be understood to mean reagent water as defined by Type IV of Specification D1193.

7.3 Stock solutions are prepared as shown in Table 1.

TABLE 1 Stock Solutions for Anaerobic Biodegradation Test

Stock Solution	Compound	Concentration, g/L	Amount, mL, added per 4 L	Concentration in media, m moles
S-1	Resazurin	0.5	8	...
S-2	$\text{KH}_2\text{PO}_4$	69.0	...	1.0
	$\text{K}_2\text{HPO}_4$	88.0	...	1.0
S-3 <sup>A</sup>	$(\text{NH}_4)_2\text{HPO}_4$	10.0	8	0.15
	$\text{NH}_4\text{Cl}$	100.0	...	3.7
	$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	60.0	...	3.0
	$\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$	20.0	...	1.0
	KCl	10.0	...	1.3
	$\text{CaCl}_2$	10.0	...	0.90
	KI	1.0	...	0.060
	$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.40	...	0.020
	$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.40	40	0.017
	$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.050	...	0.0021
	$\text{CuCl}_2$	0.050	...	0.0037
	$\text{ZnCl}_2$	0.050	...	0.0037
S-4	$\text{H}_3\text{BO}_3$	0.050	...	0.0081
	$\text{Na}_2\text{MoO}_4 \cdot \text{H}_2\text{O}$	0.050	...	0.0018
	$\text{NaIO}_3 \cdot n\text{H}_2\text{O}$	0.050	...	0.0041
	$\text{Na}_2\text{SeO}_3$	0.010	...	0.00054
	$\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$	50.0	8	0.40
	Bicarbonate	$\text{NaHCO}_3$	...	16.8 g

<sup>A</sup> S-3 may form a small amount of precipitate on standing, shake well before using.

7.4 Up to 1 mL of concentrated HCl may be added to Stock Solution S-3 to improve the solubility of salts. Shake well before use in order to distribute any undissolved material throughout the solution.

## 8. Hazards

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

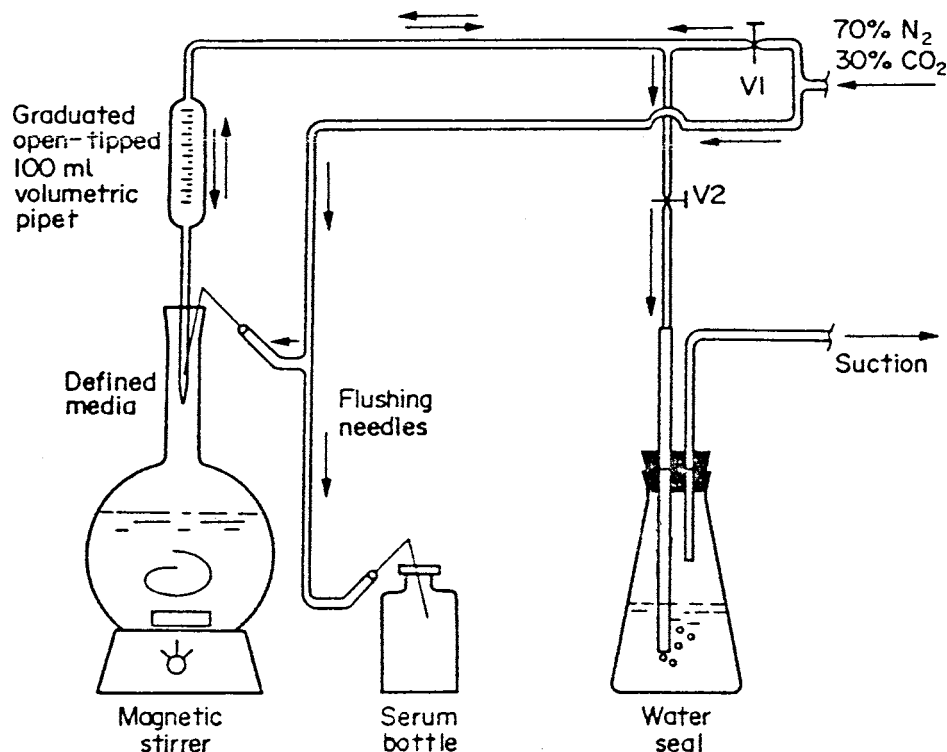


FIG. 1 Schematic Diagram of Apparatus Suitable for Maintenance of Anaerobic Conditions During Medium Preparation and Inoculation

**NOTE 1—Precaution:** This test method involves the use of sludge from a waste-treatment plant. Avoid contact with the sludge by using gloves and other appropriate protective equipment. Use good personal hygiene to minimize exposure to potentially harmful microbiological agents.

