Designation: D 6340 – 98

Standard Test Methods for Determining Aerobic Biodegradation of Radiolabeled Plastic Materials in an Aqueous or Compost Environment[1]

This standard is issued under the fixed designation D 6340; 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 These test methods directly determine the rate and degree of biological oxidation of carbon in plastic materials when placed in a composting environment containing simulated municipal solid waste or an aqueous environment under laboratory conditions.

1.2 Test Method A utilizes a mixed culture derived from the target environment (waste water, sewage sludge, compost eluant, and other environmental sources). Temperature, mixing, and aeration are monitored and controlled.

1.2.1 This method has the sensitivity to determine biodegradation at concentrations commonly found in these environments.

1.3 Test Method B starts with fresh compost and proceeds through the normal composting process to an early mature stage. Temperature, aeration; and moisture are monitored and controlled.

1.3.1 This method can determine biodegradation at levels of the plastic commonly expected in municipal solid waste.

1.4 These test methods require that the target component of the plastic material be synthesized using the radioactive isotope carbon-14. Depending upon the objective, either a portion of the components of the plastic or all of the carbon can be uniformly labeled with carbon-14. The test method will determine how that labeled portion will be metabolized and biologically oxidized by the microorganisms in the system tested.

1.5 These test methods can be applied to any carbon-14 labeled compound as well as for plastic materials that have been formulated to biodegrade in a natural aerobic environment.

1.6 The synthesis and preparation of the radiolabled plastic is beyond the scope of these methods. Carbon-14 labeled polymers may be purchased from a number of commercial labs.

1.7 There are no ISO test methods that are equivalent to the test methods in this standard.

1.8 The safety problems associated with compost and radioactivity are not addressed in this standard. It is the responsibility of the user of this standard to establish appropriate safety and health practices. It is also incumbent on the user to conform to all the regulatory requirements, specifically those that relate to the use of open radioactive sources.

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:*

D 883 Terminology Relating to Plastics[2]

D 5209 Test Method for Determining the Aerobic Biodegradation of Plastic Materials in the Presence of Municipal Sewage Sludge[3]

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

D 5338 Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions[3]

D 5512 Practice for Exposing Plastics to Simulated Compost Environment Using an Externally Heated Reactor[3]

3. Terminology

3.1 *Definitions*—For definitions of terms used in these test methods as they relate to composting, see Terminology D 883.

3.1.1 *specific activity, SA, n*—refers to the quantity of radioactivity per mass unit of compound (polymer, etc.), that is *dpmh*%.

3.2 Acronyms:

3.2.1 *Bq, n*—becquerel; SI unit where 1 curie (Ci) = $3.7 \cdot 10^{10}$ Bq.

3.2.2 *dpm, n*—disintegrations per minute, used to measure the quantity of radioactivity.

3.2.2.1 *Discussion*—The measure dpm is derived from counts per minute (cpm) where dpm = cmp-bkgd/counting efficiency. There are $2.2 \cdot 10^6$ dpm/μCi.

3.2.3 *mCi, n*—millicurie; 1/1000th of a curie (standard unit).

[1] These test methods are under the jurisdiction of Committee D20 on Plastics and are the direct responsibility of Subcommittee D20.96 on Degradable Plastics.

Current edition approved Nov. 10, 1998. Published February 1999.

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

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

3.2.4 *µCi, n*—microcurie; 1/1000th of a millicurie.

3.2.5 *MSW, n*—municipal solid waste (organic matter).

4. Summary of Test Method

4.1 Test Method A involves the characterization of the test material, the preparation of the natural mixed culture inoculum, the control of the culture environment, the collection and measurement of radioactive carbon dioxide (CO_2) over time, and the calculation and interpretation of the results. The results may be compared to those obtained from Test Method D 5209.

4.2 Test Method B involves the characterization of the test material, the preparation of the compost matrix, the control of the composting process, the collection and measurement of radioactive CO_2 over time, and the calculation and interpretation of the results. The results may be compared to those obtained from Practice D 5512 as well as Test Method D 5338.

