Designation: D 5271 – 02

Standard Test Method for Determining the Aerobic Biodegradation of Plastic Materials in an Activated-Sludge-Wastewater-Treatment System[1]

This standard is issued under the fixed designation D 5271; 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 designed to index plastic materials which are more or less biodegradable relative to a standard in aerobic activated-sludge-treatment systems.

1.2 This test method is designed to be applicable to all plastic materials that are not inhibitory to the bacteria present in the activated sludge. Compounds with toxic properties may delay or inhibit the degradation process.

1.3 This test method measures the degree and rate of aerobic biodegradation of plastic materials (including formulation additives which may be biodegradable) on exposure to activated-sludge biomass in the concentration range from 0.1 to 2.5 g/L mixed-liquor volatile suspended solids (MLVSS) under laboratory conditions.

1.4 The high MLVSS concentration relative to other biodegradation tests has the advantage of improved repeatability and increased likelihood of more rapid adaptation or acclimation of the biomass.

1.5 This test method allows for the determination of biological nitrification and the oxidation of other non-carbon components of the plastic.

1.6 This test method does not purport to determine whether or not a plastic material will pass through primary treatment to the aeration basin of an activated-sludge wastewater-treatment plant. The size or density of the plastic material may exclude it from the secondary-treatment stage of a treatment facility.

1.7 This test method is equivalent to ISO 14851.

1.8 *This standard does not purport to address all of the safety problems, 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.* For a specific hazards statement, see Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*

D 883 Terminology Relating to Plastics[2]

D 1193 Specification for Reagent Water[3]

D 1898 Practice for Sampling of Plastics[4]

D 2579 Test Methods for Total and Organic Carbon in Water[5]

D 3593 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Certain Polymers by Liquid Size-Exclusion Chromatography (Gel Permeation Chromatography GPC) Using Universal Calibration[6]

D 5209 Test Method for Determining the Aerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewer Sludge[7]

2.2 *APHA-AWWA-WPCF Standards:*[8]

2540D Total Suspended Solids Dried at 103°–105°C

2540E Fixed and Volatile Solids Ignited at 550°C

2.3 *ISO Standard:*

ISO 14851 Determination of the Ultimate Aerobic Biodegradability of Plastic Materials in an Aqueous Medium - Method by Measuring the Oxygen Demand in a Closed Respirometer[9]

3. Terminology

3.1 *Definitions:*

3.1.1 Definitions of terms applying to this test method appear in Terminology D 883.

[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.

Current edition approved March 10, 2002. Published May 2002. Originally published as D 5271 – 92. Last previous edition D 5271 – 93.

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

[3] *Annual Book of ASTM Standards*, Vol 11.01.

[4] *Annual Book of ASTM Standards*, Vol 08.02.

[5] *Annual Book of ASTM Standards*, Vol 11.02.

[6] *Annual Book of ASTM Standards*, Vol 08.02.

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

[8] *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association-American Water Works Association-Water Pollution Control Federation, 17th Edition, 1989.

[9] Available from American National Standards Institute, 25 W. 43rd St., 4th Floor, New York, NY 10036.

3.1.2 *biological nitrification*—the process by which organic nitrogen or ammonia salts are oxidized to nitrite (NO_2) or nitrate (NO_3), or both, by means of the metabolic pathways of microorganisms.

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *mixed liquid volatile suspended solids (MLVSS)*—the VSS in a completely mixed activated sludge reactor.

3.2.2 *soluble organic carbon (SOC)*—the TOC that is capable of passing through a 0.45-µm pore-size filter.

3.2.3 *theoretical biochemical oxygen demand (BODT)*—the amount of oxygen required for complete biochemical oxidation of a compound.

3.2.4 *total organic carbon (TOC)*—the concentration expressed in milligrams per litre of carbon atoms in solution as determined by Test Methods D 2579.

3.2.5 *volatile suspended solids (VSS)*—the concentration of solids expressed in milligrams per litre in solution as defined by APHA-AWWA-WPCF Standard Method 2540D.

