Designation: D7473/D7473M – 21

Standard Test Method for Weight Attrition of Non-floating Plastic Materials by Open System Aquarium Incubations[1]

This standard is issued under the fixed designation D7473/D7473M; 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 is used to determine the weight loss as a function of time of non-floating plastic materials (including formulation additives), when incubated under changing, open, marine aquarium conditions, which is representative of aquatic aerobic environments near the coasts and near the bottom of a body of water in the absence of sunlight, particularly UV and visible portions of the spectrum. The goal of this test is to obtain data that can be used to assess the potential for physical degradation of the test material. Such potential for physical degradation will be affected by real life environmental conditions.

1.2 In particular this test method does not take into consideration the possible effects of solar irradiation.

1.3 The aquarium-incubated plastic materials are examined for visual degradation and dry weight loss over time. This test does not provide information on ultimate biodegradation (that is, it is not a replacement for Test Method D6691), but it is an ASTM method for weight attrition. The standard addresses only weight loss as a function of time of the plastics materials in a marine environment and shall not be used for demonstrating ultimate biodegradation.

1.4 This test method does not provide information regarding the potential formation of microplastics due to the physical degradation of the samples.

1.5 Plastic pieces of known size and thickness are used at levels so as not to exceed the availability of micronutrients essential for and therefore limit the microbial processes.

1.6 The aquarium incubation test method allows for representative indigenous microorganisms present in seawater and marine sediment to be enriched for and carry out the biodegradation. It is recommended that the test be carried out in the geographical vicinity (latitudinal area) where the test materials are likely to be used. These Aquarium studies are conducted in indoor environments, hence any sunlight-induced effects on degradation, or biodegradation, or both, are not taken into account.

1.7 This test by itself shall not be used as the basis for claims, such as "Biodegradable in Marine Environments" since it is only a weight loss test method. This test method is solely a means for measuring a characteristic (physical degradation) under standard conditions. It does not assess the general environmental impact of plastic products.

1.8 *Units*—The values stated in either SI units or inch-pound units are to be regarded separately as standard. The values stated in each system are not necessarily exact equivalents; therefore, each system shall be used independently of the other. Combining values from the two systems has the potential to result in non-conformance with the standard.

1.9 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

NOTE 1—There is no known ISO equivalent to this standard.

1.10 *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]

D883 Terminology Relating to Plastics

D6691 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 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

3. Terminology

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

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *absence of light*—absence of electromagnetic radiation with the focus on visible and ultraviolet portions of the spectrum of sunlight or other light with similar wavelength frequencies from artificial sources.

3.2.2 *natural seawater (NSW)*—seawater unamended with any additives.

3.2.3 *indigenous microbes*—those microbes naturally occurring in a seawater or sediment sample.

3.2.4 *mesophilic*—temperature range from approximately 20 to 40°C over which microorganisms adapted to moderate conditions maintain active metabolic rates.

3.2.5 *psychrophilic*—temperature range from approximately 2 to 20°C over which microorganisms adapted to cold conditions maintain active metabolic rates.

3.2.6 *sulfate reduction*—the anaerobic microbial process whereby sulfate acting as an electron acceptor is converted to hydrogen sulfide as an end product.

3.2.7 *surface marine sediment (SED)*—the upper few millimeters to several centimeters of oceanic bottom sediments containing the natural indigenous microbial populations and ranging from oxic to potentially anoxic conditions with increasing sediment depth.

4. Summary of Test Method

4.1 This test method consists of the following:

4.1.1 Selecting, characterizing and preparing plastic materials for testing (formulation, carbon content, molecular weight, thickness and uniformity).

4.1.2 Running short-term (4 days) sterile seawater controls of the plastic materials to determine level of loss due to soluble components (plasticizers etc.). See Section 11.

4.1.3 Collection and storage in the absence of light of marine sediment from the local coastal marine environment for use in aquarium incubations.

