
HS 2001 Biodegradability Test**1. Purpose**

The biodegradability of disposable food/drink containers and bags can be determined by exposure to a controlled composting environment under laboratory conditions. The test method is designed to yield the percentage of conversion from carbon into carbon dioxide in the test material as well as their rate of conversion.

Due to a wide range of variation in the construction and operation of composting systems and their regulatory requirements, the design of this test method is not intended to simulate the environment of any particular composting system. Rather, it is expected to resemble the environment of a composting process operated under optimum conditions. A standard laboratory environment is created in which a rapid and reproducible determination of the aerobic biodegradability under controlled composting conditions can be carried out.

2. Terminology**2.1 Ultimate aerobic biodegradation**

The breakdown of an organic compound by microorganisms into carbon dioxide, water and mineral salts of any other elements present (mineralisation) plus new biomass in the presence of oxygen.

2.2 Aerobic composting

A managed aerobic process that controls the biological decomposition and transformation of biodegradable material into humus like substance called compost.

Note: Compost is an organic soil conditioner obtained by biodegradation of a mixture consisting principally of vegetable residues, occasionally with other organic material, and has a limited mineral content.

2.3 Disintegration

The physical breakdown of a material into very small fragments.

2.4 Total dry solids

The amount of solids obtained by a known volume of test material or compost and drying at about 105 °C to constant mass.

2.5 Volatile solids

The amount of solids obtained by subtracting the residue of a known volume of test material or compost after incineration at about 550 °C from the total dry solids of the same sample.

Note: The volatile solids content is an indication of the amount of organic matter present.

- 2.6 Theoretical amount of evolved carbon dioxide, ThCO₂
The maximum theoretical amount of carbon dioxide evolved after completely oxidising a chemical compound, calculated from the molecular formula and expressed as milligrams of carbon dioxide evolved per milligram or gram of test compound.
- 2.7 Lag phase
The time, measured in days, from the start of a test until adaptation and/or selection of the degrading microorganisms is achieved and the degree of biodegradation of a chemical compound or organic matter has increased to about 10% of the maximum level of biodegradation (see Annex A).
- 2.8 Maximum level of biodegradation
The degree of biodegradation, measured in per cent, of a chemical compound or organic matter in a test, above which no further biodegradation takes place during the test.
- 2.9 Biodegradation phase
The time, measured in days, from the end of the lag phase of a test until $\leq 1\%$ change of the maximum level of biodegradation has been achieved, which is the plateau phase (see Annex A).
- 2.10 Plateau phase
The time, measured in days, from the end of the biodegradation phase until the end of a test (see Annex A).
- 2.11 Inoculum
Microorganisms placed in a composting vessel to start or accelerate biological action.

3. Apparatus

- 3.1 Composting vessels, a series of at least five composting vessels, glass flasks or bottles that allow an even gas purge in an upward direction (three for the test material, one for the reference material and one for the blank). A volume of 2 to 5 litres is required. Depending on the test material, a smaller volume may be used for screening purposes.
- 3.2 Pressurised-Air System, to provide CO₂ free, H₂O-saturated air to each of the composting vessels at pre-set flow rate which would be high enough to give aerobic condition during the test.
- 3.3 Apparatus for the determination of carbon dioxide, to determine the content of carbon dioxide directly or by completely absorption in a basic solution; and to determine the dissolved inorganic carbon (DIC) (see Annex B). If carbon dioxide is measured directly, for example with a continuous infrared analyser or a gas chromatograph, exact control or measurement of the air-flow rate is required.

- Operate the gas chromatograph in conformance with ASTM Practices E260-96^[9] and E355-96^[10].
- 3.4 Gas-tight tubes, to connect the composting vessels with air supply and the carbon dioxide measurement. It should be non-permeable to carbon dioxide.
 - 3.5 Stoppers, equipped with the gas-sampling parts.
 - 3.6 Bottles, at least 5000 ml and containing carbon dioxide scrubbing solution (e.g. NaOH or Ba(OH)₂).
 - 3.7 Water baths, or any other temperature controlling equipment capable of maintaining the temperature of composting vessels at 58 °C ± 2 °C.
 - 3.8 pH meter
 - 3.9 Analytical equipment, for the determination of dry solids (at 105 °C), volatile solids (at 550 °C) (refer to APHA 2540D^[11], 2540E^[12]) and total organic carbon (TOC), for elemental analysis of the test material and; if required, for the determination of dissolved inorganic carbon.
 - 3.10 Analytical balance, with 0.1 mg accuracy to weigh test specimen.

