Designation: ~~D6691 – 09~~ D6691 – 17

Standard Test Method for Determining Aerobic Biodegradation of Plastic Materials in the Marine Environment by a Defined Microbial Consortium or Natural Sea Water Inoculum[1]

This standard is issued under the fixed designation D6691; 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~~ Scope*

1.1 This test method is used to determine the degree and rate of aerobic biodegradation of plastic materials (including formulation additives) exposed to pre-grown population of at least ten aerobic marine microorganisms of known genera or the indigenous population existing in natural seawater. The test method is conducted under controlled laboratory conditions.

1.2 This test method is designed to index polymer materials that are possibly biodegradable, relative to a positive reference material, in an aerobic environment.

1.3 This test method is applicable to all polymer materials containing at least 20 % carbon that are not inhibitory to the microorganisms present in a marine environment.

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

1.5 There is no ~~similar or equivalent ISO standard.~~ known ISO equivalent to this 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~~ safety, health, and ~~health~~environmental practices and determine the applicability of regulatory limitations prior to use.*

1.7 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D618 Practice for Conditioning Plastics for Testing
D883 Terminology Relating to Plastics
D1193 Specification for Reagent Water
D2593 Test Method for Butadiene Purity and Hydrocarbon Impurities by Gas Chromatography
D4129 Test Method for Total and Organic Carbon in Water by High Temperature Oxidation and by Coulometric Detection

3. Terminology

3.1 *Definitions of Terms Specific to This Standard*—Definitions of terms applying to this test method appear in Terminology D883.

4. Summary of Test Method

4.1 This test method consists of the following:

4.1.1 Selecting and characterizing (carbon content, molecular weight) plastic materials for testing,

4.1.2 Preparing a uniform inoculum of various isolated marine microorganisms, or obtaining a natural sea water sample (with added inorganic nutrients) for the test relying on the microbes present as the inoculum.

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[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.

***A Summary of Changes section appears at the end of this standard**

4.1.3 Exposing the test materials to the inoculum,
4.1.4 Using a respirometer to measure the total biogas (CO_2) produced as a function of time, and
4.1.5 Assessing the degree of biodegradability.

4.2 Biodegradability is assessed by determining the proportion of polymer-C converted to biogas-C. The percent of theoretical gas production, expressed as a fraction of the measured or theoretical carbon content of the test material, is reported as a function of time.

5. Significance and Use

5.1 The use of plastics aboard ships is on the rise and the use of the sea as a trash dumping site is no longer a possibility; consequently, the disposal of plastic materials while at sea remains a major issue. It is possible that biodegradable plastics will help to allay public concern by allowing for the safe disposal of plastic materials at sea. This test method has been developed to assess the rate and degree of aerobic biodegradation of plastics exposed to marine microorganisms. Aerobic biodegradation is determined by measuring the amount of biogas (carbon dioxide) produced during such an exposure.

5.2 It is acceptable to use the degree and rate of aerobic biodegradability of a plastic under the conditions of this test method to estimate the persistence of that plastic in biologically active marine environments, for example, seashore and open-ocean. However, it shall be recognized that predicting long-term environmental fate and effects from the results of short-term exposure to a simulated marine environment is difficult. Thus, caution shall be exercised when extrapolating the results obtained from this or any other controlled-environment test to disposal in the natural environment.

6. Apparatus

6.1 *Aerobic Digestion and Gas Measuring Apparatus:*

6.1.1 Biogas production can be monitored through the use of any number of respirometry systems. The respirometry system must be able to detect low levels of carbon dioxide production. A carbon dioxide sensor consisting of a single beam, nondispersive infrared device with a maximum measurement capability of 1 % carbon dioxide is recommended.

6.1.2 *Sample Bottles*—125-mL autoclave bottles with plastic, screw-on lids. The lids shall contain three entry ports for biogas collection as well as a tetrafluorethylene seal ring. These flasks as well as their lids are supplied by the various respirometry companies.

6.1.3 All components of the gas-volume measuring and collection system must be of sufficient quality to prevent gas diffusion between the system and the surrounding atmosphere.

6.2 *Water Bath or Controlled-Environment Shaker/Incubator,* capable of maintaining the temperature of the digestion flasks at 30 ± 2°C.

6.3 *Analytical Balance,* (±0.1 mg), to weigh the test materials.

