Designation: D 6692 – 01

Standard Test Method for Determining the Biodegradability of Radiolabeled Polymeric Plastic Materials in Seawater[1]

This standard is issued under the fixed designation D 6692; 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 is used to determine the degree of aerobic biodegradation of polymeric compounds utilized in plastic materials by determining the level of respiration of such radiolabeled carbon compounds to radiolabeled carbon dioxide.

1.2 The test is designed to utilize the naturally occurring microbes in seawater as the inoculum for the enrichment and subsequent mineralization (biodegradation) of the test polymer using it as a carbon and energy source resulting in a carbon dioxide as an end product.

1.3 The test method requires that the polymers to be assayed are synthesized using the radioisotope, carbon-14, and that the compound or plastic material be uniformly labeled with carbon-14.

1.4 As controls, known biodegradable compounds, such as glucose or starch, also uniformly labeled with carbon-14, are run in order to determine the biological activity of the natural population.

1.5 The concentration of added polymers shall be kept low so as not to cause limitation by oxygen, and the seawater inoculum is amended with nitrogen and phosphorus compounds to ensure that growth in not limited by these nutrients.

1.6 The safety problems and regulations associated with working with radioactive materials are not addressed in the method. It is the responsibility of the individual users to establish and ensure adherence the proper safety, health, monitoring and all regulatory practices associated with the use of radioactive compounds.

1.7 There is no similar or equivalent ISO standard.

2. Referenced Documents

2.1 *ASTM Standards:*

D 883 Terminology Relating to Plastics[2]

D 5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene By High Performance Size Exclusion Chromatography[3]

D 6340 Determining Aerobic Biodegradation of Radiolabeled Plastic Materials in an A) Aqueous or B) Compost Environment[3]

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *disintegrations per minute (DPM), n*—the degree of radioactivity of an element given as the number of decays occurring per unit time.

3.1.2 *microcurie (μCi), n*—a unit of radioactivity equal to /1,000,000 of a curie or 2.2×10^6 DPM.

3.1.3 *millicurie (mCi), n*—a unit of radioactivity equal to 1/1000 of a curie.

3.1.4 *natural seawater (NSW), n*—seawater unamended with any additives.

3.1.5 *specific activity (SA), n*—the level of radioactivity (μCi) per unit mass (for example, mg).

4. Summary of Test Method

4.1 This test method consists of the following:

4.1.1 Obtaining samples of the test polymer in which the carbon is uniformly labeled with carbon-14 and of known specific activity.

4.1.2 Collecting a sample of natural seawater (NSW) from a marine source in which the material (non-radioactive) may be ultimately disposed.

4.1.3 Incubating, over a time course, weighed samples of the polymer in sealed vessels of NSW amended with nutrients under aerobic conditions at constant temperature.

4.1.4 At varied time points, harvesting the microbially respired radiolabeled carbon dioxide in absorbent traps.

4.1.5 Determining the DPM of radiolabeled carbon dioxide in a liquid scintillation counter.

4.1.6 Based on the recovered DPM, the specific activity and the known amount of radiolabeled polymer added to the test incubation vessel calculate the amount and percentage of the polymer which was respired (biodegraded) to carbon dioxide.

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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.

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[2] *Annual Book of ASTM Standards*, Vol 08.01.

[3] *Annual Book of ASTM Standards*, Vol 08.03.

5. Significance and Use

5.1 This method allows for a definitive determination concerning aerobic microbial biodegradation occurring for plastic compounds of known uniform composition or of component materials used in the production of non-uniform (that is, blend) plastics.

5.2 The use of radioactive^{14}C materials eliminates the possibility of carbon dioxide measurement from anything other than the parent compound in question.

5.3 The use of this assay relies on the enrichment and growth of naturally occurring microbes in marine samples to biodegrade the radiolabeled plastic compounds generating cell material and carbon dioxide as the radiolabeled end products.

5.4 Time course (several days to several weeks) measurements allows for a biodegradation rate determination to be made, but caution should be exercised in extrapolating this laboratory determined rate to what may actually occur in an open system marine environment which may experience fluctuations in nutrient availability, oxygen and temperature.

5.5 It may be necessary to repeat this test more than once (depending on the season of water sampling) since microbial populations vary significantly over time and location.

5.6 The seawater inoculum may be run with a sample (ë 10 gram quantities) of marine sediment added to the seawater inoculum to increase the microbial diversity in establishing an enrichment capable of biodegrading the polymer being tested.

6. Apparatus

6.1 *Hood*, allowing for radiochemical work should be available with a face velocity of approximately 100 cubic feet/min.