4. Reagents

- 4.1 Carbon dioxide absorbent, analytical grade (e.g. NaOH or Ba(OH)₂).
- 4.2 Polyethylene, as the reference material for negative control. It should be present in the same form in which the sample is tested (e.g. polyethylene film for film samples or polyethylene pellets in case if the sample is in form of pellets).

Note: The use of the negative control is to adhere to international standards and definition on biodegradation and to demonstrate that the test sample truly exhibits significant biodegradation when compared to a known reference of non-biodegradation.

5. Test conditions (temperature and time)

Incubation shall be carried out in a dark or a diffused lighting enclosure or room maintained at a constant temperature of 58 °C ± 2 °C and free from vapour inhibitory to microorganism.

In special cases, e.g. when the melting point of the test material is low, different temperature may be chosen. This temperature shall be kept constant and within ± 2 °C during the test. Any change in temperature should be justified and clearly indicated in the result.

The normal incubation time is 180 days. It may be reduced if no significant CO₂ production in excess of the inoculum is recorded for a period of one week. The test should be regarded as completed if ≤ 1 % change of the maximum level of biodegradation has been achieved. The test period should not be longer than this specified incubation time.

6. Operating procedures

6.1 Preparation of the inoculum

6.1.1 Prepare a compost inoculum with reference to that used by Yu and Zhang ^[16]. This soil composition simulates the composition of municipal solid waste in Hong Kong ^[17]. The weights given below are on a wet-weight (normal moisture content) basis:

Component	Wet-weight, %	Remarks
Cooked rice	18	Steamed only
Cooked meat	2	Steamed and minced only, nothing added
Vegetables	15	Uncooked lettuce
Paper	15	7.5 % tissue paper and 7.5 % writing paper
Garden soil	50	25 % gardening soil and 25 % real soil

The compost inoculum should be two to four months old and well-aerated at 58 °C compost to ensure sufficient diversity of microorganisms.

Note: It is recommended that compost with sufficient porosity be used to enable aerobic conditions to be maintained as much as possible. The inoculum should be screened such that the size of particles and matters are ≤ 10 mm in diameter. Addition of structural material such as small wood particles or inert or poorly biodegradation material may prevent the compost sticking together and clogging during the test. However, the inoculum should be free from large inert materials for a homogenous inoculum.

6.1.2 Determine the total dry solids and the volatile-solids content of the inoculum (refer to the APHA test method 2540D^[11], 2540E^[12]). The total dry solids content shall be found between 50 % and 55 % of the wet solids while the volatile solids content should be not more than 15 % of the wet solids or 30 % of the dry solids. Adjust the water content, if necessary, before the compost is used by adding water or gentle drying, e.g. by aerating the compost with dry air.

6.1.3 Prepare a mixture of inoculum with 5 parts of deionised water. Mix by shaking and measure the pH immediately. It should be between 7.0 and 9.0 (refer to test method ASTM D1293-95^[1]).

Note: For further characterisation of the inoculum, suitable parameters such as the content of total organic carbon, total nitrogen or fatty acids can optionally be determined at the beginning and the end of the test.

6.1.4 Check the activity of the inoculum by measuring the carbon dioxide evolution in the blank vessels. The inoculum in the blank shall produce between 50 mg and

150 mg of carbon dioxide per gram of volatile solids over the first 10 days of the test. If the production of carbon dioxide is too high, stabilise the compost by aeration for several days before using it in a new test. If the activity is too low, use another compost for the inoculum.

6.2 Preparation of test material and reference material

- 6.2.1 Determine the total organic carbon (TOC) content of the test material and the reference material (Section 4.2) using TOC analyser and report it, preferably, as grams of TOC per gram of total dry solids. Alternatively, if the materials do not contain inorganic carbon, it is possible to determine the total carbon content by elemental analysis or simply bomb combustion method. The test material shall have sufficient organic carbon to yield carbon dioxide in an amount suitable for the determination. Normally, a minimum of 50 g of total dry solids containing 20 g of TOC is required per vessel.
- 6.2.2 Use test material and the reference material in the form of granules, powder, film or simple shapes (dump-bells). The maximum surface area of any individual piece of test material shall be about 20 mm × 20 mm.