5. Significance and Use

5.1 These test methods can provide direct and unequivocal evidence of aerobic biodegradability. This requires that the radiochemical purity of the plastic is verified using Test Method D 5296.

5.2 These methods also provide the opportunity to determine the rate of biological oxidation in a complete composting environment or aqueous environment by frequent periodic sampling of carbon dioxide.

5.3 These methods provide biodegradation data at use levels of the plastic in a full cycle composting process or an aqueous system.

6. Apparatus

6.1 *Liquid Culture Apparatus*:

6.1.1 Fig. 1 is a diagrammatic representation of a single unit for measuring the carbon-14 carbon dioxide (CO_2) production from the biodegradation of a labeled polymer in aqueous culture. It consists of a fine needle valve for the sensitive control of oxygen flow, a water and culture flask in a controlled temperature environment, a trap to remove water from the gas stream and to insure the carbon monoxide (CO) stays in the gas phase, and a CO_2 absorption column: Periodic CO_2 production over a chosen period of time can be sampled by collecting the CO_2 absorbent from the column at the end of each period by hand, or by automating the CO_2 collection.

6.1.2 Fig. 2 illustrates an eight-unit system with a semi-automated CO_2 collection system based on a timed, automated six-way valve. The gas effluent from the culture flask and acid trap is continuously passed through an absorption column and periodically switched to the next column. Just before the sixth column is due to switch, the five columns are drained and refilled. Soon after the sixth column switches, it is drained and refilled.

6.1.3 Fig. 3 represents a single unit from a fully automated CO_2 collection system where two absorption columns are alternately used to capture the CO_2. While one column is collecting CO_2 from the effluent, the other is drained into a scintillation vial, scintillation cocktail is added to the vial, and the column is refilled with the CO_2 absorbent automatically.

6.1.4 Fig. 4 is a diagrammatic representation of a sixteen-unit, fully automated system. The system is controlled by a personal computer and an 1/0 microprocessor. Valves and metering pumps are powered by electronically-controlled power supplies and relays. Reservoirs of CO_2 absorbent and scintillation cocktail serve all sixteen units. The scintillation vials are in a rack that positions the vials for each sampling period.

6.1.5 Alternative apparatus can be used if it has the capability of maintaining the appropriate temperature, controlling the oxygen flow, humidification of gas flow, and complete collection of CO_2.

6.1.6 Alternate apparatus can be manually operated or controlled by computer interface.

6.2 *Composting Apparatus*:

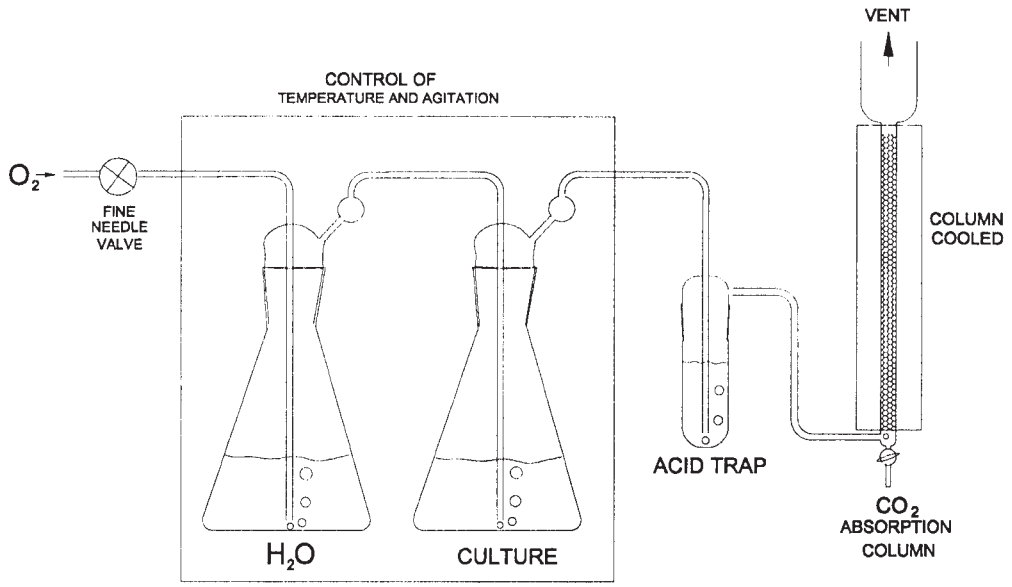

FIG. 1 Single Unit for Measuring ^{14}C CO_2 Production from the Biodegradation of a ^{14}C-Labeled Polymer