4.1.4 Having access to a continuous flow of natural seawater.

4.1.5 Exposing plastic material pieces in the absence of light to natural flowing seawater or sediment surfaces under natural flowing seawater in open tray incubations in a marine aquarium at seasonally varying water temperatures. See Section 8.

4.1.6 Harvesting plastic material pieces at varied time intervals to assess visual impacts of exposure and degradation and determining the percentage loss in dry weight and weight loss per unit area.

4.2 This aquarium incubation test method has been developed and is used to assess the rate and extent of attrition of plastic materials as a loss in dry weight during incubation exposure to indigenous marine microorganisms. The test assesses weight loss under continuous flow (open system) aquarium conditions in which microbial growth processes rely on the naturally occurring supply of nutrients (for example, nitrogen and phosphate) in the incoming seawater and use the plastic as the carbon source. Aquarium testing is more realistic of the actual marine environment than a closed flask laboratory test (that is, Test Method D6691) as it allows flushing, exposure to a diverse population of microbes, removal of metabolic end products, re-supply of oxygen, exposure to anoxic conditions in sediment, and exposure to seasonal temperature variation of the incoming seawater and natural concentration of macro- and micronutrients. This dynamic test is carried out as close to the geographical vicinity (latitudinal area) where the tested material is likely to be used in product form.

4.3 The test does not quantify the conversion of plastic organic carbon to carbon dioxide, but rather the loss in dry weight of the material over time. Therefore, other test methods, such as Test Method D6691, must be run in order to determine the maximum CO_2 production from the test sample and therefore indicate the degree of biodegradation under the more optimum conditions of the laboratory.

5. Apparatus

5.1 *Borosilicate glass beakers,* varied sizes, (250 mL to 4 L as needed for sediment).

5.2 *Autoclave* capable of steam sterilizing. The autoclave is run at 121°C for 20 min.

5.3 *Drying oven* for obtaining constant dry weight of samples

5.4 *Analytical balance,* for weighing test samples

5.5 *Access to flowing natural seawater aquarium.*

5.6 *Plastic boxes* (lids removed) with open compartments for holding samples incubated in open aquarium trays of flowing seawater.

5.7 *Nylon mesh screening,* (⅛ to ¼ in. openings).

5.8 *Opaque or transparent plastic materials in the form of film or fabric.*

6. Hazards

6.1 While there are no known specific hazards associated with this test procedure, care must be taken in handling of all samples. Latex gloves are used when handling the marine sediment.

6.2 Before preparing chemical stock solutions read the manufacturer's Safety Data Sheets.

7. Inoculum

7.1 *Natural Seawater (NSW),* as a continuous fresh supply avoiding collections sites influenced by storm water runoff or having major oil slicks on the surface. For the purposes of this standard, a major oil slick is the one that can be clearly noticed by visual inspection.

7.2 *Surface Marine Sediment (SED),* collected on or before (1 day) the day the Aquarium incubations are to be initiated. Surface sediment, preferably of a muddy nature as opposed to sand, can be collected from any coastal location at or close to the NSW source site.

8. Procedure (Open System Aquarium Incubation)

8.1 *Plastic Materials Preparation:*

8.1.1 Determine the average mil thickness of the plastic material. Cut pre-dried material in 0.5 by 0.5 in. pieces. Weigh individual pieces of the material and record weight.

8.1.2 Samples shall not be subjected to any conditions or treatments designed to accelerate weight loss prior to 8.3.2.

8.1.3 The use of a benchmark material (for example, a material whose potential for physical degradation is known) to be tested in parallel with the tested material is highly recommended.

8.2 *Aquarium Inoculation Preparation:*

8.2.1 Collect surface SED from area of the NSW site and bring to aquarium incubation site. Keep sediment in place with absence of light prior to incubation and testing. Collect enough to half fill the needed number of sections in chosen incubation containers (for example, plastic boxes with 12 separate sections and lids removed—these boxes will be placed in the flowing seawater aquarium trays). Sections of about 4 in. by 2 in. are sufficient for each sample piece. The same types of incubation containers and section size are used for the NSW incubation without any added sediment. Have enough box sections to fulfill the needed number of samples for example, 2 plastic samples × 5 time points × 3 triplicates × 2 incubation conditions—(NSW exposure alone and NSW-SED exposure) = 60 incubation container sections, in a randomized pattern.

