Designation: D7991 – 15

Standard Test Method for Determining Aerobic Biodegradation of Plastics Buried in Sandy Marine Sediment under Controlled Laboratory Conditions[1]

1. Scope

1.1 This test method determines the biodegradation level of plastic materials exposed to laboratory conditions that simulate the environment found in the sandy tidal zone.

1.2 The tidal zone, that is, the part of the coast affected by the tides and movement of the waves, is the borderline between sea and land, frequently a sandy area that is kept constantly damp by the lapping of the waves. Stony and rocky shorelines also exist.

1.3 Plastic marine debris is frequently washed up in this habitat where it must be removed in order to restore the original landscape.

1.4 It is of interest to know the biodegradation behavior of plastics when exposed to conditions simulating this habitat, because this information can help in predicting the time needed for the biodegradation of the litter.

1.5 Biodegradation is determined by measuring the CO_2 evolved by the plastic material when exposed to a sediment kept wet with salt-water in a reactor, to simulate the tidal zone.

1.6 Marine fresh-water habitats (for example, those found in brackish waters and estuaries) are not considered by this standard.

1.7 Reports shall clearly state the percentage of net CO_2 generation for both the test and reference samples at the completion of the test. Furthermore, in the laboratory reports, the results shall not be extrapolated beyond the actual duration of the test.

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

1.8 *Units*—The values stated in SI units are to be regarded as standard. No other units of measurement are included in this 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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D5988 Test Method for Determining Aerobic Biodegradation of Plastic Materials in Soil

2.2 *ISO Standards:*[3]

ISO 8245 Water quality Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)

3. Terminology

3.1 *Definitions:*

3.1.1 *tidal zone, n*—the part of the marine environment that extends from the high tide line, which is rarely inundated with water, to the low tide line, which is typically always covered with water.

3.1.1.1 *Discussion*—Synonyms are: eulittoral zone, midlittoral zone, mediolittoral zone, intertidal zone, foreshore.

4. Summary of Test Method

4.1 This test method consists of the following:

4.1.1 Selection of plastic material for the determination of aerobic biodegradation in a controlled laboratory system.

4.1.2 Obtaining sediment and seawater from the shoreline.

4.1.3 Exposing the plastic material to the wet sediment under controlled conditions.

4.1.4 Measuring CO_2 evolved as a function of time.

4.1.5 Assessing the degree of biodegradation by determining the percentage of organic carbon in the plastic material that is converted to CO_2 during the duration of the test. This percentage represents the percentage of mineralization and will

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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.

[3] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

not include the amount of carbon converted to cell biomass that is not in turn metabolized to CO_2 during the course of the test.

4.1.6 Estimating the qualitative disintegration of the test material by visual inspection at the end of the test.

5. Significance and Use

5.1 Plastic is sometimes carried by rivers or accidentally discharged by ships into the sea; this plastic can then reach different parts of the marine environment. Tides and waves also frequently deliver plastic marine debris into the sandy tidal zones.

5.2 This test method simulates the environmental conditions found in the tidal zone. Plastic debris that reaches the sandy tidal zone can settle there and become partially or totally buried by sand and kept wet by waves or tides. It is of interest to assess the biodegradation behavior of plastic materials under these conditions to predict the removal time of this waste in the environment.

5.3 This test method is applied to determine the extent of biodegradation of a plastic exposed in the laboratory to a sandy sediment kept wet with seawater. Both sediment and seawater are collected from a sandy beach in the tidal zone. If the natural microbial population present in the sediment is able to biodegrade the plastic, there will be an evolution of CO_2 as a consequence of the aerobic microbial respiration. The level of biodegradation at any given time is the ratio between the cumulative amount of the evolved net carbon dioxide and the theoretical amount produced in the case of total conversion of the organic carbon present in the plastic into carbon dioxide.

5.4 This test method does not measure the amount of organic carbon that is converted into biomass, but only the biodegradation that leads to mineralization (that is, the formation of CO_2).