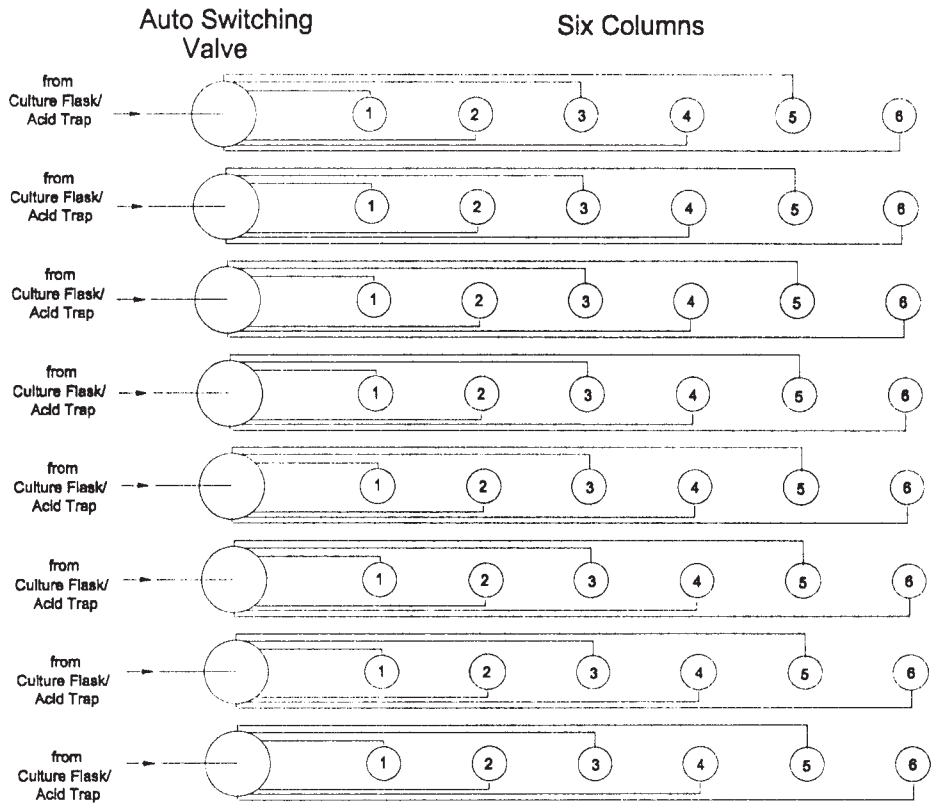

FIG. 2 Semiautomated ^{14}C CO_2 Collection System for Eight Units Over Six Sampling Periods

6.2.1 Fig. 5 is a diagrammatic representation of the radiochemical composting apparatus. The radiochemical composting apparatus consists of a glass composting vessel capped by an inert plastic surface, a controlled humidified air flow, a controlled temperature chamber, a sulfuric acid trap, and a CO_2 absorption column.

6.2.2 The composting vessel is a 1-L borosilicate glass reaction kettle with a glass flange tooled to receive an "O" ring, clamped against an inert plastic surface. Pressurized air, controlled by a needle valve, is passed through a flow meter and then either through a water trap, maintained at the same temperature as the compost, or directly to the compost (25 ± 3 cc/min). The composting vessel is fitted with a central hollow stainless steel shaft that protrudes through a perforated distributor plate at the bottom of the vessel (Fig. 5). The air is passed down the shaft to the space below the distributor plate and then passes up through the compost to the top of the compost where it exits from the vessel. The shaft contains rods projecting perpendicular from the shaft in a radiating fashion. The shaft is connected to a motor that turns the shaft at rate of about 6 r/min. The mixing motion is designed to mix, break up clumps and convey the compost upward. The resultant action tends to circulate the compost in the composting vessel and maintains an even flow of air through the compost.