8.3 *Aquarium Incubation:*

8.3.1 Have a constant supply of natural seawater that is pumped from the sea and flowed into and out of the aquarium tray. The incoming seawater is pumped directly from the sea and run through a coarse filter to reduce the amount of sedimentation of particulates over time, if necessary.

8.3.2 Place the plastic samples in the plastic boxes into individual sections, with and without sediment in the sections, recording their location. Samples placed in box sections with added sediment are placed just on top of the sediment surface with enough pressure to adhere them to the sediment. The tops of all boxes are covered with a large mesh nylon screen (⅛ to ¼ in. openings) and secured with rubber bands around the box. This prevents loss or exchange of any samples between compartment sections during incubation.

8.3.3 Place all boxes containing plastic samples into the aquarium tray. Slowly fill all the boxes with seawater before submerging them in the aquarium tray to prevent shifting of the sediment adhered samples. Cover the aquarium with opaque plastic film or fabric to keep the plastic sample(s) and sediment in dark conditions.

8.3.4 Record temperature of incoming seawater at time zero and at each sampling point. Monitor samples over time for any visible signs of degradation and harvest samples at appropriate time intervals. Report the total length of time for the test period.

9. Sample Harvesting and Processing

9.1 At selected time intervals, samples (triplicates) are removed from Aquarium box sections being careful not to loose delicate fragments if the test material has any tendency to do so. Aquarium incubation boxes are best lifted from the aquarium trays before this procedure and then replaced after samples are removed.

9.2 Sampled plastic pieces are rinsed with distilled water to remove adhered sediment particles, adhered bacterial slime if present, and sea salts and then weight recorded after drying to constant weight (35-40°C).

9.3 Note and report any blackening of the undersides of Aquarium samples, which is indicative of anoxic conditions that allow sulfate reducing microbes to play a significant role in biodegradation of the sample.

10. Correction for Soluble Components

10.1 In order to determine if significant soluble components are leached out during initial aqueous exposure, weighed pieces of plastic are incubated in pre-sterilized (autoclaved) seawater for ~96 hrs. They are then collected, dried and weighed to determine the percentage of loss, if any, due to soluble components, which would not be attributable to microbial action. The reported percentage in dry weight loss of test samples is corrected for this solubilization loss if greater than 0.5 %.

11. Calculation

11.1 Determine the percentage loss in dry weight of samples over time (average of triplicate samples). Correct for any soluble losses from sterile controls if necessary.

11.2 As microbial activity during exposure of the plastic samples is primarily a surface action, it is also important to calculate the weight loss per unit area of the plastic material. This is important when comparing materials of different mil thicknesses. A thicker material could lose as much or more weight per unit area but as it would have had a higher initial dry weight than a thinner plastic, the actual percentage of dry weight loss can actually be less than the thinner plastic. Calculate the weight loss per unit area, for example, weight loss per ½ × ½ in. piece × 4 = weight loss/square inch. Correct for any soluble losses from sterile controls if necessary, as mentioned in 10.1.

11.3 Plot percentage loss in dry weight on one y axis and weight loss per unit area on the other y axis and incubation time on the *x* axis.

12. Interpretation of Results

12.1 This test will indicate the rate and extent of physical degradation in the absence of light of a particular plastic material by assessing the loss in weight of material under marine incubations enriching for the indigenous microbes present in natural seawater and sediment. Sediment contains several orders of magnitude more bacteria than seawater so its use is intended to enhance the likelihood of microbes being present that can biodegrade the polymer materials. Note that this method does not allow one to determine or distinguish between physical or biodegradation. since it is just a weight loss as a function of time test method. Open system Aquarium conditions employ a constant supply of fresh natural seawater