An American National Standard

AMERICAN SOCIETY FOR TESTING AND MATERIALS 100 Barr Harbor Dr., West Conshohocken, PA 19428 Reprinted from the Annual Book of ASTM Standards. Copyright ASTM

# 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 D 5210; 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:

D 883 Terminology Relating to Plastics<sup>2</sup>

D 1193 Specification for Reagent Water<sup>3</sup>

D 3593 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Certain Polymers by Liquid Size-Exclusion Chromatography (Gel Permeation Chromatography—GPC) Using Universal Calibration<sup>4</sup>

### 3. Terminology

- 3.1 Definitions:
- 3.1.1 Definitions of terms applying to this test method appear in Terminology D 883.

### 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 anerobic 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.

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<sup>&</sup>lt;sup>2</sup> Annual Book of ASTM Standards, Vol 08.01.

<sup>&</sup>lt;sup>3</sup> Annual Book of ASTM Standards, Vol 11.01.

<sup>&</sup>lt;sup>4</sup> Annual Book of ASTM Standards, Vol 08.02.



- 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  $35 \pm 2$ °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 D 1193.
  - 7.3 Stock solutions are prepared as shown in Table 1.
- 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.

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	KH <sub>2</sub> PO <sub>4</sub>	69.0		1.0
	K <sub>2</sub> HPO <sub>4</sub>	88.0		1.0
	$(NH_4)_2HPO_4$	10.0	8	0.15
	NH₄CI	100.0		3.7
S-3 <sup>A</sup>	MgCl <sub>2</sub> ·6H <sub>2</sub> O	60.0		3.0
	FeCl <sub>2</sub> ·4H <sub>2</sub> O	20.0		1.0
	KCI	10.0		1.3
	CaCl <sub>2</sub>	10.0		0.90
	KI	1.0		0.060
	MnCl <sub>2</sub> ·4H <sub>2</sub> O	0.40		0.020
	CoCl <sub>2</sub> ·6H <sub>2</sub> O	0.40	40	0.017
	NiCl <sub>2</sub> , 6H <sub>2</sub> O	0.050		0.0021
	CuCl <sub>2</sub>	0.050		0.0037
	ZnCl <sub>2</sub>	0.050		0.0037
	$H_3BO_3$	0.050		0.0081
	Na <sub>2</sub> MoO <sub>4</sub> ·H <sub>2</sub> O	0.050		0.0018
	NaIO <sub>3</sub> ·nH <sub>2</sub> O	0.050		0.0041
	Na <sub>2</sub> SeO <sub>3</sub>	0.010		0.00054
S-4	Na <sub>2</sub> S⋅9H <sub>2</sub> O	50.0	8	0.40
Bicarbonate	NaHCO <sub>3</sub>		16.8 g	50.0

 $<sup>^{\</sup>rm A}\,{\rm S}\text{--}3$  may form a small amount of precipitate on standing, shake well before using.

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

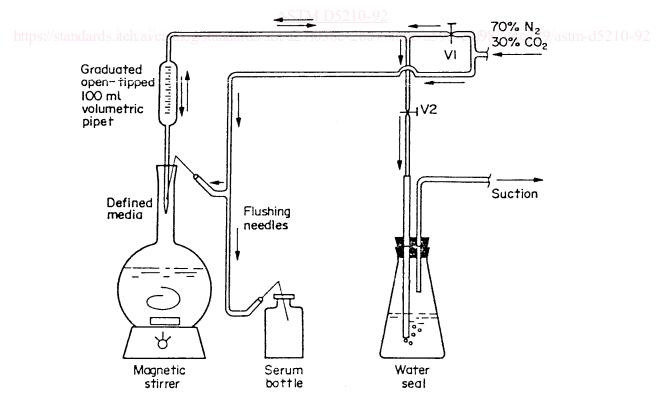


FIG. 1 Schematic Diagram of Apparatus Suitable for Maintenance of Anaerobic Conditions During Medium Preparation and Inoculation