## 9. Inoculum—Test Organisms

9.1 The inoculum consists of sludge from a well-operated anaerobic-sludge digester with a total organic solids level of at least 1 to 2 % (W/V). The sewage treatment plant should receive no more than minimal effluent from industry and the solids retention time of the digester should be 15 to 30 days. At the time of collection, filter the sludge through a 2-mm sieve or one layer of cheese cloth.

9.2 Fresh sludge may be used in this test, but it may be stored for up to two weeks at 4°C prior to use without significant loss of activity. Preferably, the sludge is anaerobically digested for another 7 to 14 days at 35°C to reduce background activity, which may interfere with the test.

9.3 Take care to minimize exposure of the sludge to oxygen during collection, handling, and storage.

## 10. Test Specimen

10.1 The test specimen should be of known weight and of sufficient carbon content to yield carbon dioxide and methane volumes that can be adequately measured by the trapping devices described in this test method. Modification of trapping devices may be made to accommodate plastic material handling.

10.2 The specimen may be in the form of films, pieces, fragments, or formed articles. Record this information in the Report section.

10.3 Optionally measure molecular weight before and after the test in accordance with Test Method [D3593](#). Record data in the Report section.

## 11. Procedure

### 11.1 Inoculum Medium:

11.1.1 Prepare the pre-reduced medium from the stock solutions in [Table 1](#). Add 8 mL of Stock Solution S-1, 8 mL of S-2, and 40 mL of S-3 to approximately 3500 mL of dechlorinated tap water, Type IV water or better, in a 4-L Erlenmeyer or Florence flask. Heat to boil while stirring with a magnetic stirrer bar and optionally sparge with a 30 % carbon dioxide and 70 % nitrogen mixture. If used, this mixture may be purchased or prepared by mixing the two gases using calibrated flow meters or other suitable devices. Readily available commercial nitrogen containing less than 10 ppm oxygen and commercial carbon dioxide containing less than 200 ppm oxygen may be used.

11.1.2 Place the flask containing the medium in an ice bath and continue gas sparging until the temperature reaches 35°C.

11.1.3 Remove the flask containing the medium, and add 16.8 g sodium bicarbonate, 400 mL of sludge inoculum, and 8 mL of Solution S-4. This volume should be approximately 4 L.

### 11.2 Test Specimen and Control:

11.2.1 Add the test specimen and control to serum bottles taking care to maintain an inert atmosphere prior to the addition of the inoculated medium. The sample weights should be accurately known.

11.2.2 Prepare the specimen bottles, blanks, and controls in triplicate.

11.2.3 Sample all bottles for analysis of soluble carbon content.

### 11.3 Filling the Test Bottles:

11.3.1 Transfer 100-mL portions of the inoculated medium anaerobically into serum bottles with a total capacity of approximately 160 mL, or proportionally larger amounts if larger bottles are used to accommodate larger plastic samples. [Fig. 1](#) illustrates an apparatus suitable for maintaining anaerobic conditions during medium preparation and transfer. Other suitable devices may be used. Valves V1 and V2 are used to control the transfer of the medium to the serum bottles. Draw inoculated medium into the pipette by suction, then move the pipette and insert the tip into a serum bottle. During these processes, continuously sparge the serum bottle and neck of the medium flask with a mixture of nitrogen and carbon dioxide of composition previously indicated.

11.3.2 Discharge the medium in the pipette into the serum bottle.

11.3.3 Insert a new butyl-rubber serum-bottle stopper into the neck of the bottle as the needle used to sparge the contents with nitrogen and carbon dioxide is withdrawn. A small amount of silicone lubricant may be used to facilitate insertion of the stopper. Hold the stopper in place with an aluminum crimp seal.

### 11.4 Incubation:

11.4.1 At the start of the incubation, release any gas pressure in each bottle to bring to atmospheric pressure.

11.4.2 Incubate the bottles in the dark at  $35 \pm 2^\circ\text{C}$  until gas evolution (biodegradation) of the test compound has stopped. This is indicated by two consecutive weeks without significant gas production in excess of that in the blank. However, if gas production is not observed from the test materials, continue incubation as long as the anaerobic cultures remain active as indicated by the production of gas from the controls.

11.4.3 Bottles containing oxidized (pink) resazurin should be discarded.

### 11.5 Analytical Measurements:

11.5.1 Make a sufficient number of measurements of gas volume in order to establish the gas production rate as a function of time. More frequent readings may be required in the early stages.

11.5.2 Measure gas production for each bottle using a syringe, or other suitable apparatus as indicated.

11.5.3 If a syringe is used to measure volume, hold the syringe in a horizontal position during measurement, taking care to keep the needle in the head space of the serum bottle. To determine gas production, allow the syringe plunger to move freely to equalize the serum flask and atmospheric pressures. If a pressure transducer is used, release gas pressure to bring to atmospheric pressure after measurement.