6.2.3 As air exits the composting vessel, it passes through a check valve and then proceeds through a sulfuric acid trap. The trap dehydrates the air and insures that the CO_2 stays in the gas phase.

6.2.4 The air then passes into a glass column filled with glass helixes and a commercial CO_2 absorber, methoxyethyl amine. The glass helixes break up the gas bubbles and provide greater surface area for the absorption (scrubbing) of CO_2.

6.2.5 The column is jacketed (has an outer glass chamber) where a refrigerant (propylene glycol) is circulated.

6.2.6 A liquid scintillation counter, capable of counting the low-energy beta emitted by the radioactive isotope carbon-14 is used to measure the quantity of radioactivity in the trapped CO_2. An instrument that can automatically measure counting efficiency and correct for quenching is preferred.

6.2.7 It is important to test the system for leaks and insure that the radioactive CO_2 does not escape from the apparatus both for accurate results and safety of personnel.

6.2.8 Vent columns to a radiochemical hood.

6.2.9 Place check valves, that will allow the air flow to travel in only one direction, between the test flasks and the acid and between the acid and absorber.

6.3 *Alternate Composting Apparatus*:

6.3.1 Alternative compost apparatus can be used if it conforms to the following requirements:

6.3.1.1 Although compost vessels can be larger than 1-L, it is generally not practical to exceed 1-L, due to the large volume of CO_2 that is produced and must be completely absorbed.

6.3.1.2 The compost temperature must be controlled.

6.3.1.3 The air supply flow must be controlled and humidified.

6.3.1.4 The air must be stripped of moisture prior to scrubbing to eliminate the two phases that occur when the scintillation cocktail is added to CO_2 absorber that has accumulated too much water.

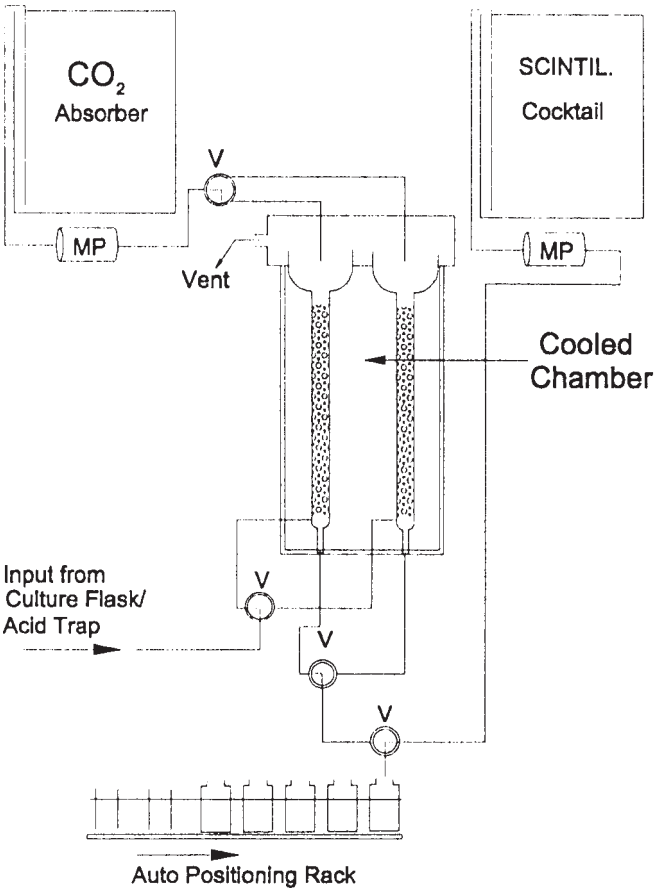

FIG. 3 Single Unit from an Automated ^{14}C CO_2 Collection System (V = valve and MP = metering pump)

6.3.1.5 The scrubbing column must be designed to permit enough air/liquid contact to completely capture the CO_2.

6.3.1.6 The efficiency of the absorption column must be verified. This can be accomplished by placing another column in series and insuring that no radioactivity is trapped in the second column.

