Designation: D 5511 – 02

Standard Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High-Solids Anaerobic-Digestion Conditions[1]

This standard is issued under the fixed designation D 5511; 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 the determination of the degree and rate of anaerobic biodegradation of plastic materials in high-solids anaerobic conditions. The test materials are exposed to a methanogenic inoculum derived from anaerobic digesters operating only on pretreated household waste. The anaerobic decomposition takes place under high-solids (more than 30 % total solids) and static non-mixed conditions.

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 found in high-solids anaerobic digesters, treating municipal solid waste **(1, 2, 3, 4)**.[2] This test method may also resemble some conditions in biologically active landfills where the gas generated is recovered and biogas production is even actively promoted, for example, by inoculation (codeposition of anaerobic sewage sludge, anaerobic leachate recirculation), moisture control in the landfill (leachate recirculation), and temperature control (short-term injection of oxygen, heating of recirculated leachate) **(5, 6, 7)**.

1.3 This test method is designed to be applicable to all plastic materials that are not inhibitory to the microorganisms present in anaerobic digesters operating on household waste.

1.4 The values given in SI units are to be regarded as the standard.

1.5 This test method is equivalent to ISO DIS15985.

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.* Specific hazards are given in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*

D 618 Practice for Conditioning Plastics 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 Chromatography[6]

D 3590 Test Methods for Total Kjeldahl Nitrogen in Water[4]

D 4129 Test Method for Total and Organic Carbon in Water by High-Temperature Oxidation and by Coulometric Detection[4]

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:*

2540 D Total Suspended Solids Dried at 103°–105°C[8]

2540 E Fixed and Volatile Solids Ignited at 550°C[8]

212 Nitrogen Ammonia[8]

2.3 *ISO Standard:*

ISO DIS 15985 Plastics— Determination of the Ultimate Anaerobic Biodegradability and Disintegration Under High-Solids Anaerobic-Digestion Conditions— Method by Analysis of Released Biogas[9]

3. Terminology

3.1 *Definitions*— Definitions of terms applying to this test method appear in 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 consists of selection and analysis of material for testing, obtaining a concentrated anaerobic inoculum from an anaerobic laboratory-scale digester, exposing the

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

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[2] The boldface numbers is parentheses refer to a list of references at the end of the text.

[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 Edition, 1989, American Public Health Association, 1740 Broadway, New York, NY 10018.

[9] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036.

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material to an anaerobic-static-batch fermentation at more than 20 % solids, measuring total carbon in the gas (CO_2 and CH_4) evolved as a function of time, and assessing the degree of biodegradability.

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 Biodegradation of a plastic within a high-solids anaerobic digestion unit is an important phenomenon because it will affect the decomposition of other waste materials enclosed by the plastic and the resulting quality and appearance of the compost after an anaerobic digestion process. Biodegradation of plastics could allow for the safe disposal of these plastics through aerobic and anaerobic solid-waste-treatment plants. This procedure has been developed to permit the determination of the rate and degree of anaerobic biodegradability of plastic products when placed in a high-solids anaerobic digester for the production of compost from municipal solid waste.

5.2 *Limitations*—Because there is a wide variation in the construction and operation of anaerobic-digestion systems and because regulatory requirements for composting systems vary, this procedure is not intended to simulate the environment of any particular high-solids anaerobic-digestion system. However, it is expected to resemble the environment of a high-solids anaerobic-digestion process operated under optimum conditions. More specifically, the procedure is intended to create a standard laboratory environment that will permit a rapid and reproducible determination of the anaerobic biodegradability under high-solids digestion conditions.

6. Apparatus

6.1 *Inverted Graduated Cylinder or Plastic Column*, in water or other suitable device for measuring gas volume. The water in contact with the gas must be at a pH of less than two during the whole period of the test to avoid CO_2 loss through dissolution in the water. The gas-volume-measuring device, as well as the gas tubing, shall be of sufficient quality to prevent gas migration and diffusion between the system and the surrounding air (see Fig. 1).

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

6.3 *Incubator*, or hot-water bath capable of maintaining the test bottles at 52°C (±2°C) for the duration of the test.

6.4 *Erlenmeyer Flasks*, with sufficient capacity for the experiment and openings of at least 7-cm diameter, set up so that no loss of gas occurs.

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

6.6 *Devices*, suitable 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 *Anaerboic Inoculum*, derived from a properly operating anaerobic digester with pretreated household waste as a sole substrate.

7.2 *Analytical-Grade Cellulose*, for thin-layer chromatography as a positive control.