6.3 Start up of the test

- 6.3.1 Set up the following composting vessels (Section 3.1):
- i) three vessels for the test material
 - ii) one vessel for the reference material (Section 4.2)
 - iii) one vessel for the blank
- 6.3.2 The amount of test mixture containing inoculum and test material used in the test will depend on the quality of the test material and the size of the composting vessels. The ratio of the dry mass of the inoculum to the dry mass of the test material shall be about 6:1. Be sure that the same amount of compost is available in each vessel. If inert material is added to prevent the compost sticking together (see note in Section 6.1.1), it should not be counted in the above ratio.
- 6.3.3 Fill about three-quarters of the volume of the composting vessels with the test mixture. Leave sufficient headspace to allow manual shaking of the test mixture. Normally, use composting vessels with a volume of about 3 litres, weigh out an amount of inoculum containing 600 g of total dry solids and an amount of test material containing 100 ± 0.5 g of dry solids and mix well. The test mixture shall have the same water content (about 50 %) as the inoculum (see Section 6.1). Adjust the moisture content of the mixture, if required, by adding water or by

aerating with dry air. Introduce the mixture into the composting vessels.

Note: It is recommended that the ratio between organic carbon and nitrogen (C/N ratio) of the test mixture be optimised so as to ensure a good composting process. The C/N ratio for the test mixture should preferably be between 20 and 40. It may be adjusted with urea, if necessary. The organic carbon content can be calculated from the TOC of the inoculum and the test material. The total nitrogen content can be measured in a representative sample of the test mixture (refer to ASTM D 3590-89^[3] or ISO 5663^[13]).

- 6.3.4 Place the composting vessels in the test environment at $58\text{ °C} \pm 2\text{ °C}$ (see Section 5) using a water bath or any other suitable temperature controlling equipment (Section 3.7) and initiate aeration using water-saturated, carbon dioxide-free air. This can be produced by passing the air through bottle (Section 3.6) filled with sodium hydroxide solution (see Annex B).

Note: Normal air, rather than carbon dioxide-free air, can be used if the carbon dioxide concentration in the exhaust air is directly measured. In this case, measurement of the carbon dioxide concentration at the inlet and outlet of each test vessel is recommended. For correction, subtract the inlet concentration from the outlet concentration (which will be much higher).

- 6.3.5 Use an optimal high flow rate (0.5 ~ 1.0 L/min) to ensure that aerobic conditions are maintained during the test throughout each composting vessel. Check the air flow rate regularly at each outlet, e.g. by using wash-bottles to ensure that there are no leaks in any part of the system.

Note: If the flow rate is too high, the concentration of CO₂ is too low to be detected by GC. On the other hand, there will be not enough O₂ for aerobic decomposition if the flow rate is too low.

Regular measurements of the oxygen concentration in the exhaust air from the composting vessels will help maintain aerobic conditions. If this is done, the oxygen concentration should not be allowed to drop below about 6 %. Oxygen levels should be closely monitored during the first week, e.g. by measuring at least twice daily. Afterwards, the measurement frequency can be reduced. Adjust air flow rates as needed.

- 6.3.6 Handle the reference material in the same way as the test material. The blank vessels containing only inoculum should have the same amount of dry solids as the vessels with the test material.