6. Apparatus

6.1 *Reactor*—Glass vessel approximately 2 to 4-L internal volume that can be sealed air-tight, such as 150-mm desiccators, with an airtight opening, large enough to allow the handling of the content. Biometer flasks are also appropriate. A suitable apparatus is shown in Figure 1 in Test Method D5988. Reactors with higher volumes can be used, if environmental conditions are not affected.

6.2 *Container for the CO_2 Absorber*—A glass beaker to be located in the headspace of the reactor and filled with 100 ml of $Ba(OH)_2$ 0.025 N or with 30 mL of KOH 0.5 N.

6.3 *Darkened Chamber or Cabinet,* in which the temperature can be maintained at a constant level within a ±2°C range.

NOTE 2—Incubator with either built in lights that can be programmed or else plug in lights that can be operated with a timer power strip can be used to better simulate the environment. The lighting in that case need to be 12:12 day/night. Details on the lighting regime, light intensity, wave length, incubator type, etc. shall be provided in the report.

6.4 *Analytical Balance,* to weigh the test specimen.

6.5 *Technical Balance,* to weigh reactors and sediment.

6.6 *pH Meter.*

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 specifications of the Committee on Analytical Reagents of the American Chemical Society where such specifications are available.[4] It is acceptable to use other grades 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 *Barium Hydroxide Solution (0.025 N),* prepared by dissolving 4.0 g anhydrous $Ba(OH)_2$/L in distilled water. Filter free of solid material and store sealed as a clear solution to prevent absorption of CO_2 from the air. It is recommended that 2 to 4 L be prepared at a time when running a series of tests. Confirm normality by titration with standard acid before use. When using $Ba(OH)_2$, however, care must be taken that a film of $BaCO_3$ does not form on the surface of the solution in the beaker, which would inhibit CO_2 diffusion into the absorbing medium. Alternatively, potassium hydroxide solution (KOH, 0.5 N) could be used and is prepared by dissolving 28 g of anhydrous KOH/L in distilled water and proceeding in the same way as for the $Ba(OH)_2$ solution.

7.3 *Hydrochloric acid,* 0.05 N HCl when using 0.025 N $Ba(OH)_2$ or 0.3 N HCl when using 0.5 N KOH.

7.4 *Sediment*—Collect seawater and sediment samples from the shoreline of a sandy beach, where the sediment is submerged in the shallow water. Collect top sediment (the layer from surface till about 20 cm depth). It is important to obtain sediment from multiple samples from the same location (at least 3). Collect the seawater with a bucket and then collect sediment samples with a shovel in separate containers overlain with water, then transfer all samples to a watertight container and quickly deliver it to the laboratory. Remove any obvious plant material, shells, pieces of driftwood, petroleum tar, and other large material. Store the sediment and seawater at approximately 4°C until use. Allow air exchange at time to avoid anaerobiosis. Use preferably within four weeks of sampling. Report the storage times. Before use, perform gravity filtration on the sediment in a funnel with a coarse filter paper to remove excess water. Sediment is ready for testing when seawater is no longer recovered from the filtration. Nitrogen sources (such as NH_4Cl or $NaNO_3$) can be added to the sediment if this is considered as a factor limiting biodegradation. These additions shall be reported in the test report.

NOTE 3—No data are available at this stage indicating that a specific nitrogen level is beneficial for the biodegradation process.

7.5 *Plastic Material*—Determine the total organic carbon both of the test material and the reference material using ISO 8245 and report it, preferably, as grams of total organic carbon per gram of total dry solids. Alternatively, provided the materials do not contain inorganic carbon, it is possible to

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[4] Reagent Chemicals, American Chemical Society Specifications, American Chemical Society, Washington, DC. For suggestions on the testing of reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BDH Ltd., Poole, Dorset, U.K., and the United States Pharmacopeia and National Formulary, U.S. Pharmacopeial Convention, Inc. (USPC), Rockville, MD.

determine the carbon content by elemental analysis. The test material shall have sufficient organic carbon to yield CO_2 in an amount suitable for the determination.

7.6 *Reference Material*—A cellulose filter paper[5] for laboratory purposes. Determine the carbon content as described in 7.5.

7.7 *Negative Control Material (optional)*—A polyethylene film. Determine the carbon content as described in 7.5.