3.3 *Abbreviations:Abbreviation:*

3.3.1 TSS—total suspended solids.

4. Summary of Test Method

4.1 This test method consists of (*1*) selection of plastic material for the determination of aerobic biodegradability, (*2*) obtaining activated sludge from a wastewater-treatment plant and preparing inoculum, (*3*) exposing plastic material to the aerated inoculum, (*4*) measuring oxygen consumed as a result of metabolism of the substrate, soluble organic carbon (SOC) consumption, nitrate and nitrite determinations (when applicable) and residual-polymer weight, and (*5*) assessing the degree of biodegradability.

4.2 Report the percent of theoretical aerobic biodegradation based on measured or calculated carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur content with respect to time and the ultimate amount of biodegradation obtained.

5. Significance and Use

5.1 The degree and rate of aerobic biodegradability of a plastic material in the environment determines to what extent and in what time period that plastic may be eliminated from certain environments. With increasing use of plastics, disposal is becoming a major issue. This procedure estimates the degree and time required to biodegrade plastics in an activated-sludge-wastewater-treatment aeration basin. This test method determines the degree of aerobic biodegradation by measuring the consumption of oxygen due to respiration of the microbial population, as a function of time when the plastic is exposed to an inoculum of activated sewer sludge in the concentration range from 30 mg/L to 1000 mg/L MLVSS. This test method is designed to measure the oxidation of plastics containing carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur, chlorine, and sodium. Changes in the molecular weight and physical characteristics of the polymer after exposure to activated-sludge inoculum can be assessed by other ASTM test methods, such as Test Method D 5209.

5.2 Activated sludge from a sewage treatment plant that treats principally municipal waste is considered to be an acceptable active aerobic inoculum available over a wide geographical area in which to test a broad range of plastic materials. When biodegradation in a specific activated-sludge-wastewater-treatment system is to be determined seed should be collected from that environment. Alternatively, soil or compost suspensions, or both can be used for inoculation, because with some plastic materials the activity of fungi is important for biodegradation.

6. Apparatus

6.1 *Respirometry Apparatus* (see Figs. 1 and 2).

6.1.1 *Cylindrical Glass Vessels*, with a volume of 0.5 to 4.0 L, capable of forming an airtight seal.

6.1.2 *Oxygen-Supply or Generation Device*, that forms an airtight seal with the cylindrical glass vessel. This may be an electrolytic cell that generates oxygen by hydrolysis, or a gaseous-oxygen supply in the form of pure oxygen.

6.1.3 *Carbon Dioxide-(CO_2) Absorbing Material*, placed in the headspace of the sealed cylindrical glass vessel. This may be KOH pellets or a KOH solution sufficient to adsorb all CO_2 generated due to substrate conversion and endogenous respiration. The CO_2 produced by 0.1 g of carbon will be adsorbed by approximately 1 g of KOH. One drop of phenolphthalein solution may be added as an indicator (the KOH solution will turn from pink to clear when the KOH is consumed). If the KOH is consumed prior to the end of the test, the test should be repeated with either a smaller sample size or additional carbon dioxide-scrubbing capacity.

6.1.4 *Oxygen Sensor/Regulator*, that measures the oxygen demand in the cylindrical glass vessel and regulates the amount of oxygen supplied to the respirometer.

6.1.5 *Recorder*, that measures and records the amount of oxygen generated or supplied to the respirometer. This may be