6.3.1.7 The CO_2 absorbent must not interfere with the accurate measurement of radioactivity in the liquid scintillation counter by incompatibility with any other reagent.

7. Reagents and Materials

7.1 Compost for this testing may be made up in accordance with either of the following recipes, municipal solid waste (MSW) or woody compost, or obtained from an active municipal solid waste or yard waste composting center.

7.1.1 Compost, designed to stimulate municipal solid waste (MSW) organic matter, is prepared by combining the following materials (on a dry matter basis): alfalfa meal, 35.7 %; shredded newspaper, 27.2 %; garden soil, 13.2 %; poplar sawdust, 10.4 %; cottonseed meal, 6.4 %; cow manure, 1.6 %; calcium carbonate; 5.3 %; sodium bicarbonate, 0.2 %. The mixture is designed to contain the biochemical ingredients found in MSW (lignified cellulose (newspaper, poplar sawdust and alfalfa meal), protein (cottonseed meal and alfalfa meal), natural inoculum (garden soil and cow manure); soluble carbohydrates and buffering capacity sufficient to maintain a neutral to slightly basic pH). The particle size of the components of the compost mix are sized to pass through a 6-mm screen. Moisture content should be adjusted (see 7.1.3) to 55 to 60 % for testing.

7.1.2 An alternate compost (used to simulate yard composting activities) was used in an ASTM-ISR study[4] and has the following composition on a dry matter basis (made up to 55 to 60 % moisture for testing): wood chips (brush), 41.4 %; chapped leaves, 25.4 %; grass clippings, 25.8 %; aged compost, 7.3 %. Different compost preparations can produce different rates of biodegradation. A yard and garden waste compost can, with certain plastics, result in slower rates of degradation.[4]

7.2 The compost is made up to 55 to 60 % moisture by measuring the moisture content of each component, calculating the moisture content of the mix, and adding water to bring the mix to near target moisture percentage. The moisture content can be checked by taking a sample of the compost, weighing it, drying it, reweighing it, and calculating the moisture from the difference in weight. Water can then be added as needed. During the experiment, testing of the moisture content should be checked weekly and maintained at 55 to 60 % by humidifying (bubbling the air through water) the air as needed.

7.3 Liquid culture inoculum are prepared from the target aqueous environment. They can be used directly, that is, sewage sludge or waste water treatment sludge, or eluant from compost, or they can be enriched by growing the mixed culture on the carbon source of interest.

7.4 Media used for liquid culture is related to the environment being simulated. For waste water or sewage sludge, a limited basal media (BAM broth minus glucose) is prepared according to the Handbook of Microbial Media.[5] This media relies on the plastic for the major source of carbon bond energy.

7.5 The carbon dioxide absorber, methoxyethyl amine[6] is used to capture CO_2 in the scrubbing columns.

7.6 A liquid scintillation cocktail designed for counting carbon-14 is required. Any commercial scintillation cocktail reagent that is compatible with the CO_2 absorber is acceptable.[7]

8. Hazards

8.1 Compost presents well-known health risks that can be avoided by the use of appropriate protective equipment such as gloves and masks.

8.2 Strong bases used for CO_2 absorption are particularly hazardous and instructions in Material Safety Data Sheets must be followed.

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[4] ISR Degradable Polymeric Materials Program, *Compilation of ISR Contractor Compost Test Report*, ASTM, 1993.

[5] R.M. Atlas, *Handbook of Microbiological Media*, CRI Press, Inc., 1993, p. 113.

[6] Carbosorb® (Packard Instrument Co.) is a strongly basic short-chain amine is a highly efficient CO_2 absorber and is compatible with many scintillation cocktails. Other organic bases can be used for trapping CO_2 but they generally lack the efficiency of methoxyethyl amine. Inorganic bases have several disadvantages for example, they are strong quenching agents, produce severe color or turbidity interference, and have low trapping capacities.

[7] Although the manufacturer of Carbosorb®, recommends their product Permaflour E+®, other scintillation cocktails are also compatible, including the Beckman Environmental Cocktail Ready Safe®.