7.8 *Test Samples*—It is preferable that the plastic material is in the form of film or plate. Cut out square-shaped samples with a dimension of approximately 5 cm. Likewise prepare square-shaped samples of reference material and negative control material. Record the mass of each sample.

NOTE 4—It is acceptable that the test material be introduced as powder. Mix the powder homogeneously with the sediment. Refer to ISO 10210 for preparation of powder from plastic materials. Furthermore, report data showing that milling has not changed the chemical structure of the test material.

NOTE 5—It is acceptable that the test material be introduced as a perforated film or plate in order to facilitate gas exchange.

8. Hazards

8.1 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.

9. Procedure

9.1 *Test Set-up*—Prepare at least the following number of reactors (6.1): a) three reactors for the test material; b) three reactors for the reference material; c) three reactors for negative control (optional); d) three reactor for the technical control (optional); and (e) three reactors for the blank.

NOTE 6—Two replicates are sufficient for screening purposes.

NOTE 7—The technical controls contain only the absorbing solution and no sediment. The ambient air which fills the headspace of all the vessels introduces CO_2 into the system. The technical controls allow accounting for and subtracting this introduced CO_2. Additionally, the technical controls indicate the air-tightness of the vessel system by showing possible infiltration of CO_2 into the sealed vessel.

9.2 *Preliminary Phase:*

9.2.1 Place an equal volume (between 200 and 600 g) of wet sediment (see 7.4) in the bottom of each reactor. In a typical case, weigh out 400 g of wet sediment and place it into the bottom of the reactor to form a homogenous layer. Do not press or compact the sediment. Introduce a container (6.2) with the CO_2 trapping solution (7.2) to each reactor. Close the reactors and locate them in a room or chamber preferably at a temperature from 15 to 25°C, but not exceeding 28°C. Monitor the CO_2 production (9.4).

9.2.2 This phase is carried out in order to: (i) verify the vitality of the sediment, as shown by the respiration level; (ii) verify that the different reactors have similar background respiration; and (iii) obtain a preliminary oxidation of excess organic matter, so as to start the test with a lower level of endogenous respiration.

9.2.3 This phase is generally carried out for one week. In the case that the CO_2 evolution of a given reactor is significantly different from its replicates, reject the diverging reactor, or in the case of multiple anomalies, restart using new sediment. Report CO_2 evolution and details of this phase on the test report.

9.3 *Start of the Test*—Open the vessels and remove about 100 g of sediment from the layer in the bottom of the reactor. Transfer it in a clean container. Smooth the surface of the residual sediment with a spatula but do not apply pressure. Place 100 mg of plastic material (7.5) or of reference material (7.6) onto the surface of the sediment. The blank reactor will not include any sample. Cover the plastic or reference material with the withdrawn sediment , forming a homogenous layer. Close the vessels tightly. Select a temperature preferably between 15 to 25°C, but not exceeding 28°C, and maintain the selected temperature at ±2°C.

NOTE 8—If the test material is introduced as a powder, mix it homogenously with the sediment

9.4 *CO_2 Analysis:*

9.4.1 The CO_2 produced in each reactor will react with $Ba(OH)_2$ and precipitate as barium carbonate ($BaCO_3$). The amount of CO_2 produced is determined by titrating the remaining $Ba(OH)_2$ with 0.05 N hydrochloric acid to a phenolphthalein end-point or by using an automatic titrator. Because of the static incubation, the ($BaCO_3$) builds up on the surface of the liquid and must be broken up periodically by shaking the container gently to ensure continued absorption of the evolved CO_2. This problem can be avoided by using KOH instead of $Ba(OH)_2$, which does not form a precipitate.

9.4.2 The container for the CO_2 absorber must be removed and titrated before its capacity is exceeded. The period of time will vary with sediments and test materials and increases slowly as the carbon content of the sediment is reduced (a recommended frequency of every 3 to 4 days for the first 2 to 3 weeks and every 1 to 3 weeks thereafter). At the time of removal of the containers, weigh the reactor to monitor moisture loss from the sediment. Allow the reactor to sit open approximately 15 min so that the air in the reactor is refreshed before replacing 100 mL of fresh $Ba(OH)_2$ and resealing the reactor. Add distilled or deionized water periodically to the sediment to maintain the initial weight of the reactor, if needed.