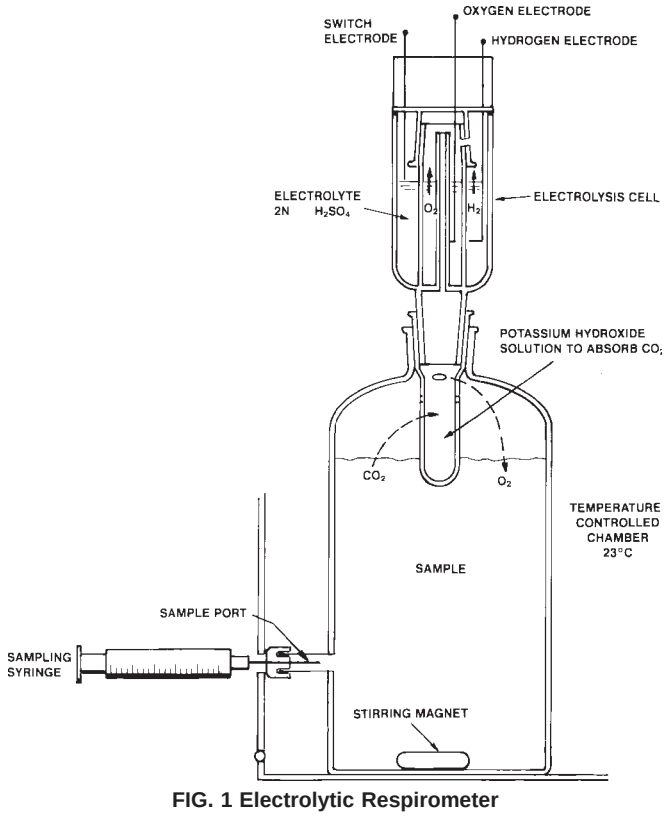

FIG. 1 Electrolytic Respirometer

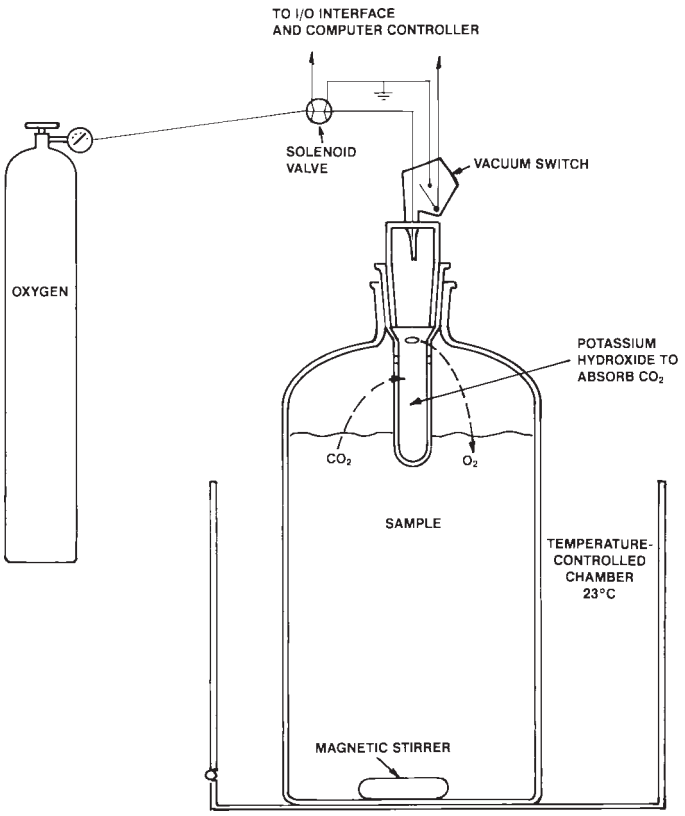

FIG. 2 Pneumatic Computerized Respirometer

an analog- or a digital-recording device such as a strip-chart recorder or a computer.

6.1.6 A means of stirring the solution to maintain the MLVSS in suspension. This may be a magnetic stir bar, mechanical stirrer, or agitation by circulating the gaseous or liquid phases.

6.2 *Analytical Equipment*, to measure soluble organic carbon before and after the experiment is concluded (Test Methods D 2579). Optionally, analytical equipment such as an ion chromatograph to measure the nitrite and nitrate concentration at the beginning and end of the run may be used.

6.3 *pH Meter*:

6.4 *Analytical Balance*, to weigh the test specimen before and after exposure.

6.5 *Membrane Filters* (0.45-µm pore size) that do not generate or absorb SOC.

6.5.1 The filter can be shown to not generate SOC by comparing the TOC of high-quality water before and after it passes through the filter.

6.5.2 The filter can be shown not to absorb SOC by comparing the TOC of a centrifuged sample to the TOC of a filtered sample.