11.5.4 Determine methane and carbon dioxide production by using analytical methods suitable for the detection and quantification of these gases, such as gas chromatography with an appropriate detector.

11.5.5 At the cessation of gas evolution, as defined in 11.4.2, sample aqueous phase for measurement of soluble organic content for mass balance calculation.

11.5.6 Strain the contents of the flask to isolate insoluble polymer remaining which should be washed, dried, and weighed for mass balance calculation. Molecular weight may also be obtained.

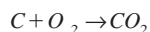
## 12. Calculation

12.1 Determine by calculation or elemental analysis the total organic carbon in the test specimen. This determines the quantity of specimen added to the serum bottle and the size and volume requirements for the gas-volume-measuring device employed in this test method, and the theoretical gas-volume evolution for total biodegradation.

12.2 Calculate the cumulative-average (of the three results) gas volume from the anaerobic biodegradation of the test material by subtracting the cumulative-average gas volume production of the blank controls.

12.3 Calculate the percent of theoretical gas volume produced by dividing the cumulative average gas volume of the test material by the theoretical maximum gas production and multiplying by 100.

12.4 The maximum theoretical gas production (carbon dioxide plus methane) from an organic chemical is calculated as follows, based on 1 mmol (= 12 mg) of organic carbon added as the sample; chemical transformation:



One millimole of gaseous carbon occupies 22.4 mL at NTP, at 35°C (reaction temperature), and volume, corrected for vapor pressure at 35°C, is as follows:

$$22.4 \times \frac{308}{273} \frac{760}{760 - 41} \text{ mL}^5 = 26.71 \text{ mL} \quad (2)$$

(Correction will also be needed for atmospheric pressure variation during test.) This is the theoretical volume of gas that can be generated per metre mole of carbon added to the serum bottle.

## 13. Interpretation of Results

13.1 Information on the toxicity of the plastic material may be useful in interpreting low or negligible biodegradability. (Prior knowledge may indicate toxicity at a certain level of material in this test and this level should not be exceeded. In the absence of information, a low result may be an indication that lower levels of material should be evaluated to avoid possible toxicity effects. Care should be taken to stay within material levels that yield sufficient gas evolution and to measure with accuracy so that biodegradability can be assessed.)

13.2 When investigating a plastic material, a reference or control substance known to biodegrade under anaerobic conditions (for example, cellulose, starch) is necessary in order to check the activity of the inoculum. If less than 70 % biodegradation is observed with the reference, (on the basis of CO<sub>2</sub> and CH<sub>4</sub>), the test must be regarded as invalid and should be repeated with fresh inoculum.

13.3 The plateau level of gas production in this test method, together with the weight of specimen remaining and the measured dissolved organic carbon content, will indicate by carbon balance the degree of biodegradability of the plastic material in this test method.

13.4 The wettability of the plastic material may influence the results obtained, hence the procedure may be limited to comparing plastic materials of similar chemical structure.

## 14. Report

14.1 Report the following data and information:

14.1.1 Information on the inoculum, including source, percent volatile solids, date of collection and use, storage time and conditions, handling and potential acclimation to the test material,

14.1.2 Carbon content of the plastic material,

14.1.3 Record cumulative gas evolution over time until plateau is reached and display graphically as not only end result, but lag phase and slope (rate) are important,

14.1.4 Report analysis of gas as percent methane and percent carbon dioxide at each gas reading,

14.1.5 The percent of theoretical gas evolution along with the form of plastic material, that is, sheet, powder, pellet, etc. for both the test material and the standard control,

14.1.6 The standard deviation for each replicate set of bottles (at least three),

14.1.7 Temperature range of test,

14.1.8 If a more rigorous mathematical treatment of the data is required, the cumulative gas-evolution-versus-time data can be fitted to a non-linear regression model to generate rate constants for mineralization and final extent of biodegradation at infinite time (asymptote, if no plateau is reached)<sup>4, 5</sup>

14.1.9 Molecular weight of the plastic material before and after the test, if measured, and

14.1.10 The weight loss of the specimen and the soluble organic carbon content of the inoculum before and after the exposure period.

## 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 anaerobic; biodegradation; degree (of biodegradation); municipal; plastics; sewage; sludge

<sup>4</sup> Correction for vapor pressure of water at 35°C to correct for wet gas.

<sup>5</sup> Larson, R. J., and Brothansfor, J., Fate Chem. Aquat. Environ., Proc. Workshop, American Society Microbial, Washington, DC, 1980, pp. 67–88.

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