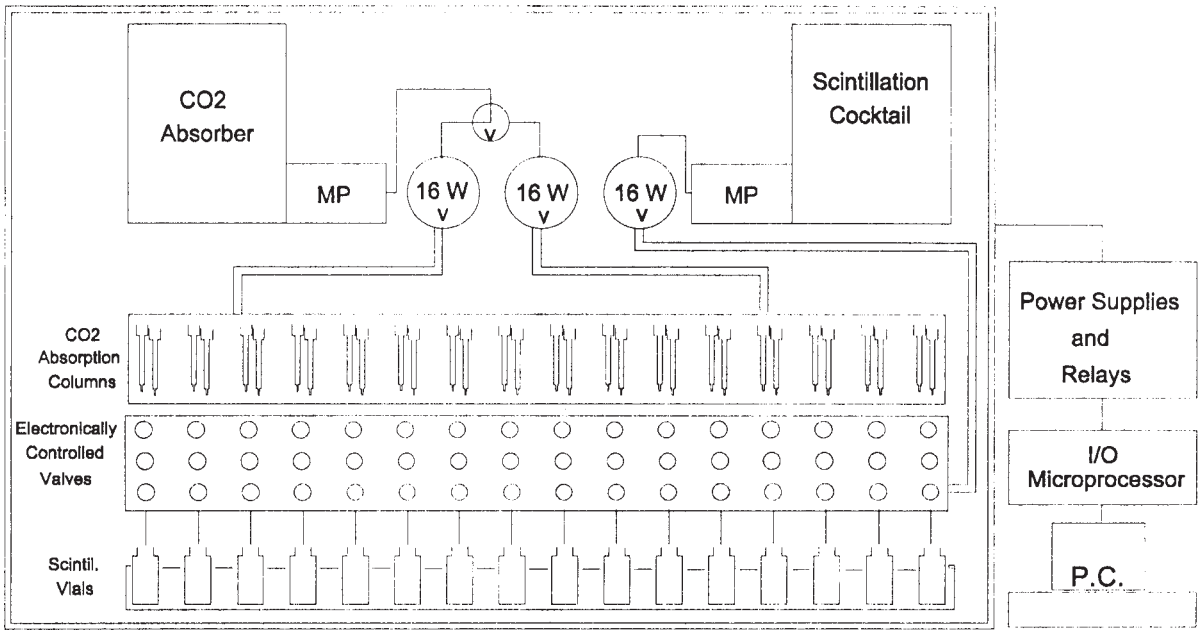

FIG. 4 Fully Automated ^{14}C CO_2 Collection System for Sixteen Units (MP = metering pump, v = valves, 16Wv = sixteen way valves)