6.6 *Centrifuge*, capable of at least 1 000 *g* but less than 100 000 *g* at the tip of the centrifuge tube.

$$g = \frac{\omega^2 r}{981} \quad (1)$$

where:

g = acceleration, cm/s^2,

ω = angular velocity, rad/s,

$\omega = \dfrac{\text{r/min}}{60 \text{ s/min}} \times 2\pi$ rad, and

r = radius to tip of centrifuge tubes, cm.

7. Reagents and Materials

7.1 *High-Quality Water* (see Specification D 1193), free of toxic substances (copper, in particular), with low-carbon content (<2.0 mg/L SOC) and with resistivity >18 MΩ/cm. The water must never contain more than 10 % of organic carbon introduced by the test material.

7.2 *Phenophthalein Solution*, 1 % phenolphthalein in ethanol.

7.3 *Stock Solutions*, for test medium, each made up in high-quality water. For simulating a natural environment use the standard test medium. If a test material is used at higher concentrations (up to 2000 mg/L), use the optimized test medium which contains higher buffering capacity and nutrient concentrations.

7.3.1 *Standard Test Medium*

7.3.1.1 *Allylthiourea*, $CH_2CHCH_2NHCSNH_2$, 2.3 g/L.

7.3.1.2 *Calcium Chloride*, $CaCl_2.2H_2O$, 36.4g/L.

7.3.1.3 *Ferric Chloride*, $FeCl_3.6H_2O$, 0.25 g/L.

7.3.1.4 *Magnesium Sulfate*, $MgSO_4.7H_2O$, 22.5 g/L.

7.3.1.5 *Phosphate Buffer*, KH_2PO_4, 8.5 g/L; K_2HPO_4, 21.75 g/L; $Na_2HPO_4.2H_2O$, 33.4 g/L; and NH_4Cl, 0.5 g/L.

7.3.2 *Optimized Test Medium*

7.3.2.1 *Allylthiourea*, $CH_2CHCH_2NHCSNH_2$, 2.3 g/L.

7.3.2.2 *Calcium Chloride*, $CaCl_2.2H_2O$, 36.4 g/L.

7.3.2.3 *Ferric Chloride*, $FeCl_3.6H_2O$, 0.25 g/L.

7.3.2.4 *Magnesium Sulfate*, $MgSO_4.7H_2O$, 22.5 g/L.

7.3.2.5 *Phosphate Buffer*, KH_2PO_4, 37.5 g/L; $Na_2HPO_42H_2O$, 87.3 g/L; and NH_4Cl, 2.0g/L.

7.3.2.6 *Trace-Element Solution (optional)*, dissolve in aqueous HCl (25%, 7.7 mol/L) in the following sequence: 70 mg $ZnCl_2$, 100 mg $MnCl_2.4H_2O$, 6 mg H_3BO_3, 190 mg $CoCl_2.6H_2O$, 3 mg $CuCl_2.2H_2O$, 240 mg $NiCl_2.6H_2O$, 36 mg $Na_2MoO_4.2H_2O$, 33 mg $Na_2WO_4.2H_2O$ and 26 mg $Na_2SeO_3.5H_2O$, and make up to 1000 mL with water.

7.3.2.7 *Vitamin Solution (optional)*, dissolve in 100 mL of water 0.6 mg biotine, 2.0 mg niacinamide, 2.0 mg *p*-aminobenzoate, 1.0 mg pantothenic acid, 10.0 mg pyridoxal hydrochloride, 5.0 mg cyanocobalamine, 2.0 mg folic acid, 5.0 mg riboflavin, 5.0 mg DL-thioctic acid and 1.0 mg thiamine dichloride or use a solution of yeast extract in 100 mL water. Filter the solution for sterilization using membrane filters.

7.3.3 *Sodium Hydroxide*, 1 N, NaOH, 40 g/L.

7.3.4 *Sulfuric Acid*, 1 N, H_2SO_4, 28 mL/L.