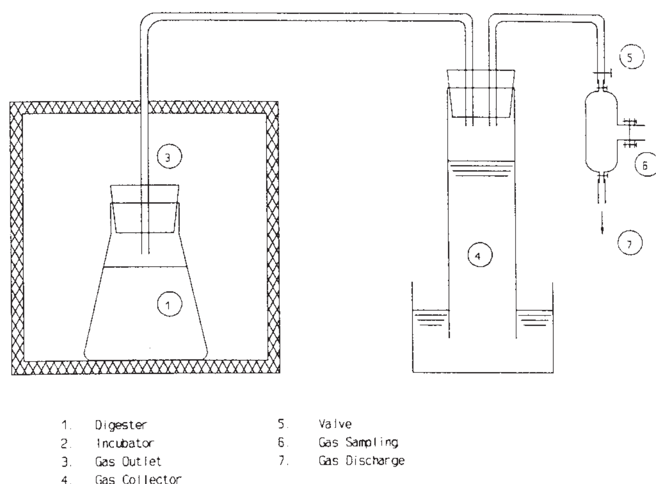
7.3 *Polyethylene*, as a negative control. It should be in the same form as the form in which the sample is tested (for example, film polyethylene for film samples, pellets of polyethylene if the sample is in the form of pellets, etc.).

8. Hazards

8.1 The procedure given in this test method involves the use of an inoculum composed of 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 garments. 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 The biological reactor is not designed to withstand high pressures; operate it at close to ambient pressure.



1. Digester
2. Incubator
3. Gas Outlet
4. Gas Collector
5. Valve
6. Gas Sampling
7. Gas Discharge

FIG. 1 Test Setup

8.4 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.

8.5 The methane produced during this 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 must be derived from a properly operating anaerobic digester functioning with a pretreated household waste as a sole substrate. The pretreated household waste should come from an existing waste treatment facility treating municipal solid waste, where through sorting, shredding, sieving, or other means, a fairly homogeneous organic fraction is produced of less than 60 mm. The digester should be operating for a period of at least four months on the organic fraction, with a retention time of a maximum of 30 days under thermophilic conditions (52 ± 2°C). Gas-production yields should be at least 15 mL at standard temperature and pressure of biogas per gram of dry solids in the digester and per day on the average for at least 30 days.

9.1.1 The inoculum should be derived preferably from a digester operating under dry (>20 % total solids) conditions, or can be derived from a wet fermentation whereby the anaerobically digested sludge is dewatered through centrifugation, with a press or through drying at a maximum temperature of 55°C to a dry-solids content of at least 20 %.

9.2 The prepared inoculum should undergo a short post-fermentation of approximately seven days at the same operating temperature from which it was derived. This means that the inoculum is not fed but 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.

9.2.1 The most important biochemical characteristics of the inoculum shall be as follows:

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

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

9.2.1.3 NH_4^+-*N*—Between 0.5 and 2 g/kg wet weight (in accordance with APHA Test Method 212 and Test Method D 3590).

9.3 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 accurately measured by the trapping devices described. Add more test specimen when low biodegradability is expected, up to 100 g on a dry weight basis of the test specimen.

10.2 The test specimen may be in the form of films, powder, pellets, formed articles, or in the form of a dog bone and conforming to Practice D 618. The test set-up should be able to handle articles that are 100 by 50 by 4 mm thick.

11. Procedure

11.1 *Inoculum Medium*:

11.1.1 Remove enough inoculum (approximately 15 kg) from the post-fermentation vessel and mix carefully and consistently by hand in order to obtain a homogeneous medium.

11.1.2 Test three replicates each of a blank (inoculum only), positive control (thin-layer chromatography cellulose), negative control (polyethylene), and the test substance being evaluated.

11.1.2.1 Manually mix 1000 g wet weight (at least 20 % dry solids) of inoculum in a small container for a period of 2 to 3 min with 15 to 100 g of volatile solids of the test substance or the controls for each replicate. (Determine dry solids and volatile solids in accordance with APHA Standards D 2540, E 2540, and Test Method D 1888).

11.1.2.2 For the three blanks containing inoculum only, manually mix 1000 g of the same inoculum in a small container for a period of 2 to 3 min with the same intensity as was done for the other vessels containing test substance or controls.

11.1.2.3 Determine the weight of the inoculum and test substance added to each individual Erlenmeyer flask accurately.

11.1.2.4 If formed plastic articles are added, a specific number of articles may be added and retrieved at the end of the digestion period.