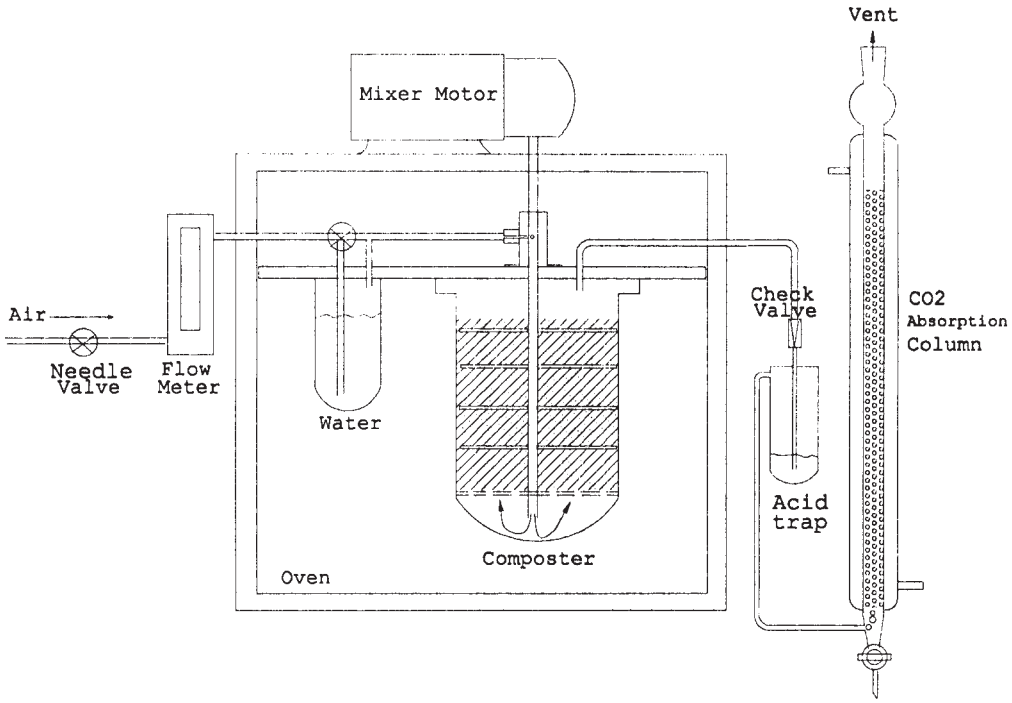

FIG. 5 Automated Radiochemical Composting Apparatus

8.3 Radioactive carbon-14 compounds must be handled in accordance with federal and state regulations.

9. Radiolabeled Preparations

9.1 The utility of tagging the carbon in a molecule or a portion of the molecule with the radioactive isotope of carbon, lies in the ability to detect and distinguish that carbon-14 isotope from a virtual sea of carbon-12 in the compost. This ability to follow or trace that carbon due to its radioactivity requires that the radiochemical purity of the compound be established, that is, verify that only the compound of interest is labeled and no label is present in any other material. This verification requires an analytical technique that can separate the polymer and determine that the radioactivity resides solely in the polymer, and that the structure (NMR analysis) and

molecular weight distribution (see D 5296) are the same as the polymer intended for commercial production.

9.2 Thickness of polymer film can be an important variable for any biodegradation test. Thus, the thickness should be measured by a micrometer and included with any data describing biodegradation rates of polymeric material. Further, in the preparation of such films, take care to produce a film of uniform thickness.

9.3 The radiolabeled plastic should be prepared with an SA of at least 7000 dpm/mg. ASA of 10 000 dpm/mg provides sufficient counts for extended test.

10. Test Method A—Aqueous Environment

10.1 Prepare incubation flasks by adding 75 mL of limited BAM, 2 to 5 mL of inoculum and 50 to 100 mg (>500 000 dpm) of the radiolabeled plastic sample to a 125-mL Erlenmeyer flask.

10.2 Pass oxygen through the water flask and the incubation flask (each maintained at 58 ± 5°C) at a rate of 15 mL/min ± 2 mL/min.

10.3 Shake the flasks at 100 ± 10 rotations/oscillations per min.

10.4 The gasses leaving the incubation flask pass through the acid trap and then through the CO_2 absorption column containing 4 to 5 mL of methoxyethyl amine cooled to >15°C or through a column containing 8 to 10 mL of methoxyethyl amine without cooling.

10.5 Switch the gas flow to another absorption column in intervals from 4 to 8 h, depending on the objectives of the experiment and the methoxyethyl amine from the original column is drained into a scintillation vial.

10.6 Add 10 to 15 mL of scintillation cocktail to the scintillation vial and count the sample in the liquid scintillation counter set to measure the beta radiation of carbon-14.

11. Test Method B—Compost Environment

11.1 Uniformly distribute the radiolabeled plastic sample, cut in pieces approximately 2 cm^2(for film), in approximately 300 g of compost as the compost vessel is filled. Addition of 200 to 300 mg of plastic is sufficient for each composting vessel. Comparative samples should have similar surface areas.