7.4 Prepare the standard test medium (7.3.1) so that it contains the following stock solutions in 1 L of high-quality water: 1 mL ferric chloride solution, 1 mL magnesium sulfate solution, 1 mL calcium chloride solution, and 10 mL phosphate buffer solution.

7.4.1 If nitrification inhibition is desired add 20 mL allylthiourea solution. Allylthiourea can only inhibit nitrification during short periods, because it is biodegradable. However,

with low inoculum concentration (about 1% v/v) nitrification will not occur, even during long incubation periods without inhibitor.

7.5 Prepare the optimized test medium (7.3.2) so that it contains the following stock solutions in 1 L of high-quality water: 1 mL ferric chloride solution, 1 mL magnesium sulfate solution, 1 mL calcium chloride solution, 1 mL trace-element solution, 1 mL vitamin solution, and 100 mL phosphate buffer solution.

8. Hazards

8.1 This test method includes the use of hazardous chemicals. Avoid contact with chemicals and follow manufacturers' instructions and Material Safety Data Sheets.

8.2 This test method includes the use of hazardous material from a waste-treatment plant. Avoid contact with the sludge by using gloves and other appropriate protective equipment. Follow good laboratory practices and use good personal hygiene to minimize exposure to harmful microbial agents.

9. Inoculum Test Organisms

9.1 The source of the test organisms is activated sludge freshly sampled from a well-operated municipal-sewage treatment plant. This sewage-treatment plant should receive no toxicants that inhibit or show adverse effects to the biomass. Alternatively, soil or compost suspensions, or both can be used for inoculation, because with some plastic materials the activity of fungi is important for biodegradation. A mixture of inocula from these sources may be used.

NOTE 1—If biodegradation in a specific treatment system is to be tested, activated sludge from that municipal or industrial sewage treatment plant preferably should be used and referenced in the results.

9.2 Aerate activated sludge in the laboratory for 4 h. Determine the VSS in accordance with APHA-AWWA-WPCF Standard Methods 2540D and 2540E. Use within 72 h.

9.3 Adaptation of the biomass may be desirable to provide increased reproducibility. The adaptation conditions and period must be reported in 14.1.1.

9.4 Calculate the amount of seed-bacteria MLVSS to be added to each vessel:

$$\text{volume of } MLVSS, \text{mL} = \frac{\text{desired } VSS \times \text{media volume}}{\text{measured } VSS \text{ (see 9.2)}} \qquad (2)$$

NOTE 2—Desired VSS must fall within the range from 30 to 1000 mg/L. (For example, if the desired VSS = 500 mg/L and the media volume was to be 1000 mL, if 2500 mg/L was the measured VSS, then 200 mL of MLVSS would be required.) When biodegradation processes in a natural environment are to be simulated or when a carbon balance determination is to be carried out, an inoculum concentration of 30 mg/L suspended solids is recommended.

9.5 Suspend 10 g of non-sterile, fertile soil or compost from a composting plant treating predominantly organic waste in 100 mL or test medium. Allow suspension to settle for about 30 min. Decant and filter the supernatant liquid through a coarse porous filter. Add to the test vessels to obtain a concentration of 1% v/v to 5% v/v in the test medium.

10. Test Specimen and Reference Materials

10.1 The test specimen should be of known mass and contain at least 60 mg/L. Use lower concentrations only if the sensitivity of the respirometer is adequate. The maximum amount of test material is limited by the oxygen supply to the respirometer and the test material used. When using the optimized medium, the test material concentration shall be such that the TOC does not exceed 2,000 mg/L.

10.2 The test specimens may be in the form of films, pieces, fragments, or formed articles.

10.3 Determine the carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur content of the sample by an appropriate analytical technique if it is not known from the formulation.

10.4 Optionally, record molecular weight of the polymer in the appropriate section.

10.5 Use analytical-grade cellulose for thin-layer-chromatography as a positive reference in order to check the activity of the inoculum.

10.6 A reference negative control substance of polyethylene in the same form as the test substance may be used to determine any physical effects, of the plastic added, on the operation of the test.