7. Reagents and Materials

7.1 All chemicals shall be of American Chemical Society (ACS) reagent-grade quality.

7.2 Type IV distilled water shall be prepared in accordance with Specification D1193.

7.3 Marine agar per litre consists of the following:

Bacto tryptone	5.0 ± 0.1 g
Bacto yeast extract	2.5 ± 0.1 g
Bacto dextrose (glucose)	1.0 ± 0.1 g
Bacto agar	15.0 ± 0.1 g

7.4 Marine broth per litre consists of the following:

Peptone	5.0 ± 0.1 g
Yeast extract	1.0 ± 0.1 g
Ferric citrate	0.1 ± 0.1 g
Sodium chloride	19.4 ± 0.1 g
Magnesium chloride, dried	5.9 ± 0.1 g
Sodium sulfate	3.24 ± 0.1 g
Calcium chloride	1.8 ± 0.1 g
Potassium bromide	0.08 ± 0.1 g
Strontium chloride	34.0 ± 0.1 mg
Boric acid	4.0 ± 0.1 mg
Sodium silicate	4.0 ± 0.1 mg
Sodium fluoride	2.4 ± 0.1 mg
Ammonium nitrate	1.6 ± 0.1 mg
Disodium phosphate	8.0 ± 0.1 mg

7.5 *Marine Solution*—Shall be either 7.5.1 or 7.5.2.

7.5.1 *Refer to* Table 1. All of the components must be mixed with 1 L of Type IV distilled water, until all of the salts have dissolved and then sterilized.

TABLE 1 Components of Minimal Marine Solution

Substance	Formula	MW, g/mol	Concentration, g/L
Ammonium chloride	NH_4Cl	53.49	2.00 ± 0.05
Synthetic sea salt	. . .	. . .	17.50 ± 0.05
Magnesium sulfate, 7-hydrate	$MgSO_47H2O$	246.48	2.0 ± 0.05
Potassium nitrate	KNO_3	101.1	0.5 ± 0.05
Potassium phosphate	K2HPO4 · 3H2O	228.2	0.1 ± 0.05

7.5.2 Natural sea water with inorganic nutrients (0.5 g/L of NH_4Cl and 0.1g/L of $KH_2(PO_4)$.

7.6 *Reference Materials*—Cellulose, chitin and Kraft paper, or all three, can act as the positive control and solitary inoculum as the negative control. Reference materials shall be provided in the same form as the test specimens, that is, powders, films, foams, and so forth. Sodium bicarbonate (100 mg) and sodium sulfite (100 mg) in an acidic water solution (100 mL) shall be tested also to ensure that the CO_2 sensors of the respirometry apparatus are functioning properly.

7.7 Microorganisms shall be selected on the basis of ability to degrade various biodegradable polymers, starches, cellulosics, and bacterial polyesters. Table 2 shows the composition of the synthetic sea salt solution.

7.8 It is important that sampling for the natural sea water be from a site not influenced by sewage outflow, chemical dumping, waste water discharge areas or oil slicks in the water. Also, do not take the samples from a river estuary having significant tidal flow characteristics as it is possible that this will not be representative of natural sea water.

8. Hazards

8.1 All microorganisms present the possibility of disease and shall be handled with due caution. Hands shall be washed before and after exposure. Latex gloves and safety glasses shall be used along with a mouth cover. All spills containing organisms shall be cleaned with germicidal/antibacterial agents, and all old cultures shall be autoclaved before being discarded.

8.2 This test method requires the use of hazardous chemicals. Avoid contact with chemicals and follow the manufacturer's instructions and Material Safety Data Sheets.

8.3 All purchased media also can be hazardous. Read all safety instructions.

TABLE 2 Composition of Synthetic Sea Salt Solution at Approximate Salinity of 34 ppt, Production Variance of ±5 %

Ion	Concentration, mg/L
Chloride	19251
Sodium	10757
Sulfate	2659
Magnesium	1317
Potassium	402
Calcium	398
Carbonate/bicarbonate	192
Strontium	8.6
Boron	5.6
Bromide	2.3
Fluoride	1.0
Iodide	0.22
Lithium	0.18
Copper	trace (<0.03)
Iron	trace (<0.03)
Nickel	trace (<0.04)
Zinc	trace (<0.02)
Manganese	trace (<0.01)
Molybdenum	trace (<0.01)
Cobalt	trace (<0.05)
Vanadium	trace (<0.04)
Selenium	trace
Lead	trace (<0.005)
Arsenic	trace (<0.0002)
Cadmium	trace (<0.02)
Chromium	trace (<0.0006)
Aluminum	trace (<0.04)
Tin	trace
Antimony	trace
Rubidium	trace
Barium	trace (<0.05)
Mercury	none
Nitrate	none
Phosphate	none