6.4 Incubation period

- 6.4.1 Measure the amount of carbon dioxide evolved from the exhaust air of each composting vessel at intermediate time intervals directly using a gas chromatograph, a TOC analyser or an infrared analyser. Alternatively, measure the cumulative carbon dioxide evolved as dissolved inorganic carbon (DIC) after absorption in sodium hydroxide solution by titration method. In any case, measure the carbon dioxide evolved at least once a day at time intervals of about 24 hours for the first two weeks and twice per week later on until the plateau phase is reached.
- 6.4.2 Shake the composting vessels weekly to prevent extensive channeling and to ensure uniform attack of the microorganisms on the test material.
Note: It is recommended that the air-supply system and the carbon dioxide measurement system be disconnected before shaking the composting vessels.
- 6.4.3 Ensure that the humidity of the test mixture in the composting vessels is neither too high nor too low by visual observation. No free-standing water or clumps of material should be present. Very dry conditions are, typically, revealed by the absence of condensate in the headspace of the composting vessel. Moisture can also optionally be measured by suitable instruments. In this case, the moisture content should be kept at about 50 %. The desired moisture content is achieved by increasing flow rate or inserting copper sulphate crystal. A more drastic change in the moisture content can be obtained by adding water or drainage via the air inlet. The weekly shaking of the composting vessels is helpful in ensuring an even distribution of moisture. If adjustments are made, the carbon dioxide evolution should be monitored closely.
- 6.4.4 During the weekly agitation of the composting vessels and at the end of the test period, record any visual observations with regard to the appearance of the compost, such as structure, moisture content, colour, fungal development and disintegration of the test material.
- 6.4.5 Incubate the composting vessels for a period of 180 days. The incubation period can be shortened if the plateau phase is reached earlier or the percentage biodegradation of the test sample meets the criteria of $\geq 60\%$. The incubation period should not be longer than 180 days.
- 6.4.6 Measure the pH at regular intervals, as at the start of the test (see Section 6.1.3). Measure the pH by diluting the inoculum sample on a 5:1 w/w ratio of distilled water to compost inoculum or residue. Mix them by shaking manually and

measure immediately.

Note: If the pH is less than 7.0, biodegradation could be inhibited due to acidification of the compost by rapid degradation of an easily degradable test material. In this case, measurement of the volatile fatty acids spectrum is recommended to check for souring of the contents of the composting vessel (refer to ASTM D 2908-91^[2]). If more than 2 g of volatile fatty acids per kilogram of total dry solids has been formed, then the test must be regarded as invalid due to acidification and inhibition of the microbial activity. To prevent acidification, give more air or change the composition of the compost by reducing the food residue or add more compost to all vessels or repeat the test using, for example, less test material or more compost.

6.5 End of the test

6.5.1 Record any visual observations with regard to the appearance of the test material to determine its degree of disintegration.

Note: It is recommended that further investigations be carried out with any test material remaining, such as measuring physical properties, chemical analysis and photography.

7. Results

7.1 Calculation of the theoretical amount of carbon dioxide

Calculate the theoretical amount of carbon dioxide ThCO₂, in grams per vessel, which can be produced by the test material using equation (1):

$$\text{ThCO}_2 = C_{\text{TOT}} \times \frac{44}{12} \times W \quad (1)$$

where:

C_{TOT} is the proportion of total organic carbon in the total dry solids in the test material, in grams per gram

W is the weight of dry solid sample used in each vessel, in grams.

44 and 12 are the molecular mass of carbon dioxide and atomic mass of carbon respectively

7.2 Calculation of the percentage biodegradation

The amount of released carbon dioxide can be measured by indirect (e.g. titration) and direct analysis (e.g. GC and IR) method. A brief description on using GC to analyse the carbon dioxide content is attached in Annex E.

From the cumulative amounts of carbon dioxide released, calculate the percentage

biodegradation D_t of the test material for each measurement interval using equation (2):

$$D_t = \frac{(\text{CO}_2)_T - (\text{CO}_2)_B}{\text{ThCO}_2} \times 100 \% \quad (2)$$

where:

$(\text{CO}_2)_T$ is the cumulative amount of carbon dioxide evolved in each composting vessel containing test material, in grams per vessel

$(\text{CO}_2)_B$ is the cumulative amount of carbon dioxide evolved in the blank vessel, in grams per vessel

ThCO_2 is the theoretical amount of carbon dioxide which can be produced by the test material, in grams per vessel

If the differences between the individual results are less than 20 %, calculate the average percentage of biodegradation. If this is not the case, use the values for each composting vessel separately.

Use the same equation to calculate the degree of biodegradation of the reference material.

8. Expression of results

Samples claimed to be biodegradable should have ≥ 60 % of the total organic carbon (TOC content) being converted to carbon dioxide by the end of test period.