NOTE 9—Distilled/deionized water can adsorb atmospheric CO_2 and become more acidic. To avoid this, boil water and then allow it to cool before use.

NOTE 10—The minimum water content is what the sediment retains after filtration. The initial mass (wet sediment) shall be kept constant by adding distilled or deionized water.

9.4.3 It is possible that the CO_2 evolution will plateau when all of the accessible carbon has been oxidized.

9.4.4 The maximum test duration is typically set at two years, though it is recommended to terminate the test at the CO_2 evolution plateau point. When a constant level of CO_2 release is attained (plateau phase reached) and no further biodegradation is expected, the test is considered to be completed. The test duration can also be less, at the discretion of

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[5] Laboratory filter paper Whatman n. 42 has been found satisfactory for this purpose.

the user. If possible, retrieve the residual test material from the sediment and determine mass loss. Alternatively, the user can extract the residual test material from the sediment with an appropriate solvent, if possible, and quantify.

NOTE 11—Longer durations are possible but this can lead to a higher error in measurements. The practitioner who wishes to extend the duration needs to arrange extra controls to exclude errors and describe them in the final report.

10. Calculation

10.1 *Amount of CO_2 Produced*—The first step in calculating the amount of CO_2 produced is to correct the test material reactors for endogenous CO_2 production. The blank reactors correct for CO_2 potentially produced through endogenous microbial respiration or biodegradation of organic matter in the sediment. The amount of CO_2 produced by a test material is determined by the difference (in mL of titrant) between the experimental and blank containers. The next step is to convert volume of HCl in mL titrated into mg of CO_2 produced.

10.1.1 *$Ba(OH)_2$ Used as a CO_2 Absorber*—When CO_2 enters the absorber containers, it reacts in the following manner:

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3\downarrow + H_2O \tag{1}$$

The $BaCO_3$ formed is insoluble and precipitates. The amount of $Ba(OH)_2$ remaining in solution is determined by titration of the 100 mL with HCl according to the following equation:

$$Ba(OH)_2 + 2\ \ HCl \rightarrow BaCl_2 + 2H_2O \tag{2}$$

From the above two equations, it can be seen that 1 mmol of CO_2 is produced for every 2 mmol of HCl titrated. This means that the number of mmol of CO_2 produced:

$$\text{mmol}\ \ CO_2 = \frac{\text{mmol}\ \ \text{HCl}}{2} \tag{3}$$

The normality of HCl used is 0.05 N. Substituting for mmol gives:

$$\text{mmol}\ \ CO_2 = \frac{(0.05\ \text{N}) \times (\text{mL of HCl})}{2} \tag{4}$$

To convert to mg CO_2, the value must be multiplied by the molecular weight of CO_2 which is 44:

$$\text{mg}\ \ CO_2 = \frac{((0.05) \times \text{mL titrated})}{2} \times 44 = 1.1\ \ \text{mL of HCl titrated} \tag{5}$$

Thus, to convert mL of HCl to mg CO_2, the former is multiplied by 1.1.

10.1.2 *KOH Used as CO_2 Absorber*—The evolved CO_2 will react with KOH in following manner:

$$2KOH + CO_2 \rightarrow K_2CO_3 + H_2O \tag{6}$$

K_2CO_3, the product of reaction (Eq 6) is soluble and does note precipitate.

The fresh KOH solution, where no CO_2 has been absorbed, can be titrated with HCl as:

$$KOH + HCl \rightarrow KCl + H_2O\text{, at pH 7} \tag{7}$$

The KOH solutions used as CO_2 absorbers will have both unreacted KOH and K_2CO_3 in accordance with (Eq 6). During titration both chemical species will react with HCl, as follows:

$$KOH + HCl \rightarrow KCl + H_2O\text{, at pH 7} \tag{8}$$

$$K_2CO_3 + HCl \rightarrow KHCO_3 + KCl\text{, at pH 8.5} \tag{9}$$

The pH shifts of reactions (Eq 7) and (Eq 8) are superimposed and cannot be distinguished. Only a single end point in the range of pH between 7 and 8, corresponding to the two reactions, can be identified by using a suitable indicator.