11. Procedure

NOTE 3—This procedure is written for a 1000-mL media volume in a 1200-mL vessel. The procedure should be scaled proportionally to the volume desired. A control blank that includes all components except the test specimen and a positive control such as starch or cellulose shall be run with each set. A negative control such as polyethylene is optional. It is recommended that all specimens and controls be run at least in triplicate.

11.1 Add approximately 600 mL of high-quality water to each vessel. Allow the water to warm to room temperature (23 ± 2°C) with gentle mixing.

11.2 To each vessel add: 1 mL ferric chloride solution, 1 mL magnesium sulfate solution, 1 mL calcium chloride solution, and 10 mL phosphate buffer solution. If nitrification inhibition is desired add 20 mL allylthiourea solution.

NOTE 4—If the pH at the end of the run has varied more than 1 unit additional buffering may be required.

11.3 Add a representative sample (see Practice D 1898) to be tested to each vessel; then adjust the pH to 7.0 ± 0.2 pH units using 1 *N* sulfuric acid or 1 *N* sodium hydroxide solutions. The sample should be in the form in which it will be introduced to the activated-sludge environment.

11.4 Add the volume of MLVSS calculated in 9.4 to achieve the desired VSS concentration. Add sufficient high-quality water to bring the media volume to 1000 mL.

11.5 Stir the sample such that the bottom of the vortex is approximately 10 to 20 % of the vessel height from the bottom. If necessary adjust the pH of the media to 7.0 ± 0.2 pH units. The pH of he cells should be adjusted using 1 *N* sulfuric acid or 1 *N* sodium hydroxide solutions.

11.6 Remove a known volume of sample from each cell of sufficient size to perform a soluble organic carbon analysis and optional nitrite/nitrate analysis. Samples should be immediately filtered through a 0.45-µm pore-size membrane. Record the volume removed and use this to adjust the solution volume reported in 14.1.5.

11.7 Assemble the respirometry apparatus and begin to monitor oxygen consumption. Oxygen consumption should be recorded at least every 4 h.

11.8 Maintain cell temperature at 23 ± 2°C, in dark or diffused light.

NOTE 5—Activated-sludge-aeration basin temperatures typically range from 10 to 25°C, depending upon geographical location, season, and treatment-system size. Since temperature may have an effect on the degree and rate of plastic degradation the test may be modified to evaluate biodegradation at nonstandard temperatures. Any additional nonstandard temperatures used to evaluate biodegradation should be reported in 14.1.5.

11.9 Allow the run to proceed for a minimum of 4 to 6 weeks and a maximum of 6 months. The sample is completely metabolized when the oxygen consumption of the sample minus the oxygen consumption of the control is equivalent to the theoretical oxygen consumption due to the substrate (13.3). Once the sample has been at least 90 % metabolized the run may be terminated if the oxygen consumption has leveled off and not changed significantly over 72 h. If an acclimation period of more than 72 h is required report this in 14.1.5.

11.10 Measure and record the pH at the end of the test.

11.11 Remove a known volume of sample from each cell of sufficient size to perform a soluble-organic-carbon analysis and optional nitrite/nitrate analysis. Immediately filter samples through a 0.45-µm pore-size membrane.

11.12 Filter the contents of each vessel through a 50 to 100-mesh pore-size membrane to remove remaining insoluble plastic, which is washed, dried and weighed. Molecular weight may also be measured.

11.13 The test is considered valid if the degree of biodegradation of the reference material is > 60 % at the end of the test, and the BOD of the blank at the end of the test does not exceed an upper limiting value obtained by experience (this value depends on the amount of inoculum and is, for example, in the case of 30 mg/L dry matter, about 60 mg/L).

12. Calculation

12.1 If not known determine the chemical composition (C_c H_h O_o N_n P_p S_s Na_{na} X_x, where X = chlorine, bromine, and fluorine) of the test material from elemental analysis. This allows the theoretical quantity of biochemical oxygen demand to be calculated as follows:

$$\frac{BODT,\ \text{mg}\ O_2}{\text{mg test material}} = \frac{16[2c + 0.5(h - x - 3n) + 3s + 4p + 0.5na - o]}{\text{molecular weight}} \quad (3)$$

NOTE 6—This calculation implies that carbon is oxidized to carbon dioxide, hydrogen to water, phosphorus to PO_4, sulfur to an oxidation state of + 6, and halogens are eliminated as hydrogen halides and nitrogen as ammonia.