11.1.3 Add the mixtures to a 2-L wide-mouth Erlenmeyer flask and gently spread and compact the material evenly in the flask to a uniform density.

11.1.4 After placing the Erlenmeyer flask in a water bath or incubator, connect it with the gas-measurement or gas-collection device.

11.1.5 Record room temperature and atmospheric pressure prior to turning on the heating system of the incubator or water bath.

11.2 *Incubation*:

11.2.1 Incubate the Erlenmeyer flasks in the dark or in diffused light at 52°C (±2°C) for a period of normally 15 days. The incubation time may be extended until no significant gas production in excess of the blank has been recorded during one week or until the positive reference has been degraded for more than 70 %, depending on the methanogenic activity of the inoculum.

11.2.2 Control the pH of the water used to measure biogas production to less than two by adding HCl.

11.3 *Analytical Measurements*:

11.3.1 Make a sufficient number of measurements of gas volume in order to establish the gas production as a function of time. More frequent readings in the early stages may be required, with less frequent readings needed as time progresses.

11.3.2 Determine 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, in accordance with Practices E 260 and E 355.

11.3.3 Verify the quality of the inoculum by analyses for pH, volatile fatty acids, and total Kjeldahl nitrogen (in accordance with Test Methods D 1293 and D 3590 and Practice D 2908).

11.4 At the end of the digestion period, allow the setup to cool to room temperature for 8 h and determine the following parameters:

11.4.1 Total gas-volume production produced during the test,

11.4.2 Gas composition at the end of the test,

11.4.3 Weight loss of each vessel, and

11.4.4 Room temperature and atmospheric pressure at the end of the test.

12. Calculation

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

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

Each mmole (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 mmole 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. Correct also for water 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 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:

$$\%\ biodegradation: \frac{mean\ C_g\ (test) - mean\ C_g\ (blank)}{C_i} \times 100 \quad (2)$$

where:

C_g = amount of gaseous carbon produced, g, and

C_i = amount of carbon in test compound added, g.

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

$$S_e = SQRT((s^2_{test}/n1) + (s^2_{blank}/n2)) \times 100/C_i \quad (3)$$

where:

$n1$ and $n2$ = number of replicate test and blank digesters respectively, and

s = standard deviation of the total gaseous carbon produced.

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

$$95\ \%\ CL = \%\ biodegradation \pm (t \times s_e) \quad (4)$$

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 in interpreting whether the plastic material falls within the scope of this test method.

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 after 15 days, and the deviation of the percentage of biodegradation for the reference material in the different replicates less than 20 % of the mean value) is not observed within the duration of the test method, then the test method 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; mesophilic or thermophilic; pH; volatile fatty acids (in milligrams per kilogram); NH_4^+-N in grams per kilogram); percent of dry solids; percent volatile solids; date of collection and use; storage time and conditions; and handling and potential acclimation to the test material,

14.1.2 Carbon content of the plastic material, the positive control and the negative control, and maximum theoretical gas production (carbon dioxide and methane),

14.1.3 Record and display graphically the cumulative gas production over time, and

14.1.4 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.

15. Precision and Bias

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

15.2 Preliminary results for within-laboratory repeatability testing are presented in Table 1. These data represent four different determinations of the degradation of thin-layer chromatography cellulose as the positive reference material under

TABLE 1 Results from Within-Laboratory Testing for the Anaerobic Biodegradability of Cellulose Under the High-Solids Anaerobic Conditions at 52°C

	Biodegradability After Ten Days	Standard Deviation	95 % Confidence Limit
Run 1	86.7 %	0.3 %	2.4 %
Run 2	85.6 %	2.2 %	4.5 %
Run 3	86.2 %	1.1 %	5.4 %
Run 4	84.2 %	4.5 %	9.0 %
Mean of Four Runs	85.7 %	. .	...
Mean Variance With 95 % Confidence Limit	...	5.1 %	10.2 %

thermophilic conditions (52°C). The average degradation of cellulose after ten days in the test method is 85.7 %, with a mean variance of 5.1 % and a 95 % confidence limit interval of ±10.2 % for the four runs. All four runs were carried out within a twelve-month period by the same operators. Figs. 2-4 represent the results of the first run in which a biodegradability of 86.7 % was obtained as the mean for the three replicates containing cellulose as the positive control, with a standard deviation of 0.3 % and a 95 % confidence limit interval of 2.4 %. Fig. 2 represents the gas production of three replicates with inoculum only as the blanks, while Fig. 3 gives a view of the total biogas production after cellulose as a positive reference has been added. Fig. 4 represents the net biogas production from the cellulose after the average biogas production coming from the inoculum has been subtracted.

