Designation: D7473 – 12

Standard Test Method for Weight Attrition of Plastic Materials in the Marine Environment by Open System Aquarium Incubations[1]

This standard is issued under the fixed designation D7473; 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 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 will predict real world experiences based on the extent and rate of biodegradation data of the same materials obtained from the laboratory Test Method D6691. The aquarium incubated films are examined for visual degradation and dry weight loss over time. This test is not a replacement to Test Method D6691, but rather an additional ASTM method for weight attrition. The standard addresses weight loss of the plastics in a marine environment and cannot be used for demonstrating biodegradation for which Specification D7081 needs to be used.

1.2 Plastic film 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 biodegradation process.

1.3 The aquarium incubation test method allows 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 film is likely to be used and potentially disposed of in the marine environment if biodegradable criteria are met. These Aquarium studies are conducted in indoor environments, hence any sunlight-induced effects on degradation, or biodegradation, or both, are not taken into account.

1.4 Prior to conducting this aquarium test method (weight loss data) for the verification of biodegradability, Test Method D6691 shall be run on the same materials to establish quantitative levels of the plastic organic carbon oxidation and levels of carbon dioxide recovered there from. If Test Method D6691 achieves 30 % mineralization, then apply this Aquarium test and perform it. If the results from Test Method D6691 do not achieve 30 % mineralization, then aquarium incubation testing need not be done and the material shall be considered non-biodegradable in the marine environment.

1.5 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.

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.*

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

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

D7081 Specification for Non-Floating Biodegradable Plastics in the Marine Environment

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.

[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.

Current edition approved May 1, 2012. Published June 2012. DOI:10.1520/D7473–12.

[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.

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*—the upper few millimetres to several centimetres 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 films for testing (formulation, carbon content, molecular weight, film thickness and uniformity).

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

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4.1.3 Collecting 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 film 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 film 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.

4.1.7 The film material is related for its attrition and weight loss in this realistic open system aquarium incubation, to the prior determination of its organic carbon biodegradability to CO_2 based on the outcome of Test Method D6691 testing of the same film.

4.2 Conventional plastics are not allowed to be disposed of at sea, and yet the use of such materials aboard ships has increased in recent years. A technological goal is to develop a test method for plastics, designed to biodegrade safely in the marine environment (conversion to carbon dioxide by means of microbial metabolism). These can be used in place of conventional plastics which will fulfill the criteria for allowing them to be disposed of in the marine environment. This aquarium incubation test method has been developed and is used to assess the rate and extent of attrition of biodegradable plastics 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. The test is carried out as close to the geographical vicinity (latitudinal area) where the test film 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, Test Method D6691 must be run prior to this test in order to determine the maximum CO_2 production from the test film and therefore indicate the degree of biodegradation under the more optimum conditions of the laboratory but which are less realistic of the actual marine environment.

4.4 Conducting Test Method D6691 initially as a closed system test in the laboratory will determine if the plastic items meet criteria of acceptable biodegradability to the pass level for mineralization specified in 1.4 and if so, the open system aquarium test is warranted. The rate of biodegradation can be expected to be faster under laboratory conditions compared to the Aquarium test since the latter is conducted under changing and often colder temperatures and a more limited supply of nutrients relative to the available carbon.

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,* (±0.1 mg) 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 plastic 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 Material Safety Data Sheets.

7. Inoculum

7.1 *Natural Seawater (NSW),* as a continuous fresh supply avoiding collections sites influenced by storm water runoff or have major oil slicks on the surface.

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 *Film Preparation:*

8.1.1 Determine the average mil thickness of the film. Cut pre-dried film in 0.5 by 0.5 in. pieces. Weigh individual pieces of film 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.

8.2 *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 film samples × 5 time points × 3 triplicates × 2 incubation conditions—(NSW exposure alone and NSW-SED exposure) = 60 incubation container sections.

8.3 *Aquarium Incubation:*

8.3.1 Have a constant supply of natural seawater flowing into and out of the aquarium tray. The incoming seawater can be run through a coarse filter to reduce the amount of sedimentation of particulates over time if necessary.

8.3.2 Place the film 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 inch 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 sample films 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 films. Cover the aquarium with opaque plastic film or fabric to keep the plastic sample and sediment in dark conditions.

8.3.4 Record temperature of incoming seawater at zero time and at each sampling point. Monitor samples over time for any visible signs of degradation and harvest samples at appropriate time intervals over a maximum of 180 days. Report the total length of 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 film 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 film 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 are indicative of anoxic conditions that allow sulfate reducing microbes to play a significant role in biodegradation of the films.

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 film are incubated in pre-sterilized (autoclaved) distilled water 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 film 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 films is primarily a surface action it is also important to calculate the weight loss per unit area of film. This is important when comparing films of different mil thicknesses. A thicker film could lose as much or more weight per unit area but as it would have had a higher initial dry weight than a thinner film, the actual percentage of dry weight loss can actually be less than the thinner film. Calculate the weight loss per unit area e.g. 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.

11.4 Have data from Test Method D6691 run on the same film available for comparison.

12. Interpretation of Results

12.1 This test will indicate the rate and extent of biodegradability in the absence of light of a particular polymer film 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 test film. The aquarium open system incubations are aimed at supplementing (not replacing) the results of Test Method D6691 by determining if said degradable film is actually biodegraded under more realistic, yet less optimum conditions. Open system Aquarium conditions employs constant supply of fresh natural seawater which is seasonally variable and thus allowing microbes of both mesophilic and psychrophilc character to play a role. The constant flow insures oxygenated seawater and for the films placed on sediment, anaerobic processes (e.g. sulfate reduction) can play a role in the biodegradation. The absence of light during the Aquarium incubation eliminates the potential of polymer photodegradation processes that can accelerate biodegradation and reduce the presence of light sensitive microorganisms that have the potential to play a role in the plastic biodegradation process. The levels of major nutrients, nitrogen and phosphate, are only available at that present in the NSW, yet they are constantly replenished at this level through the inflowing NSW.

12.2 The percentage loss in dry weight and loss in dry weight per unit area can be used in conjunction with data from Test Method D6691 to determine biodegradation behavior or potential in the marine environment.

13. Report

13.1 Mil thickness of the polymer film.

13.2 Formulation and identification of film.

13.3 Location and season of NSW and SED source.

13.4 Aquarium conditions of NSW (temperature, prefiltration, etc.).

13.5 Soluble, non-biodegradable, losses of polymer film exceeding 0.5 %.

13.6 Graphic display of incubation time vs. percentage loss in dry weight and weight loss per unit area from both tests using natural sea water (NSW) as well as natural seawater with surface marine sediment (NSW + SED) as inocula.

NOTE 2—All test results shall be presented and not just the end points.

13.7 Definitive conclusions of biodegradability cannot be made as Test Method D6691 must also be performed.

14. Precision and Bias

14.1 *Precision*—For triplicate polyhydroxyalkanoate (PHA) samples incubated in natural sea water (NSW) a mean average of 83.9 % ± 1.0 of dry weight loss and grams/unit area was 0.0228 g ± 0.0006 was determined.[3] The reproducibility of this test method is being determined and will be available on or before December 2012.

[3] Contractors Report 2010 to U.S. Army Labs entitled: "Microbial Biodegradation of Polymeric Plastic Substitutes in the Marine Environment—Inter-Laboratory Aquarium Study. Woods Hole, MA."