Compile tables (listed in Annex C to D) containing the measured and calculated data on the test material, the reference material and the blanks for each measurement. Plot the cumulative amount of carbon dioxide evolved for each composting vessel containing blank, test material and reference material as a function of time. Plot a biodegradation curve (percentage biodegradation as a function of time) for the test material and the reference material (see Annex A). Use mean values if the differences between the individual values are less than 20 %. If this is not the case, plot biodegradation curves for each composting vessel.

Read from the plateau phase of the biodegradation curve the mean degree of biodegradation and report it as final result.

If the test material consists of discrete pieces, describe qualitatively the degree of disintegration of the material.

9. Validity of results

The test is considered valid if:

- a) The inoculum in the blank has produced more than 50 mg but less than 150 mg of carbon dioxide per gram of volatile solids (mean value) after 10 days of incubation.

10. References

1. ASTM D1293-95, Standard Test Methods for pH of Water, ASTM, Philadelphia, PA, USA.
2. ASTM D2908-91, Standard Practice for Measuring Volatile Organic Matter in Water by Aqueous-Injection Gas Chromatography, ASTM, Philadelphia, PA, USA.
3. ASTM D3590-89 (1994), Standard Test Methods for Total Kjeldahl Nitrogen in Water, ASTM, Philadelphia, PA, USA.
4. ASTM D4129-98, Standard Test Method for Total and Organic Carbon in Water by High Temperature Oxidation and by Coulometric Detection, ASTM, Philadelphia, PA, USA.
5. ASTM D5338-98, standard Test Method for Determining Aerobic Biodegradation of Plastic Materials Under Controlled Composting Conditions, ASTM, Philadelphia, PA, USA.
6. ASTM D5509-94, Standard Practice for Exposing Plastics to a Simulated Compost Environment, ASTM, Philadelphia, PA, USA.
7. ASTM D6002-96, Standard Guide for Assessing the Compostability of Environmentally Degradable Plastics, ASTM, Philadelphia, PA, USA.
8. ASTM D6400-99, Standard Specification for Compostable Plastics, ASTM, Philadelphia, PA, USA.
9. ASTM E260-96, Standard Practice for Packed Column Gas Chromatography, ASTM, Philadelphia, PA, USA.
10. ASTM E355-96, Standard Practice for Gas Chromatography Terms and Relationships, ASTM, Philadelphia, PA, USA.
11. APHA 2540D, Total Suspended Solids Dried at 103 to 105°C, Standard Methods for the Examination of Water and Waste water, 17th Edition, 1989, American Public Health Association (APHA), 1740 Broadway, New York, USA.
12. APHA 2540E, Fixed and Volatile Solids Ignited at 550°C, “Standard Methods for the Examination of Water and Waste water”, 17th Edition, 1989, American Public Health Association (APHA), 1740 Broadway, New York, USA.
13. BS ISO 5663:1984, Water quality – Determination of Kjeldahl Nitrogen – Method after mineralisation with selenium, British Standard Institution, London.
14. BS ISO 8245: 1999, Water quality – Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC), British Standard Institution, London.
15. BS ISO 14855:1999, Determination of ultimate aerobic biodegradability and disintegration of plastic materials under controlled composting conditions – Method by analysis of evolved carbon dioxide, British Standard Institution, London.
16. Jian Yu, F. Zhang, ‘The Performance, Degradability and Material Property of Disposable Food Containers’, Department of Chemical Engineering, The Hong

Kong University of Science & Technology, (1999).

17. John K. O. Chung, 'Monitoring of Solid waste in Hong Kong 1998', Facilities Planning Group, Environmental Protection Department, HKSAR Government, 1999.