The adsorbed CO_2 can be determined by subtracting from the H+ equivalents needed to neutralize the original KOH solution and the H+ equivalents needed to neutralize the reactions (Eq 8) and (Eq 9). In practice:

$$\begin{aligned}\text{mmol}\ \ CO_2 = [&\text{mL HCl consumed (Eq. 2)}\\ &-\ \text{mL HCl consumed in (Eq. 8)}\\ &-\ \text{mL HCl consumed in (Eq. 9)}\ \ \text{end point}]*\text{N HCl}\end{aligned} \tag{10}$$

where N is the normality of the HCl solution.

If an endpoint titrator is available the mmol of CO_2 can be determined, without using an indicator, with a further reaction. A further addition of HCl makes HCl react with KHCO3, produced with reaction (Eq 9):

$$KHCO_3 + HCl \rightarrow H_2CO_3 + KCl\text{ at pH 4} \tag{11}$$

The number of equivalent consumed in reaction (Eq 11), and therefore in reaction (Eq 9), corresponds to the K_2CO_3 produced during reaction (Eq 6) that in turn corresponds to the absorbed CO_2. Consequently, 1 mole of $KHCO_3$ corresponds to 1 mole of CO_2 reacted in (Eq 1):

$$\text{mmol}\ \ CO_2 = \text{mmol HCl consumed in (11) end point} \tag{12}$$

Therefore:

$$\text{mmol}\ \ CO_2 = \text{mL HCl consumed in (11)* N HCl} \tag{13}$$

where N is the normality of the HCl solution.

The amount of CO_2 expressed in milligrams is finally obtained as follows:

$$\text{mg}\ \ CO_2 = \text{mmol}\ \ CO_2{*}44 \tag{14}$$

NOTE 12—A discussion on the use of KOH in place of $Ba(OH)_2$ is reported in Modelli et al.[6]

10.2 *Percentage of Biodegradation*—The percentage of biodegradation is the ratio between the evolved CO_2 and theoretical CO_2 ($ThCO_2$). The $ThCO_2$ is:

$$ThCO_2 = \text{specimen (mg)} \times \text{TOC (\%)} \times \frac{44}{12} \tag{15}$$

where:

TOC (%) = TOC of the plastic material (or reference material) divided by 100,
44 = molecular weight of CO_2, and
12 = molecular weight of C.

Therefore:

$$\%\ \text{Biodegradation} = \frac{\text{mg}CO_2\text{produced}}{ThCO_2} \times 100 \tag{16}$$

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[6] Modelli A., Calcagno B., Scandola M. "Kinetics of Aerobic Polymer Degradation in Soil by Means of the ASTM D5988-96 Standard Method," *J. Environ. Polym. Degr.*19997(2): 109-116

11. Validity Criteria

11.1 The reference material is necessary in order to check the activity of the sediment. If, after 180 days, limited biodegradation (<60 %) is observed for the reference, the test must be regarded as invalid and needs to be repeated using fresh sediment.

12. Report

12.1 Report the following data and information:

12.2 Information on the sediment, including source, pH, ash content, TOC, C:N ratio, date of collection, storage conditions, handling, and potential acclimation to test material.

12.3 TOC of the plastic material and reference material.

12.4 Form of the plastic materials.

12.5 Cumulative average CO_2 evolution over time to plateau (or termination), reported and displayed graphically.

12.6 Residual weight of the test material, if determined.

12.7 Percent of theoretical aerobic biodegradation for each plastic material tested and the reference material.

12.8 Temperature range of the test and lighting conditions.

12.9 pH of the sediment, initially and finally.

13. Precision and Bias

13.1 Precision and bias statements for this test method cannot be made at this time. They will be developed during future round-robin testing.

14. Keywords

14.1 aerobic; biodegradation; marine; sandy sediment; tidal

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