6.2 *Micro Balance*, (accurate to ± 0.1 mg) should be available for weighing out radiolabeled materials.

6.3 *Liquid Scintillation Counter*, is needed for determination of the DPM of the recovered radiolabeled carbon dioxide. A multiple or single sample counter can be used.

6.4 *Oxidizer*, capable of combusting weighed samples of the test material to CO_2 may be necessary if the specific activity reported by the manufacturer needs to be verified.

6.5 *Autoclave*, capable of steam sterilizing seawater is necessary for preparing sterile (negative) controls which are used for volatilization calculations. The autoclave is run at 121°C for 20 min.

7. Reagents and Materials

7.1 *Purity of Reagents*—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specification of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

7.2 *Examples of Reagent Descriptions are as Follows*:

7.2.1 *Ammonium Chloride (NH_4Cl)*, make a 5 % solution, filter sterilize and add to the natural seawater at a 1:100 dilution to give a final concentration of 0.5g/L.

7.2.2 *Potassium Phosphate (KH_2PO_4)*, make a 1 % solution, filter sterilize and add to the natural seawater at a 1:100 dilution to give a final concentration of 0.1g/L.

7.2.3 *Methyl Benzethonium Hydroxide*, a 1.0 molar solution in methanol.

7.2.4 *Hydrochloric Acid (HCl)*, a 4.0 N solution.

7.3 *Serum Bottles (120 ml)*, autoclaved and used for incubation vessels.

7.4 *Buna-n Rubber Serum Bottle Seals (20 mm)*, with a tetrafluoroethylene inner surface (flange type)—see footnote[4].

7.5 *Aluminum Seals and Crimper*, for flange type serum bottle seals.

7.6 *Sleeve Type Red Rubber Serum Stoppers*.

7.7 *Natural Seawater (NSW)*, collected on or before (1 to 2 days) the day the assay is to be initiated. There are no limitations on the collection of this seawater. Avoid collection sites that are directly influenced by storm water runoff or that have major oil slicks on the surface.

7.8 *Plastic Center Wells*, see footnote.

7.9 *Filter Paper Pieces*, (0.5 by 5 mm—cut and fluted).

7.10 *Scintillation Vials*.

7.11 *Scintillation Cocktail*.

7.12 *Syringes and Needles*.

8. Hazards

8.1 This test method requires the use of hazardous materials including hazardous chemicals such as acid and base, hypodermic needles, and radioisiotopes.

8.2 When using chemicals such as the carbon dioxide absorbent (caustic) instructions in the Material Data Safety Sheets must be followed.

8.3 The technique involves the use of radioactive^{14}C compounds and the protocols of Federal and State safe handling and disposal of radioisotopes must be followed.

9. Radiolabeled Test Materials

9.1 The purity of the radioactive compound to be tested shall be established to verify that it is the only radiolabeled compound and that^{14}C carbon is not present in any other material. This verification shall come from the manufacturer who synthesized the compound and also who determines its specific activity (SA).

9.2 If necessary, the specific activity of the radiolabeled compound may be verified by combustion of weighed samples using an oxidizer instrument. A liquid scintillation counter is used for counting the recovered DPM of the combusted sample and the specific activity calculated in accordance with combusted (DPM) (1 mCi)/ (weigh of sample in gram) (2.2×10^9 DPM).

9.3 The test material shall be in a form such as a large grain powder or some shredded form (for example, 1 mm in length) and of uniform thickness which will allow for easy weighing and transfer.

9.4 The radiolabeled compound shall have a specific activity of at least 0.1 µCi/mg and not exceed 5 to 10 µCi/mg. It is suggested that 5.0 and not to exceed to 10.0 mg of radiolabeled compound be incubated per 50 ml of NSW.

[4] The only known supplier of the tetrafluoroethylene faced serum bottle seals is Wheaton, Millville, NJ (product # 24100-175). The only known supplier of the plastic center wells is Kontes Scientific Glassware Co. Vineland, NJ (product #882320-0000).

10. Procedure (Aerobic Natural Seawater)

10.1 Samples of uniformly 14-carbon radiolabeled polymer (5.0 to 10.0 mg) shall be used. If uniformly labeled compounds cannot be synthesized, then selectively labeled carbon polymers shall be used. For samples containing selective labeling, the carbon site(s) of the label shall be determined and stated.

10.2 The samples shall be weighed out in a hood under low exhaust velocity using a micro balance and placed in sterilized 120 ml serum bottles.

10.3 The equivalent amount of radioactivity (µCi) added shall be determined from the weight of material based on the known specific activity (supplied by manufacturer of the radiolabeled polymer).