Annex A – Examples of graphical representation of carbon dioxide evolution and biodegradation curves

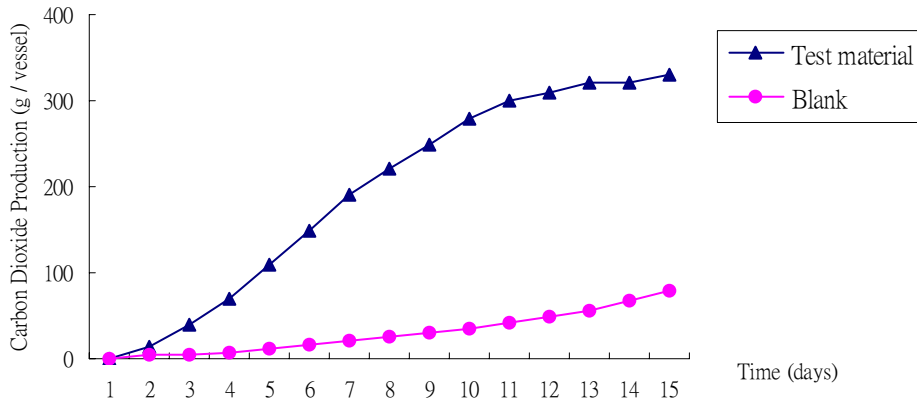
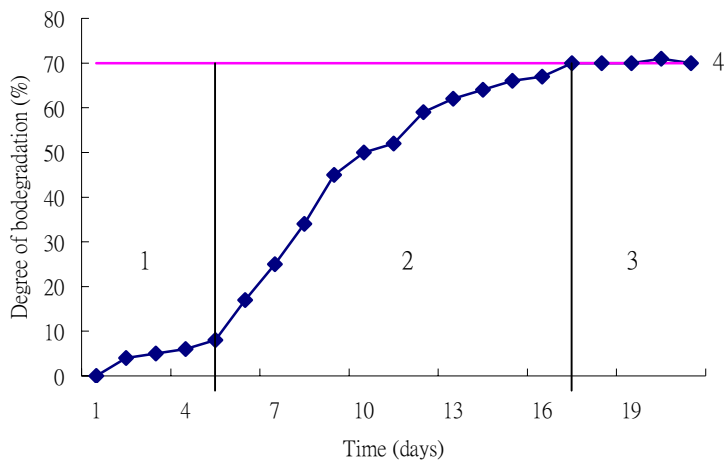


Figure F.1 – CO₂-evolution curve



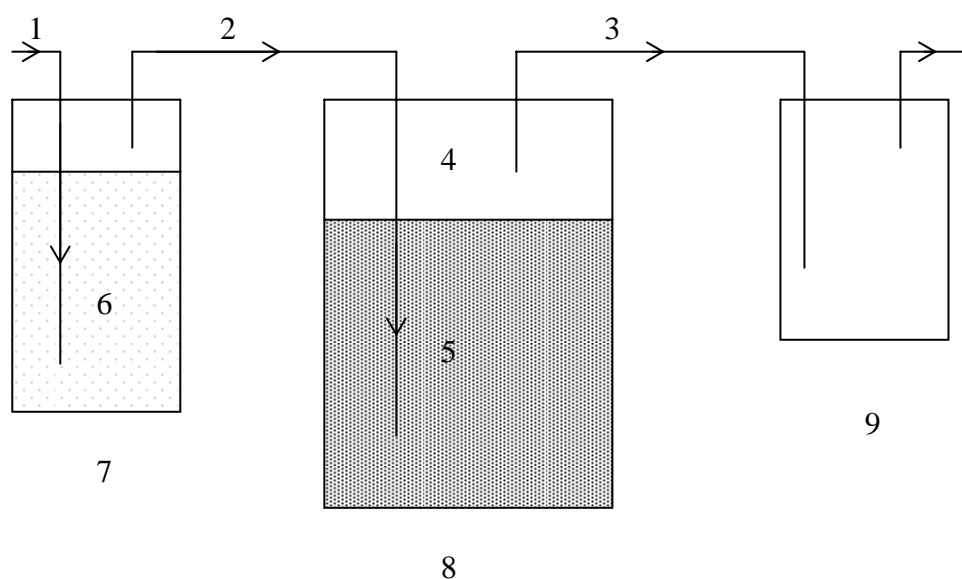
Key:

- 1 Lag phase
- 2 Biodegradation phase
- 3 Plateau phase
- 4 Mean degree of biodegradation

Figure F.2 – Biodegradation curve

Annex B - Principle of test system

Synthetic air free from carbon dioxide or compressed air is supplied at a constant low pressure. If compressed air is used, the carbon dioxide is removed by passing the air through a suitable carbon dioxide absorption system. If a solution of sodium hydroxide in water is used as the absorption system, the air is humidified at the same time. A second trap containing barium hydroxide can be used to indicate the absence of carbon dioxide (optional).