12.1.1 *Theoretical Oxygen Consumed Due to Nitrogen*:

NOTE 7—In case of nitrogen-containing compounds the nitrogen may be eliminated as ammonia, nitrite, or nitrate corresponding to different theoretical biochemical-oxygen demands. If allylthiourea is not added nitrification may occur. Nitrification consumes oxygen in a two-step process.

(1) Nitrite production by *Nitrosomonas* $NR_3 + O_2 \rightarrow NO_2$

(2) Nitrite production by *Nitrosomonas* $NO_2 + O_2 \rightarrow NO_3$

To calculate the amount of oxygen consumed by nitrification the initial and final sample must be analyzed for nitrite and nitrate.

12.1.1.1 Calculate oxygen consumed due to partial nitrogen oxidation to nitrite as follows:

$$\frac{BOD_{NO_2}\ \text{mg}\ O_2}{\text{mg test material}} = \frac{48\ n}{\text{molecular weight}} \quad (4)$$

12.1.1.2 Calculate oxygen consumed due to complete nitrogen oxidation to nitrate as follows:

$$\frac{BOD_{NO_3}\ \text{mg}\ O_2}{\text{mg test material}} = \frac{64\ n}{\text{molecular weight}} \quad (5)$$

12.1.2 *Maximum Theoretical Oxygen Demand Due to Aerobic Biodegradation of the Substrate*:

12.1.2.1 If nitrification does not occur the maximum theoretical oxygen consumption due to substrate = BODT.

12.1.2.2 If complete nitrification occurs the maximum theoretical oxygen consumption due to substrate = BODT + BOD_{NO_3}.

12.2 Determine the amount of oxygen consumed in the metabolism of the test specimen by subtracting the oxygen consumed by the control blank from the oxygen consumed by the sample.

NOTE 8—If the apparatus in Fig. 1 is used the device is affected by barometric pressure fluctuations. Therefore, the barometric pressure at the beginning and the end of the run should be measured and the oxygen consumption adjusted according to the following formula:

Add the following correction to the oxygen consumption;

$$\text{mg}\ O_2\ \text{correction} = \frac{13.01\ VP}{T} \quad (6)$$

where:

V = volume of air space in reactor and oxygen generator that is sealed off from the atmosphere, mL,

P = barometric pressure change from beginning to end of run (in. of mercury), and

T = temperature, K (273 + °C).

12.3 Calculate the percent of theoretical aerobic-biological-oxygen demand.

12.3.1 If nitrification does not occur calculate the percent theoretical biological-oxygen demand as follows:

$$\text{Theoretical } BOD,\ \% = \frac{\text{oxygen consumed, mg}\ O_2}{BODT \times \text{mg substrate}} \times 100 \quad (7)$$

12.3.2 If complete nitrification occurs calculate the percent theoretical biological-oxygen demand as follows:

$$\text{Theoretical } BOD,\ \% = \frac{\text{oxygen consumed, mg}\ O_2}{(BODT + BOD_{NO_3}) \times \text{mg substrate}} \times 100 \quad (8)$$

13. Interpretation of Results

13.1 Information on toxicity of the plastic material may be useful in the interpretation of low results.

13.2 If at least 60 % biodegradation is not observed with the reference, the test must be regarded as invalid and should be repeated using fresh inoculum.

13.3 The plateau level of oxygen consumption minus the control blank, together with the weight of specimen remaining and the measured soluble-organic-carbon content, will suggest the degree of biodegradability of the plastic material. The percent of theoretical biochemical oxygen demand can also be calculated.