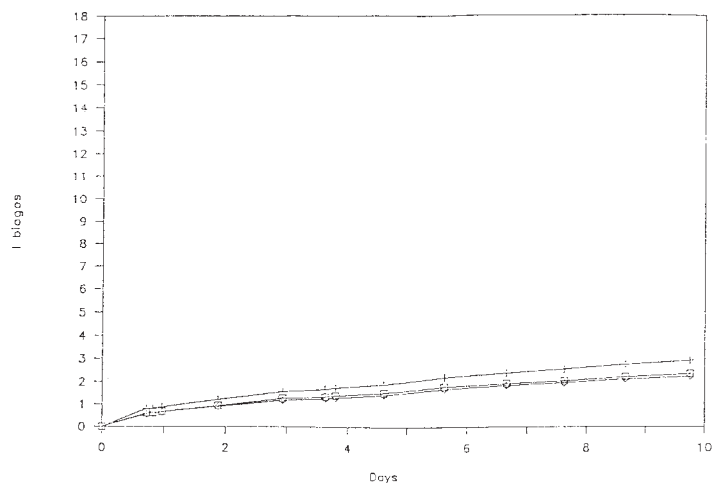


FIG. 2 Representative Plot Showing Total Biogas Production from Inoculum for Three Replicate Specimens Tested in a Single Run

16. Keywords

16.1 anaerobic biodegradation; anaerobic digestion; biodegradation; high-solids digestion; landfill; plastics test method

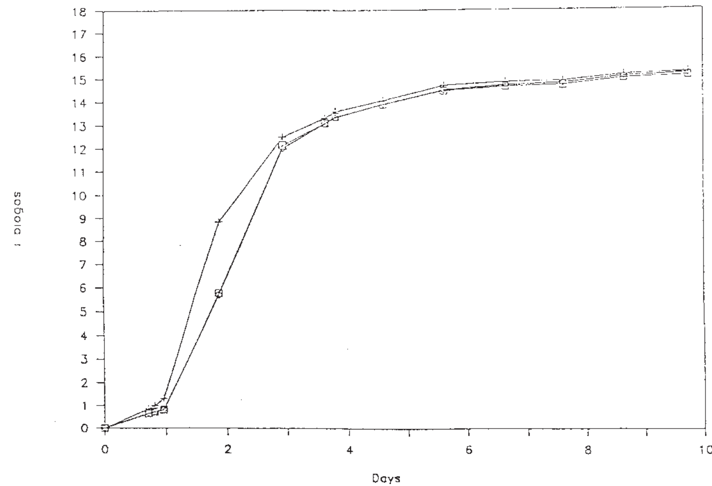


FIG. 3 Representative Plot Showing Total Biogas Production from Inoculum Plus Cellulose for Three Replicate Specimens Tested in a Single Run

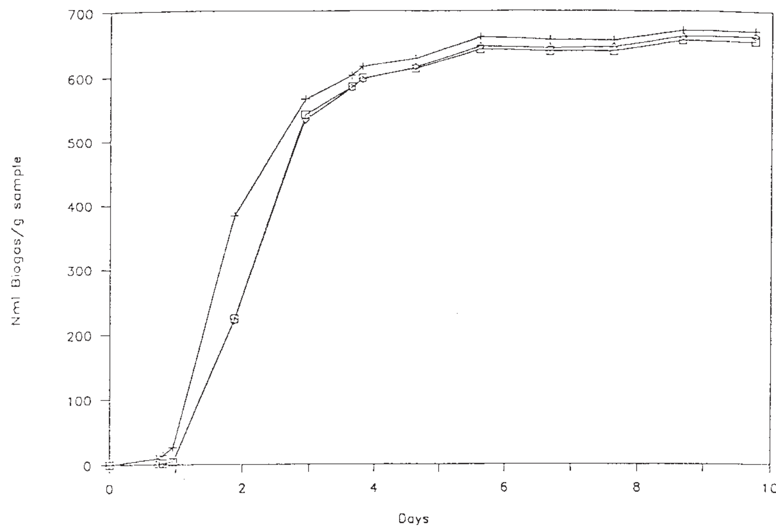


FIG. 4 Representative Plot Showing Net Biogas Production from Cellulose for Three Replicate Specimens Tested in a Single Run

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