Where:

- | | |
|---|---------------------------------------|
| 1 | Air |
| 2 | CO ₂ -free air |
| 3 | Exhaust air |
| 4 | Headspace |
| 5 | Test mixture |
| 6 | NaOH solution |
| 7 | CO ₂ -removal system |
| 8 | Composting system |
| 9 | CO ₂ -determination system |

Note: The composting system should be air-tight and maintained in oven, water bath or any other appropriate temperature controlling equipment. If a water bath is used, a stirrer/submerged air pump should be installed to ensure thorough thermal mixing.

The air used to aerate the test mixture in the composting vessels should preferably be introduced at the bottom of the vessel and distributed as evenly as possible. If biodegradation takes place, carbon dioxide is produced and swept out in the exhaust air.

The CO₂ in the exhaust air can be measured directly, e.g. with a continuous infrared analyser or a gas chromatograph. In this case, exact metering or measurement of the gas flow is necessary. Depending on the measurement instrument, it may be necessary to remove water from the air, e.g. by cooling. If several composting vessels are connected up to a single measuring instrument, a suitable gas switch may be required.

The exhaust air from each composting vessel can be absorbed in a carbon dioxide trap containing e.g. 20 g/l solution of sodium hydroxide in water and the CO₂ measured as dissolved inorganic carbon (DIC), e.g. in a suitable TOC analyser (refer to ASTM D4129-98^[4] or ISO 8245^[14]) or titrate with hydrochloric acid to yield the amount of CO₂ produced.

Annex C - Biodegradability - Test Report

Test material: _____

Reference material: _____

Weight of test material: _____

Weight of reference material: _____

Origin of compost: _____

Age of compost: _____

Volume of test vessels: _____

Method of CO₂ determination: _____**Test results**

	Mean biodegradation calculate from CO₂ evolved %	Test duration /days	Observations
Test material			
Reference material			

Validity criteria

Mean CO₂ production in the blank vessels after 10 days in the range
50 mg to 150 mg CO₂/g volatile solids?

Yes No

Annex D - Biodegradability - Degree of biodegradation calculated from CO₂ evolution

Test material/reference material: _____

TOC: _____ g/g ThCO₂: _____ g/vessel

Date	Day	(CO ₂) _B g/vessel	(CO ₂) _{t1} g/vessel	(CO ₂) _{t2} g/vessel	(CO ₂) _{t3} g/vessel	D _{t1} %	D _{t2} %	D _{t3} %	D _{t, mean} %

Total dry sample weight: _____

(CO₂)_B = measured cumulative CO₂ production by blank(CO₂)_t = measured cumulative CO₂ production by test material or reference material at time t

Calculation:
$$D_t = \frac{(CO_2)_t - (CO_2)_B}{ThCO_2}$$

$$D_{t, mean} = \frac{D_{t1} + D_{t2} + D_{t3}}{3}$$

Annex E Calculation of the amount of carbon dioxide using GC method

Concentration of CO₂ measured in gas chromatography (GC) is usually expressed in ppm (parts per million) or ppb (parts per billion)

For manual sampling

Record flow rate of air Q (m³s⁻¹) in the test system (preferably at the CO₂ removal system)

Find sampling volume V_0 (m³) (usually volume of syringe)

Record concentration measurement C (ppm/ppb) indicated by GC spectrum

Calculate amount of CO₂ in sampling volume V_I (m³) = $C V_0$

Calculate mole of CO₂ in sampling volume N_I (mol) = V_I / V_A ($V_A = 2.241 \times 10^{-2} \text{ m}^3 \text{ mol}^{-1}$)

Hence calculate the mass of CO₂ in sampling volume m_I (g) = $N_I \times 44 \text{ gmol}^{-1}$

Find volume of air flowing through the system during the sampling interval V_{air} (m³) = $Q \times t$

Where t (s) is the time interval of sampling

Calculate ratio of sampling volume to volume of air $R = V_0 / V_{air}$

R is also the ratio of CO₂ sampled to total CO₂ emitted during the time interval

Therefore total mass of CO₂ emitted during sampling interval m_2 (g) = m_I/R