13.3.1 If no nitrification occurs, the percent theoretical oxygen demand is equal to the following times 100.

$$\frac{\bar{X}, \text{mg } O_2 \text{ consumed} - \bar{X}, \text{mg } O_2 \text{ uptake by controls}}{BODT, \text{mg } O_2 \times \text{mg substrate}} \quad (9)$$

13.3.2 If complete nitrification occurs the percent theoretical oxygen demand is equal to the following times 100.

$$\frac{\bar{X}, \text{mg } O_2 \text{ consumed} - \bar{X}, \text{mg } O_2 \text{ uptake by controls}}{(BODT, \text{mg } O_2 + BOD_{NO_3}) \times \text{mg substrate}} \quad (10)$$

13.4 The wettability of the plastic material may influence the results obtained, and hence the procedure may be limited in comparing plastic materials of different chemical structure.

14. Report

14.1 Report the following information:

14.1.1 Information on the inoculum, including source, percentage volatile solids, date of collection, storage, handling, and potential acclimation to test material.

14.1.2 Carbon content and elemental formula of the plastic material.

14.1.3 Cumulative oxygen consumption at the end of the run of all vessels, including blank controls, positive controls, and negative controls. These data should also be displayed graphically over time from the start to the end of the run (see Fig. 3).

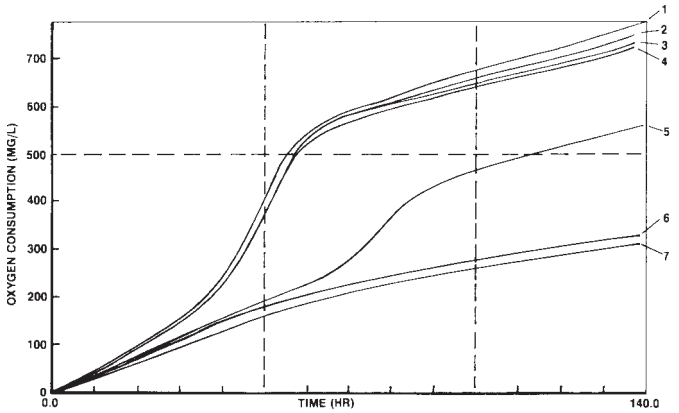

FIG. 3 Plot of Oxygen Consumption Versus Time

14.1.4 Results for all soluble-organic-carbon measurements and nitrite and nitrate measurements where applicable.

14.1.5 All test conditions, including vessel volume, solution volume, VSS concentration, temperature range, initial pH value, final pH value, acclimation time period where applicable, and addition of allylthiourea if nitrification was inhibited.

14.1.6 Percent of theoretical aerobic-biological-oxygen demand for each plastic material tested, including the standard control polymer.

14.1.7 If a more mathematical treatment of the data is required, the cumulative oxygen consumption versus time data can be fitted to a nonlinear regression model to generate rate constants for mineralization and a final extent of biodegradation at infinite time (asymptote, if no plateau is reached, O_2, % = asymptote $(1 - e^{-k \text{ (inclusive time } - \text{ lag)}})$).

14.1.8 Dimensions and form in which each sample was tested and the molecular weight of the plastic material when measured.

14.1.9 Molecular weight of the remaining polymeric material after the test (see Test Method D 3593, optional).

15. Precision and Bias

NOTE 9—Round-robin interlaboratory testing of this test method is being completed under the jurisdiction of Subcommittee D20.96 on Degradable Plastics.

15.1 *Precision*—It is not practicable to specify the precision of the procedure in this test method because of insufficient testing under the specified conditions on an interlaboratory basis. A single laboratory reports the precision to be compound and apparatus dependent, with a standard deviation ranging from 2.75 to 10.2 %.

15.2 *Bias*—It is not practicable to specify the bias of the procedure in this test method because of insufficient testing under the specified conditions on an interlaboratory basis. A single laboratory reports the bias of the test method to be compound and apparatus dependent, with the oxygen uptake ranging from 85 to 105 % of the theoretical oxygen demand when the compound is completely biodegradable.

16. Keywords

16.1 aerobic; biodegradation; municipal; nitrification; plastics; sewage; sludge; waste treatment