10.4 For each test polymer a minimum set of six bottles shall receive a 50 ml inoculum of recently collected (1 to 2 days) and pre-aerated (bubbled with air for 20-30 minutes) NSW which has been supplemented with NH_4Cl and KH_2PO_4.

10.5 Another set of six bottles (minimum) for each polymer shall receive the same inoculum of seawater and an addition of 10 grams of aerated sediment, and supplemented with NH_4Cl and KH_2PO_4.

10.6 The bottles are crimp sealed using tetrafluoroethylene faced rubber serum closures and shaken at 100 to 200 RPM. The 70 cc headspace of air ensures that aerobic conditions prevail over the course of incubation.

10.7 Control bottles containing weighed polymer shall receive 50 ml of sterilized seawater (filter sterilized or autoclaved) and allowed to incubate for 48 hours before being assayed for volatile radioactive material which may be trapped by the CO_2 collection procedure. Any radioactive counts recovered by this procedure shall be subtracted from actual test values before calculation.

10.8 Analysis of the radioactive samples shall consist of measuring the amount of radioactive polymer that had been mineralized to $^{14}CO_2$ at various time points. Individual bottles shall be sacrificed at respective time points (for example, days to weeks). Sampling points can be determined by how fast the respiration rate to CO_2 is proceeding. The buna seal shall be quickly replaced with a new sleeve type red rubber stopper holding a plastic center well with a fluted filter paper wick saturated with 0.3 ml Methyl Benzethonium Hydroxide (or other appropriate carbon dioxide trapping agent).

10.9 The samples shall be injected through the serum stopper with 4N HCl sufficient to bring the pH down to 2.5 to 3.0 followed by shaking (200 rpm) for 6 hours during which time the $^{14}CO_2$ was trapped in the filter paper wick. The necessary amount of acid (ë 0.1 to 0.5 ml) to achieve the desired pH shall be predetermined on an equivalent non-radioactive aliquot of NSW.

10.10 After the shaking period, the bottles are opened, the outer surface of the center well shall be wiped and the whole center well cup containing the filter paper is snipped off and placed in a scintillation vial containing 10 ml of scintillation cocktail. The vial is shaken and then counted on a liquid scintillation counter to determine the DPM of radiolabeled CO_2 trapped.

10.11 The amount of polymer that was respired to CO_2 shall be determined knowing the weight of substrate added, the specific activity, and the amount of activity recovered as $^{14}CO_2$. Based upon the recovered (corrected for any volatilization in sterile controls) DPM the actual µCi of polymer substrate respired to CO_2 is calculated and the percent of added polymer carbon respired is determined.

11. Calculation

11.1 *Determining the Total Amount of Radioactivity Added to Each Vessel*:

Total DPM added to each incubation vessel = t-DPM

Total microcuries added to each vessel = t-µCi

where:

one microcurie (µCi) of radioactivity = 2.2×10^6 DPM,

W = weight of polymer added to vessel in mg,

SA = specific activity in µCi/mg (from manufacturer), and

t-µCi = (SA × W).

11.2 *Determining the Percentage of Added Polymer Respired to CO_2*:

Recovered DPM in a test CO_2 collection wick at time t = rt-DPM

Recovered volatile DPM in control CO_2 collection wick at 48 h = rv-DPM

Recovered corrected total DPM at time t = c-DPM (where c-DPM = rtDPM- rv-DPM)

Recovered corrected total microcuries at time t = c-µCi

where:

c-µCi = c-DPM/2.2×10^6)

Percent polymer mineralization to CO_2 at time t = (c-µCi/t-µCi) × 100

12. Interpretation of Results

12.1 This test will specifically measure the amount of carbon for a particular polymer which is respired to carbon dioxide as a result of microbial biodegradation. The resulting percentage of oxidation (as a respiration percentage) indicates the level of biodegradation and will always be less than 100 % since the calculation does not take into account the amount of polymer carbon that is converted into new cellular biomass. Also the final respiration percentages will vary with the particular compound as is known for metabolizable soluble organics (for example, glucose, acetate) as well. The level of biodegradation is optimized by addition of nutrients and in fact may be substantially slower in the actual environment due to limitation by these nutrients.

13. Report

13.1 Specific activity of the polymer.

13.2 Graphic display of percent mineralization for the polymer in comparison to negative (sterile) and positive controls.

13.3 Average and standard deviation of replicates if there are sufficient numbers.

14. ISO Equivalence

14.1 There is no ISO equivalent method at the present time.

15. Precision and Bias

15.1 Data on the Precision and Bias of the test method between laboratories will be determined by a round-robin test

with a target date of August 2002. Experiments with radiolabeled starch are being carried out in triplicate to provide repeatability statement and will be supplied with the round robin test data.