11.2 Partially fill acid traps with concentrated sulfuric acid so as to accommodate the water removed from the airflow.

11.3 Keep the compost vessel and humidifying trap in a 58 ± 5°C chamber. Record the temperature of each composter daily and ensure that it is within 5°C of the chamber. Raise the temperature of the air to 58°C by passing it through a coil in the heated chamber prior to entering the water trap (humidifying trap). Bypass these humidifying traps during the first 2 to 3 days due to the production of large amounts of metabolic water. Sample the composters at 3 days and weekly thereafter to determine the percent moisture of the compost. If the percent moisture drops to 50 % pass the incoming air through the water traps. Control moisture in the compost at 50 to 60 %.

11.4 Fill the CO_2 absorption columns with >200 mL of methoxyethyl amine. Drain and measure the volume, and place a 2-mL aliquot in a scintillation vial with 15-mL scintillation fluid. Count in a Beta counter calibrated to count carbon-14.

NOTE 1—It is important in the early days of the composting process to monitor the viscosity of methoxyethyl amine by observing the bubble flow. The large quantity of CO_2 evolving during this period can cause the methoxyethyl amine to solidify. Usually a daily collection and refill of methoxyethyl amine will be sufficient. In order to reduce the volatility of this short-chain amine, the column can be cooled by circulating refrigerant at temperatures< 15°C through the jacket of the column. If the absorption column is not cooled, an additional 20 % of the absorbant must be used. Generally, absorption columns are completely drained and refilled once a day.

NOTE 2—Occasionally two phases develop due to larger quantities of water trapped in the methoxyethyl amine, and these two phases can be blended into one by the addition of several millilitres of methanol. During prolonged composting trials (over 20 days) the sampling interval can be extended depending upon the objectives of the experiment.

11.5 In order to test whether the oxidation of the carbon in the plastic could occur chemically under these composting conditions, it may be necessary to use a sterile control. This is not necessary if the chemistry of the compound being tested is well documented and it is known that chemical oxidation does not occur under these composting conditions.

12. Calculation

12.1 Total dpm in a composting vessel at the start of the test (ST, dpm) is calculated as follows:

$$ST, \text{ dpm} = (SA)(LP) \quad (1)$$

where:
SA = specific activity in dpm/mg, and
LP = mg of labeled plastic added to the vessel.

12.2 To calculate the quantity of radioactivity (dpm) detected in CO_2 absorbent, measure the volume of methoxyethyl amine drained from the absorption column. Then, measure the radioactivity in a 2-mL aliquot. Employ the following equation:

$$C, \text{ dpm} = [(Al - B)/Cv]/2 \quad (2)$$

where:
C, dpm = total dpm per collection,
Al = dpm in the 2-mL aliquot,
B = background dpm, and
Cv = millilitres from each column collection.

12.3 Calculate the cumulative percent production of carbon-14 carbon dioxide ($^{14}CO_2$) or percent of plastic biologically oxidized (P_x, %) using the following equation:

$$P_x, \% = \Sigma_{1-n}(C, \text{ dpm/ST, dpm})\ 100 \quad (3)$$

13. Interpretation of Results

13.1 The $^{14}CO_2$ measured in these test methods are a direct indication of the oxidation of the sample. However, the extent and the rate of oxidation are related to the compost mixture made for that individual test and the form of the sample. Although different batches of compost can produce different results, the compost formula for the simulation of MSW in these test methods will generally give repeatable results. This is due to the selection of common feed ingredients that are standardized in the trade and tend to have a consistent composition. Depending upon the objectives of the test, it is generally wise to include within each test series a standard preparation of known degradation rates and to make test comparisons.

14. Report

14.1 Report the following information:

14.1.1 Data regarding compost temperature and moisture,

14.1.2 Daily (or periodic) production of $^{14}CO_2$,

14.1.3 Cumulative periodic summation of $^{14}CO_2$,

14.1.4 Graphic display of $^{14}CO_2$ summation data comparing treatments, and

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

15. Precision and Bias

15.1 Measurement of radioisotope labeled (carbon-14) polymer is inherently non-biased since radioactivity is a direct measure of the polymer carbon. However, an accurate true value is determined only when detection efficiencies are high, interferences are avoided, and proper calibration is performed. This can be accomplished routinely with present scintillation counting techniques.

15.2 Data on the precision and bias of these test methods between laboratories are being determined by a round-robin test, and will be available on or before December 2003.